www.btvb.org

Official Journal of
SISET
Società Italiana per lo Studio dell'Emostasi e della Trombosi

BLEEDING, THROMBOSIS AND VASCULAR BIOLOGY

XXVII Congresso Nazionale SISET

Perugia | 2-5 novembre 2022

btvb

pagepress

Bleeding, Thrombosis and Vascular Biology

SISET - Società Italiana per lo Studio dell'Emostasi e Trombosi

The XXVII National Congress of the Italian Society on Hemostasis and Thrombosis (SISET), to be held in Perugia, November 2-5, 2022 is the first congress in presence since 2018.

SISET was founded 52 years ago and held its first Congress in Parma, in 1970.

This will be a four-day important scientific event the *motto* of which is "Hemostasis: the present and the future".

This conference will include plenary, educational and state of the art sessions delivered by the most distinguished Italian scientists in the field of hemostasis and thrombosis and by several prominent international scientists. They will present and discuss the most recent findings and the current knowledge on the pathophysiology, diagnostics and treatment of hemorrhagic and thrombotic disorders.

A series of lectures will deal with the new horizons in basic and clinical research including genomics, transcriptomics, artificial intelligence and new drug developments.

A large space will be dedicated to the copious original research produced by Italian scientists in the last year, with many sessions of oral communications and posters, presented by young clinicians and researchers.

This year, all these valuable scientific contributions are published in the current special issue of BTVB, the new, open access, international official scientific journal of SISET, with a prestigious international editorial board.

BTVB represents a high quality international forum for research on basic, translational and clinical aspects of hemostasis, thrombosis, vascular biology and related fields. We are all proud that the most recent Italian scientific research is now presented in preview in the journal.

Paolo Gresele
President of the SISET

XXVII Congresso Nazionale SISET

Perugia

2-5 novembre 2022

INDEX

Selected oral communications

OC001

D-DIMER AND REDUCED DOSE APIXABAN FOR EXTENDED TREATMENT AFTER UNPROVOKED VENOUS THROMBOEMBOLISM. THE APIDULCIS STUDY

C. Legnani[1], G. Palareti[1], D. Poli[2], W. Ageno[3], E. Antonucci[1], E. Bucherini[4], S. Testa[5], O. Paoletti[5], A. Chistolini[6], A. Serrao[6], I. Martinelli[7], P. Bucciarelli[7], A. Falanga[8], A. Tosetto[9], L. Sarti[10], D. Mastroiacovo[11], B. Cosmi[12], A. Visonà[13], R.C. Santoro[14], N. Zanatta[15], E. Grandone[16], L. Bertù[3], V. Pengo[17], L. Caiano[3], P. Prandoni[1], for the Apidulcis study group

[1]Fondazione Arianna Anticoagulazione, Bologna; [2]Malattie Aterotrombotiche, AOU Careggi, Firenze; [3]UOC Pronto Soccorso, Medicina d'Urgenza e Centro Trombosi ed Emostasi, ASST dei Sette Laghi, Varese; [4]SS Medicina Vascolare e Angiologia, Medicina 2, AUSL Romagna, Ravenna; [5]Centro Emostasi e Trombosi, UUOO Laboratorio Analisi chimico-cliniche e microbiologiche, ASST Cremona, Cremona; [6]Dipartimento di Medicina Traslazionale e di Precisione Sapienza Università di Roma, Roma; [7]Centro Emofilia e Trombosi ABianchi Bonomi, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano; [8]Divisione di Immunoematologia e Medicina Trasfusionale & Centro Emostasi e Trombosi, ASST Papa Giovanni XXIII, Bergamo; [9]UOC Ematologia, Centro Malattie Emorragiche e Trombotiche (CMET), AULSS 8Berica Ospedale SBortolo, Vicenza; [10]Centro per la diagnosi e la sorveglianza della malattia tromboembolica, UO Medicina interna d'urgenza, Azienda Ospedaliero Universitaria Policlinico di Modena, Ospedale Civile Baggiovara, Modena; [11]UOSD Angiologia e Diagnostica Vascolare, Dipartimento Medico, Ospedale Civile SS Filippo e Nicola, Avezzano (AQ); [12]UO di Angiologia e Malattie della Coagulazione, Azienda Ospedaliero Universitaria di Bologna, Policlinico SOrsola-Malpighi, Bologna; [13]UOC Angiologia, Dipatimento di Medicina Clinica, Azienda ULSS 2Marca Trevigiana, Ospedale San Giacomo Apostolo, Castelfranco Veneto (TV); [14]Centro Emostasi e Trombosi, Dipartimento Emato-Oncologico, Presidio Ospedaliero deLellis, AzOsp "Pugliese-Ciaccio", Catanzaro; [15]UOSD di Angiologia, Ospedale di Conegliano, Conegliano (TV); [16]UO Emostasi e Trombosi, Centro Ricerca Poliambulatorio Giovanni Paolo II, IRCCS Ospedale Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG); [17]Clinica Cardiologica, Azienda Ospedaliera di Padova, Padova, Italy

Background and Aims: D-dimer assay may be useful to stratify patients with venous thromboembolism (VTE) for the risk of recurrence. This approach was never evaluated after direct oral anticoagulants are available. With this multicenter, prospective cohort study we wanted to assess the usefulness of an algorithm incorporating serial D-dimer testing to avoid extended treatment if results are negative and to give apixaban 2.5 mg twice daily if positive. **Methods**: 732 outpatients, from 49 Italian centers, aged 18 to 74 years, anticoagulated for at least 12 months after a first unprovoked VTE, were included. Patients underwent D-dimer testing with commercial assays and pre-established cutoffs. If the first D-dimer, during anticoagulation, was negative anticoagulation was stopped and testing repeated after 15, 30 and 60 days. At first positive result, patients received apixaban for 18 months; those with negative results were left without anticoagulation, and all underwent follow-up for 18 months. **Results**: D-dimer resulted persistently negative in 286 patients (39.1%) and positive in the remaining (n=446, 60.9%). During the entire follow-up of 869 years, 26 primary outcomes occurred in the 732 subjects who correctly followed the study protocol (12 proximal DVTs with or without PE, 10 isolated PEs, and 4 MB events), for an incidence rate of 3.0% person-years (95%CI, 1.9-4.4). The study was prematurely stopped

when a planned interim analysis found an unacceptable (according to a pre-established stopping rule) higher prevalence of recurrences in patients who remained off anticoagulation compared to those receiving reduced dose apixaban. Twenty-one primary events (19 of efficacy) occurred in patients (14 males) with negative D-dimer (6.2% person-years, 95% CI, 3.9-9.5), and 5 (3 of efficacy; 4 males) in those receiving apixaban after positive D-dimer results (0.9% person-years; 95% CI, 0.3-2.2). The unadjusted HR for primary outcomes between the two groups of patients was 6.6 (95% CI, 2.7-19.8), and the adjusted HR was 8.2 (95% CI, 3.2-25.3). The rate of primary outcomes in patients with negative D-dimer was significantly lower (p=0.0362) when the index event was associated with weak risk factors and in young (aged <51 years) patients (p=0.0460). Though no other difference reached statistical significance, it seems useful to note that recurrences occurred less in women whose index event was associated with hormonal therapy. Nine events (incidence of 33.3% person-years) occurred in the 40 subjects who did not correctly follow the algorithm. **Conclusions**: In patients anticoagulated for >12 months after a first unprovoked VTE, the decision whether to further extend anticoagulation should not be based on D-dimer testing. Reduced dose apixaban is highly effective and safe against recurrences in patients with positive D-dimer.

OC002

THROMBOTIC EVENTS ASSOCIATED WITH THROMBOCYTOPENIA AFTER COVID-19 VACCINATION IN ITALY: A REPORT FROM THE SISET VAX COVID-19 DATABASE

M.P. Donadini[1], E. Tarasconi[1], L. Bertù[1], E. Antonucci[2], L. Contino[3], V. De Stefano[4], L. Russo[5], G. Tiscia[6], A. Tripodi[7], P. Gresele[8]

[1]Centro di ricerche per le malattie tromboemboliche e le terapie antitrombotiche, Dipartimento di Medicina e Chirurgia, Università dell'Insubria Varese; [2]Fondazione Arianna Anticoagulazione, Bologna; [3]S.S.DHaemostasis and Thrombosis Center, Internal Medicine Department, "SS Antonio e Biagio" Hospital, Alessandria; [4]Sezione di Ematologia, Università Cattolica, Fondazione Policlinico AGemelli IRCCS, Roma; [5]Division of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo; [6]U.O.SDipartimentale e di Ricerca Emostasi e Trombosi; Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo; [7]IRCCS Ca' Granda Maggiore Hospital, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano; [8]. University of Perugia, Department of Medicine and Surgery – Section of Internal and Cardiovascular Medicine, Italy

Background and Aims: Vaccine induced immune thrombotic thrombocytopenia (VITT) is a very rare and life-threatening complication occurring after adenoviral vector-based COVID-19 vaccination. However, thrombocytopenia may also arise from other causes than VITT in some patients diagnosed with a thrombotic event (TE). Aim of the study was to collect relevant information on thrombosis associated with thrombocytopenia occurring after COVID-19 vaccination. **Methods**: SISET VAX COVID-19 is an ongoing national, multicenter, prospective registry that was set up by the Italian Society on Thrombosis and Haemostasis, enrolling consecutive adult patients objectively diagnosed with any TE occurring within 30 days after receiving any COVID-19 vaccine dose, with or without thrombocytopenia. Primary objective was the characterization of TEs, in terms of type and extension of thrombosis (venous, arterial, microvascular), diagnosis, treatment and adverse events (thrombosis progression/early recurrence, major bleeding and death) during 30-day follow-up. The study was approved by the national ethics committee for studies on COVID-19 and by participating centers. Fondazione Arianna Anticoagulazione provided support for web-based dataset implementation, data management and approval procedures. **Results**: From June 3rd, 2021, to March 20th, 2022, 237 patients from 41 centers were included in the registry. This report focuses on 38 patients (16%) who had concomitant thrombocytopenia. The mean age was 58 years (standard deviation (SD) 15) and female/male proportion was 18/20. TE occurred after a mean of 11.7 days (SD 7.8) after the latest vaccine dose (30 after 1st or unique dose, 4 after 2nd dose, 4 after 3rd dose), that was represented by ChAdOx1 nCOV-19 (Astrazeneca) in 25 patients, Ad26.COV2.S (Janssen) in 2, BNT162b2 (Pfizer/BioNTech) in 8 and mRNA-1273 (Moderna) in 3. TEs involved a single venous site in 19 patients, a single arterial site in 6 patients and multiple sites in 13 patients (4 only venous multiple sites, 9 combined venous and arterial sites). In 22/38 patients (57.8%) a diagnosis of VITT was reported, supported in 17 patients by positive antibody assay against PF4/heparin (16 patients) and/or platelet activation assay (6 patients). All these patients had received Astrazeneca (21) or Janssen (1) vaccine; 2 patients had been exposed to heparin in the past. TE involved at least one unusual site in 16/22 patients. The median platelet count at nadir was 33 × 109/L (Interquartile range 22-50). Patients diagnosed with VITT were treated with anticoagulants (16 patients fondaparinux, 6 argatroban and 6 DOAC) and received immunoglobulin (21), high-dose steroids (17) and plasma exchange (2). (Table 1). During 30-day follow-up, 9 patients (40.9%) experienced thrombosis progression and 4 (18.2%) died. For the remaining 16/38 patients (42.1%), thrombocytopenia was attributed to other causes than VITT: immune thrombocytopenic purpura (2), disseminated intravascular coagulation (2), thrombotic thrombocytopenic purpura (1), systemic infection (1), myelodysplastic syndrome (1), iatrogenic (1), no specific causes(8). **Conclusions**: In this national registry on TEs occurring after vaccination against COVID-19, a diagnosis of VITT was extremely rare, was reported only after adenoviral vector-based COVID-19 vaccines in both sexes and was associated

with significant thrombosis progression and mortality. About 40% of reported TEs with thrombocytopenia was not attributable to VITT.

Table 1. Treatment of patients with VITT.

	No adverse short-term outcome (11)	Adverse short-term outcome (11)
Anticoagulants (any), n	11	11
Parenteral non-heparin anticoagulants, n	10	8
Iv immunoglobulin, n	11	10
High dose steroids, n	9	8
Plasma exchange, n	2	0

OC003

TRANSPLANTATION OF FETAL LIVER AND ADULT BONE MARROW CELLS FOR PHENOTYPIC CORRECTION OF HEMOPHILIA A

A. Cottonaro[1], S. Merlin[1], S. Akula[1], V. Kalandadze[1], S. Buzzi[1], E. Borroni[1], T. Garcia-Leal[2], L.J. Serrano[2], A. Liras[3], M.J. Sanchez[2], A. Follenzi[1]

[1]Department of Health Sciences, University of Piemonte Orientale, Novara, Italy; [2]Centro Andaluz de Biología del Desarrollo (CABD), CSIC, UPO, Seville, Spain; [3]Department of Genetic, Physiology and Microbiology, Biology School, Complutense University of Madrid, Spain

Background and Aims: We previously showed that adult-derived FVIII-producing cells, *i.e.* liver sinusoidal endothelial cells (LSECs) and hematopoietic stem cells (HSCs), can be used for the treatment of adult HA mice. However, after transplantation in busulfan-conditioned newborn mice, adult LSEC/HSC cannot efficiently engraft compared to murine fetal liver (FL) hemato/vascular cells from day 11-13 of gestation (E11-E13) that strongly reconstitute the hematopoietic compartment and showed multi-organ endothelial reconstitution potential and FVIII secretion ability. The aim is to investigate the ability of FL cells in repopulating the hemato/vascular compartment following transplantation in newborn HA mice without preconditioning. **Methods**: We transplanted adult BM or FLE11-E13 cells from GFP mice into newborn and adult HA mice pre-treated (+BU) or not (noBU) with busulfan. The engraftment level and FVIII activity was assessed starting from 4w after transplantation and followed-up for 18m. Bleeding phenotype correction was verified by hemarthrosis induction and bleeding assay. **Results**: In all BU-conditioned groups we observed long term (18m) stable GFP+ blood engraftment (>60%) with up to 16% FVIII activity following FL and BM cells transplantation. Interestingly, without pre-conditioning we observed lower but stable engraftment (≤12%) and consequent FVIII activity (1-4%) after FL cells transfer, while BM cells did not engraft in noBU mice. Tail bleed challenge and induced hemarthrosis experiments further confirmed the phenotypic correction in all mice receiving FL/BM+BU and in FLnoBU mice. Additionally, we observed an increased survival rate in corrected mice (80%) compared to HA controls (30%). None of the transplanted mice developed anti-FVIII or anti-GFP antibodies. **Conclusions**: Transplantation of HSCs from adult BM and FL may provide a novel, more stable and highly promising preclinical model for pediatric HA treatment. Our results show FL cells having higher engraftment ability, thus paving the way for studies aimed at maximizing the engraftment and proliferation of donor FVIII-corrective cells while minimizing/avoiding harming pre-conditioning regimens.

OC004

EXPRESSION AND LOCALIZATION OF COAGULATION FACTOR XI AND ITS RECEPTOR APOER2 IN HUMAN PLATELETS OF HEALTHY SUBJECTS AND ASSESSEMENT OF ITS CONTRIBUTION TO PLATELET PROCOAGULANT ACTIVITY

A. Becchetti[1], M. Brambilla[1], P. Canzano[1], M. Conti[1], M. Camera[1,2]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy; [2]Department of Pharmaceutical Sciences, Università degli Studi di Milano, Milan, Italy

Background and Aim: Coagulation Factor XI (FXI) is an important member of the contact pathway; its activity is critical for thrombus growth and stability, but plays a secondary role in haemostasis. It circulates in plasma and binds platelets through Apolipoprotein E Receptor 2 (ApoER2). The binding makes FXI more accessible for activation and induces signal amplification that promotes platelet activation. Furthermore, FXI is stored in platelets and can be secreted upon activation. Not all platelets have a role in haemostasis: indeed only 30% of platelets express both phosphatidylserine and Tissue Factor (TF) and bind coagulation factors. Thus, the aims of this study were 1) to clarify whether FXI and ApoER2 are present in all platelets or only in a subpopulation and 2) to assess intracellular localization through pharmacological approach; 3) to assess the contribution of platelet-associated FXI to thrombin generation (TG). **Methods**: Platelet-associated FXI and ApoER2 expression were evaluated in 25 healthy subjects by whole blood (WB) flow cytometry under basal conditions and upon stimulation. Platelet FXI associated thrombin generation (TG) was assessed by CAT assay. TG was evaluated in presence and absence of FXI-blocking antibody in FXI depleted plasma or in plasma pool CTI. **Results**: Only a platelet subpopulation expresses FXI on the surface (21.4±7.7%) and ApoER2 behaved similarly (26±10%), they colocalized in 15% of platelets. They are also intracellularly stored in 50% of platelets. Stimulation with different agonists results in a further significant increase in the number of positive platelets and in the amount of antigens/platelet (FXI: ADP, 29±15.7%; TRAP-6, 30.3±14.7%; U46619, 29.4±13.9% p=0.04 compared to resting condition; ApoER2; ADP,

45.1±15.3%, TRAP-6, 45.1±8.6%, U46619, 48.6±16.7% p=0.031 compared to resting condition). This upregulation was blunted when stimulation was performed in the presence of the ApoER2 inhibitor RAP and also in the presence Colchicine. This last finding highlights the localization of both FXI and ApoER2 in the open canalicular system (OCS). To assess the contribution of platelet-FXI to TG, we evaluated platelet TG in FXI-depleted plasma. TG was delayed compared to autologous plasma sample (Lagtime: autologous plasma:11±2min, FXI-depleted plasma17.3±1min; p=0.0022). In the presence of an anti-FXI antibody, Lagtime was further slowed indicating that platelet FXI is functionally active and plays a key role in sustaining the amplification phase in absence of plasma FXI (baseline: 17.3±1min, +αFXI Ab 31.5±1min; p=0.0313). Moreover, when TG was performed in plasma pool CTI, thus FXI could be activated only by TF-generated thrombin, Lagtime in presence of αFXI antibody was prolonged compared to basal condition (baseline: 23.7±6.6min, +αFXI Ab: 36.4±11min; p=0.05) supporting the evidence that FXI has a pivotal role in platelet TG. Similarly, inhibition of ApoER2 also results in a decreased TG. **Conclusions**: All together these data indicate that: 1) FXI and ApoER2 are expressed by a subpopulation of platelets and partially co-localized; 2) they are intracellularly stored in OCS; 3) platelet FXI is functionally active and plays a key role in sustaining the amplification phase of platelet TG.

OC005

SURGICAL PROCEDURES IN SUBJECTS WITH HEMOPHILIA A AND INHIBITORS TREATED WITH EMICIZUMAB: A SINGLE CENTRE EXPERIENCE

S. Linari[1], L. Pieri[1], C. Carulli[2], F. Demartis[1], R. Fjerza[1], M.C. Di Stefano[1], G. Castaman[1]

[1]Centre for Bleeding Disorders and Coagulation, Department of Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [2]Orthopaedic Clinic, Orthopedic Traumatologic Centre, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

Background and Aims: The management of surgery in persons with hemophilia A and inhibitors (PWHAi) has always been a major challenge and typically requires the use of activated prothrombin complex concentrates (aPCC) or recombinant activated FVII (rFVIIa). The recent widespread use of the bi-specific monoclonal antibody emicizumab for long-term prophylaxis in PWHAi has required a reassessment of the antihemorrhagic perioperative treatments with by-passing agents. The aim of this study is to report our experience in management of surgical procedures in PWHAi on emicizumab. **Methods**: Between January 2018 and April 2022, nine PWHA and high titre inhibitors underwent 31 surgical procedures (14 major surgeries, 17 minor surgeries). At the time of surgery inhibitor titer was >5BU in 5/9 patients and >500 BU in two of them. All major surgeries were planned to be managed with rFVIIa in addition to emicizumab prophylaxis. All patients were treated with 2-3 bolus of 90 µg/kg rFVIIa every 3 hours at the beginning of surgery till wound suturing, followed by FVIIa 90 µg/kg every 4 hrs during the first 2 days, every 6 hrs on days 3-4, every 8 hrs on days 5-7, twice a day on days 8-14 and daily (before the rehabilitation, when required) on days 15-20, then rFVIIa was discontinuated. Adjunctive intravenous tranexamic acid (TA) 1 g every 12 hours for 7 days was also administered. Thromboprophylaxis was not prescribed. Minor surgeries were managed without rFVIIa prophylaxis, except for 2 potentially complicated tooth extractions. A bolus of rFVIIa (90 µg/kg) was infused before procedure, followed by oral TA (1 g every 8 hours for 5 days). **Results**: No thrombotic events, thrombotic microangiopathy, significant changes of thrombophilia/microangiopathy markers were observed. Bleeding was as expected, apart from retroperitoneal toilet, which however was controlled without additional rFVIIa injections. **Conclusions**: Major surgery with a regimen of emicizumab and rFVIIa in PWHAi has been safely and efficaciously performed. This represents the first series of major surgery ever reported in this setting using an internal developed protocol. Minor surgeries can be safely performed without prophylaxis with rFVIIa. A multidisciplinary team and strict monitoring are recommended in management of surgical procedures in PWHAi treated with emicizumab.

Table 1.

Major surgery	N	Age of patient(yrs)	rFVIIa use	TA use	Post-surgical bleeding	Transfusion requirements	Days of hospitalization
Total knee replacement	1	56	yes	yes	no	no	12
Total knee replacement revision	1	49	yes	yes	no	no	15
Total hip replacement	1	59	yes	yes	no	2 RBCs	12
Total hip replacement revision	2	49/ 61	yes	yes	yes	4 RBCs in 49 years old patient	23 / 15
Amputation of a thigh	1	56	yes	yes	no	no	12
Muscle biopsy (thigh pseudotumor)	1	62	yes	yes	no	no	4
Thigh pseudotumor excision	2	62	yes	yes	no	no	15
Iliac wing biopsy	1	58	yes	yes	no	no	41
Lumbar arterial embolization to treat ileopsoas hematoma	1	58	yes	yes	no	no	41
Ureteral pig-tail position	1	58	yes	yes	no	no	41
Retroperitoneal hematoma toilet	1	56	yes	yes	yes	5 RBC	41
Partial nefrectomy	1	64	yes	yes	no	no	7
Minor surgery							
Dental extraction	2	32/56	yes	yes	no	no	0
Hemorroidal ligature	2	56/56	no	no	no	no	0
Hyaluronic acid joint injections	11	59/42	no	no	no	no	0
Cataract	2	58/69	no	no	no	no	0

OC006

THE HUMAN NPM1 MUTATION A IMPAIRS PROPLATELET FORMATION AND PLATELET FUNCTION

L. Bury[1], S. Momi[1], E. Falcinelli[1], E. Varasano[2], B. Falini[2], P. Sportoletti[2], P. Gresele[1]

[1]Section of Internal and Cardiovascular Medicine, Department of Medicine and Surgery, University of

Perugia, Perugia, Italy; [2]Section of Hematology and Clinical Immunology, Centro di Ricerche Emato-Oncologiche, Department of Medicine and Surgery, University of Perugia, Perugia, Italy

Background and Aims: Nucleophosmin (NPM1) is a multifunctional phosphoprotein which shuttles between the nucleus and the cytoplasm. NPM1 mutations, the most common of which is the type A variant, result in the aberrant localization of the protein in the cytoplasm and characterize a specific type of acute myeloid leukemia (AML) reported in 20-30% of cases. A conditional mouse model expressing the human NPM1 mutation A in hematopoietic cells previously created did not develop AML and showed reduction of platelet count, increased mean platelet volume and prolonged bleeding time. In this study we aimed to get a deeper insight into the role of NPM1 in megakaryocyte (MK) differentiation, platelet formation and function using the conditional mouse model carrying the NPM1 mutation A. **Methods**: MK were isolated by marrow cells and cultured in the presence of TPO. Proplatelet formation was evaluated by fluorescence microscopy. NPM1 was assessed in ultrapurified control platelets by PCR and Western blotting. Platelet life-span was assessed by flow cytometry upon injecting mice with an anti-GPIX mAb conjugated with DyLight 488. Platelet glycoprotein expression was assessed by flow cytometry. P-Selectin and JON/A binding upon platelet activation by convulxin (CVX) or thrombin were assessed by flow cytometry. Dense granule content and release were assessed by flow cytometry in platelets labelled with green fluorescent mepacrine. Clot retraction was assessed in PRP additioned with thrombin. Thrombus formation was evaluated *in vitro* on a collagen-coated surface under flow. Tail bleeding time and photochemically-induced femoral artery thrombosis were also studied *in vivo*. **Results**: Ultrapurified control human and mouse platelets express NPM1 protein but not NPM1 mRNA which is found in MK. The number of circulating platelets was significantly lower and platelets were larger in Npm1-mutA mice compared to control mice ($227\pm41x10^3/\mu l$ *vs* $696\pm76x10^3/\mu l$; mean platelet diameter 3.7±1 µm *vs* 2.9±0.6 µm;$p<0.05$). The percentage of MK differentiated from bone marrow cells was comparable in Npm1-mutA and control mice, however MK from Npm1-mutA mice showed strongly defective proplatelet formation. Surface expression of platelet glycoproteins $\alpha_{IIb}\beta_3$ and GPVI was reduced, expression of CD9 was increased in Npm1-mutA mice. A significant impairment of JON/A binding and P-selectin expression was found upon activation, showing defective integrin $\alpha_{IIb}\beta_3$ activation and α-granules release. Also, secretion of d-granules was impaired. Clot retraction was significantly reduced (~60%). Perfusion of Npm1-mutA blood over a collagen-coated surface revealed that the total surface area covered by platelets was significantly decreased compared with wild type mice, both at high and low shear rates. Tail bleeding time was slightly, but significantly, prolonged (610±208 sec *vs* controls 200±89 sec, $p<0.05$). Photochemical damage-induced femoral artery thrombosis was significantly delayed, occurring in 11.1±1.2min in control mice and in 30±0.8min in Npm1-mutA ($p<0.05$). **Conclusions**: Unexpectedly, given its recognized function of shuttle between nucleus and cytoplasm, platelets express NPM1, which plays a significant role in platelet formation and function. These findings suggest an NPM1-mediated regulation of genes coding for proteins involved in several aspects of platelet generation and function, that is lost in the presence of a mutant NPM1, and for which further studies are warranted.

OC007

PLATELETS TRANSFECTED WITH A KRASG12D-SILENCING SIRNA REDUCE PANCREATIC TUMOR MASS *IN VIVO* IN MICE

M. Malvestiti, G. Purgatorio, E. Cesari, S. Momi, P. Gresele

Sezione di Medicina Interna e Cardiovascolare, Dip. Medicina e Chirurgia, Università di Perugia, Italy

Background and Aims: Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest solid cancers with a dismal 7% 5-year survival rate and is projected to become the second leading cause of cancer-related mortality by the end of this decade. Recent exome sequencing studies have provided a detailed genetic profile of PDAC, with mutational activation of the KRAS oncogene found in ~95% of patients. In addition, there is compelling evidence that aberrant KRAS protein function is critical for PDAC growth and progression. The extraordinarily complex PDAC tumor microenvironment (TME) is believed to be a crucial cause of drug resistance and the major hurdle to overcome for any PDAC therapeutic. Moreover, no effective drugs targeting KRAS have entered clinical use so far. Small-interfering RNAs (siRNAs) harbor tremendous therapeutic potential because they offer highly-selective, reversible control of gene expression thus potentially silencing pathogenic oncogenes. However, utilization of siRNAs *in vivo* has been limited by their short circulating half-life due to degradation by serum nucleases and limited tumor uptake. A crosstalk between blood platelets and pancreatic cancer cells has been previously characterized, thus aim of our study was to verify whether platelets can efficiently deliver therapeutic siRNAs to PDAC TME *in vitro* and *in vivo*. **Methods**: We co-incubated human platelets transfected with KRASG12D siRNA with Panc-1 cells (PDAC cell line) for 24 and 48 hours in order to assess the target gene silencing in Panc-1 cells by q-PCR analysis. Then, human KRASG12D-siRNA-transfected platelets were intravenously infused in NSG (immunotolerant of human cells) mice 7 days after the subcutaneous injection of $1x10^6$ Human Panc-1 cells. A control group of mice infused with non-targeting scrambled-siRNA (NC) transfected platelets was

studied in parallel. A total of 6 siRNA-transfected platelet infusions were administered every 2 days starting from day 7 to day 20 in each mouse. $45x10^6$ human siRNA-transfected platelets were transfused each time. Human platelets were isolated from 6 different healthy donors. Tumor burden was quantified following animal sacrifice at day 20. During the experiment human platelet count in mouse blood, by measuring the fraction of circulating platelets expressing human CD41, and animal weight were constantly monitored. **Results**: *In vitro* a strong reduction of KRASG12D expression in Panc-1 cells (-92.86±0.11% *vs* control) after co-incubation for 48 hours with KRASG12D-siRNA-transfected platelets was obtained while a non-significant reduction was found after 24 hours. KRASG12D-siRNA-transfected platelets-infused mice showed a reduced tumor burden compared with the NC-transfected platelet-infused group, as shown by a statistically significant decrease of tumor volume and tumor weight (Figure 1). No significant differences in the two experimental groups were found regarding body weights and circulating human transfected platelets (3.18±0.83% *vs* 3.99±0.51% at day 11). **Conclusions**: Our data show that platelets transfected with a siRNA targeting KRASG12D and co-incubated with PDAC cells *in vitro* are able to strongly silence the tumor cell KRAS oncogene. The repeated transfusion of human siRNA-transfected platelets in mice in which PDAC was subcutaneously injected significantly reduced tumor progression. Platelet transfected with siRNAs targeting specific oncogenes may represent an innovative approach for chemotherapy resistant cancers.

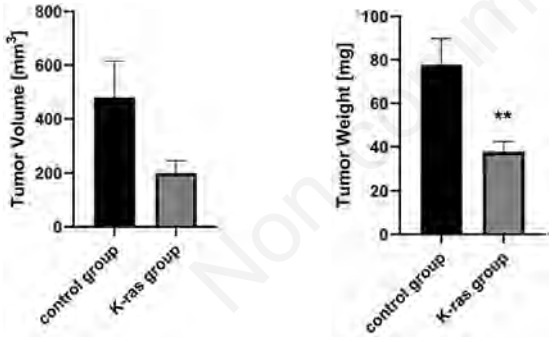


Figure 1.

OC008

OXIDATIVE-MEDIATED STRUCTURAL FIBRINOGEN MODIFICATIONS: A NEW LINK BETWEEN IMMUNITY AND ATHEROTHROMBOSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

M. Becatti[1], G. Emmi[2], F.R. Argento[1], E. Fini[1], A. Mannucci[1], A. Bettiol[2], M. Nicastro[3], A. Vaglio[4], N. Taddei[1], C. Fiorillo[1], D. Prisco[2]

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Firenze, Italy; [2]Department of Experimental and Clinical Medicine, University of Firenze, Firenze, Italy; [3]Clinical Immunology, Allergy and Advanced Biotechnologies Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy; [4]Department of Biomedical, Experimental and Clinical Sciences "Mario Serio," University of Firenze, Firenze, Italy; Nephrology and Dialysis Unit, Department of Pediatrics, Meyer Children's Hospital, Firenze, Italy

Background and Aims: Cardiovascular events are the major determinant of morbidity and mortality in systemic lupus erythematosus (SLE), particularly in patients with lupus nephritis. A number of studies suggested a pivotal role for oxidation in promoting vascular and renal damage in SLE, though the precise mechanisms are unclear. This study aimed therefore to investigate the potential role of oxidative-mediated structural/functional fibrinogen alterations in the pathogenesis of atherothrombosis in SLE. **Methods**: We enrolled 144 adult SLE patients and 90 matched controls. We assessed specific plasma oxidative stress markers and, in purified fibrinogen fractions obtained from SLE patients and control subjects, both fibrinogen oxidative modifications and its functional/structural parameters. In particular, thrombin-catalyzed fibrin polymerization and fibrin resistance to plasmin-induced lysis were evaluated. Fiber diameter and clot porosity were assessed by stimulated emission depletion (STED) super-resolution microscopy. Moreover, NADPH oxidase expression in renal biopsies from patients with active proliferative lupus nephritis was estimated by confocal microscopy. **Results**: Compared to controls, SLE patients exhibited an increased lymphocyte, monocyte and neutrophil reactive oxygen species (ROS) production, mainly due to neutrophil NADPH oxidase (42432±18998 *vs* 13234±4223 RFU/s, p=0.001), paralleled by an increase in plasma lipid peroxidation, a reduction in antioxidant defenses, and an enhanced fibrinogen oxidation (294 (256-329) *vs* 163 (121-184) RFU, p <0.0001). SLE patients also exhibited marked alterations in fibrinogen structure and clot architecture: this latter was mainly characterized by reduced porosity and by a tight fibrin network with filaments of decreased average size. Furthermore, significant alterations in fibrinogen functional features, namely thrombin-catalyzed fibrin polymerization and fibrin susceptibility to plasmin-induced lysis were more evident in SLE than in controls. Interestingly, fibrinogen structural and functional modifications significantly correlated with redox parameters, which in turn correlated with SLE disease activity. NADPH oxidase subunit p22phox was overexpressed in renal biopsies from patients with active proliferative lupus nephritis. **Conclusions**: collectively, these data suggest that ROS induce fibrinogen structural/functional modifications in SLE; this may represent a new pathogenetic mechanism underlying atherothrombosis in SLE.

OC009

LOW PLATELET COUNT HAS A STRONG WORSENING IMPACT ON OUTCOME IN PATIENTS WITH ACUTE CORONARY SYNDROMES: FROM THE START ANTIPLATELET REGISTRY.

G. Guglielmini[1], M. Del Pinto[2], P. Calabrò[3], P. Pignatelli[4], G. Patti[5], V. Pengo[6], E. Antonucci[7], P. Cirillo[8], T. Fierro[1], G. Palareti[7], R. Marcucci[9], P. Gresele[1] and the START Antiplatelet Registry Group

[1]Department of Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia; [2]Division of Cardiology, Perugia Hospital; [3]Department of Translational Medical Sciences University of Campania "Luigi Vanvitelli", Naples; [4]Department of Clinical, Internistic, Anesthesiologic and Cardiovascular Sciences, Sapienza University of Rome; [5]Department of Traslational Medicine, University of Eastern Piedmont, Maggiore della Carità Hospital, Novara; [6]Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova; [7]Arianna Anticoagulazione Foundation, Bologna; [8]Division of Cardiology, Department of Advanced Biomedical Sciences, "Federico II" University, Naples; [9]Department of Experimental and Clinical Medicine, University of Florence; Italy

Background and Aims: Some previous observations suggest that a low platelet count is associated with an increased risk of adverse outcome in patients with acute coronary syndromes (ACS). However, most of the information comes from post-hoc analyses of randomized clinical trials, many of which performed several years ago. **Objectives**: Our aim was to assess the impact of low platelet count on cardiovascular outcome and treatment decisions in patients hospitalized for ACS in a current real-life setting in Italy. **Methods**: START-ANTIPLATELET is a multicenter registry enrolling patients admitted to Italian coronary care units (CCU) for ACS and treated by PCI or medical therapy. Baseline clinical characteristics and treatment at discharge were recorded and follow-up reassessments were repeated at 6-months, one year and yearly thereafter. Low platelet count was defined as a platelet count at admission lower than 150k/µL. Major cardiovascular and cerebrovascular adverse events (MACCE) were defined as cardiovascular death, non-fatal MI, TIA and ischemic stroke, target vessel revascularization (TVR) and major arterial ischemic events. Net adverse cardiac and cerebrovascular events (NACE) were MACCE plus major bleeding. Data were analyzed using the IBM SPSS 25 software, categorical variables were compared using the Fisher exact test while continuous variables were compared with the t-test or with the Mann-Whitney U test, as appropriate. Clinical event rates were analyzed with the Kaplan-Meier method and compared with Logrank statistics. **Results**: Among 1890 enrolled patients presenting with an ACS, 1.9% had a platelet count lower than 100k/µl and 9.9% lower than 150k/µl. Women were 11.2% of patients with <150k/ul platelets and 17.2% of those with <100k/µl *versus* 28.2% of the population with normal platelets. Patients with <150k/µl platelets were older (71±10.9 *vs* 65.6±12.5 years, p<0.0001) and had higher incidence of non-valvular atrial fibrillation (17.1%*vs* 8.6%, p=0.02), previous ischemic stroke (22.5% *vs* 8.7%, p=0.01) and previous PCI (14.6% *vs* 7.8%, p=0.001) than patients with a normal platelet count. At hospitalization patients with <150k/µl had significantly lower hemoglobin levels (12.9±2.1 *vs* 13.6±1.9 g/dL, p<0.001), hematocrit (38.6±6% *vs* 40.8±5.4%, p<0.0001) and glomerular filtration rate (73.9±41.2 *vs* 86.5±32.9 ml/min, p=0.001). Clinical presentation and type of revascularization were similar in normal and low platelet count patients, however the latter were discharged more frequently on single antiplatelet therapy (15.2% *vs* 8.9%, p=0.028). After two years follow-up, MACCE-free survival time was significantly shorter in patients with platelet count <100k/µl (13.2 *vs* 21.0 months, p=0.005; HR=9.96, 95% CIs 2 to 49.1) as well as for patients with platelet count <150k/µl (17.7 *vs* 21.0 months, p=0.001; HR=2.9, 95% CIs 1.5 to 5.5). Also NACE occurred significantly more frequently in patients with low platelet count (<150k/µl=15.2% *vs* 8.4%, p=0.02). **Conclusions**: Low platelet count identifies a subgroup of ACS patients at significantly increased risk of adverse outcome. Patients admitted to CCU for ACS with a low platelet count should be treated with special care to prevent excess adverse outcome.

OC010

A FACTOR X VARIANT WITH ENGINEERED ACTIVATION PEPTIDE AS INNOVATIVE BY-PASSING AGENT FOR HAEMOPHILIA

E. Tonetto, R. Tarantino, M.F. Testa, L. Peretto, F. Bernardi, M. Pinotti, A. Branchini

Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

Background and Aims: In haemophilia with inhibitors, by-passing agents hold the promise of enhancing thrombin generation by increasing the conversion of FX to its activated form FXa. However, FXa, which should be the ideal candidate for therapeutic purposes, is rapidly inhibited and displays very short half-life *in vivo*. Hence, the availability of a by-passing FX molecule would be of great relevance, with a theoretically low immunogenicity because of the presence of circulating FX in patients. The aim of this study is to develop a novel hyper-activable engineered FX variant with by-passing activity ($FX^{HA\text{-}BP}$, patent application in progress) able to correct the defective intrinsic pathway in haemophilia plasma with inhibitors. **Methods**: FX activation peptide (AP) was

rationally engineered by site-directed mutagenesis of wild-type FX (FXwt) and the resulting engineered variants ($FX^{HA\text{-}BP\text{-}1\text{-}4}$) were transiently expressed in Human embryonic Kidney (HEK) 293 cells. Secreted FX protein levels were evaluated by ELISA. Activity was assessed by PT-based (FX-deficient plasma) and aPTT-based (haemophilia A plasma) assays. **Results**: The $FX^{HA\text{-}BP}$ variants were transiently transfected in HEK293T and the antigen levels assessed by ELISA. The $FX^{HA\text{-}BP}$ variants showed appreciable secreted levels, with higher values for $FX^{HA\text{-}BP3}$ (65% of FXwt) and $FX^{HA\text{-}BP2}$ (40%) than $FX^{HA\text{-}BP1}$ and $FX^{HA\text{-}BP4}$ (20%). Intriguingly, all FX variants efficiently shortened coagulation times in PT-based assays in FX-deficient plasma and showed a specific activity (activity/antigen ratio) slightly increased (~1.6, $FX^{HA\text{-}BP2}$ and $FX^{HA\text{-}BP3}$; ~2.5, $FX^{HA\text{-}BP1}$ and $FX^{HA\text{-}BP4}$), as compared to FXwt. Upon intrinsic activation in aPTT-based assays, addition of $FX^{HA\text{-}BP1\text{-}3}$ variants to haemophilia A plasma resulted in a graded shortening of coagulation times ($FX^{HA\text{-}BP1}$, 155±1 sec; $FX^{HA\text{-}BP2}$, 150±2 sec; $FX^{HA\text{-}BP3}$, 151±5 sec), while addition of equal amounts of FXwt resulted in negligible effects (165 sec) as also observed for the negative control (medium from untransfected cells) used as baseline. Noticeably, the $FX^{HA\text{-}BP4}$ variant efficiently restored coagulation, with coagulation times (124±3 sec) significantly different from those of the other $FX^{HA\text{-}BP1\text{-}3}$ variants as well as of control FXwt and baseline. These data demonstrated the by-passing activity of the $FX^{HA\text{-}BP4}$ variant even at the relatively low concentrations obtained after transient expression. **Conclusions**: The approach of rational engineering on FX AP resulted in FX variants with improved functional properties, with the relevant ability of by-passing the defective intrinsic pathway in haemophilia plasma. In particular, the $FX^{HA\text{-}BP4}$ variant, the most promising by-passing molecule, will be further engineered to further improve its by-passing activity as well as its half-life, with the aim of developing an extended half-life by-passing variant that might represent a novel therapeutic tool for haemophilia with inhibitors.

OC011

CONTACT PATHWAY ACTIVATION AND RISK OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH PANCREATIC CANCER: THE PROSPECTIVE SENECA STUDY

E. Campello[1], F.T.M. Bosch[2], C. Bulato[1], S. Gavasso[1], M.W. Henderson[3], C. Bassi[4], N. Martinelli[5], N. van Es[2], A. Ilich[3], H.R. Büller[2], N. Key[3], P. Simioni[1]

[1]. Department of Medicine, University of Padova, Italy; [2]. Department of Vascular Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands; [3]. UNC Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [4]. Unit of General and Pancreatic Surgery, G.B. Rossi Hospital, Verona, Italy; [5]. Department of Medicine, University of Verona, Italy

Background and Aims: Patients with pancreatic cancer are at very high risk of venous thromboembolism (VTE). Activation of the contact system and the intrinsic pathway may play an important role, but their exact contribution to hypercoagulability in pancreatic cancer remains uncertain. We aim to assess the association between intrinsic pathway activation as measured by complexes of the contact system enzymes with their serpins and VTE risk in patients with pancreatic cancer. **Methods**: Patients with advanced or metastatic pancreatic cancer who were scheduled for palliative chemotherapy were included. Blood was drawn at baseline and patients were followed for a maximum of 180 days. The main outcome was VTE, composing of deep-vein thrombosis of upper or lower extremities, pulmonary embolism, and splanchnic vein thrombosis. Contact system complexes (PKa:C1, FXIIa:C1, FXIa:C1, FXIa:α1at, FXIa:AT, and FIXa:AT) were measured with ELISA and as well as known biomarkers for VTE. Discrimination of complexes and biomarkers was assessed by areas under the receiver operating characteristic curve (AUROC) and subdistribution hazard ratios (SHR), with death not related to VTE as a competing risk. **Results**: Ninety-three patients with complete 6-month follow-up were included. The mean age was 66 years (7.8 SD) and 50 (53.8%) were male. Sixteen (17.2%) patients developed VTE. The AUROC was highest for FXIa:α1at (AUC 0.75; 95% CI 0.62-0.87) (Table 1). When contrasting the upper quartile with lower quartiles, the following biomarkers of the intrinsic pathway were significantly associated with VTE in univariable analyses: FXIa:α1at (SHR 3.53; 95% CI 1.34-9.30) and FXIa:AT (SHR 3.62; 95% CI 1.38-9.51). Log-transformed FXIa:α1at (SHR 1.51; 95% CI 1.05-2.18) was also significantly associated with VTE when assessed as a continuous variable (Table 1). **Conclusions**: Markers of contact pathway activation, such as FXIa:α1at and FXIa:AT complexes, were strongly associated with VTE risk in patients with pancreatic cancer. These results help to provide insight in the mechanism of thrombosis in pancreatic cancer and may aid in refining VTE prediction.

Table 1. Competing risk analysis for all venous thromboembolic events versus no events during six months of follow-up, with death as a competing risk.

	VTE* N=16	No events* N=77	AUROC (95%)	SHR (95%CI) Highest quartile†	SHR (95%CI) per log increase
D-dimer (ng/ml)	1556 (807 – 2992)	616 (454 – 1068)	0.69 (0.54 – 0.85)	**5.13 (1.94 – 13.6)**	**1.86 (1.22 – 2.84)**
TFPI (ng/ml)	80.0 (58.8 – 92.0)	78.0 (65.0 – 94.0)	0.49 (0.32 – 0.64)	0.71 (0.25 – 2.53)	0.66 (0.22 – 2.02)
TAT (ng/ml)	1.85 (1.55 – 2.42)	1.70 (1.50 – 2.20)	0.56 (0.39 – 0.74)	1.45 (0.51 – 4.14)	1.89 (0.50 – 7.16)
PAP (ng/l)	37.2 (35.2 – 39.8)	35.9 (34.5 – 38.2)	0.59 (0.43 – 0.75)	1.91 (0.71 – 5.14)	5.15 (0.48 – 55.7)
F1+2 (nM)	0.94 (0.68 – 0.96)	0.94 (0.89 – 0.97)	0.48 (0.33 – 0.63)	0.42 (0.10 – 1.83)	1.02 (0.02 – 55.2)
PKa:C1 (pM)	23.5 (18.2 – 28.2)	21.1 (16.9 – 27.3)	0.53 (0.37 – 0.70)	1.39 (0.50 – 3.92)	0.98 (0.40 – 2.16)
F12:C1 (pM)	6.02 (4.44 – 7.05)	5.22 (4.32 – 6.48)	0.59 (0.44 – 0.74)	1.39 (0.50 – 3.92)	1.08 (0.63 – 1.73)
F11:C1 (pM)	0.63 (0.53 – 0.85)	0.57 (0.38 – 0.85)	0.58 (0.43 – 0.73)	1.00 (0.31 – 3.04)	1.43 (0.83 – 2.47)
F11:α1at (pM)	1.02 (0.84 – 1.58)	0.74 (0.58 – 0.94)	0.75 (0.62 – 0.87)	**3.53 (1.34 – 9.3)**	**1.51 (1.05 – 2.18)**
F9:AT (pM)	0.73 (0.66 – 0.93)	0.64 (0.52 – 0.76)	0.65 (0.51 – 0.79)	1.99 (0.73 – 5.44)	1.26 (0.79 – 2.01)
F11:AT (pM)	0.59 (0.45 – 0.75)	0.46 (0.37 – 0.59)	0.66 (0.52 – 0.81)	**3.62 (1.38 – 9.51)**	1.48 (0.89 – 2.49)
MVTF (nM)	0.01 (0.01 – 0.02)	0.01 (0.00 – 0.01)	0.54 (0.40 – 0.68)	0.68 (0.25 – 1.85)	1

*Data in median and interquartile range for all variables. †Subdistribution hazard ratio for highest quartile versus three lower quartiles combined. ‡Unable to log transform zero values. Abbreviations VTE: venous thromboembolism; AUC: area under the curve; SHR: subdistribution hazard ratio; TFPI: tissue factor pathway inhibitor; TAT: Thrombin–antithrombin complex; PAP: plasmin-alpha 2-antiplasmin complex; F1+2: prothrombin fragment F1+2

OC012

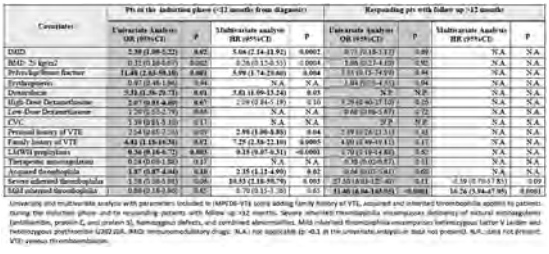
IMPACT OF INHERITED AND ACQUIRED THROMBOPHILIA ON THE THROMBOTIC RISK IN MULTIPLE MYELOMA

F. Di Landro, T. Za, F. Bartolomei, A. Ciminello, S. Betti, S. Ceglie, F. Ramundo, P. Arcuri, L. Fianchi, L. Pagano, E. Rossi, V. De Stefano

Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica, Fondazione Policlinico AGemelli IRCCS, Roma, Italy

Background and Aims: Venous thromboembolism (VTE) is a common complication of multiple myeloma (MM). The IMPEDE VTE score is a validated risk assessment model for prophylaxis; however, despite antithrombotic prophylaxis, VTE incidence in MM pts remains high, prompting the need for a more tailored antithrombotic strategy. In this monocentric retrospective study, we analyzed the impact on VTE risk of the IMPEDE VTE variables; moreover, we included family history of VTE and inherited or acquired thrombophilia as additional risk factors. **Methods**: We included in the study only MM pts investigated for inherited and acquired thrombophilia. We considered major VTE as the primary outcome: deep vein thrombosis, pulmonary embolism, and cerebral and splanchnic vein thrombosis. We analyzed the parameters included in the IMPEDE VTE score, including as additional risk factors for VTE a family history of VTE, inherited thrombophilia (*i.e.*, antithrombin, protein C and S deficiencies, factor V Leiden [FVL], and prothrombin G20210A mutation [FII GA]), and acquired thrombophilic alterations (*i.e.*, hyperhomocysteinemia, antiphospholipids, and transitory disease-related deficiency of natural anticoagulants). We included as candidate predictors for VTE in the multivariate Cox proportional-hazards regression model only the covariates with a significance level of $p<0.1$ at the univariate analysis. **Results**: We investigated 292 MM pts (M/F 163/129, median age 65 years, range 28-89). During the induction phase (first 12 months from diagnosis), 182 pts received immunomodulatory agents (IMIDs). We recorded 41 VTE events. Out of the IMPEDE VTE parameters, no patient was of Asian ethnicity. We failed to confirm the role of body mass index ≥25 kg/m2, central venous catheter, low-dose Dexamethasone (DEX), erythropoietin stimulating agent and therapeutic anticoagulation as risk factors for VTE; we confirmed the prognostic impact of IMIDs, pelvic/hip/femur fractures, doxorubicin, high dose DEX, personal history of VTE and antithrombotic prophylaxis. Family history of VTE and acquired thrombophilia were significant risk factors for VTE; inherited thrombophilia did not enhance the risk of VTE (Table 1). Then we considered the pts with an observation time >12 months (N=236): a personal history of VTE and mild inherited thrombophilia (*i.e.*, heterozygous FVL and FII G/A) were independent risk factors for VTE with an hazard ratio (HR) HR of 4.5 and 5.7, respectively. From this population, we further analyzed only the pts with responding MM (N=170), and we recorded 9 VTE events; mild thrombophilia was confirmed as a risk factor for VTE (HR 16.2) (Table 1). **Conclusions**: The risk of thrombosis is enhanced during the first year from diagnosis of MM by the following factors: IMIDs, pelvic/hip/femur fractures, doxorubicin, high dose DEX, familial and personal history of VTE, and acquired thrombophilia. After the first year from diagnosis, heterozygous FVL and FII G/A and personal history of VTE were independent risk factors for VTE. Considering only pts with responding MM, mild inherited thrombophilia was confirmed to be a strong risk factor for thrombosis. We suggest that during the first phase of active disease, the moderate risk associated with inherited thrombophilia was obscured by the stronger clinical or biochemical risk factors dependent on the MM burden disease. Conversely, the constitutional risk due to inherited factors becomes more critical during a stable phase of the disease.

Table 1.



Oral communications

OC013

DEFECTIVE NITRIC OXIDE PRODUCTION AND INCREASED REACTIVE OXIGEN SPECIES GENERATION BY PLATELETS FROM COVID-19 PATIENTS

E. Petito[1], G. Guglielmini[1], E. De Robertis[2], E. Giglio[1], C. Becattini[1], E. Falcinelli[1], L. Franco[1], L. Saccarelli[2], F. Gori[3], G. Vaudo[4], V. Cerotto[5], U. Paliani[6], P. Gresele[1]

[1]Section of Internal and Cardiovascular Medicine; [2]Division of Anaesthesia, Analgesia, and Intensive Care, [3]Section of Anesthesia, Intensive Care, and Pain Medicine, Department of Medicine and Surgery, University of Perugia, Perugia, Italy; [4]Unit of Internal Medicine, Terni University Hospital; [5]Section of Anesthesia, Intensive Care and Pain Medicine, Department of Emergency and Urgency; [6]Division of Internal Medicine, ASL [1]Umbria, 06012, Città di Castello, Italy

Background and Aims: Oxidative stress, an imbalance between reactive oxygen/nitrogen species (ROS/RNS) and the antioxidant potential, contributes along with the cytokine storm to COVID-19 pathogenesis (*PMID: 34836751*). A hyperactivated platelet phenotype is strongly involved in the thrombotic complications of COVID-19 (*PMID: 35027697*). ROS and NO produced by activated platelets play a pivotal and opposite role in regulating platelet function: ROS potentiate (*PMID: 32660144*), while NO suppresses platelet activation (*PMID: 17901370*). To our knowledge no studies have assessed platelet ROS and NO production in COVID-19. Our aim was to analyze platelet ROS and NO production in COVID-19 patients, comparing it to healthy controls and critically ill non COVID-19 patients. **Methods**: Venous blood was collected from 24 COVID-19 patients hospitalized in ordinary wards (non ICU), 24 age- and sex-matched healthy subjects (HS), 11 COVID-19 patients hospitalized in intensive care unit (ICU), and 21 age- and sex-matched patients hospitalized in ICU not for COVID-19 (non COVID-19 ICU). NO and ROS generation by platelets was assessed by flow cytometry using two specific fluorescent probes, DAF-FM diacetate (*PMID: 34233447*) and DCF (*PMID: 26197385*), normalizing the fluorescence intensity for platelet number. **Results**: Platelets from HS and non COVID-19 ICU patients showed a dose-dependent and comparable increase of NO production in response to collagen (Figure 1A). On the contrary, platelets from COVID-19 patients, both ICU and non ICU, did not show any increase of NO production in response to increasing collagen concentrations, showing an almost complete suppression of platelet NO production (Figure 1A). This suppression appears to be selective to COVID-19 patients, because NO generation by platelets from critically ill patients hospitalized in ICU not for COVID-19 did not differ from that of HS (Figure 1A). Moreover, platelets from COVID-19 patients showed a significant increase in ROS production compared with HS platelets but this increase was also evident in non COVID-19 ICU patients (Figure 1B). **Conclusions**: Our study shows a strikingly defective NO production by platelets from COVID-19 patients, independent from disease severity. Platelets from non COVID-19 ICU patients instead released NO normally, and significantly more than platelets from COVID-19 patients, thus showing a selective SARS-CoV-2 infection-related defect of platelet NO production. We also show an increase in ROS production by COVID-19 platelets, evident with the lowest collagen dose, indicating that COVID-19 platelets are in a hyperreactive state independently from disease severity. However, for this parameter platelet hyperreactivity was also evident in platelets of patients hospitalized in ICU for reasons different from COVID-19, suggesting that the acute inflammatory reaction of the critically ill patient associates with enhanced platelet ROS production. Platelets may play a central role in the previously reported decreased NO bioavailability in COVID-19 (PMID: 34020311). Therapeutic restoration of platelet

NO production may represent a target for treatments potentially leading to better clinical outcome in COVID-19 patients.

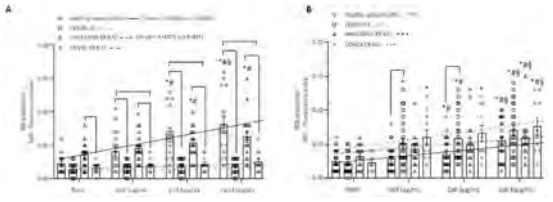


Figure 1. Nitric oxide (A) and ROS production (B) by resting and activated platelets. (*p<0.05 vs their own basal; # p<0.05 vs their own Coll 1 µg/mL; § p<0.05 vs their own Coll 5 µg/mL). Lines report slopes of the concentration-response curves.

OC014

TOLL-LIKE RECEPTOR 4-DEPENDENT AND INDEPENDENT PLATELET-DEPENDENT THROMBOSIS IN SARS-COV-2 INFECTION

V. Cammisotto[1], R. Carnevale[2,3], S. Bartimoccia[2], C. Nocella[1], V. Castellani[4], L. Loffredo[1], S. Sciarretta[2,6], A. Coluccia[5], S. Romano[5], G. Ceccarelli[7], A. Oliva[7], M. Venditti[7], F. Pugliese[4], C. Mastroianni[7], O. Turriziani[8], M. Leopizzi[1], G. D'Amati[9], P. Pignatelli[1,3], F. Violi[1,3]

[1]Department of Clinical Internal, Anaesthesiological and Cardiovascular Sciences, Sapienza University of Rome; [2]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome; [3]Mediterranea Cardiocentro-Napoli; [4]Department of General Surgery and Surgical Speciality Paride Stefanini, Sapienza University of Rome; [5]Laboratory affiliated with the Institute Pasteur Italy – Cenci Bolognetti Foundation, Department of Drug Chemistry and Technologies, Sapienza University of Rome; [6]IRCCS Neuromed; [7]Department of Public Health and Infectious Diseases, Sapienza University of Rome; [8]Department of Molecular Medicine, Laboratory of Virology, Sapienza University of Rome; [9]Department of Radiological, Oncological and Pathological Sciences, Sapienza University of Rome, Italy

Background and Aims: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with an increased risk of venous and arterial thrombosis but the underlying mechanism is still unclear. **Methods**: We performed a cross-sectional analysis of platelet function in 25 SARS-CoV-2 and 10 healthy subjects (HS) by measuring Nox2-derived oxidative stress and thromboxane (Tx) B2 and investigated if administration of monoclonal antibodies against the Spike (S) protein of SARS-CoV-2 affects platelet activation. Furthermore, we investigated *in vitro* if the S protein of SARS-CoV-2 or plasma from SARS-CoV-2 enhanced platelet activation (PA). **Results**: *Ex vivo* studies showed enhanced platelet Nox2-derived oxidative stress and TxB2 biosynthesis and under laminar flow platelet-dependent thrombus growth in SARS-CoV-2 compared to controls; both effects were lowered by Nox2 and Toll-like receptor 4 (TLR4) inhibitors. Two hours after administration of monoclonal antibodies a significant inhibition of PA was observed in SARS-CoV-2 patients compared to untreated ones. A docking simulation analysis suggested that TLR4 binds to S protein via three receptor-binding domains; furthermore, immunoprecipitation and immunofluorescence showed S protein-TLR4 co-localization in platelets from SARS-CoV-2. Plasma from SARS-CoV-2 patients enhanced PA and Nox2-related oxidative stress, an effect blunted by TNFα inhibitor; this effect was recapitulated by an *in vitro* study documenting that TNFα alone promoted PA and amplified the platelet response to S protein via p47phox up-regulation. **Conclusions**: The study identifies two TLR4-dependent and independent pathways promoting platelet-dependent thrombus growth and suggests inhibition of TLR4 or p47phox as a tool to counteract thrombosis in SARS-CoV-2.

OC015

TF-POSITIVE SMALL EXTRACELLULAR VESICLES CONTRIBUTE TO THE PROCOAGULANT PHENOTYPE IN COVID-19 PATIENTS

P. Canzano[1], M. Brambilla[1], R. Frigerio[2], M. Cretich[2], A. Becchetti[1], M. Conti[1], M. Camera[1,3]

[1]Centro Cardiologico Monzino, IRCCS; [2]National Research Council of Italy (SCITEC-CNR); [3]Deptof Pharmaceutical Sciences, Università degli Studi di Milano, Italy

Background and Aims: Coronavirus-2 (SARS-CoV-2) infection causes a sustained prothrombotic state driven by a massive Tissue Factor (TF) expression in circulating platelets, leukocytes and microvesicles. Whether also circulating small extracellular vesicles (sEVs), in addition to large microvesicles (MVs), can contribute to this hypercoagulable scenario through TF expression is not yet known, mainly due to methodological issues in detecting and sizing the smallest vesicles. Therefore, aim of this study was to characterize TF expression and activity in circulating sEVs, compared to that of MVs, of COVID-19 patients during acute phase infection and after symptom remission. **Methods**: M*Vs* and sE*Vs* were isolated by plasma differential centrifugation from 10 COVID-19 patients enrolled at acute phase infection (T0) and at six-month-follow-up (T1). Ten healthy subjects (HS) were analyzed as controls. sE*Vs* were counted by Nano Tracking Assay. In sE*Vs* TF expression was analyzed within CD9/CD63/CD81^{pos} events by imaging flow cytometry (IFC) and ExoView™ microarray, while TF activity by FXa generation assay. TF expression and activity in M*Vs* were evaluated for comparison. **Results**: By IFC analysis COVID-19 patients at T0 have about 1.5- and 4-fold higher number of TF^{pos}-sE*Vs*

and -MVs, respectively, compared to HS, with a trend toward reduction to physiological levels at T1. By microarray analysis sE*Vs* behaved similarly (36±12 and 25±10 TF^{pos}-spots at T0 and T1, respectively; p=0.0281). sEVs-associated TF is functionally active thus able to partially support FXa formation as sE*Vs* preincubation with TF-neutralizing antibody reduced FXa generation by about 30%. However, although sE*Vs* number is significantly higher compared to that measured for M*Vs* (~600-fold in HS), functional activity of sEV is one-third lower compared to that of MVs. **Conclusions**: These data suggest that, in COVID-19 patients, the altered procoagulant phenotype could also be supported by TF carried by sEVs, although their functional activity is significantly lower than that of MVs.

OC016

FLOW CYTOMETRY AND MICROSCOPY ASSAY TO IDENTIFY PLATELET ACTIVATING ANTIBODIES AFTER CHADOX1 NCOV-19 VACCINE

C.M. Radu, C. Bulato, E. Campello, S. Gavasso, L. Spiezia, D. Tormene, P. Simioni

Department of Medicine, Thrombotic and Hemorrhagic Diseases Unit, University of Padua, Italy

Introduction: Vaccination against severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) is the most important countermeasure to fight the ongoing Covid-19 pandemic. The vaccine ChAdOx1 nCov-19 (AstraZeneca) is a recombinant adenoviral vector encoding for the spike glycoprotein of SARS-Cov-2. Vaccine-induced thrombotic thrombocytopenia (VITT) is a fatal coagulation disorder after ChAdOx1 nCov-19 vaccination, characterized by deep venous thrombosis, pulmonary embolism and cerebral venous sinus thrombosis in combination with thrombocytopenia. The cause of VITT is due to platelet activating antibodies against platelet factor 4 (PF4) potentially complexed with an unknown co-factor making this protein immunogenic. These features make VITT to clinically mimics autoimmune heparin-induced thrombocytopenia (HIT). **Aims**: To evaluate by CytoFLEX SRT, ImageStream flow cytometers and confocal microscopy the presence of pathogenic antibodies directed against platelets in a patient with fatal VITT and cerebral venous thrombosis after adenovirus vaccine ChAdOx1 nCOV-19. **Methods**: The laboratory results are show a patient platelet count of 20.000 per cubic millimeter and the presence of anti-PF4 antibodies detected by enzyme-linked immunosorbent assay. The patient had not previously been exposed to heparin. We analyzed the presence of platelet activating antibodies by CytoFLEX SRT flow cytometry. Fresh platelet rich plasma (PRP) from healthy donors was incubated with VITT patient serum without and in the presence of low and high doses of heparin to inhibit PF4-dependet reaction. Samples were stained using the following antibodies: anti-CD62P-selectin-PE and anti-Annexin-V-APC as platelet activation markers and anti-CD41-PC7 as specific platelet glycoprotein. The same samples were stained with anti-IgG-FITC and anti-PF4 and analysed with the ImageStream image cytometer. Patient tissue samples, lung and brain, were analyzed by confocal microscopy for the presence of PF4-IgG complexes. **Results**: Platelet activation was determined by the expression of membrane α-granule glycoprotein P-selectin (CD62P+) and by the presence of phosphatidylserine phospholipids (Annexin-V+). The serum from the patient with VITT was able to activate the donor platelets demonstrated by the high expression of CD62P+/Annexin-V+. In PRP from healthy subjects, in the presence of VITT patient serum and low doses of heparin, the CD62P+/Annexin-V+ expression was decreased and at high doses of heparin, the signal was further reduced. The ImageStream cytometer demonstrated the presence of IgG-PF4 complexes on the platelet surface. Furthermore, the same complexes were observed by confocal microscopy in the patient tissues. **Conclusions**: The flow cytometry results confirmed the presence of platelet activating antibodies and that the mechanism of action is similar to that observed in patients with HIT. Flow cytometry could be a useful assay to identify the real incidence of VITT due to the presence of platelet activating antibodies after vaccination with ChAdOx1 nCOV-19. In addition, ImageStream cytometry demonstrated the presence of IgG-PF4 complexes on the platelet surface. Tissues analyses demonstrate the presence of IgG-PF4 complexes. The exact mechanism by which vaccination with ChAdOx1 nCOV-19 is associated with these platelet-activating antibodies is still unknown.

OC017

PLATELET-DERIVED EXTRACELLULAR VESICLES CAUSE NEUTROPHIL ACTIVATION IN SYSTEMIC SCLEROSIS

N. Maugeri, G.A. Ramirez, A. Monno, A. Capobianco, S. Franchini, M.E. Bianchi, P. Rovere-Querini, A.A. Manfredi

San Raffaele Scientific Institute and Vita-Salute San Raffaele University, Italy

Background and Aims: Platelet extracellular vesicles (Plt-EVs) expressing the damage-associated molecular pattern prototypic signal, High Mobility Group Box 1 (HMGB1) accumulate in the blood of patients with systemic sclerosis (SSc) and contribute to autoimmunity, fibrosis and vascular inflammation. A poorly understood defect in critical players in sprouting angiogenesis, angiopoietins and the angiopoietin receptor, Tie2 is also an hallmark of SSc. Neutrophils respond to angiopoietins and other stimuli by undergoing activation and upregulating the angiopoietin2, Tie2 expression. **Methods**: Human

studies: We analyzed Tie2 expression by neutrophils in of 39 patients with SSc and 43 sex- age- matched healthy donors (HD) by flow cytometry. Tie2 expression was confirmed by immuno-gold electron microscopy in purified neutrophils of 20 patients, *In vitro* experiments: purified neutrophils were purified and challenged with recombinant HMGB, or plt-E*Vs* from the plasma of patients with SSc. Animal model: Plt-EV were purified from the plasma of patients or HD and injected in the tail vein of NSG mice. After 18 hours blood samples were analyzed by flow cytometry and histology of lungs assessed. Neutrophil ablation was induced by treating mice with liposome-encapsulated clodronate before Plt-EV injection. The HMGB1 competitive antagonist, Box A and low molecular weight heparin (LMWH) were used as inhibitors of HMGB1 in *in vitro* and *in vivo* experiments. **Results**: Most neutrophils in SSc blood expressed Tie2 (84.7±2.4% *vs* 8.3±1.1% in HD; p <0.0001), regardless of the extent of fibrosis or vascular or systemic inflammation. HMGB1 is a putative candidate signal supporting neutrophils Tie2 overexpression, as it plays a critical role in neutrophil activation and is present as an EV-associated bioactive molecule in the blood of SSc patients. Our data support such a role, as HMGB1 and HMGB1+-Plt-EV purified from the plasma of patients with SSc induced upregulation of Tie2 *in vitro* and the event was abrogated by HMGB1 antagonists, Box A and LMWH. Furthermore, 18 hours after intravenous infusion of EV purified from the plasma of SSc patients (but not from the plasma of healthy donors) the fraction of Tie2-expressing neutrophils in the blood increased dramatically. BoxA and LMWH prevented the neutrophil Tie2 upregulation (>85 and 95% respectively). Histological analysis revealed fibrosis associated with massive infiltration of the lung by Tie2+ neutrophils. Neutrophil ablation by liposome-encapsulated clodronate prevented SSc Plt-EV-induced lung fibrosis, thus causally linking neutrophil activation, Tie2 neutrophil-expressing pulmonary infiltration and tissue fibrosis. **Conclusions**: Particulate HMGB1 expressed on Plt-E*Vs* could be a player and a pharmacological target in SSc.

OC018

NEUTROPHIL ACTIVATION AND NETS RELEASE DRIVER THROMBOSIS IN VACCINE-INDUCED IMMUNE THROMBOTIC THROMBOCYTOPENIA

C. Nocella[1], S. Bartimoccia[2], V. Cammisotto[1], V. Castellani[3], M. Leopizzi[4], G. D'Amati[4], M. Dominici[5], R. Marcucci[6], M. De Michele[7], R. Carnevale[2,8], P. Pignatelli[1,8], F. Violi[8]

[1]Department of Clinical Internal, Anaesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy; [2]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy; [3]Department of General Surgery and Surgical Speciality Paride Stefanini, Sapienza University of Rome, Rome, Italy; [4]Department of Radiological, Oncological and Pathological Sciences, Sapienza, University of Rome, Rome, Italy; [5]Interventional Cardiology Unit, Santa Maria Hospital, Terni, Italy[6]Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italy; [7]Stroke Unit, Emergency Department, Sapienza University of Rome, Italy; [8]Mediterranea Cardiocentro-Napoli, Naples, Italy

Background and Aims: Vaccine-induced immune thrombocytopenia and thrombosis (VITT) is a new syndrome associated with the ChAdOx1 nCoV-19 adenoviral vector vaccine against severe acute respiratory syndrome coronavirus 2. Like heparin-induced thrombocytopenia (HIT), this condition has been associated with the development of anti-platelet factor 4 antibodies, leading to the neutrophil-platelet aggregate formation and neutrophil extracellular traps (NETs) release. However, the precise molecular mechanisms governing its occurrence are yet to be established. **Methods**: We studied patients with ischemic stroke after ChAdOx1 nCoV-19 vaccination, patients with ischemic stroke from other causes (IS) and healthy subjects (HS). We analyzed NETs release and coagulation by measuring in plasma and cerebral thrombi citrullination of histone H3 (CitH3) and tissue factor (TF), respectively. Furthermore, we investigated *in vitro* if plasma post-ChAdOx nCoV-19 vaccination enhanced NETs release. **Results**: *In vivo* studies showed enhanced circulating levels of CitH3 and TF in patients with ischemic stroke after ChAdOx1 nCoV-19 vaccination compared to IS and HS groups. *In vitro* study showed that plasma from post-ChAdOx nCoV-19 vaccination increased NETs release and Protein Arginine Deiminase 4 (PAD4) expression by neutrophils; this effect was more evident in the presence of platelets and blunted by PAD4 inhibitor, heparin, and anti-FCγRII antibody. Finally, immunohistochemistry analysis showed a much stronger staining intensity for the antibody against PAD4 in a cerebral thrombus from a patient after ChAdOx1 nCoV-19 vaccination compared to that from IS patient. **Conclusions**: This study provides evidence that in VITT patients PAD4 overactivation is responsible for NETs release at the level of thrombus growth, thereby its inhibition may represent a novel approach to the treatment of VITT.

OC019

A GLOBAL SCORE OF MODIFIABLE CARDIOVASCULAR RISK FACTORS IN INDIVIDUALS FREE FROM CARDIOVASCULAR DISEASE: LONGITUDINAL FINDINGS FROM THE MOLI-SANI STUDY

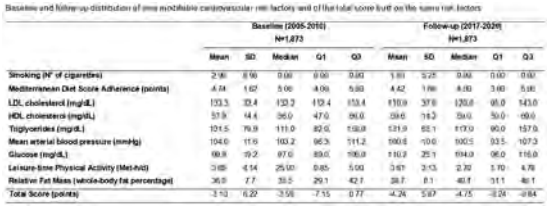
M. Persichillo[1], M. Bonaccio[1], S. Costanzo[1], A. De Curtis[1], S. Magnacca[3], F. Bracone[1], T. Panzera[1], C. Cerletti[1], M.B. Donati[1], G. de Gaetano[1], L. Iacoviello[1,2], A. Di Castelnuovo[3] on behalf of the Moli-sani Study Investigators

[1]Department of Epidemiology and Prevention, IRCCS NEUROMED, Pozzilli (IS); [2]EPIMED Research Center,

Department of Medicine and Surgery, University of Insubria, Varese; [3]Mediterranea Cardiocentro, Napoli, Italy

Background and Aims: Cardiovascular (CV) disease, the leading cause of death globally, is largely preventable through targeting modifiable risk factors. Scores of modifiable CV risk factors able to collect the combined effect of all the included components were mainly developed in US populations, or do not include nutritional aspects. Beside the effect of specific prevention strategies, the CV risk profile based on modifiable CV risk factors may change in time, but data dealing with this variation over long periods are scarce. Based on data from an Italian population, we aimed a) to compute an algorithm based on common modifiable CV risk factors, including diet and b) to assess the variation at individual level of 9 common modifiable CV risk factors, and of the global score based on them, after 12.7 years of follow-up. **Methods**: We analyzed data on 1,873 participants from a sub-cohort of the larger Moli-sani Study (enrolled 2005-2010) that was re-examined in 2017-2020. Individuals were randomly recruited from those in the original cohort with longer follow-up and were free from CV disease. The panel of modifiable CV risk factors included smoking habit, adhesion to Mediterranean diet, LDL, HDL, triglycerides, mean arterial pressure, glucose, relative fat mass (proxy of body fat percentage) and leisure-time physical activity. A global score of modifiable CV risk factors was calculated as a weighted sum of the nine standardised risk factors. Weights reflect the strength of the risk factor/outcome association. To derive the algorithm, we used data on 16,656 men and women (aged ≥45 years) from the population of the Moli-sani Study free from CV disease, and tested the combined association of the 9 risk factors with a composite outcome of fatal and non-fatal CV events. **Results**: In multivariable Cox regression analysis adjusted for non-modifiable risk factors, one unit more in the score was associated with a hazard ratio for CV events equal to 1.07 (95%CI: 1.06 to 1.08). The re-analysed sample consisted of 1,873 individuals, 55% women, 57 (SD=7) years of age. In 12.7 median years of follow-up, an improvement in the CV risk profile was observed as number of cigarettes, mean arterial pressure and LDL were reduced, and HDL increased (Table 1). On the contrary, a worsening in the CV risk profile was detected as adherence to Mediterranean diet was reduced and relative fat mass and glucose was increased (Table 1). Mean values of triglycerides and physical activity remained unchanged (Table 1). Because of these variations, the global score of modifiable CV risk factors diminished of nearly 1 point (Table 1). **Conclusions**: In the last 13 years, lipid profile and smoking habit of a large Italian population, originally free from CV disease, improved, whereas adherence to Mediterranean diet, fat distribution and glycaemic control worsened. These findings can help setting strategies for cardiovascular prevention. We propose an algorithm based on nine modifiable CV risk factors, easily to replicate and predictive of fatal and non-fatal CV events. The score can be managed to design and evaluate the efficacy of a preventive approach, to compare different strategies and to communicate effectiveness of a preventive action, thus increasing compliance to the strategy itself.

Table 1.



OC020

HIGH, BUT NOT LOW-, DOSE ATORVASTATIN PREVENTS THE INCREASE OF CIRCULATING MMP-2 LEVELS IN PATIENTS WITH NSTE-ACUTE CORONARY SYNDROMES

F. Paciullo[1], E. Falcinelli[1], M. Del Pinto[2], T. Fierro[1], P. Gresele[1]

[1]Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy; [2]Division of Cardiology, Perugia Hospital, Italy

Background and Aims: High-dose statins improve cardiovascular outcomes in patients with acute coronary syndromes (ACS). The beneficial effect of statins are considered to be largely due to their lipid lowering action but, especially upon very short term administration, they may also be the consequence of pleiotropic actions. Metalloproteinases (MMP), a family of enzymes degrading extracellular matrix, are released in blood during acute coronary syndromes and have been reported to contribute to atheroma unstabilization and recurrent cardiovascular events. In particular, it has been recently reported that in patients with ACS high MMP-2 levels are associated with the degree of myocardial injury, measured as high-sensitivity troponin T levels, and can predict the severity of outcome. Moreover, in a cohort of patients with AMI MMP-2 levels predicted all-cause mortality. In the present study we investigated the effect of atorvastatin at high *vs* standard doses on the early increase of circulating MMP-2 levels in patients with NSTE-acute coronary syndrome (ACS). **Methods**: In this single center study we prospectively collected clinical and laboratory data from patients with a definite diagnosis of NSTE-ACS. Patients were divided in three groups according to the statin regimen: atorvastatin 80 mg/die from day 1 (high-dose group); atorvastatin 20 mg from day 1 (low-dose group); atorvastatin 20 mg starting later than 96 hours after admission (non statin group). Dose regimens were chosen by the caring cardiologist based on LDL levels at hospital admission. Each patient was clinically evaluated on admission and after 3, 5 and

30 days from the event, and blood samples for the measurement of MMP-2 were collected. Peripheral blood was collected in parallel also from a group of age- and sex-matched healthy controls (HC). Plasma MMP-2 levels were measured by zymography. Serologic, clinical and instrumental parameters were compared in three groups. Data are reported as mean±SEM. **Results**: 40 consecutive patients were enrolled, 13 patients in the high dose group, 14 in the low-dose group and 13 in the non statin group. Characteristics of patients in the three groups were comparable (age: 67±2, 70±3, 69±3; sex (F): 38%, 21%, 15%; prevalence of risk factors: 70%, 50%, 50%; hours from symptom onset: 20±8, 24±6, 11±7, respectively) Peripheral blood plasma MMP-2 levels at enrollment were significantly higher in NSTE-ACS patients than in HC (median 918±72 ng/ml *vs* 682±97 ng/ml, $p<0.01$) but did not differ significantly between the three patient groups. High-dose atorvastatin significantly reduced plasma MMP-2 levels at 3 days compared to other strategies (High-dose=before: 986.4±90ng/ml day3:781±81ng/ml; Low-dose=before: 842.4±148ng/ml day3:895.2±120 and non statin=before:930.2±1259 day3: 1033±123ng/ml, $p<0.05$, respectively, for high-dose *vs* low-dose and *vs* non statin regimen) (Figure 1). **Conclusions**: High-dose, but not low-dose, statin reduces the increase of circulating MMP-2 triggered by ACS. Given that high circulating MMP-2 levels in patients with ACS can predict poor prognosis and are associated with increased incidence of cardiovascular events, this reduction may contribute to explain the protective effect of high dose statin after ACS on recurrent cardiovascular events.

OC021

INFLAMMATION, HAEMOSTASIS AND EXTRACELLULAR MATRIX IN THE STRATIFICATION OF BLEEDING AND THROMBOTIC RISK OF ATRIAL FIBRILLATION ON ORAL ANTICOAGULANT THERAPY: INSIGHTS FROM STRAT-AF STUDY

F. Alfano[1], A.M. Gori[1], F. Cesari[1], E. Salvadori[2], M. Berteotti[1], B. Giusti[1], A. Kura[1], G. Cassioli[1], E. Lotti[1], D. Poli[1], A. Poggesi[2], R. Marcucci[1]

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [2]NEUROFARBA Department, Neuroscience Section, University of Florence, Florence, Italy

Background: In anticoagulated atrial fibrillation patients, the validity of models recommended for the stratification of the risk ratio between benefits and hemorrhage risk is limited. We hypothesize that biological markers – both circulating and neuroimaging-based – and their possible interaction, might improve the prediction of bleeding risk in atrial fibrillation patients under treatment with any type of oral anticoagulant. **Methods**: The Strat-AF study is an observational, prospective, single-center hospital-based study enrolling patients with atrial fibrillation, aged 65 years or older, and with no contraindications to undergo magnetic resonance imaging, referring to Center of Atherothrombotic Disease of our University Hospital (AOU Careggi) for the management of oral anticoagulation therapy. Recruited patients are evaluated by means of a comprehensive protocol, with clinical, cerebral magnetic resonance imaging and circulating biomarkers assessment. The main outcomes are the evaluation of cerebral microangiopathy using MRI (lacunar infarcts, non-lacunar infarcts, microbleeds, hyperintensity of the white matter, SVDs [small vessel disease score]) and the onset of ischemic or hemorrhagic stroke related to the levels of circulating biomarkers of inflammation (IL-6, IL-8, TNFα, IL-4, IL-10, CCL2, CXCL10, ICAM-1, VCAM-1, VEGF), haemostasis (PAI-1, CLT, vWF) and extracellular matrix remodeling (MMP-2,-7,-8,-9,-12, TIMP-1,-2,-3,-4). Starting from September 2017, 191 patients (mean age 78.1±6.7, range 65-97; 65.3% males) were enrolled. 56 patients (29.3 %) were on vitamin K antagonists, and 135 (70.7 %) were on direct oral anticoagulants. Follow-up clinical evaluation and brain MRI were performed at 18 months. **Results**: At multivariate analysis, adjusted for age, sex, CHA2DS2-VASc, HAS-BLED and type of anticoagulant, independent predictors were: low levels of vWF and elevated levels of TIMP-2 for microbleeds [OR: 0.62 95% CI (0.42-0.89), p=0.011 and OR: 1.25 (1.01-1.77), p=0.049 respectively]; elevated levels of IL-6 and TIMP-4 for hyperintensity of white matter [OR: 1.55 (1.01-2.58), p=0.049 and OR: 1.44 (1.01-2.07), p=0.047 respectively]; elevated levels of MMP-2 and of TIMP-1 and TIMP-2 for lacunar infarcts [OR: 1.33 (1.01-1.89), p=0.049, OR: 1.48(1.01-2.18), p=0.048 and 1.50 (1.05-2.16), p=0.028 respectively]; elevated levels of TIMP-1, TIMP-2, TIMP-3 for the appearance of at least 1 new lesion according to the SVD score [OR: 1.48 (1.01-2.19), p=0.046, OR: 1.53 (1.05-2.23), p=0.028 and OR: 1.58 (1.09-2.31), p=0.016 respectively]. **Conclusions**: The Strat-AF study may be an essential step towards the exploration of the role of a combined clinical biomarker or multiple biomarker models in predicting stroke risk in atrial fibrillation and might sustain the incorporation of such new markers in the existing stroke prediction schemes by the demonstration of a greater incremental value in predicting stroke risk and improvement in clinical outcomes in a cost-effective fashion.

OC022

CIRCULATING BIOMARKERS AND BLOOD-BRAIN-BARRIER LEAKAGE: RESULTS FROM THE REPERFUSION INJURY IN ISCHEMIC STROKE (RISK) STUDY

A. Bertelli[1], A. Kura[1], B. Piccardi[2], F. Arba[2], G. Barbieri[1], R. De Cario[1], A. Sodero[2], B. Giusti[1], A.M. Gori[1], R. Marcucci[1], P. Nencini[2]

[1]Dept of Experimental and Clinical Medicine, University

of Florence, Florence, Italy; [2]NEUROFARBA Department, Neuroscience Section, University of Florence, Italy

Aim: To evaluate the effect of circulating inflammatory mediators and matrix metalloproteinases (MMP) on blood-brain-barrier (BBB) leakage in the acute phase of stroke. **Methods**: Observational prospective single-centre hospital-based study that included consecutive acute ischaemic stroke patients treated with intravenous thrombolysis, endovascular treatment or both. Circulating biomarkers were taken before and after 24 hours from acute interventions. We assessed pre-treatment BBB leakage with CT perfusion by using Ktrans within the ischaemic area. BBB disruption was evaluated with Ktrans maps automatically provided by Olea Sphere within the ischemic core and the ischemic penumbra. We evaluated independent associations between BBB leakage and biomarkers levels at baseline, 24 hours and their relative variations [(Δ=24hours level-baseline level)/baseline level] adjusting for age, sex, baseline stroke severity, type of treatment and onset to treatment time. **Results**: We included 100 patients, mean (±SD) age was 75.9±11.6, 51 (51%) males, median (IQR) NIHSS was 17 (10-22). Endovascular treatment alone was performed in 47 (47%) patients, intravenous thrombolysis in 25 (25%) patients, and 28 patients (28%) received both treatments. Baseline biomarker levels were not significantly different among the 3 types of treatment. We did not find any association between baseline biomarker values and BBB leakage. BBB leakage was correlated with 24h MMP-2 (Rho 0.22, p=0.045), 24h IL-12 (Rho 0.26, p=0.017), and with ΔIL-12 levels (Rho 0.22; p=0.047). When we dichotomized BBB values according to the mean for the whole population ("low" BBB disruption with values up to 0.63; "high" BBB disruption with values higher than 0.63), patients with higher BBB had significantly higher levels of 24h MMP-7 (p=0.010), 24h MMP-9 (p=0.019) and 24 h IL-12 (p=0.010). At multivariate logistic regression analysis, adjusted for the above mentioned variables, higher BBB leakage was significantly associated with 24h IL-12 levels [OR=1.81 (CI 1.06-3.08), p=0.030], 24h MMP-7 [OR=2.80 (CI 1.28-6.14), p=0.010] and 24h MMP-9 [OR=1.89 (CI 1.17-3.04), p=0.009]. Modified Rankin disability score (mRS) was dichotomized into good (mRS 0–2) (n=45) or poor (mRS 3–6) (n=55) outcome. Patients with poor outcome had significantly higher levels of baseline IL6, CCL2 and CXCL10 [IL6:199 (131-350) pg/mL *vs* 145 (112-206) pg/mL, p=0.0401; CCL2: 10.74 (0.48-1.27) ng/mL *vs* 0.52 (0.35-0.76) ng/mL, p=0.005 and CXCL10: 1.4 (1.1-2.3) ng/mL *vs* 0.9 (0.6-1.6) ng/mL, p 0.025] than patients with mRS 0-2. IL6/IL4 and IL6/IL10 ratios were significantly higher in patients with poor outcome [1.28 (0.91-2.33) *vs* 0.86 (0.59-1.10), p<0.001; 0.74 (0.48-1.27) *vs* 0.52 (0.34-0.76), p=0.020] than in patients with mRS 0-2. At multivariate logistic regression analysis, the ratios IL6/IL4 and IL6/IL10 were significantly associated with three-month mRS 3–6 [OR: 2.48 (1.01-10.11), p=0.049 and OR:2.99(1.05-8.54), p=0.040 respectively]. **Conclusions**: Early BBB leakage was associated with 24 hours IL12, MMP-7 and MMP-9 levels. Unbalanced pro-/anti-inflammatory ratios were associated with poor outcome assessed by three-month mRS. Our results support a possible link between BBB disruption and consequent inflammatory cascade in acute ischemic stroke and suggests the pathogenetic role of inflammation into 3 month neurological disability.

OC023

ENHANCED PLATELET REACTIVITY IN GESTATIONAL DIABETES

G. Guglielmini[1], E. Falcinelli[1], E. Piselli[1], A.M. Mezzasoma[1], L. Alfonsi[2], C. De Luca[2], V. Fino[2], E. Torlone[3], P. Gresele[1*], S. Gerli[2*]

*[1]Division of Internal and Cardiovascular Medicine; [2]Division of Obstetrics and Gynecology; [3]Division of Endocrinology and Metabolism; Department of Medicine and Surgery, University of Perugia, Perugia, Italy; *these authors share senior authorship*

Background and Aims: Gestational diabetes mellitus (GDM) is a disorder of carbohydrate metabolism which occurs in around 14% of pregnancies. While platelet hyperresponsiveness in type-2 diabetes mellitus (T2DM) has been well characterized and shown to play a crucial role in cardiovascular complications, this aspect has been little studied in GDM. Aim of our study was to evaluate platelet activation *in vivo*, *ex vivo* platelet hyperreactivity and endothelial function in GDM compared with normal pregnancy. **Methods**: For the GDM group inclusion criteria were glucose intolerance confirmed by a pathologic oral glucose tolerance test performed between 16 and 18 weeks of gestation. For the control pregnant (CP) group the only inclusion criterion was a physiological pregnancy. Groups were balanced for age and gestational age. Exclusion criteria were: pregestational T2DM, hypertension, preeclampsia, BMI>30, pregestational hypothyroidism, hemorrhagic diathesis, platelet abnormalities, platelet count <100,000 cells/µl, or thrombotic events within 6 months. All tests were conducted at 26-28 weeks of gestation (T1), at 34-36 weeks (T2) and 8 weeks post-partum (T3). *In vitro*: collagen-, ADP-, Arachidonic Acid (AA)-, epinephrine- U46619- and TRAP-6-induced LTA; PFA-100® closure time, platelet activation antigens and platelet production of reactive oxygen species (ROS) and nitric oxide (NO) upon *in vitro* stimulation. *In vivo*: Endothelial function measured using the EndoPAT™ System. **Results**: 46 pregnant women, 23 GDM and 23 CP, were enrolled. Low dose collagen-, ADP- and AA- induced platelet aggregation increased significantly from T1 to T2, both in CP and GDM women, to decrease then at T3. However, GDM showed a significantly higher LTA at T2 compared with CP which persisted at T3. EC50 for collagen LTA was significantly lower in GDM. Epinephrine- U46619- and TRAP-6-induced platelet aggregation increased significantly throughout pregnancy in both CP and GDM

women, but then decreased at T3 in CP, while GDM women maintained a significantly higher platelet reactivity. PFA-100 C-EPI closure time was significantly shorter in GDM than in CP at all the observation time points. Platelet adhesion to a collagen-coated surface under high-flow was significantly higher in GDM than in CP. The expression of P-Sel or PAC-1 upon stimulation with ADP or TRAP was significantly higher in GDM compared with CP at T1 and T2. Platelet sensitivity to the inhibitory effect of NO was significantly lower in GDM compared to CP. Also, platelet NO production was significantly lower in GDM than in CP women at T1 and further decreased at T2. Arterial stiffness by the EndoPAT™ System was significantly higher in GDM at all time points. **Conclusions**: Our data show that GDM generates a condition of platelet hyperreactivity. A particularly evident defect is represented by a lower sensitivity of platelets to the inhibitory action of NO and by a reduced platelet and endothelial NO production, while platelet oxidative stress is increased, contributing to the hyperreactivity state of platelets in GDM. In conclusion GDM generates a platelet hyperreactivity condition which tends to worsen as pregnancy progresses, and persists for at least 2 months after delivery. Platelet hyperreactivity may contribute to long term cardiovascular complications in GDM patients.

OC024

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) LEVELS AND ABNORMALLY HIGH ANKLE-BRACHIAL INDEX IN ATRIAL FIBRILLATION

P. Pignatelli[1,4], D. Menichelli[1], G. Galardo[2], V. Cammisotto[1], S. Bartimoccia[1], R. Carnevale[3,4], D. Pastori[1]

[1]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy; [2]Department of General Surgery, Surgical Specialties and Organ Transplantation "Paride Stefanini," Sapienza University of Rome, Rome, Italy; [3]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy; [4]Mediterranea Cardiocentro, Naples, Italy

Background and Aims: High ankle-brachial index (ABI) has been associated with increased risk of worse outcomes in the general population. Few data on atrial fibrillation (AF) do exist. Experimental data suggest that proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) contribute to vascular calcification but clinical data on this association are lacking. To investigate the relationship between circulating PCSK9 levels and abnormally high ABI in patients suffering from AF. **Methods**: We analysed data from 579 patients included in the prospective ATHERO-AF study. An ABI ≥1.4 was considered as high. Patients with an ABI <0.9 were excluded. PCSK9 levels were measured coincidentally with ABI measurement. We used an optimized cut-off of PCSK9 >1150 pg/ml obtained from ROC curve analysis. All-cause mortality according to the ABI value was also analysed. **Results**: 115 (19.9%) had an ABI ≥1.4. The mean age was 72.1 years and 42.1% of patients were women. Patients with ABI ≥1.4 were older, more frequently male and diabetic. Multivariable logistic regression analysis showed an association between ABI ≥1.4 and serum levels of PCSK9 >1150 pg/ml (Odds Ratio 1.835, 95%CI 1.133-2.970, p=0.014). During a median follow up of 41 months, 112 deaths occurred. At multivariable Cox regression analysis, ABI ≥1.4 was associated with an increased risk of mortality (Hazard Ratio 1.676, 95%CI 1.073-2.617, p=0.023). **Conclusions**: In AF patients, PCSK9 levels relate to an abnormally high ABI, which is in turn associated with an increased mortality risk. This is the first clinical evidence of an association between PCSK9 and vascular calcification in AF patients

OC025

HEAD-TO-HEAD COMPARISON OF THE EFFECT OF PLATELET- AND MONOCYTE-DERIVED MICROVESICLES ON ENDOTHELIAL FUNCTIONS

M. Brambilla[1], P. Canzano[1], M. Talmon[2], L. Fresu[2], S. Brunelleschi[2], E. Tremoli[3], M. Camera[1,4]

[1]Centro Cardiologico Monzino I.R.C.C.S., Milan, Italy; [2]Dept of Health Sciences, University of Piemonte Orientale, Novara, Italy; [3]Maria Cecilia Hospital, Cotignola, Italy; [4]Dept of Pharmacological and Biomolecular Sciences,Università degli Studi di Milano, Milan, Italy

Background and Aims: Circulating microvesicles (MVs) are recognized as both biomarkers and mediators of inflammation and endothelial dysfunction in many pathological conditions, including cardiovascular diseases. The two main MV-subsets mediating these effects are those derived from monocytes and platelets (PLT), although the specific contribution of each of them is not well defined. Thus, aim of this study was to assess how M*Vs* released from PLT and monocytes influence processes involved in the vessel damage -*i.e.* oxidative stress, inflammation, leukocyte-endothelial adhesion. **Methods**: PLT and monocytes isolated from healthy subjects (HS, n=10) were stimulated with TRAP-6 (20 μM) and LPS (10μg/ml) to release M*Vs* that were isolated, characterized by flow cytometry, and added to the culture medium of human vascular endothelial cells (hECV). Superoxide anion production, inflammatory markers (IL6, TNFalpha, NF-κB mRNA expression) and hECV adhesiveness after M*Vs* challenge were evaluated. M*Vs* spontaneously released from cells of acute myocardial infarction patients (AMI; n=10) were used to verify whether the *in vivo* setting reflected the *in vitro* activation. **Results**: Incubation of hECV with M*Vs* released by HS-activat-

ed PLT and monocytes triggered an oxidative burst (3-fold increase) in a MV concentration-dependent manner. Monocyte-M*Vs* doubled IL6, TNFalpha, and NF-κB mRNA expression and monocyte-endothelial adhesion. These functions were only slightly influenced by PLT-MVs. These effects were associated with an antigenic signature, *i.e.* procoagulant and PLT-Psel+ and monocyte-CD16+ MVs, reflecting the *in vitro* stimulation and also found in AMI MVs. Interestingly however, AMI-monocyte-M*Vs* supported adhesion of monocytes to hECV as observed with HS-M*Vs* but not the oxidative stress. Conversely, AMI PLT-M*Vs* sustained not only redox state but also the inflammatory phenotype (two-fold increase). **Conclusions**: These data provide evidence that M*Vs* derived from activated PLT and monocytes differently affect endothelial behavior. These functional effects are partially shared also by M*Vs* spontaneously released by platelets and monocytes from AMI patients and support the antiplatelet treatment in this clinical setting.

OC026

PLEIOTROPIC EFFECTS OF ANTICOAGULANT THERAPIES: IS THERE A DIFFERENCE BETWEEN AVK AND DOAC?

F. Alfano[1], A.M. Gori[1], F. Cesari[1], E. Salvadori[2], B. Bandinelli[1], B. Giusti[1], A. Bertelli[1], A. Kura[1], E. Lotti[1], R. De Cario[1], A. Poggesi[2], R. Marcucci[1]

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [2]NEUROFARBA Department, Neuroscience Section, University of Florence, Florence, Italy

Background: Atrial fibrillation (AF) is the most common supraventricular arrhythmia. Emerging evidence suggests a significant role of inflammation in the pathogenesis and in the maintenance of AF. We hypothesize a different role of anticoagulant therapy (AVK *vs.* DOACs) in reducing the levels of inflammatory biomarkers in AF. **Methods**: The Strat-AF study is an observational, prospective, single-center hospital-based study enrolling patients with atrial fibrillation, aged 65 years or older, and with no contraindications to undergo magnetic resonance imaging, referring to Center of Atherothrombotic Disease of our University Hospital (AOU Careggi) for the management of oral anticoagulation therapy. Recruited patients are evaluated by means of a comprehensive protocol, with clinical and circulating biomarkers assessment. One of the main outcomes is the evaluation of the grade of inflammation in patients treated with AVK *vs.* DOACs. In order to evaluate the grade of inflammation, we calculated the ratios between pro- and anti-inflammatory cytokines (IL6/IL4, IL6/IL10, TNF-alpha/IL4, TNF-alpha/IL10). The pro-/anti-inflammatory ratios were divided into tertiles and were used to calculate a score of inflammation. Patients with ratios in the third tertile were assigned as patients with an elevated grade of inflammation not balanced by anti-inflammatory cytokines; patients with ratios in the other tertiles were assigned as patients with a low level of inflammation. Starting from September 2017, 191 patients (mean age 78.1±6.7, range 65-97; 65.3% males) were enrolled. 56 patients (29.3 %) were on vitamin K antagonists, and 135 (70.7 %) were on direct oral anticoagulants. **Results**: Patients treated with DOACS had higher ratio values than patients treated with AVK [IL6/IL4: 0.15 (0.07-0.35) *vs* 0.05 (0.03-0.25), p=0.001; IL6/IL10: 0.70 (0.28-5.49) *vs* 0.54 (0.18-1.42), p=0.090; TNF-alpha/IL4: 0.16 (0.12-0.31) *vs* 0.13 (0.08-0.31), p=0.055; TNF-apha/IL10: 0.97 (0.25-5.23) *vs* 0.75 (0.51-2.62), p=0.105]. According to pro-anti-inflammatory ratio tertiles, 28/135 DOAC patients (20.7%) had an elevated grade of inflammation not balanced by anti-inflammatory cytokines, whereas in AVK group only 5.4% (3/53) were classified as patients with high levels of inflammation (p=0.009). At multivariate logistic regression analysis, adjusted for age, sex, CHA2DS2-VASc, HAS-BLED, AVK treatment resulted an independent and protective predictor for having a high grade of inflammation not balanced by anti-inflammatory cytokine levels [OR=0.27 (0.08-0.97), p=0.045]. **Conclusions**: These result from Strat-AF study may be an essential step towards the exploration of the role and the contribute of anticoagulant therapy in reducing inflammation-related biomarkers in AF patients. Inflammation has also been associated with endothelial dysfunction, coagulation cascade activation and thrombogenesis. Thus, inflammation may contribute to both the occurrence/maintenance of AF and its thromboembolic complications. These results might sustain the incorporation of such new markers in the existing stroke prediction schemes by the demonstration of a greater incremental value in predicting stroke risk and improvement in clinical outcomes in a cost-effective fashion.

OC027

IN VITRO CHARACTERIZATION OF PATIENT-SPECIFIC ENDOTHELIAL COLONY-FORMING CELLS TO INVESTIGATE THE ROLE OF ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF UNPROVOKED VENOUS THROMBOEMBOLISM

A. Cancellara[1], M. Bacci[2], L. Bertolani[1], R. Ciceri[1], E. Romualdi[3,4], V. Pessi[4], M. Fantuzzi[4], C. Lodigiani[2], M.P. Donadini[4,5], S. Della Bella[1], F. Calcaterra[1*], D. Mavilio[1*]

[1]Unit of Clinical and Experimental Immunology, Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy; Humanitas Clinical and Research Center - IRCCS, Rozzano, Milan, Italy; [2]Center for Thrombosis and Haemorragic Diseases, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy; [3]UO Medicina 2, Ospedale di Circolo e Fondazione Macchi, ASST Sette

*Laghi, Varese, Italy; [4]Università dell'Insubria, Dipartimento di Medicina e Chirurgia, Varese, Italy; [5]Centro Trombosi ed Emostasi, Ospedale di Circolo e Fondazione Macchi, ASST Sette Laghi, Varese, Italy; *Equally contributed*

Background: Venous thromboembolism (VTE) is characterized by a multifactorial pathogenesis that often occurs in the presence of provoking factors; major risk factors induced provoked VTE (pVTE) whereas weakly provoked (wpVTE) is associated with minor ones. Nevertheless, up to 50% of VTE events are defined as "unprovoked" (uVTE) since their pathogenesis is largely unknown, with a high risk of recurrence. In this regard, the presence of endothelial alterations deserves to be explored as endothelial dysfunction (ED) is known to trigger thrombus formation. Endothelial colony-forming cells (ECFCs) are the true endothelial progenitors proposed as cell therapy product or liquid biopsy in vascular disorders. **Aims**: To investigate whether ECFCs in VTE patients show alterations that may reflect a perturbation of the endothelial compartment involved in uVTE pathogenesis. **Methods**: ECFCs were isolated and expanded from peripheral blood mononuclear cells obtained from 53 VTE patients (16 uVTE, 21 wpVTE, 16 pVTE) and 20 sex- and age-matched healthy donors (HDs). ECFCs were analyzed for efficiency of isolation, cell viability and growth. Endothelial function was assessed at baseline by analyzing: a) platelet deposition and fibrin formation under flow conditions, assessed in thrombogenesis assay by confocal microscopy; b) thrombin formation assessed by thrombin generation assay (TGA) on ECFCs - a modification of Hamker's method. Expression levels of the adhesion molecule CD106 (VCAM-1) and the pro-coagulant molecule Tissue Factor (TF) were also assessed, by flow cytometry. **Results**: ECFCs were isolated from the three subgroups of VTE patients with a similar efficiency as HDs in term of frequency and number of colonies. In pVTE, a trend in the reduction of the frequency of subjects who gave origin to ECFCs was observed. Time of appearance was longer in VTE patients with the difference being significant only in wpVTE patients. VTE ECFCs showed a higher rate of early senescence (passage≤2), with wpVTE group being characterized by the highest frequency. By performing *in vitro* functional assays, we observed: a) an increased platelet deposition and fibrin formation in VTE patients than in HDs as assessed by thrombogenesis assay; b) a reduction of the lag phase and an increase in thrombin formation in VTE patients being more pronounced in uVTE patients as assessed by TGA on ECFCs. CD106 and TF were barely expressed on ECFCs in basal condition in all analyzed groups and only a slight increase in their expression was observed in ECFCs obtained by wpVTE patients. **Conclusions**: Our results indicate alterations in the growth and in functionality of VTE ECFCs thus confirming their use to investigate ED role in uVTE pathogenesis. A deeper characterization of ECFCs, still ongoing, may highlight the molecular mechanisms involved in ED in uVTE and to disclose whether they are associated with uVTE only are shared with secondary VTE.

OC028

FROM COAGULATION TO ANGIOGENESIS: POTENTIAL ROLE OF FVIII IN ENDOTHELIAL FUNCTIONALITY

A. Cucci[1], C. Olgasi[1], C. Borsotti[1], G. Walker[1], I. Molineris[2], F. Anselmi[2], S. Assanelli[1], C. Sgromo[1], S. Oliviero[2], A. Follenzi[1]

[1]Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy; [2]Italian Institute for Genomic Medicine (IIGM), Università degli Studi di Torino, Torino, Italy

Background and Aims: Hemophilia A (HA) is a rare X-linked bleeding disorder caused by absence or reduced activity of coagulation FVIII. The extent of clinical manifestations of HA patients depends on the severity of the FVIII deficiency. The main clinical manifestation is spontaneous bleeding episodes that primarily involve hemarthroses and intracranial hemorrhages. The factors that initiate hemorrhage are not known, and the onset of hemorrhage is often a random event, occurring either spontaneously or after minimal injury. Standard replacement therapies are ineffective in preventing the bleeding episodes. It is well established that FVIII is mainly secreted by endothelial cells (ECs), but the impairment of vessel stability in HA patients and a correlation between FVIII and endothelial functionality has never been explained. Therefore, we aimed to explore the role of FVIII in endothelial stability and identify significant differences in HA and healthy ECs. **Methods**: iPSCs-derived ECs, differentiated from HA patients or healthy donors, were used as EC model. HA-ECs were transduced with a lentiviral vector (LV) carrying the B domain deleted form of FVIII under the control of the vascular endothelial cadherin promoter (LV-VEC.FVIII). To evaluate differences in healthy, HA ECs and LV-VEC.FVIII-transduced HA ECs, RNA-Seq analysis was carried out and modulated genes were validated by qPCR. Finally, *in vitro* and *in vivo* endothelial functional assays were performed to investigate HA ECs impairment. **Results**: RNA-Seq analysis revealed different gene expression profile among healthy, HA and LV-VEC.FVIII HA ECs, particularly highlighting the down regulation of several genes in HA compared to healthy ECs, suggesting an impairment in HA ECs stability. LV-VEC.FVIII HA ECs showed an intermediate transcriptional profile indicating only a partial correction after LV transduction. Endothelial functional *in vitro* experiments demonstrated a reduced capability of HA ECs, showing a weakening in vessel-formation capability, migration potential and permeability when compared to healthy ECs. The impaired phenotype was attenuated in LV-VEC.FVIII transduced HA ECs, suggesting the potential involvement of FVIII. Finally, *in vivo* experiments in a HA mouse model demonstrated an altered permeability and tubulogenesis potential of HA vessels compared to wild type mice. **Conclusions**: These preliminary results, if confirmed in primary ECs, can provide new insights into the possible

extra-coagulative role of FVIII and it can be crucial to understand the key molecular targets missing in HA patients at the cellular level impairing EC functionality. This information can lead to new therapeutic approaches resulting in a safer and more efficient treatment of HA.

OC029

THE ROLE OF MICROENVIRONMENT IN MORPHOLOGICAL AND TRANSCRIPTIONAL CHANGES OF BRAIN MICROVASCULAR ENDOTHELIAL CELLS IN WILD TYPE AND HEMOPHILIC MICE

S. Assanelli, C. Sgromo, P.E. Di Simone, C. Borsotti, C. Olgasi, A. Follenzi

Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy

Background and Aims: Hemophilia A (HA) is a recessive X-linked bleeding disorder caused by the deficiency of the coagulation factor VIII (FVIII). Clinical manifestations are bleeding episodes that primarily consist in hemarthroses and intracranial hemorrhages (ICHs). Standard therapies are ineffective in preventing the bleeding episodes that can occur without any clear cause. The manifestation of ICHs is often spontaneous, and mainly occurs in patients that do not adhere to the prophylaxis regimen with a mortality around 20%. Although the cause of the development of ICHs is still unknown, a correlation between the severe form of HA and ICHs in children has been described, emphasizing the importance of prophylaxis in reducing the risk of ICHs. Additionally, clinical evidence indicate a general microvascular endothelial impairment in HA patients and that HA untreated patients show a pro-inflammatory environment with higher levels of cytokines in the plasma compared to healthy controls. Thus, we aim to investigate morphological and transcriptional differences in brain microvascular endothelial cells (BMECs) isolated from wild type (WT) and HA mice and to define if WT or HA microenvironment can influence the phenotype of BMECs. **Methods**: To isolate murine BMECs from WT and HA C57BL/6 mice brains were removed from 8 weeks old mice, and they were mechanically disaggregated. After enzymatic digestion of the tissues, myelin was eliminated, and endothelial cells were separated from the debris through a Percoll® gradient. FACS analysis was performed to evaluate endothelial markers expression. BMECs were then cultured in endothelial medium with FBS, WT and HA murine serum. After 5 days of culture, qPCR and immunofluorescences were performed to verify the expression of endothelial markers and to reveal the presence of other cell types. **Results**: Isolation of BMECs was optimized for both WT and HA mice. Most of the live isolated cells were endothelial (CD31+CD45-): the number of isolated CD31+ cells was not different between WT and HA mice in 3 independent experiments (WT CD31+%: 70.3±11; HA CD31+%: 70.6±5, p value=0.9). Microglia contaminants (CD45+CD11b+CD31-) were eliminated by 5 days of culture in endothelial medium, with the persistency of only 5% of pericytes (αSMA+ cells). No significant morphological differences were appreciated between WT and HA BMECs when cultured in FBS. However, qPCR analysis indicated that the absence of FVIII secreted by BMECs modulates the expression of endothelial markers. On the contrary, cells cultured with WT or HA serum showed morphological and transcriptional changes with an increased rate of growth of WT BMECs cultured in WT murine serum compared to HA cells cultured in HA murine serum. **Conclusions**: These preliminary data indicate that WT and HA BMECs have similar morphology and rate of growth if cultured in FBS but they showed a partially different gene expression profile. This difference is marked when cells are cultured in WT or HA serum suggesting that HA pro-inflammatory microenvironment could induce a disorganized endothelium. These preliminary data need to be further confirmed and could subtend a possible impaired vascular functionality in HA which could justify the spontaneous ICHs in HA patients.

OC030

$P2Y_{12}$ DEPENDENT MODULATION OF PLATELET-ASSOCIATED TISSUE FACTOR AND INTRACELLULAR LOCALIZATION

M. Brambilla[1], P. Canzano[1], G.E. Rovati[2], M. Conti[1], A. Becchetti[1], A. Loffreda[3], M. Cattaneo[4], E. De Candia[5], G.M. Podda[4], C. Pinna[2], E. Tremoli[6], M. Camera[1,2]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy; [2]Dept of Pharmaceutical Sciences, Università degli Studi di Milano, Milan, Italy; [3]Experimental Imaging Center, San Raffaele Scientific Institute, Milan, Italy; [4]Unità di Medicina II, ASST Santi Paolo e Carlo, Dept of Scienze della Salute, Università degli Studi di Milano, Milan, Italy; [5]Dept of Traslational Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy; [6]Maria Cecilia Hospital, Cotignola, Italy

Background and Aims: ADP is stored at high concentrations in platelet dense granules and it enables platelet activation and aggregation by binding to two purinergic receptors on the platelet surface: $P2Y_1$, triggering platelet activation, and $P2Y_{12}$, which amplifies the process potentiating platelet secretion through membrane exposure of several intracellular proteins, including Tissue Factor (TF). The contribution of $P2Y_1$ and $P2Y_{12}$ receptors in platelet TF modulation is still unknown. The aims of the study were to assess: 1) the involvement of P2Y1 and $P2Y_{12}$ receptors in ADP-induced TF exposure on the platelet surface in healthy subjects' platelets, exploiting platelets from a $P2Y_{12}$-deficient patient; 2) TF intracellular localization through immunogold-electron

microscopy and pharmacological approach, taking advantage from the study of two Grey Platelet Syndrome (GPS) patients' platelets. **Methods**: TF^+- and P-$selectin^+$-platelets were evaluated by whole blood (WB) flow cytometry. *In vitro* experiments were performed on 10 healthy subjects (HS; n=5 males and n=5 females; mean age 39±6y) and WB was incubated with AR-C69931MX (1pM–100nM) or MRS-2500 (1pM–100nM) and stimulated with ADP (10µM). To assess actin and microtubular polymerization involvement in TF and P-selectin expression, platelets were preincubated with cytochalasin D (10µM) or colchicine (10µM). Immunogold labelling and electron microscopy were also performed to define platelet TF localization. Two patients with GPS syndrome (males, aged 31y and 15y) and a subject with inherited severe $P2Y_{12}$ deficiency (male, aged 65y) were also studied to confirm our hypothesis. **Results**: Flow cytometry analysis of WB from HS showed that $P2Y_{12}$-inhibitor AR-C69931MX, but not $P2Y_1$-inhibitor MRS-2500, concentration-dependently prevented ADP-stimulated TF exposure. To confirm the exclusive involvement of $P2Y_{12}$ in TF expression, we analyzed blood of a $P2Y_{12}$-deficient patient. Upon ADP stimulation, no increase of TF expression on the platelet surface was observed, but TF was readily detectable after stimulation with U46619 (+1.7 fold) or TRAP-6 (+1.4 fold). Unlike what observed for TF, the membrane exposure of P-selectin, a protein stored in platelet alpha-granules, was significantly modulated by both $P2Y_1$ and $P2Y_{12}$. This finding led us to hypothesize a different cellular localization of these proteins. Inhibition of open canalicular system (OCS) externalization, by preincubation with cytochalasin D or colchicine, impaired ADP-induced TF but not P-selectin expression, supporting TF storage within OCS as indicated also by immunogold labelling and electron microscopy analysis. Furthermore, we confirmed this hypothesis in patients with GPS syndrome, a disease characterized by large platelets lacking normal alpha-granules. Flow cytometry analysis showed significantly reduced levels of P-$selectin^+$-platelets (about -65%) compared to HS. Conversely, platelet-associated TF expression in GPS patients was comparable to that of HS, despite the alpha-granule defect. **Conclusions**: This study shows for the first time 1) the unique involvement of $P2Y_{12}$ receptor in ADP-induced TF exposure and 2) the TF intracellular localization that is stored in the OCS and exposed upon stimuli-induced OCS externalization. All together our findings add new insights into the regulation of the procoagulant activity of platelets highlighting that as for phosphatylserine, also TF exposure is a $P2Y_{12}$ dependent mechanism.

OC031

THROMBIN GENERATION IS ASSOCIATED WITH STROKE BUT NOT WITH CORONARY HEART DISEASE IN A GENERAL ADULT POPULATION: THE MOLI-SANI STUDY

S. Costanzo[1], R. de Laat-Kremers[2], A. Di Castelnuovo[3], A. De Curtis[1], T. Panzera[1], M. Ninivaggi[2], C. Cerletti[1], G. de Gaetano[1], L. Iacoviello[1,4], M.B. Donati[1], B. de Laat[2,5], for the Moli-sani Study investigators

[1]Department of Epidemiology and Prevention, IRCCS Neuromed, Pozzilli, IS, Italy; [2]Department of Data Analysis and Artificial Intelligence, Synapse Research Institute, Maastricht, the Netherlands; [3]Mediterranea Cardiocentro, Napoli; [4]Research Center in Epidemiology and Preventive Medicine (EPIMED), Department of Medicine and Surgery, University of Insubria, Varese, Italy; [5]Department of Functional Coagulation, Synapse Research Institute, Maastricht, the Netherlands

Background and Aims: Thrombin is the key enzyme in the coagulation process. It leads to clot formation by promoting activation of platelet and coagulation factors. On the other hand, thrombin bound to thrombomodulin activates protein C, an inhibitor of the coagulation cascade. The Thrombin generation (TG) assay assesses the potential of plasma to form thrombin, and it is useful for detecting hyper-or-hypocoagulability tendency at the individual level. To date a limited number of studies have investigated the relationship of TG with the risk of coronary heart disease (CHD) and stroke, with inconsistent results. The possible association of both low and high values of the TG parameters with the incidence of CHD and stroke was therefore evaluated in a large cohort of an Italian adult population. **Methods**: We analysed data on 20,109 individuals (53.3% women, mean age 54.8 ±11.5 years) enrolled in the Moli-sani study (2005-2010), without a history of cardiovascular disease or use of vitamin K antagonists at baseline, for whom TG data were available. TG curves were measured in each sample at 5 pM tissue factor by the calibrated automated thrombinography method (Synapse Research Institute, The Netherlands). The whole cohort was followed-up to December 2015 for fatal or non-fatal CHD and stroke events; the latter were ascertained by record linkage to the regional hospital discharge form and death registries and validated by checking medical records or death certificate, respectively. Multivariable hazard ratio (HR) and 95% confidence interval (CI) for outcomes were estimated by Cox-proportional hazard models, categorizing TG parameters in quintiles (the third acted as the reference) including a large panel of cardiovascular risk factors as possible confounders. **Results**: Over a median follow-up of 8.2 years, a total of 571 (2.8%) CHD and 183 (0.9%) stroke events were ascertained. No association between any TG parameter and CHD was found. In contrast, individuals in the lowest or the highest endogenous thrombin potential (ETP) quintiles showed an increased risk of total stroke compared to the third one: multivariable HR=1.63 (95%CI: 1.03-2.58) and HR=1.75 (95%CI: 1.06-2.89), respectively (Table 1). Dose-response analyses by use of restricted cubic spline functions, confirmed the U-shaped relationship between ETP and risk of total stroke (P value for overall association 0.032; P value for non-linear association 0.013). Preliminary, under-

powered analyses by stroke subtypes showed U-shaped association of ETP with ischemic (N=95), but a negative linear link of ETP with haemorrhagic (N=50) events. **Conclusions**: In comparison with median values, both lower and higher levels of ETP, corresponding to the total amount of thrombin formed during the assay, are associated with an increased risk of total stroke. The thrombin dualistic role in the coagulation cascade could be an explanation of the U-shaped relationship observed with total (particularly ischemic) stroke. Further confirmation of a relationship between TG and CHD/stroke would not only contribute to the knowledge on the physio-pathological processes leading to thrombosis, but also suggest new preventive approaches.

Table 1.

Rate of events and multivariable hazard ratios for fatal and non-fatal CHD and stroke associated with endogenous thrombin potential (ETP) quintiles.

Fatal and non-fatal stroke , 183/20,190	N	Rate of event	HR[a] (95%CI)	HR[b] (95%CI)
ETP, nM x min				
Q1 (244-1421)	4,038	60 (1.49%)	**1.72 (1.09-2.71)**	**1.63 (1.03-2.58)**
Q2 (1422-1605)	4,038	36 (0.89%)	1.18 (0.72-1.95)	1.16 (0.70-1.91)
Q3 (1606-1787)	4,038	27 (0.67%)	*Reference*	*Reference*
Q4 (1788-2054)	4,038	23 (0.53%)	0.95 (0.54-1.65)	0.95 (0.54-1.65)
Q5 (2052-5293)	4,038	37 (0.92%)	**1.71 (1.04-2.81)**	**1.75 (1.06-2.89)**
P-value for overall association			**0.023**	**0.030**
Fatal and non-fatal CHD, 571/20,190	**N**	**Rate of event**	**HR[a] (95%CI)**	**HR[c] (95%CI)**
ETP, nM x min				
Q1 (244-1421)	4,038	136 (3.37%)	1.06 (0.82-1.37)	1.11 (0.85-1.43)
Q2 (1422-1605)	4,038	118 (2.92%)	1.05 (0.81-1.36)	1.07 (0.82-1.39)
Q3 (1606-1787)	4,038	105 (2.60%)	*Reference*	*Reference*
Q4 (1788-2054)	4,038	106 (2.63%)	1.14 (0.87-1.49)	1.13 (0.86-1.48)
Q5 (2052-5293)	4,038	106 (2.63%)	1.30 (0.99-1.71)	1.25 (0.96-1.65)
P-value for overall association			0.35	0.58

[a]Adjusted for sex, age. [b]Adjusted for sex, age, diabetes, hypertension, education, adhesion to Mediterranean diet, heart failure and atrial fibrillation. [c]Adjusted for sex, age, diabetes, hypertension, dyslipidaemia, body mass index, waist-to-hip ratio and education.

OC032

THROMBOPLASTIN CALIBRATION REVISITED TO LOOK FOR POSSIBLE REVISION OF THE WORLD HEALTH ORGANIZATION RECOMMENDATIONS

E. Scalambrino[1], M. Clerici[1], V. Chantarangkul[1], F. Peyvandi[1,2], A. Tripodi[1]

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano and Fondazione Luigi Villa, Milano, Italy; [2]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milano, Italy

Background and Aims: Monitoring vitamin K antagonists (VKA) entails frequent measurement of the prothrombin time (PT) with results expressed as international normalized ratio (INR) to be used for dose adjustment. To this end thromboplastin calibration is essential to determine the international sensitivity index (ISI) required to calculate the INR. The procedure for calibration recommended by the World Health Organization (WHO) calls for selection of patients on stable anticoagulation in the range from 1.5 to 4.5 INR. These patients are difficult to be recruited as the conventional therapeutic intervals for VKA is from to 2.0 to 3.0 INR. A possible solution could be including in the calibration patients with less intense anticoagulation. We sought to investigate the impact of the above simplified procedure on the following parameters of calibration: (i) ISI; (ii) coefficient of variation (CV) of the calibration and (iii) INR. **Methods**: We used eight data sets of previous calibrations of a rabbit thromboplastin (Sclavo PT Ready Quick, Dasit, Milano, Italy) in combination with the coagulometer CSA 5100, Sysmex, Kobe, Japan. The above calibrations were performed against the rabbit thromboplastin WHO standard (coded RBT/16) and included patients on anticoagulation as required by WHO and with INR from 1.5 to 4.5. Parameters of calibration as determined with the full data sets were identified as "full calibrations" and were considered as reference. Each of the data sets were then used to recalculate the calibration parameters after including patients with INR <4.0, <3.5 or <3.0, which were identified as "trimmed calibrations" and compared to those from the "full calibrations". **Results**: Results are in Table 1.

Table 1.

Calibration parameters for eight *full* vs *trimmed* calibrations. *Full* calibrations represent the inclusion of 60 patients stabilized on VKA and 20 healthy subjects. *Trimmed* calibrations represent the inclusion of patients on VKA with INR up to < 4.0, < 3.5 or < 3.0. VKA, vitamin K antagonists. ISI, international sensitivity index. MNPT, mean normal prothrombin time. CV, coefficient of variation. N, numbers of patients included in the calibrations.

Calibration		Full	Trimmed < 4.0	Trimmed < 3.5	Trimmed < 3.0
1	ISI	1.14	1.15	1.17	1.16
	MNPT *(sec)*	12.3			
	CV *(%)*	1.3	1.4	1.4	1.8
	N. patients	59	53	44	29
2	ISI	1.10	1.10	1.11	1.12
	MNPT	13.3			
	CV *(%)*	1.5	1.6	1.7	1.9
	N. patients	59	57	49	33
3	ISI	1.13	1.13	1.12	1.15
	MNPT	13.0			
	CV *(%)*	1.7	1.7	1.9	2.2
	N. patients	59	57	46	35
4	ISI	1.07	1.06	1.06	1.06
	MNPT	13.1			
	CV *(%)*	1.2	1.2	1.2	1.4
	N. patients	60	59	56	36
5	ISI	1.14	1.14	1.16	1.16
	MNPT	12.1			
	CV *(%)*	1.3	1.4	1.4	1.7
	N. patients	59	53	44	29
6	ISI	1.10	1.10	1.11	1.12
	MNPT	13.3			
	CV *(%)*	1.6	1.6	1.8	1.9
	N. patients	59	57	49	34
7	ISI	1.13	1.13	1.12	1.15
	MNPT	13.0			
	CV *(%)*	1.7	1.7	1.8	2.1
	N. patients	59	57	46	33
8	ISI	1.08	1.08	1.08	1.08
	MNPT	12.8			
	CV *(%)*	1.2	1.2	1.3	1.4
	N. patients	60	59	56	37
Overall Mean	ISI	1.11	1.11	1.12	1.13
	MNPT	12.9			
	CV *(%)*	1.4	1.5	1.6	1.8
	N. patients	59	57	49	33

There was marginal variation of the ISI, CV and INR that can hardly be considered of practical significance. CV was the most affected parameter, which increased from the full to the trimmed <3.0 calibration, but never exceeded the 3% cut off value recommended by WHO. **Conclusions**. Should the results of this pilot study be

confirmed for calibrations of other thromboplastin/coagulometer combinations, the revision of the WHO recommendations to include patients with INR from 1.5 to 4.0 is warranted.

OC033

EXTENDEND-HALF-LIFE RECOMBINANT FVIII PRODUCTS (EHL-FVIIIS): ONE STAGE COAGULATION ASSAY OR CHROMOGENIC ASSAY?

M. Sacco[1], S. Lancellotti[2], M. Tardugno[1], R. De Cristofaro[1,2]

[1]Catholic University, Translational Medicine and Surgery Department Rome; [2]Fondazione Policlinico Universitario AGemelli, IRCCS, Center for Hemorrhagic and Thrombotic Diseases and Haemophilia Center, Rome, Italy

Background and Aims: The extended-half-life recombinant FVIII products (EHL-FVIIIs) have been introduced to improve the FVIII pharmacokinetic profile (increasing about 1.5 fold the half-life of FVIII) reducing the infusions number in hemophilic patients and the annual bleeding rates with less frequent dosing and higher trough plasma concentrations. These products are structurally and functionally heterogeneous; in fact, chemical coupling of Polyethylene glycol (PEG) increases hydrodynamic volume to prevent FVIII clearance, while the fusion with other proteins, such as the Fc fragment of IgG1 or albumin, protects FVIII from lysosomal sorting and degradation. These different strategies lead to several laboratory discrepancies in FVIII coagulant (FVIII:C) measurement by chromogenic substrate assay (CSA) and one-stage clotting assay (OSA) inducing an altered FVIII recovery with an over/under-estimating by more than 30%. Similar discrepancies were observed in patients receiving ReFacto® (B-Domain-Deleted FVIII) a decade ago and has been resolved with the use of specific ReFacto Laboratory Standard. The laboratory assays used should obtain the recovery of FVIII:C closely in accordance with the assay used to demonstrate the product efficacy during clinical trials. The aim of our study is to evaluate, with four different types of assays, FVIII:C and recovery of spiked EHL FVIIIs in FVIII-deficient human plasma. **Methods**: The EHL-FVIII products tested were: Damoctocog alpha-pegol, Rurioctocog alpha-pegol, Turoctocog alpha-pegol and Efmoroctocog alfa. All EHL-FVIIIs were reconstituted according to the manufacturer's instructions and serial dilutions (1/0.5/0.1/0.05/0.025 UI/dL) were obtained by using HemosIL® FVIII Deficient Plasma (Werfen). For each product, FVIII:C was then tested through OSA (Werfen) by using two different APTT reagents containing different plasma activators (colloidal silica and Ellagic Acid), EHL-FVIII-calibrated OSA methods and two different commercially available CSA (Chromogenix Coamatic® Factor VIII and Trinichrom FVIII Stago). Each sample was also tested using thrombin generation assay (TGA) by ST Genesia system – Stago which measured the generated thrombin in fluorescence. The parameters considered were normalized: lag-time, peak height, endogenous potential of active thrombin (ETP), velocity index, time to peak and start tail. **Results**: The classical OSA assay could show significant discrepancies between the certificated and real EHL-FVIII concentrations. The determination of Efmoroctocog alfa by OSA provides a value two-fold higher than the expected level. The calibrated-OSA assay is more accurate in determining the values of all EHL-FVIIIs, showing in the spiked experiments a drug recovery from 92% to 101%. On the contrary, the CSA, despite has been used to determine the potency of all products, reported a CV more than 30%. Noteworthy, Damoctocog alpha-pegol levels were significantly underestimated by one of the CSA tested, underestimating the nominal value up to 50%. TGA showed a significant functional difference between EHL-FVIII products. The thrombogram obtained from spiked EHL-FVIIIs, demonstrated lower sensitivity at concentration <0.1 IU/dL. **Conclusions**: In this scenario, a standard calibration curve for each EHL-FVIII product could avoid inaccurate laboratory results, which might heavily impact on patient management.

OC034

COAGUCHEK• PRO II PERFORMANCE EVALUATION TO ASSESS DIRECT ORAL ANTICOAGULANT (DOAC) ACTION. THE DOAC-CHECK STUDY

C. Legnani[1], M. Cini[1], S. Testa[2], A. Tosetto[3], C. Dellanoce[2], S. Bellesso[3], G. Carli[3], I. Nichele[3], L. Lissandrini[3], S. Zorzi[1], E. Antonucci[1], G. Palareti[1]

[1]Arianna Anticoagulazione Foundation, Bologna; [2]Haemostasis and Thrombosis Center, Laboratory Medicine Department, Cremona Hospital, Cremona; [3]Department of Hematology, San Bortolo Hospital, Vicenza, Italy

Background and Aims: Direct oral anticoagulants (DOACs) do not require routine laboratory monitoring, which is suggested in specific conditions, especially emergencies. Specific tests for DOAC are available, though implemented in few laboratories. PT and PTT may be useful for a rough evaluation of anticoagulation level but are performed in laboratory, and results are delayed by sample transportation and handling. Conversely, POCT for PT and PTT measurements are currently used. The DOAC-CHECK Study, an observational, national, multicentre, no-profit study, aimed to evaluate weather CoaguChek® Pro II (Roche Diagnostics) use can provide reliable information in DOAC treated patients. The study, promoted by Fondazione Arianna Anticoagulazione, was supported by

Roche Diagnostics and Fondazione Cassa di Risparmio di Bologna. **Methods**: The study was carried out in two FCSA (Italian Federation of Thrombosis Centers) centers. PT and PTT were performed by CoaguChek® Pro II using capillary blood; at the same time venous samples were collected for measuring DOAC levels by chromogenic assay (Diagnostica Stago). Since data to define relevant DOAC concentrations are currently limited we choose 3 different concentration thresholds for our analysis (30, 50 and 100 ng/mL). Using the ROC curves, for each test/DOAC threshold, as the ideal cut-off point was selected the one that yielded a sensitivity of at least 95% (misprediction percentage <5%, considered sufficiently safe for clinical application) associated to the highest possible specificity, to avoid false-negative results but simultaneously to identify the largest number of patients eligible for emergency treatment such as thrombolysis or emergency surgery. **Results**: 512 patients were enrolled; 222 received Apixaban, 120 Edoxaban, 111 Rivaroxaban, and 59 Dabigatran. For Edoxaban and Rivaroxaban both for CoaguChek® Pro II PT and PTT a sensitivity >95% corresponded to satisfying specificity values, especially at the 50 and 100 ng/mL threshold DOAC concentrations; the NP*Vs* resulted in the range 90-100% both for CoaguChek® Pro II PT and PTT at all DOAC thresholds. Conversely, CoaguChek® Pro II PT and PTT could identify patients with Apixaban concentrations above the pre-defined thresholds, but only at the expense of a high number of false-positive cases, thus excluding from thrombolysis/surgery a too large number of patients. CoaguChek® Pro II PT and PTT do not seem to be useful for identifying Dabigatran concentrations higher than the pre-defined threshold; acceptable results were obtained only for CoaguChek® Pro II PTT at the threshold of 100 ng/mL Dabigatran concentration. **Conclusions**: Our results suggest that CoaguChek® Pro II can be used to qualitatively identify relevant concentrations of Edoxaban and Rivaroxaban, but not of Apixaban and Dabigatran. However, as the suggested cut-offs were determined retrospectively, further evaluation in a prospective clinical trial, ideally in emergency situations, is warranted to investigate the clinical safety of this approach.

OC035

ADAMTS-13/VON WILLEBRAND RATIO: A PROGNOSTIC BIOMARKER FOR PORTAL VEIN THROMBOSIS (PVT) IN COMPENSATED LIVER CIRRHOSIS. A PROSPECTIVE OBSERVATIONAL STUDY

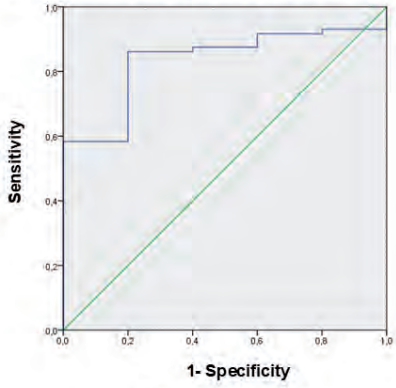
M. Sacco[1], M. Tardugno[1], S. Lancellotti[2], A. Ferretti[2], F.R. Ponziani[3], L. Riccardi[3], M.A. Zocco[3], A. De Magistris[3], F. Santopaolo[3], M. Pompili[3], R. De Cristofaro[1,2]

[1]Catholic University, Translational Medicine and Surgery Department Rome; [2]Fondazione Policlinico Universitario AGemelli, IRCCS, Center for Hemorrhagic and Thrombotic Diseases and Haemophilia Center, Rome; [3]Fondazione Policlinico Universitario AGemelli, IRCCS, Department of Digestive, Endocrine and Metabolic System, Institute of Internal Medicine and Geriatrics, Rome, Italy

Background: Portal vein thrombosis (PVT) represents one of the most fearsome comorbidity of cirrhosis and its prevalence is higher in patients with decompensated disease. These patients are characterized by a fragile hemostatic balance with a decrease of both procoagulant and anticoagulant factors, synthesized by hepatocytes, together with an increase in endothelial-derived factors such as Factor VIII (FVIII) and von Willebrand factor (VWF). Simultaneously, the pro-fibrogenic transformation of liver's stellate cells causes a progressive decrease of ADAMTS-13 levels, while VWF multimers secretion by endothelial cells is strongly enhanced. This unbalance induces an accumulation of ultra-large VWF multimers in sinusoidal circulation where PVT in intra- and extra-hepatic branches could occur, especially in decompensated cirrhosis. A decreased portal vein flow velocity <15cm/sec2, thrombophilia factors and portal hypertension are well-known risk factors predisposing to PVT but it is still unclear how the primary and secondary haemostatic abnormalities are involved in PVT development. Thus, the identification of biomarkers acting as predictors of PVT in compensated cirrhotic patients could have a significant clinical impact allowing to identify patients at risk who could eventually undergo prophylactic therapy. This observational prospective clinical trial on compensated cirrhotic patients (ClinicalTrial.gov: NCT03322696) could identify ADAMTS-13/VWF:GpIbR ratio as a key predictive factor of PVT development in cirrhotic patients. **Methods**: The final 79/118 enrolled subjects were followed for 48 months and underwent Doppler-ultrasound liver examination and clinical (Child-Pugh and MELD-score) and hematochemical (PT%, APTT%, AT%, PC%, PS%, LAC, fibrinogen, FVIII:C, D-dimer) assessment. VWF antigen and VWF:GpIbR were measured by chemiluminescence assays; ADAMTS-13:activity by a FRET-based-assay; both FV Leiden G1691A and the prothrombin G20210A polymorphisms by using the RT-PCR; statistical analyses by the SPSS-software. **Results**: Five patients presented PVT, the cumulative incidence of PVT in this cohort was: 2.5, 3.8, and 6.3% at 1, 2 and 3 years after the enrollment. These patients showed a statistically significant reduction of ADAMTS-13/VWF:GpIbR ratio compared to patients without PVT [mean value: 0.30 (0.28-0.32) *vs* 0.69 (0.61-0.77). Univariate analysis showed that at the enrollment visit ADAMTS-13 levels, ADAMTS-13/VWF:GpIbR ratio, PS, PC, Platelets count, INR were significantly correlated with PVT. The ADAMTS-13/VWF:GpIbR ratio sensitivity and specificity as PVT predictor, 86% and 80% respectively, were assessed by a ROC curve (Figure 1), highlighting the AUC equal to 0.833 and a cut-off-value=0.40, below which the risk to develop a PVT complication increases (OR 14.6, 95% C.I.:1.36-157.2, p=0.027) as also confirmed by a Cox-regression-analysis, where the

risk to develop a PVT rises exponentially after 30th month-follow-up in patients with a ADAMTS-13/VWF:GpIbR ratio <0.4 compared to patients with ratio >0.4 [HR:7.7 (C.I.; 2.2-26.7, p=0.001]. **Conclusions**: In compensated cirrhosis a ADAMTS-13/VWF:GpIbR ratio <0.4 could be a useful predictive biomarker for the identification and stratification of patients at risk of PVT development. Further studies are needed to confirm these findings even in moderate and severe clinical setting where new treatment strategy could be used for preventing and treating this complication of cirrhosis.

ROC curve and the corresponding results of the sensitivity and specificity characteristics of the ADAMTS-13/VWF:GpIbR ratio as predictor of PVT.



Area Under the Curve

Test Result

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,833	,067	,013	,702	,964

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 1.

OC036

BIOMARKERS OF HYPERCOAGULABILITY AND PREDICTION FOR EARLY DISEASE PROGRESSION AND MORTALITY IN PATIENTS WITH METASTATIC GASTROINTESTINAL CANCER

C. Giaccherini[1], M. Marchetti[1], C. Verzeroli[1], L. Russo[1], S. Gamba[1], C.J. Tartari[1], S. Bolognini[1], C. Ticozzi[1], F. Schieppati[1], A. Santoro[2], F. De Braud[3], G. Gasparini[4], F. Petrelli[5], F. Giuliani[6], A. D'Alessio[7], M. Minelli[8], R. Labianca[9], A. Falanga[1,10] on behalf of the HYPERCAN Investigators*

[1]Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo; [2]Oncology Unit, IRCCS National Cancer Institute, Milan; [3]Oncology Unit, IRCCS Humanitas Institute, Rozzano, Milan; [4]Oncology Unit, Hospital San Filippo Neri, Rome; [5]Oncology Unit, Hospital Treviglio-Caravaggio, Treviglio, Bergamo; [6]Oncology Unit, IRCCS Cancer Institute Giovanni Paolo II, Bari; [7]Medical Oncology and Internal Medicine, Policlinico San Marco, Zingonia, Bergamo; [8]Oncology Unit, Hospital San Giovanni Addolorata, Rome; [9]DIPO, ASST Papa Giovanni XXIII, Bergamo; [10]School of Medicine, University of Milan-Bicocca, Milan, Italy

Background and Aims: Gastrointestinal (GI) cancers represent 26% of the global cancer incidence and more than one-third of all cancer-related deaths. The clinical benefits of treatments developed over years differ critically among individuals and, in many cases, patient's prognosis remains poor. Due to the reciprocal interaction between coagulation and cancer, the biomarkers of the hemostatic system could be useful in monitoring prognosis and in the choice of the most appropriate treatment. In a prospective cohort of patients with newly diagnosed metastatic GI cancer, we aimed to evaluate whether pre-chemotherapy hemostatic biomarkers may predict for 6 months disease progression (6m-DP), and for 1-year overall survival (1-year OS). **Methods**: Prospectively enrolled patients with metastatic GI cancer from the HYPERCAN study were analyzed. At enrollment, before starting any curative chemotherapy, plasma samples were collected and tested for thrombin generation (TG) potential and D-dimer, fibrinogen, and prothrombin fragment 1+2 (F1+2) levels. DP and mortality were monitored during follow-up. **Results**: A cohort of 626 GI cancer patients (age: 66 y [26-87]), 462 with colorectal and 164 with gastric cancers, was available for analysis. After 6 months from the start of chemotherapy, DP occurred in 148 patients, providing a cumulative incidence of 24.8% (CI 95% 21.4-28.4) with death as competing risk. Baseline D-dimer levels were significantly (p=0.001) higher in these patients compared to those without DP. Gastric cancer, pre-chemotherapy D-dimer, ETP, and the presence of more than 1 metastatic sites were identified as independent risk factors for 6m-DP by multivariate Fine-Gray proportional hazard regression analysis. After 1-year, overall survival (OS) was 75.7% (CI 95% 71.9-79.0). Patients who died showed higher baseline D-dimer (p<0.001), F1+2 (p=0.004), fibrinogen (p=0.023), and TG ETP (p<0.01) values compared to surviving. Multivariate Cox regression identified independent risk factors for 1-year OS the followings: gastric cancer, D-Dimer, ETP, ECOG-PS, and advanced cancer stage at diagnosis. D-dimer and TG ETP levels were dichotomized using ROC curves and a score was created accordingly for classification of patients at low, intermediate, or high risk of early DP. As a result, having at least one or both parameters above the specific threshold was a risk factor not only for 6m-DP (intermediate *vs* low: SHR=2.1 [1.3-3.4]; high *vs* low: SHR=2.3 [1.4-3.9]), but also for 1-year mortality (intermediate *vs* low: HR=2.7 [1.5-4.8]; high *vs* low: HR=5.5 [3.1-9.8]). **Conclusions**: Our results showed that, in newly diagnosed metastatic GI cancer patients, pretreatment TG potential and D-dimer appear as promising candidate biomarkers to predict for 6-month DP and 1-year OS. In this setting, the role of TG as a prognostic biomarker emerges for the first time in a large prospective cohort of GI cancer patients.

OC037

ASSOCIATION BETWEEN NON-O BOOD TYPE AND EARLY UNEXPLAINED RECURRENT SPONTANEOUS ABORTION IN WOMEN WITH AND WITHOUT THROMBOPHILIA

L. Spiezia, A. Poretto, E. Borella, G. Turatti, M. Marobin, C. Simion, E. Campello, D. Tormene, P. Simioni

Thrombotic and Hemorrhagic Diseases Unit, Padova University Hospital, Padova, Italy

Background and Aims: The association between inherited thrombophilias (IT) and recurrent spontaneous abortion (RSA) has been studied extensively without reaching definitive conclusions. We retrospectively evaluated whether non-O blood type – the commonest prothrombotic risk factor – might be associated with a higher risk to develop early unexplained RSA in women with and without IT. **Methods**: N. 238 consecutive women with a history of early (*i.e.* before 12 weeks of gestation) objectively unexplained RSA (*i.e.* ≥3 consecutively) tested for IT at the laboratory of Thrombotic and Hemorrhagic Diseases Unit, Padova University Hospital, Padova, Italy between December 2011 and December 2021 were considered for enrollment. A group of healthy women, age-matched, with ≥1 normal pregnancy and no history of adverse pregnancy outcomes acted as controls. All participants were tested for antithrombin, protein C and S deficiency, prothrombin G20210A, FV Leiden and MTHFR mutations and were ABO genotyping. **Results**: N. 216 (mean age at the time of first abortion 36 yrs, range 24-43 yrs) women were enrolled. No significant differences between cases and controls according to age and BMI ($p > 0.05$ in each comparison) were observed. Non-O blood type was significantly more prevalent in cases than controls ($p < 0.05$). Adjusted Odds ratio (aOR) of RSA in non-O *vs.* O blood type was 1.37 (95% CI, 1.04 to 2.18); aOR of RSA in women with *vs.* without IT was 2.06 (95% CI, 1.08 to 3.61). Interestingly, we found concomitant IT and non-O in n. 29 (13%) cases and n. 16 (7%) controls, aOR 2.52 (95% CI, 1.12 to 5.47). **Conclusions**: We found a significant higher prevalence of non-O blood group or IT in women with unexplained RSA. Their combined presence further increases RSA risk. Larger studies are warranted to validate our findings.

OC038

PREGNANCY IN PATIENTS WITH MECHANICAL HEART VALVES: FOETO-MATERNAL OUTCOME IN THE POPULATION MONITORED BY ANTICOAGULATION SERVICE IN SALAM CENTRE FOR CARDIAC SURGERY-EMERGENCY NGO – KHARTOUM – SUDAN

N. Erba[1,2,4], S. Abdelwahab[2], S. Gatti[1], M. Langer[1,5], L. Chatenoud[3], G. Portella[1], D. Poli[4], S. Testa[4], R. Baiocchi[1]

[1]Emergency NGO ONLUS, Milan, Italy; [2]SALAM Centre for Cardiac Surgery, Khartoum, Sudan; [3]Department of Public Health, Laboratory of Clinical Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy; [4]Federazione Centri per la Diagnosi Della Trombosi e la Sorveglianza delle Terapie Antitrombotiche (FCSA), Milan, Italy; [5]Università degli Studi, Milan, Italy

Background and Aim: Pregnancy on anticoagulants after mechanical heart valve replacement presents a high risk of complications for both mother and baby. Moreover, pregnancy is a prothrombotic condition itself. A safe and effective anticoagulation regimen for both mother and foetus doesn't exist. The most effective drugs to prevent valve thrombosis are Vitamin K Antagonists (VKAs): the dosage needs to be adjusted with frequent INR checks. VKAs can have embryopathic and teratogenic effect; moreover they can cause foetal bleeding. Patients in follow-up and anticoagulant treatment at the Salam Centre for Cardiac Surgery in Sudan live scattered in a large area, with limited access to the Center. For all these reasons pregnancy is discouraged and contraception and therapeutic abortion recommended, but this guidance frequently goes unheeded. The aim is to analyze pregnancy outcomes in women with mechanical valve prosthesis followed by the Anticoagulant Clinic (AC) of Salam Centre. **Methods**: Data were collected from all pregnancies recorded among patients followed by our AC from April 2017 until November 2021. Death, valve thrombosis and major bleeding occurred during pregnancy till 6 weeks after its conclusion have been considered as maternal outcomes; miscarriage, intrauterine foetal death and neonatal death have been considered as foetal outcomes. **Results**: 307 pregnancies in 253 women were assessed. All patients had mechanical heart valve prostheses: 15 aortic valve prostheses (AVPs), 163 mitral prostheses (MVPs) and 75 combined MVPs and AVPs. Out of 307 pregnancies, there were 15 maternal deaths (4.9%), 24 thrombotic events (7.8%) and 22 major bleedings (7.2%). The cause of death often is unknown. Thrombosis leading to death was more frequent than hemorrhage (33.3% *vs.* 6.7%). Out of 24 thrombotic events, 23 were valve thrombosis. Major bleeding occurred mainly after miscarriage/therapeutic abortion compared to post-partum (12.9%, 12%, 3.9% respectively; $p=0.019$). Fifty pregnancies (16.3%) were terminated by therapeutic abortion. Only 133/237 (56.1%) mothers had at least one live baby. Nearly one out of five mothers (19.8%) repeatedly tried to have a child, but only 41 babies were born live after their collective 101 pregnancies. Information about VKAs embrio-foetopathy in our population was not available. **Conclusions**: To become a mother is a natural desire all over the world. In medium- and low-income countries, the importance placed on women having children, and the social pressure on them to do so, is very strong. In this context, limitations to family planning are not easily accepted, even if for para-

mount medical reasons. In our population maternal mortality and major complications during pregnancy are at an unacceptable level: this is related to the presence of the mechanical valve at first, but also to low compliance to anticoagulant therapy and scarcity of tertiary healthcare services. Safeguarding mothers' lives must become our principal commitment, and solutions should be context-related. First, more efforts should be made to improve women's awareness about health issues related to pregnancy. Standardized counseling, focused on self-engagement, can strengthen alliance between patients and caregivers, improve adherence to therapy and contraception, and ensure timely communication about pregnant status. At last but not at least, a nationwide obstetric support network should be implemented to meet this complex and growing health demand.

OC039

IMPACT OF TRIAGE PRIORITY CODE ON THE MANAGEMENT OF CHILDREN AND ADULTS WITH INHERITED BLEEDING DISORDERS IN THE EMERGENCY DEPARTMENT

C. Ferraris[1], L. Franceschi[1], E. Castagno[2], B. Pollio[3], I. Ricca[3], R. Albiani[3], A. Borchiellini[4], C. Dainese[4], A. Catalano[5], R. Cerchio[2], C. Bondone[2]

[1]Department of Public Health and Pediatrics, University of Turin, Turin, Italy; [2]Department of Pediatric Emergency, Regina Margherita Children's Hospital - A.O.UCittà della Salute e della Scienza di Torino, Turin, Italy; [3]Transfusion Medicine, Department of Diagnostic, Regional Center for hemorragic and thrombotic diseases in childhood, Regina Margherita Children's Hospital - A.O.UCittà della Salute e della Scienza di Torino, Turin, Italy; [4]Hematology Division, Regional Center for hemorragic and thrombotic diseases, A.O.UCittà della Salute e della Scienza di Torino, Turin, Italy; [5]Department of Clinical and Biological Sciences, University of Turin, Orbassano (TO), Italy

Background and Aims: Haemophilia and von Willebrand disease are the most common inherited bleeding disorders in the Emergency Department (ED). Early replacement of the lacking clotting factor is recognized as the cornerstone for better outcome, based on consensus of experts but lacking high-level evidence [1,2]. For this reason, in most pediatric EDs children with known inherited bleeding disorders usually receive high triage priority code even for not serious events. Our aim was to investigate the impact of triage priority code on the management of children with inherited bleeding disorders admitted to ED and their outcome, compared to adults. **Methods**: All the children <18 years and adults with inherited bleeding disorders referred to the Pediatric ED and General ED of our Institution between January 2015 and June 2021 were retrospectively reviewed. We included patients with haemophilia A and B, von Willebrand disease and other rare inherited bleeding disorders. Their demographic profile, reason for admission to the ED, triage priority code, length of stay in the ED, time between triage and the first replacement dose of lacking clotting factor, and outcome were investigated. Descriptive analysis is reported. Significance was set at $p<0.05$. **Results**: Overall, 60 children were admitted 210 times (median age: 8.00 years): 41 with haemophilia A, 7 haemophilia B, 9 von Willebrand disease, and 3 other rare bleeding inherited disorders (2 with factor VII deficiency, 1 with factor XI deficiency). On the same period, 58 adults were admitted 115 times (median age: 38.00 years): 40 with haemophilia A, 9 haemophilia B, 8 von Willebrand disease, and 1 factor V deficiency. Triage priority code was significantly higher in children, with red and yellow high priority codes attributed respectively to 190/210 (90.5%) admissions of children and 42/115 (36.5%) admissions of adults ($p<0.001$). Suspected spontaneous bleeding was reported in 70/210 pediatric admissions (33.3%) and 59/115 adult admissions (51.3%). Median time between triage and the first replacement dose of clotting factor was 49.00 minutes (IQR 23.00-85.00) in children and 157.00 minutes (78.00-242.50) in adults ($p <0.001$). Compared to adults, children showed significantly shorter median time since triage to clinical examination (15 minutes *vs* 47 minutes, respectively; $p<0.001$), shorter median length of stay in the ED (106 minutes *vs* 311 minutes, respectively; $p<0.001$), and lower prevalence of hospitalization (8.1% *vs* 15.2%, respectively; $p=0.049$). **Conclusions**: The upgrade of triage priority code for children with inherited bleeding disorders was associated to earlier administration of factor, shorter length of stay in the ED, and lower hospitalization rate, which represent indirect indicators of better outcome. Our observation provides evidence supporting the recommendations not to keep patients with inherited bleeding disorders waiting in the ED and to provide early replacement of lacking clotting factors.

References:

1 Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, *et al.* WFH Guidelines for the Management of Hemophilia, 3rd edition. Haemophilia 2020;26(Suppl 6):1-158.
2 Dunkley S, Lam JCM, John MJ, Wong RSM, Tran H, Yang R, *et al.* Principles of haemophilia care: The Asia-Pacific perspective. Haemophilia 2018;24: 366-75.

OC040

LPA KRINGLE IV TYPE 2 DETECTION: QPCR OR DDPCR?

G. Barbieri[1], T. Capezzuoli[1], M. Giannini[1], G. Cassioli[1], F. Cesari[2], R. Marcucci[3], A.M. Gori[3], B. Giusti[3], E. Sticchi[3]

[1]University of Florence, Department of Experimental

and Clinical Medicine, Florence; [2]Atherothrombotic Diseases Center, Careggi Hospital, Florence; [3]University of Florence, Department of Experimental and Clinical Medicine, Atherothrombotic Diseases Center, Careggi Hospital, Florence, Italy

Background: Evidence to support the role of lipoprotein (a) [Lp(a)] as a risk factor for atherothrombosis continue to increase. Lp(a) levels strongly differ among individuals, and many studies have shown the relationship between variants in the *LPA* gene, encoding apolipoprotein (a), and the increase in Lp(a) levels, closely related to the increase in cardiovascular risk. The major genetic determinant of these levels is a copy number variation (CNV) polymorphism, consisting of a variable number of repeats of a 5 kb region that includes exons 4 and 5 of the gene, encoding the protein domain Kringle IV type 2 (KIV2). Determination of KIV2 repeat can be used to indirectly assess Lp(a) levels and to evaluate its involvement in modulating cardiovascular risk. However, the peculiar structural characteristics of this variant constitute a significant challenge to the development of accurate methods for its detection. In this study, we compared quantitative real-time PCR (qPCR) and digital droplet PCR (ddPCR) in KIV2 repeats determination. **Methods**: 70 patients with probable/certain diagnosis of Familial Hypercholesterolemia according to the Dutch Lipid Clinic Network score were analysed. Demographic and laboratory/clinical characteristics of the study population were collected. CNV values were obtained with qPCR using the 7900HT Sequence Detection System and with ddPCR using the QX200 Droplet Generator and reader system. Telomerase reverse transcriptase gene (TERT) was used as a single-copy reference gene in both techniques. To make CNV values comparable within and between different plates, three internal controls (CNV: 41<*C1*<47, *C2*=50, 61<*C3*<68) were used. **Results**: In the whole cohort, qPCR analysis showed median values of repeats of 28.37 [IQR: 20.25-40.21], while ddPCR of 10.55 [IQR: 9.44-12.26]. Correlation analysis between the two methods was slightly significant, because of the greater dispersion of data obtained by qPCR compared with ddPCR. Control sample C2, used as a reference sample for qPCR and estimated to have around 50 repeats, was confirmed to have a mean value of 54.85±1.11 with ddPCR analysis. Measurement of the other two internal controls (C1, C3) showed a wider data dispersion with qPCR *vs* ddPCR (mean±SD: 36.99±11.25 *vs* 44.28±2.74 and 92.78±25.20 *vs* 65.02±3.53, respectively). Spearman's rho test showed an inverse proportional correlation between Lp(a) levels of each patient and the CNV polymorphism, as expected, but higher and significant when evaluated with ddPCR despite qPCR (R=-0.393, p<0.001 *vs* R=-0.198, p=0.044, respectively). Dividing patients in two groups based on Lp(a) concentration (300 mg/L as cut off), a significant lower number of repeats of the KIV2 domain emerged among patients with greater levels of Lp(a) compared with the other group in both methods but with strongly evidence with ddPCR than in qPCR (P<0.001 and P=0.024, respectively). **Conclusions**: Data obtained by this study show the limitations in terms of stability and sensitivity of qPCR, enhancing the more accurate and less variable results obtained with ddPCR, increasingly widespread method in diagnostics. The complex nature of this kind of polymorphism still makes difficult to find an effective method to determine the exact number of repeats of the KIV2 domain, nevertheless it is important to refine the techniques, since Lp(a) measurement has been shown to provide clinically significant improved cardiovascular risk reclassification.

OC041

EFFECTS OF TRINITRO-RESVERATROL A NITRIC OXIDE-RELEASING DERIVATIVE OF RESVERATROL, ON PLATELET ACTIVATION

G. Guglielmini[1], E. Berardi[1], F. Messina[2], M.C. Marcotullio[2], P. Gresele[1]

[1]Division of Internal and Cardiovascular Medicine, Department of Medicine and Surgery; [2]Department of Pharmaceutical Sciences, University of Perugia, Italy

Background and Aims: Resveratrol (RSV), a polyphenol of red wine, has been considered as a crucial contributor to the «French paradox» which showed a protective effect of moderate wine consumption against cardiovascular events. RSV stimulates endothelial nitric oxide (NO) synthase by complex mechanisms thus enhancing NO production. In conditions of high atherosclerotic risk, in which the antioxidant potential of plasma is strikingly impaired, the bioavailability of NO is reduced diminishing his cardiovascular protective action, thus the use of NO-releasing compounds can have relevant therapeutic effects. Aim of the present study was to evaluate whether a newly synthetized nitro derivative of RSV may act as a NO-donor and display an antiplatelet effect. **Methods**: 3,5,4'-tri-oxy resveratrol (TN-RSV) was synthesized at the Department of Pharmaceutical Sciences of the University of Perugia. Trans-Resveratrol (RSV) was obtained from Sigma Aldrich. Collagen-induced aggregation was assessed by LTA on washed platelets and followed for 5 min. At the end samples were centrifuged at 12,000xg for 30sec and surpernatant stored at -80°C for subsequent NOx assay. NOx were measured using a colorimetric non-enzymatic assay (Griess Reaction Assay). Platelet reactive oxygen species (ROS) production was measured by flow-cytometry using the fluorescent probe H2DCFDA. Washed platelets were preincubated for 40 min with increasing concentrations (0.1 to 5µM) of RSV, TN-RSV or their vehicles and stimulated with collagen (50µg/µl, 3min). Platelet adhesion to collagen under flow conditions was assessed in a parallel plate perfusion chamber. Citrated whole blood was preincubated with RSV, TN-RSV or their vehicles, perfused over a collagen-coated coverslip at high

shear rate (3000sec-1) for 5 min, then coverslips were stained and surface coverage measured (IMAGE J). **Results**: RSV exerted a mild inhibitory effect on platelet aggregation with a J-shaped dose-response curve and a maximal inhibitory effect at 0.5µM (-33.9±4.9%). On the contrary, TN-RSV induced strong inhibition of collagen-induced platelet aggregation, already significant at 0.1µM and maximal at 0.5µM (-52.4±16.6%,p=0.02). NOx increased dose-dependently in the supernatant of collagen-stimulated platelets preincubated with RSV. With TN-RSV the release of NOx was significantly higher than with RSV over the entire concentration range tested (at 0.1µM RSV=+21.4±3.4% *vs* TN-RSV=+62.5±7.3%,p=0.03) maintaining similar difference up to 5µM. Preincubation of citrated whole blood with RSV decreased platelet adhesion to collagen. TN-RSV 0.1µM also inhibited platelet adhesion, but more strongly and its effect was enhanced by increasing the incubation time (at 40min RSV=-55.2%±10.3 *vs* TN-RSV=-83.9±2.1%, p=0.02). Collagen-induced platelet ROS formation was reduced by RSV with a J-shaped curve, with a significant maximal inhibition at 0.5µM, while TN-RSV inhibited it more strikingly and dose-dependently (at 0.5µM RSV=-57.1±3.9% *vs* TN-RSV=-66.1±1.8%%, p=0.04). **Conclusions**: We synthesized a novel RSV derivative with the capacity to release NO, property that adds to the beneficial activities of resveratrol the ability to act as a direct source of NO, with a synergistic inhibitory effect on platelet actation. The addition of a NO-donating moiety to RSV may enhance its effectiveness in disease conditions of *in vivo* peroxidation and strong atherosclerosis-related inflammation, such as those found in high-risk patients with acute coronary syndromes.

OC042

DISENTANGLING THE ASSOCIATION BETWEEN DEPRESSION AND CARDIOVASCULAR RISK: FINDINGS FROM THE MOLI-SANI STUDY

F. Bracone[1], A. Gialluisi[1,2], S. Costanzo[1], F. Santonastaso[2,3,4], A. Di Castelnuovo[5], S. Orlandi[1], S. Magnacca[5], A. De Curtis[1], C. Cerletti[1], M.B. Donati[1], G. de Gaetano[1], L. Iacoviello[1,2], on behalf of the Moli-sani Study Investigators

[1]Department of Epidemiology and Prevention, IRCCS NEUROMED, Pozzilli, Italy; [2]EPIMED Research Center, Department of Medicine and Surgery, University of Insubria, Varese, Italy; [3]Human Technopole, Viale Rita Levi Montalcini 1, 20157Milan, Italy; [4]European School of Molecular Medicine, University of Milan, 20122Milan, Italy; [5]Mediterranea Cardiocentro, Napoli, Italy

Background and Aims: Major Depressive Disorder (MDD) is a mental illness associated with chronic conditions like cardiovascular disease. Circulating inflammation has been proposed as a potential mechanism underlying this link, although its consequences on comorbidities are not well elucidated. We aimed to disentangle the relationship between depression, inflammation and cardiovascular risk by investigating the role of specific biomarkers, gender, and symptoms domains in this link. **Methods**: We performed gender-stratified multivariable Cox regressions of first hospitalization (median follow-up 7.28 years) or mortality for cardiovascular disease (CVD) and cerebrovascular disease (CeVD) (median follow-up 8.24 years) *vs* depression severity, in an Italian population cohort (N=13,191; age≥35 years; 49% men), adjusting for sociodemographic, health and lifestyle factors. We tested potential mediation of C-reactive protein, granulocyte-to-lymphocyte ratio (GLR), platelet and white blood cell counts (WBC). Depressive symptoms were assessed through an alternative validated version of the Patient Health Questionnaire (PHQ9-6). To investigate specific domains of depressive symptoms, we derived latent variables tagging somatic and cognitive symptoms, through a polychoric factor analysis. First hospital admissions were recorded by direct linkage with the Molise regional registry of hospital discharge records, updated to December 31st, 2015. Overall and cause-specific mortality was assessed through interrogation of the Italian mortality (ReNCaM) registry and linkage with the Moli-sani database through unique identifier codes for each participant, updated to December 31st, 2015. **Results**: In 6,689 women, N=630, N=88 and N=48 events for CVD, CeVD hospitalizations and CVD deaths occurred, respectively. In 6487 men, CVD, CeVD hospitalizations or CVD death events were 1,020, 138 and 93, respectively. Depression severity was similarly associated with CVD hospitalization in both men and women (Table 1). On the contrary, CeVD hospitalizations and CVD mortality relative risk was higher only in men with severe depression (Table 1). In multivariable models reciprocally adjusting for both factors, somatic depressive symptoms resulted associated with CVD hospitalization or death, and cognitive symptoms with CeVD hospitalization events (Table 1). In the association between depression severity and CVD hospitalizations, an explanatory effect of the leukocyte biomarkers of inflammation were revealed, with WBC and GLR explaining 2.4% (P=0.01) and 2.0% (P=0.03) of the association. A mediation role for WBC (13.7%, p=0.01) and GLR (10.4%, p=0.11) was also detected for CVD mortality risk. C-reactive protein and platelet count showed negligible mediation roles. **Conclusions**: We further characterized the link between depression and related CVD risks, revealing a prominent explanatory role of immune components of inflammation, in particular granulocytes and lymphocytes, and highlighting the importance of gender and of somatic depressive symptoms in this link. These findings point to the existence of pathways other than inflammation through which depression may play a detrimental effect on cardiovascular system, possibly related to gender-specificity and symptom clusters.

OC043

COVID-19 CONVALESCENT PLASMA IN PATIENTS WITH SARS-COV2 INFECTION: FOCUS ON IMMUNOSUPPRESSED OR NEOPLASTIC PATIENTS

A.M. Palladino[1,2], C.J. Tartari[1], L. Russo[1], C. Giaccherini[1], S. Gamba[1], S. Bolognini[1], C. Ticozzi[1], M. Marchetti[1], O.C. Cretu[1], F. Schieppati[1], L. Barcella[1], F. Zunino[1], A. Falanga[1,2]

[1]Division of Immunohematology and Transfusion Medicine, Papa Giovanni XXIII hospital, Bergamo; [2]Medicine and Surgery, University of Milan-Bicocca, Milan, Italy

Background and Aims: Patients treated with immunosuppressive and corticosteroid drugs are at increased risk for morbidity and mortality due to SARS-CoV2 infection. Passive immunization therapy with convalescent plasma has been successfully used in prior viral pandemics. For these reasons, from the beginning of the COVID-19 pandemic our hospital has been actively involved in collection of hyperimmune plasma from convalescent patients and in the administration of the same plasma to hospitalized patients for SARS-CoV2 infection. In this setting, in a cohort of COVID-19 plasma recipients, we aim to evaluate the association of convalescent plasma treatment with clinical and laboratory outcome. **Methods**: Adult patients with SARS-CoV2 infection diagnosed with moderate to severe acute respiratory distress syndrome were considered for COVID-19-convalescent plasma (CCP) treatment. Blood samples were collected before and after 7 days from CCP administration and tested for anti-SARS-CoV2 Nucleocapsid (anti-N) and Spike protein (anti-S) IgG antibodies, markers of inflammation [*i.e.* fibrinogen, reactive protein C (CRP), lactate dehydrogenase (LDH), lymphocytes], hypercoagulability (*i.e.* D-dimer) and organ functionality (*i.e.* AST, ALT, creatinine, GFR). **Results**: From April 2020 to April 2022, 334 hospitalized COVID-19 patients [103M/231F, age range 67 (41-86) years] were treated with CCP, (84 ICU and 250 not-ICU patients). Among them, 125 were patients under immunosuppressive therapy (68 with cancer, 56 with diabetes or other comorbidities). In the overall group (n=334), treatment with CCP led to a significant increase in anti-SARS-CoV2 antibodies, both anti-N [3.5 (0.06-7.74) *vs* 5.74 (0.25-7.95), p<0.001] and anti-S [63.15 (7.13-397.15) *vs* 123 (34-400) p<0.001], especially in ICU patients. Inflammatory parameters (*i.e.* CRP, LDH and ferritin) significantly decreased after CCP treatment in the overall cohort of patients, including those under immunosuppressive therapies. In particular, anti-N SARS-CoV2 antibodies titre positively correlated with platelet counts (r=0.127 p<0.05) and negatively correlated with ferritin levels (r=-0.505 p<0.01), also after adjustment for age and gender. Interestingly, in the sub-group of neoplastic patients (N=68), D-dimer and fibrinogen, measured before CCP treatment, were significantly associated with mortality (fibrinogen b=0.709 p<0.01 and D-dimer b=0.065 p<0.05). Only four out of 334 patients (1.1%) treated with CCP developed adverse events (*i.e.* anaphylaxis/hypersensitivity Grade 1-2) with a resolution in a day after CCP infusion suspension. **Conclusions**: CCP treatment led to an increase in antibody levels in recipients, especially in ICU patients with severe COVID-19. The low incidence of adverse events supports the role for CCP administration, especially in patients with severe inflammation and low antibody levels at admission.

OC044

A PLATELET LIPIDOMICS SIGNATURE IN PATIENTS WITH COVID-19

E. Petito[1], L. Goracci[2], A. Di Veroli[2], E. Falcinelli[1], C. Bencivenga[2], E. Giglio[1], C. Becattini[1], E. De Robertis[3], G. Vaudo[4], P. Gresele[1]

[1]Division of Internal and Cardiovascular Medicine; [2]Department of Chemistry, Biology and Biotechnology; [3]Division of Anaesthesia, Analgesia, and Intensive Care, Department of Medicine and Surgery, University of Perugia, Perugia, Italy; [4]Unit of Internal Medicine, Terni University Hospital, Terni, Italy

Background and Aims: Besides respiratory system involvement, venous thromboembolism is a severe complications of COVID-19, largely due to the strong derangement of hemostasis, with platelets playing a central role (*PMID: 35027697*). Great attention has recently been devoted to lipid alterations in COVID-19, because viruses reprogram cellular lipid metabolism in order to fuel their replication. Lipidomics studies in COVID-19 patients have been performed mainly in plasma and serum (*PMID: 33571544*). To the best of our knowledge, platelet lipidomics have not been examined despite the central role played by platelets in COVID-19 complications. Aim of our study was to preliminarily explore whether platelet lipidomics are altered in COVID-19 patients compared to age- and sex-matched healthy subjects, analyzing lipidomic profile of ultrapurified platelets. **Methods**: Eight hospitalized COVID-19 patients and eight age- and sex-matched healthy controls were enrolled. Platelets were ultrapurified by negative selection using Dynabeads™ CD45 from peripheral venous blood collected in trisodium citrate 3.2% (0.109 M, 1/10 v/v) (*PMID: 30408636*). Platelet lipids were extracted by LC-MS/MS using an untargeted lipidomics approach (*PMID: 25381612*) and analyzed by the Lipostar software (*PMID: 28471643*). **Results**: No significant differences in clinical and demographic characteristics were observed between patients and controls, except for neutrophil count, neutrophil to lymphocyte ratio (NLR) and lymphocyte count which were significant-

ly higher or lower, respectively, in COVID-19 patients. The final profile included 341 lipids covering five categories (sterols, sphingolipids, glycerophospholipids, glycerolipids, fatty acyls) and about 20 subclasses. A full separation of the lipidomics profile of COVID-19 platelets from that of healthy controls was observed (*Figure 1A*). In particular, a significant decrease of ether phospholipids (*Figure 1B-D*) and dihydro-Ceramide (*Figure 1E*), and increased levels of ganglioside GM3 (*Figure 1F*) were observed in platelets from COVID-19 patients compared to healthy controls. **Conclusions**: Our study shows for the first time that platelets from COVID-19 patients display a peculiar lipidomics signature distinguishing them from healthy controls, and suggests that altered platelet lipid metabolism may play a role in viral spreading and in the thrombotic complications of COVID-19. In particular, ether lipids are synthesized in peroxisomes (*PMID: 28523433*), and SARS-CoV-2 decreases the number and alters the structure of peroxisomes (*PMID: 34010015*), thus reshaping the host cell environment in a way more suitable for its replication, given that peroxisomes are signaling platforms for antiviral innate immunity (*PMID: 34696946*).

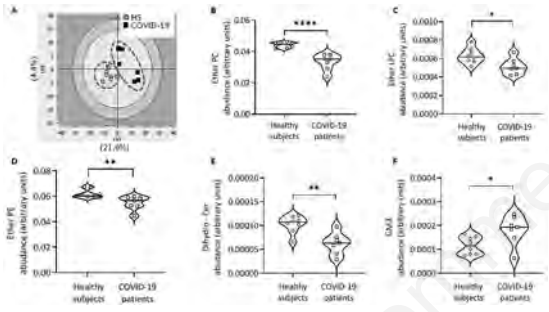


Figure 1. Untargeted lipidomic analysis of platelet samples. A) Partial Least Squares Discriminant Analysis (PLS-DA) scores plot based on the controls (gray circles)/COVID-19 (black squares) classification. B-F) Variation of the lipid classes in COVID-19 patients compared to healthy subjects: B) ether phosphatidylcholine (PC), C) ether lysophosphatidylcholine (LPC), D) ether phosphatidylethanolamine (PE), E) Dihydro-Ceramide (Dihydro-Cer), F) ganglioside GM3.

OC045

COVID-19 HD TRIAL: RESULTS OF THE INTERIM ANALYSIS

M. Marietta[1], P. Mighali[2], V. Coluccio[1], F. Banchelli[3], R. Vicini[2], A. Gozzi[2], P. Vandelli[2], R. D'Amico[3] on behalf of the COVID-19 Study Group*

[1]*Struttura Semplice Malattie della Coagulazione, Azienda Ospedaliero-Universitaria di Modena;* [2]*Servizio Formazione, Ricerca e Innovazione, Azienda Ospedaliero- Universitaria di Modena;* [3]*Università di Modena e Reggio Emilia, Cattedra di Statistica Medica, Dipartimento di Scienze Mediche e Chirurgiche Materno-Infantili e dell'Adulto, Modena; *COVID-19HD Study Group: DAschieri, AUSL Piacenza; LBrugioni, AOU Modena; EClini, AOU Modena; MCodeluppi, Piacenza Hospital; DImberti, Piacenza Hospital; GDolci, AOU Modena; E.Franceschini, AOU Modena; C.Mussini, University of Modena and Reggio Emilia; GPinelli, AOU Modena; APietrangelo, University of Modena and Reggio Emilia; LSarti, AOU Modena; MSilva, AUSL Piacenza, Italy*

Background and Aims: COVID-19 is associated with a severe procoagulant status, leading to a substantial incidence of venous thromboembolism (VTE), despite standard prophylaxis with Low Molecular Weight Heparin (LMWH). Several Randomized Controlled Trials (RCT) have addressed the issue of whether higher-than standard prophylactic doses of LMWH might improve the prognosis of COVID-19 patients. On these ground we designed the COVID-19 trial (Eudract N. 2020-001972-13), aimed at comparing the efficacy and safety of two doses of Enoxaparin in non-critically ill COVID-19 pts. **Methods**: COVID-19 HD is a multicenter, randomized, controlled, open label, two arms study aimed at assessing whether Enoxaparin at dose of 70 IU/kg twice daily is more effective than Enoxaparin 4000 IU once day to prevent clinical worsening, and similar in terms of major bleeding risk, in COVID-19 adult hospitalized patients with pneumonia and coagulopathy (D-dimer >4 times the upper level of normal reference range or Sepsis-Induced Coagulopathy (SIC) score >4), not requiring invasive mechanical ventilation (IMV). The Primary Efficacy Endpoint was clinical worsening during hospital stay, defined as the occurrence of death, acute Myocardial infarction, VTE, or need for escalating the oxygen support. This outcome was analyzed as a binary outcome as well as a time-to-event one. Primary safety endpoint was major bleeding, defined according to ISTH. A sample size of 300 pts was planned. The first patient was enrolled on June 30th, 2020. Because of the low accrual rate due to the changed epidemiological scenario, an interim analysis was performed on September 2021, after the randomization of 132 patients, with the agreement of the Data Safety Monitoring Board. **Results**: Of the 132 pts examined, 96 were males and 36 females; 54.8% had 0-1 comorbidities, and 45.2% 2 or more. The mean age was 59.9 yrs (SD 10.8), and only 10 pts. were older than 75 yrs. The mean BMI was 30.6 (SD 5.0). The two groups were well balanced in terms of demographic and clinical characteristics.The primary end-point occurred in 25.3% of pts. allocated to the low dose and in 12.3% of those to the high dose arm (Relative Risk 0.48, 95% C.I. 0.22-1.04, P=0.055). When the primary outcome was examined as a time-to-event one, the analysis of survival curves showed that most of events occurred within 5 days from of hospitalization. This correspond to an incidence rate of 20.8% /pts/week in the low dose and of 8.9%/pts/week in the high dose, with an Incidence Rate Ratio of 0.43 (95% C.I 0.18-0.99, P=0.042). No major bleeding was reported in either group. **Discussion**: In this analysis, we found that the use of Enoxaparin 70 IU/kg twice

daily was associated to a trend toward a lower risk of clinical worsening in non-critically ill hospitalized COVID-19 pts. Moreover, the time-to event analysis showed a statistically significant lower incidence rate of the primary outcome in the high-dose arm. Both treatments proved to be safe, with no major bleeding reported in either group. Although preliminary, these findings are in keeping with those of the larger ATTACC, ACTIV-4a, and REMAP-CAP Multiplatform RCTs. A limit of this study is the low accrual rate, which lengthened randomization for several months, thus including pts. infected by different SARS-CoV-2 variants, and possibly with different clinical characteristics of the disease. The data from the COVID-19 HD will be included in two large ongoing metanalysis, whose results will hopefully provide a better evidence about the efficacy and safety of high doses of LMWH in preventing clinical worsening in hospitalized, non-critically ill COVID-19 pts.

OC046

LONG COVID-19 SYNDROME: INSIGHTS INTO THE CONTRIBUTION OF PLATELET ACTIVATION TO PULMONARY IMPAIRMENT

M. Conti[1], P. Canzano[1], M. Brambilla[1], A. Becchetti[1], P.G. Agostoni[1], M. Pengo[2], E. Tortorici[2], M.E. Mancini[1], D. Andreini[1], A. Bonomi[1], G.F. Parati[2], M. Camera[1,3]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy; [2]Istituto Auxologico Italiano, Milan, Italy; [3]Department of Pharmaceutical Sciences, Università degli Studi di Milano, Italy

Background and Aims: Long-COVID-19 syndrome (LCS) is defined as symptoms persisting beyond initial phase of infection. Among them, pulmonary fibrotic damage remains in 25-30% of COVID-19 patients at 3-6 month-follow-up. We documented that acute COVID-19 patients have massive platelet activation characterized by the formation of platelet-leukocyte aggregates (PLA), that may be involved in the pulmonary microthrombi found in autoptic specimens, and by a prothrombotic phenotype. No data are currently available on contribution of platelet activation to residual pulmonary impairment and procoagulant potential in LCS patients. Thus, aims of this study was to characterize platelet activation, microvesicle (MV) profile, platelet thrombin generation capacity (pTGC) in LCS patients at 6-month-follow-up (6mo-FU) compared to acute COVID-19 infection patients. **Methods**: Twentyfour 6mo-FU COVID-19 patients with established LCS defined according to their residual pulmonary impairment assessed by Cardiopulmonary Exercise Testing (CPET) and 64-rows-CT scan evaluation were enrolled. Platelet activation (P-selectin, Tissue Factor [TF] and PLA) and MV profile were evaluated by flow cytometry; pTGC by calibrated automated thrombogram. Fortysix patients enrolled during acute COVID-19 infection and 46 healthy subjects (HS) were used for comparison. **Results**: Dispnea in LCS patients was confirmed by CPET showing compromised alveolus-capillary membrane diffusion and residual pulmonary impairment. TF^{pos}-platelet and -MV levels were 3- and 2-fold lower at 6mo-FU compared to acute phase, being comparable to HS, as well as pTGC. At 6mo-FU, the MV profile (total number and derived from different cells) returned to physiological levels. Conversely, although lower than that measured in acute phase, a 2.5-fold higher platelet P-selectin expression and PLA formation was observed at 6mo-FU compared to HS. Interestingly, a significant correlation between PLA formation and residual pulmonary impairment was observed. **Conclusions**: These data strengthen the hypothesis that the presence of PLA in the bloodstream, and thus also in the pulmonary microcirculation, may contribute to support pulmonary dysfunction still observed in LCS patients.

OC047

EFFICACY AND SAFETY OF DIFFERENT HEPARIN REGIMENS FOR PREVENTION OF VENOUS THROMBOEMBOLISM IN HOSPITALIZED PATIENTS WITH COVID-19: A META-ANALYSIS

M. Graziani, C. Vedovati

Medicina Interna Vascolare-Stroke-Unit, Università degli Studi di Perugia, Perugia, Italy

Background: Venous thromboembolism (VTE) is common in patients with coronavirus disease-2019 (COVID-19). The optimal heparin regimen remains unknown and should balance thromboembolic and bleeding risks. The aim of this study is to evaluate the efficacy and safety of standard or higher heparin regimens for the prevention of VTE in patients hospitalized due to COVID-19. **Materials and Methods**: We performed a systematic literature search; studies reporting on hospitalized patients with COVID-19 who received standard heparin prophylaxis *vs.* high (intermediate or therapeutic) heparin regimens were included if outcome events were reported by treatment group and more than 10 patients were included. Primary study outcome was in-hospital VTE. Secondary study outcomes were major bleeding (MB), all-cause death, fatal bleeding and fatal pulmonary embolism. **Results**: Overall, 30 studies (10637 patients) were included. Venous thromboembolic events occurred in 5.4% and in 8.3% of patients who received heparin prophylaxis with at high-dose or standard-dose, respectively (RR 0.72, 95% CI 0.56-0.93, I2 52.2%). MB was significantly higher in patients who received high- compared to the standard-dose (3.8% *vs* 2.1%, RR 1.89, 95% CI 1.41-2.53, I2 18.1%). Sub-analyses showed a slight benefit associated with high-dose heparin in patients admitted to non-intensive care unit (ICU) but not in those to ICU. No significant differences were observed

for mortality outcomes. **Conclusions**: Heparin prophylaxis at high-dose reduces the risk of VTE, but increased the risk of MB compared to the standard-dose. No clinical benefit for heparin high-dose was observed for ICU setting, but its role in the non-ICU deserves further evaluation.

OC048

PLATELET AGGREGATION IN MILD COVID-19: A PERSPECTIVE SINGLE CENTER STUDY

C. Simion, E. Campello, L. Spiezia, D. Tormene, F. Dalla Valle, C. Bulato, M. Fadin, S. Gavasso, C.M. Radu, G. Saggiorato, F. Sartorello, P. Zerbinati, P. Simioni

General Medicine and Thrombotic and Hemorrhagic Diseases Unit, Department of Medicine – DIMED, University-Hospital of Padova, Italy

Background and Aims: COVID-19 is associated with a peculiar coagulopathy characterized by increased risk of arterial and venous thromboembolism (VTE). Little is known about the role of platelets (PLT) function in the pathophysiology of COVID-coagulopathy: mild thrombocytopenia and PLT hyperactivity have been described, particularly in later stages; defects of PLT functional responses were also reported. The aim of our study is to evaluate PLT function in in-patients with mild COVID-19 at admission and post-discharge through impedance aggregometry. **Methods**: We enrolled all consecutive adults admitted to our Internal Medicine Unit between November 2020 and May 2021 for mild COVID-19. Exclusion criteria were ongoing antiplatelet therapies, severe thrombocytopenia ($<50x10^9$/L) and previous semi-intensive or intensive care unit (ICU) stay. Clinical characteristics, blood chemistry, coagulation panel and Multiplate® aggregometry were collected within 48h after admission, at discharge and 30-day post discharge. Clinical outcomes were incidence of venous or arterial thrombosis, any bleeding complications, transfer to semi-intensive or ICU, in-hospital mortality, 30-day post-discharge hospitalization and mortality. **Results**: Out of 247 patients admitted, 168 were enrolled (median age 77 years [IQR 64–84], F 56.4%). At admission, median PLTs count was within normal range ($240x10^9$/L [151-258], n.v. 150-$450x10^9$/L), while levels of von Willebrand factor (vWF) antigen (247% [225-373], n.v. 42-170%) and FVIII (176.1% [135.7-227.9], n.v. 50-150%) were increased. Using impedance aggregometry, we observed normal PLTs aggregation: ASPI 53 AUC [29-72] (n.v. 21-112), ADP 63 AUC ([42-84], n.v. 48-119) and reduced thrombin-driven aggregation: TRAP 53 AUC ([29-72], n.v. 86-159) (Figure 1). During hospitalization, bleeding occurred in 15 (8.9%) patients, particularly 3 muscle hematomas and worsening anemia requiring iron supplementation or transfusion. We reported 3 cases (1.8%) of arterial thrombosis (1 acute coronary syndrome, 2 strokes) and 28 cases (16.6%) of VTE, mostly distal and peri-catheter deep venous thrombosis (28.6% each). 41 patients (24.4%) were transferred to semi-ICU or ICU, while 15 subjects (8.9%) died, mainly because of respiratory or multiorgan failure and septic shock. None of bleeding or thrombotic events were associated with PLTs count or function. 62 underwent PLTs study at discharge, showing a slightly increased of median PLTs count ($282x10^9$/L [211-386]) and PLT aggregation still normal: ASPI 69 AUC [29-72], ADP 67 AUC [45-87) and TRAP 88 AUC [63-116]. Two patients (1.2%) died within 15 days post-discharge (for unkown reasons) and 9 (5.4%) had a re-hospitalization within 30 days (1 for rectorrhagia, 1 for a proximal DVT of the leg). None of these events were associated with PLTs count or function. 30 days after discharge, PLT count was within the normal range ($292x10^9$/L [221-356]), while vWF antigen (217% [157-245]) and FVIII (179.9% [122.9-236.8]) still increased. Aggregometry test showed normal PLT function: ASPI 57 AUC [38-71], ADP 59 AUC [46-72] and TRAP 85 AUC [74-95]. Among patients who were retested post-discharge, neither arterial or VTE nor bleedings occurred. **Conclusions**: According to our findings, mild COVID-19 patients present with normal PLTs count and reduced thrombin-driven platelet aggregation. This latter was restored at discharge. No clinical events were associated with PLTs function.

Box-and-whisker plot: the line represents the median. The normal reference ranges of AUC are highlighted by a coloured area. **Graphics A-D**: results of platelet count and impedance aggregometry in COVID-19 patients at admission, at discharge and 30 days-post discharge. **Graphics E-H**: results of platelet count and impedance aggregometry in COVID-19 patients according to overall outcomes.

Figure 1.

OC049

MONTELUKAST REPURPOSING IN COVID-19 PATIENTS

M. Brambilla[1], P. Canzano[1], A. Becchetti[1], M. Conti[1], G.E. Rovati[2], M. Camera[1,2]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy; [2]Department of Pharmaceutical Sciences, University of Milan, Milan, Italy

Background and Aims: Sustained platelet activation, thrombosis, vascular damage, fibrotic response as well as inflammatory overload are typical features of COVID-19 pathology. Common denominator in these processes are leukotrienes (LTs). Elevated levels of LTE4 have been detected in bronchoalveolar lavage of COVID-19 patients so that the use of LT receptor antagonists as a potential therapeutic for COVID-19 patient treatment has been hypothesized. A first phase III randomized double-blind clinical trial testing montelukast in COVID-19 patients has been indeed proposed. Thus, aim of this study was to investigate whether montelukast affects the expression of the major markers of platelet activation such as tissue factor (TF), P-selectin, as well as the formation of platelet-leukocyte aggregates and microvesicle (MV) release observed in COVID-19 syndrome. **Methods**: Blood from healthy subjects (HS; n=4-6) was plasma-depleted and reconstituted with plasma pools (n=3) from COVID-19 patients (4 patients/pool) or from the same HS blood donors. To assess the effect of montelukast on cell activation, blood from HS was preincubated for 30 minutes with the drug. Circulating cell-associated TF expression, platelet activation markers and MV release were analyzed by flow cytometry. **Results**: Plasma from COVID-19 patients significantly increased (4-fold) the number of TFpos- and P-selectinpos-platelets of HS recapitulating the platelet activation status of COVID patients. Montelukast prevented platelet activation induced by plasma from COVID-19 patient and it reduced the formation of circulating monocyte- and granulocyte-platelet aggregates, decreasing the number of those TFpos by 4-times. Finally, it completely inhibited the release of TFpos circulating MVs, reducing by more than 2-times those derived from platelets. **Conclusions**: Our data indicate that leukotrienes contribute to sustain platelet activation occurring in the COVID-19 patient, which can however be prevented by treatment with montelukast. Until results from ongoing trials will be available, our data provide the molecular basis by which the drug may be effective in the treatment of COVID-19.

OC050

SARS-COV2 ASSOCIATED VENOUS THROMBOEMBOLISM - A LONG TERM FOLLOW-UP

A. Maino[1], M. Landolfo[1], S. Conci[2], E. Vettorato[2], C. Susanna[2], S. Magnoni[3], F. Boccafoglio[4], D. Peterlana[1]

[1]Dipartimento di Medicina Interna, U.Odi Medicina Interna, Ospedale Santa Chiara, Azienda Provinciale per i Servizi Sanitari (APSS), Trento, Italy; [2]U.O di Medicina Interna, Ospedale Santa Maria del Carmine, Azienda Provinciale per i Servizi Sanitari (APSS), Rovereto, Italy; [3]U.Odi Anestesia e Rianimazione, Ospedale Santa Chiara, Azienda Provinciale per i Servizi Sanitari (APSS), Trento, Italy; [4]U.Odi Pneumologia, Ospedale Santa Chiara, Azienda Provinciale per i Servizi Sanitari (APSS), Trento, Italy

Background and Aims: Venous thromboembolism (VTE) is associated with acute SARS-CoV2 infection, especially in cases with severe interstitial pneumonia, and influences its short-term prognosis. Data on long-term consequences and follow-up of SARS-CoV2 associated VTE are still lacking. Therefore, we aims to investigate long term recurrences, resolution rates and outcome of SARS-CoV2 associated VTE. **Methods**: We conducted a cohort study of all SARS-CoV2 patients consecutively admitted for severe pneumonia to the intensive and sub-intensive care units at the Azienda Provinciale per i Servizi Sanitari, from March 21st to May 4th 2020, who developed an episode of VTE (either pulmonary embolism, PE, or deep vein thrombosis, DVT). During hospitalization, all patients were screened for DVT and, when appropriate, for PE. After discharge, we followed patients with confirmed VTE for thrombotic and haemorrhagic events at three, six and 12 months. Thrombus resolution and cardiac involvement were evaluated by venous and cardiac ultrasound. The post-COVID19 functional status scale (PCFS) was used to investigate residual symptoms and dysfunction. Thrombophilia screening (including factor V Leiden, prothrombin G20210A mutation, antiphospholipid antibodies, protein S, protein C and antithrombin levels) was performed at least one month after termination of anticoagulation. Descriptive results are presented by mean and standard deviation where appropriate. **Results**: As shown in Figure 1, of 218 patients admitted for severe SARS-CoV2 pneumonia, 35 (16%) had VTE (mean age 66±11 years, 27 males). Five patients died during hospitalization (all males, mean age 68±11 years). Of the 30 remaining (mean age 65±12, Brescia SCORE at admission 2.8±0.9, duration of hospitalization 41±15 days), 13 (43%) had PE and 17 (57%) DVT, of which 13 (43%) had isolated calf thrombosis. At 12 months, all patients had complete resolution of the DVT, and none had signs of pulmonary hypertension at the cardiac ultrasound. Thrombophilia screening was performed in 16 patients, and showed negative results. Anticoagulant therapy (22/30 low molecular weight heparin; 5/30 direct oral anticoagulant, 1/30 fondaparinux) was stopped after three months in most patients with DVT (13/17, 76%) and six months in most patients with PE (7/13 54%). Only six patients continued anticoagulation for 12 months (6/20, 20%). After a mean follow-up of 14.8±1.2 months, 6 (20%) minor bleeding and no VTE recurrence occurred. Seven patients (54%) reported no limitations or symptoms at the PCFS, and the remain-

ing reported mild to moderate limitations. **Conclusions**: We did not observe recurrences of SARS-CoV2 associated VTE. Our preliminary data suggest that stopping anticoagulation after a standard treatment period of three (for DVT) or six (for PE) months might be a reasonable approach in SARS-CoV2 related VTE. Nevertheless, the risk of bleeding might be not negligible.

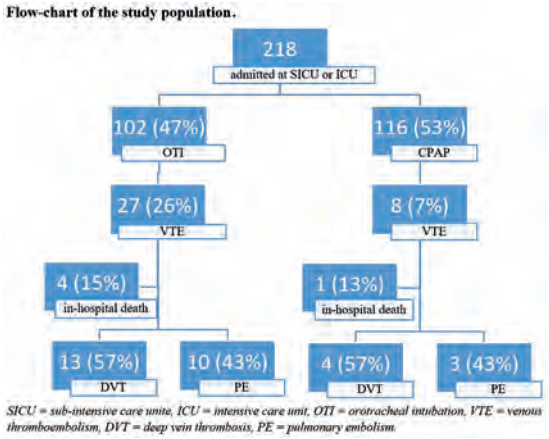


Figure 1.

OC051

DIAGNOSTIC STRATEGIES FOR PULMONARY EMBOLISM IN PATIENTS HOSPITALIZED FOR COVID-19: ROLE OF CLINICAL PREDICTION RULES

M.C. Vedovati, F. Balducci, C. Becattini

Internal, Vascular and Emergency Medicine - Stroke Unit, University of Perugia, Perugia, Italy

Background: Diagnostic strategies for pulmonary embolism (PE) in patients already facing respiratory failure due to COVID-19 is challenging. The use of the conventional diagnostic algorithm and clinical prediction rules (CPR) for PE is controversial in these patents. **Methods**: We conducted a retrospective cohort study in patients with COVID-19 infection, with the aim to evaluate the accuracy of currently available CPRs to assess the risk for venous thromboembolism (VTE) in medically ill patients and algorithms for the diagnosis of PE. Consecutive patients >18 years hospitalized at Santa Maria della Misericordia Hospital (Perugia, Italy) from March 2th, 2020, to September 29th, 2021 were included if they had: 1) confirmed diagnosis of COVID-19 with a molecular testing; 2) chest CT angiography performed for clinical suspicion of PE during the hospital stay. The study outcome was the accuracy of currently available CPRs for PE diagnosis (Wells and Geneva) and for VTE-risk stratification in medically ill patients (IMPROVE, IMPROVEDD and Padua score) to predict the diagnosis of PE as confirmed by a contrast-enhanced CT lung scan. **Results**: During the study period, 74 COVID-19 patients who had CT angiography for PE clinical suspicion were included (mean age 68 years, male 64.9%). Thirteen patients (17.6%) had PE confirmed at CT. No significant differences were observed for comorbidities, antithrombotic treatment and mortality between the two groups. D-dimer resulted significantly higher in patients with compared to patients without PE. Poor discrimination was observed for Wells and Geneva scores (AUC 0.596, 95% CI 0.413-0.779, and AUC 0.603, 95% CI 0.439-0.767, respectively), without substantial differences adding d-dimer at conventional cut-off (Table 1). The IMPROVEDD score had the highest discriminative power among CPRs for VTE (AUC 0.699, 95% CI 0.539-0.860). Scores' performance improved by increasing the D-dimer cut-off at level of 2000 ng/ml: among diagnostic scores, Wells showed the best discrimination (AUC 0.806, 95% CI 0.674-0.939, negative predictive value 97%); among CPRs for VTE the IMPROVEDD confirmed its accuracy (AUC 0.769, 95% CI 0.633-0.904, negative predictive value 94%). **Conclusions**: The accuracy of the currently used diagnostic and predictive scores for PE or VTE in COVID-19 patients is poor. D-dimer improves the diagnostic accuracy of these scores; most of all, it seems to allow a diagnostic strategy with a high negative predictive value, so we can rule out a consistent part of the patients with a low risk of PE.

Table 1.

Clinical prediction rule:	AUC	95% CI	AUC	95% CI
diagnosis of PE				
Wells + d-dimer	0.599*	0.437-0.761	0.806°	0.674-0.939
Geneva + d-dimer	0.595*	0.426-0.763	0.698°	0.555-0.841
risk for VTE				
IMPROVE	0.615	0.446-0.783	-	-
IMPROVEDD	0.699*	0.539-0.860	0.769°	0.633-0.904
Padua score	0.656	0.495-0.816	0.758°	0.622-0.893

* D-dimer at conventional cut-off; ° D-dimer at cut-off 2000 ng/ml

OC052

IMPACT OF COVID-19 PANDEMIC ON PULMONARY EMBOLISM CHARACTERISTICS: A MULTICENTRIC 24-MONTH PERSPECTIVE STUDY

P. Sterpone[1], I. Abatangelo[2], A. Raza[3], F. Piacentino[4], L. Tofanelli[5], W. Ageno[6,7], F. Pavesi[2], S. Birocchi[2], M.P. Donadini[6,7], G.M. Podda[2]

[1]Internal Medicine Residency Program, School of Medicine, University of Insubria, Varese; [2]Medicine II Unit, ASST Santi Paolo e Carlo, Department of Health Sciences, University of Milan, Milan; [3]School of Medicine, University of Insubria, Varese; [4]Department of Diagnostic and Interventional Radiology, Ospedale di Circolo, ASST Sette Laghi, Varese; [5]Radiology Unit,

ASST Santi Paolo e Carlo, Milan; [6]Research Center on Thromboembolic Disorders and Antithrombotic Therapies, University of Insubria, Varese; [7]Thrombosis and Heamostasis Center, Ospedale di Circolo, ASST Sette Laghi, Varese, Italy

Background: COVID-19 is associated with intense systemic inflammation and abnormal coagulation profile leading to an increased incidence of venous thromboembolic events (VTE). We reported that the incidence of Pulmonary Embolism (PE) during COVID-19 pandemic was significantly increased of about 20% as compared to pre-COVID-19 pandemic. Few data are available on PE characteristics in COVID-19 patients. Nevertheless, it was suggested that occlusions of pulmonary arteries observed in COVID-19 patients are caused by pulmonary thrombi originating in loco, as a result of a thrombo-inflammatory process. Our study aimed to evaluate the clinical, laboratory, and radiological characteristics of PE in patients with or without COVID-19 **Methods**: We conducted an observational multicentric cross-sectional study on consecutive patients diagnosed with PE at admission or during hospital stay at Azienda Socio Sanitaria Territoriale (ASST) Sette Laghi (Varese) and ASST Santi Paolo e Carlo (Milan) during 24 months, from12 months before (pre-COVID period) up to 12 months after (COVID period) February 21st2020, when the first Italian case of COVID-19 was diagnosed. Patients' clinical and laboratory characteristics were collected. Thoracic Computed Tomography images (TC) were revised and data on the distribution and extent of PE were collected and quantified by means of the Qanadli Index. Non-COVID-19 patients (ie those of the pre-COVID period and those of the COVID period who tested negative for SARS-CoV-2) were compared to COVID-19 patients. **Results**: During the study period 772 patients were identified with acute PE (336 in the PRE-COVID period and 436 in the post COVID period), of whom 88 had COVID-19. In patients with PE and COVID-19, male sex was more common than in non-COVID-19 patients (52% *vs* 41%; p-value 0.01). Presence of risk factors for VTE was more frequent in patients without COVID-19: previous VTE and active cancer were significantly less common in COVID-19 patients compared to non-Covid-19 patients (3.5% *vs* 11.6% p 0.02; 4% *vs* 24.4% p<0.0001 respectively). Interestingly, median Troponin T and nT pro BNP levels were lower (15 *vs* 56 ng/L p-value 0.04 and 424 *vs* 1501 pg/ml p-value 0.01 respectively) in COVID-19 patients. In COVID-19 patients the median value of Qanadli Index was inferior (4 *vs* 7 p<0.0008), right ventricular dilatation was less frequent (4.3 *vs* 11.5 % p 0.07) and distal obstruction was more common compared to negative patients. **Conclusions**: In COVID-19 patients the classic risk factors for VTE are less frequent, possibly reflecting that COVID-19 is itself a strong risk factor. The different characteristics of COVID-19 associated PE suggest a difference in pathophysiological mechanisms. Furthermore, our study found a predominantly distal distribution of PE with a significantly lower burden in terms of severity indicators and clot extension as compared to non-COVID-19 patients. All these data together suggest a potential role of lung infection causing local pulmonary artery thrombosis instead of typical VTE with clot embolization. Further research is needed to better understand PE in COVID-19 patients.

OC053

IMPACT OF ROTATIONAL THROMBOELASTOMETRY (ROTEM) ON CLINICAL COURSE AND OUTCOMES OF COVID -19 CRITICALLY ILL PATIENTS: A RETROSPECTIVE SINGLE CENTRE OBSERVATIONAL STUDY.

M. Pazzi[1], E. Gemmi[1], D. Conti[1], L.A. Zanofino[1], L. Tofani[2], D. Prisco[3], V. Pavoni[1], R. Marcucci[4]

[1]SOC Anestesia e Rianimazione Ospedale Santa Maria Annunziata, Azienda USL Toscana Centro; [2]Department of Statistics, Computer Science, Applications, University of Florence; [3]Department of Experimental and Clinical Medicine, University of Florence; [4]Center for Atherothrombotic disease, AOU Careggi, Dept of Experimental and Clinical medicine, University of Florence, Italy

Background and Aims: Recently a close correlation between prothrombotic laboratory alterations and COVID-19 severity has been suggested and increased D-Dimer (DD) levels have been reported as a negative prognostic factor. Although Viscoelastic tests (VETs) have been used to detect hypercoagulability (characterized by acceleration in clot formation time (CFT) and increased maximum clot firmness (MCF), their usefulness in clinical management of critically ill COVID-19 patients has yet to be elucidated. Our study aimed to identify the relationship between ROTEM profiles and adverse outcomes (mortality, needing for Mechanical Ventilation (MV), Venous Thromboembolism (VTE), COVID-19 Associated Coagulopathy (CAC)) in critically ill COVID-19 patients. **Methods**: 100 consecutive patients admitted to the intensive care unit (ICU) with severe COVID-19 pneumonia, between October 1, 2020 May 31, 2021 were enrolled in Florence, Santa Maria Annunziata Hospital. (Table 1). **Results**: We enrolled a population with a significantly higher prevalence of male (80%), hypertension and diabetes. As concerns standard coagulation tests and ROTEM profile, a prothrombotic pattern was documented, with high DD (>6 VN) and fibrinogen levels. A modest increase of MCF in EXTEM and FIBTEM was instead depicted probably due to early administration of "intermediate" LMWH dosage before ICU admission in moderate respiratory failure patients. Regarding ICU mortality, statistically significant differences were found between DD values and platelet counts. ROTEM analysis highlighted any hypercoagulability differ-

ence but Maximum Lysis (ML) values resulted statistically different in EXTEM and INTEM. (Table 1) A sub-group analysis demonstrated hypofibrinolysis in MV patients too. However, as concerns MV rate, the univariate analysis showed a possible association with hypercapnia (OR 1.11, 1.03-1.2, p=0.0053) and leukocytosis (OR 1.12, 1.02-1.23, p=0.0181). Patients with VTEs (n=20) showed higher DD values (10732±1703 ng/ml *vs.* 3773±747 ng/ml, p=0,010319); conversely no correlation was highlighted in terms of ROTEM hypercoagulability or hypofybrinolisis. No statistical difference was found between CAC (n=29) *vs* no CAC (n=71) patients. Lastly, survival analysis allowed us to identify hypofibrinolysis (ML EXTEM; HR 0,87, 0,81-0,95, p=0,0011and INTEM; HR 0,9, 0,84-0,97, p=0,0083)as a possible risk factor. **Conclusions**: In our COVID-19 population, NOT-survivors had higher DD values, lower platelet counts and a more pronounced hypofibrinolytic ROTEM pattern than survivors. Hypofibrinolysis occurs more frequently in patients requiring MV, although it does not result as a possible risk factor for intubation. Similarly, hypercoagulability is a common ROTEM feature but it appears to increase neither VTE risk, intubation rate, or mortality probably due to early administration of intermediate-dose LMWH. Therefore, we could hypostasize that hypofibrinolysis, but not hypercoagulability, resulted in worse outcomes in terms of mortality risk, MV and VTE. 29% of patients met CAC criteria but no significant difference in mortality, MV rate, VTE and ROTEM parameters was revealed. Further studies are highly recommended in order to define the usefulness of VETs as a tool for multiparametric prognostic assessment. However, based on current data, ROTEM analysis could represent a helpful tool for predicting ICU admission risk and worst outcome as well as bleeding risk in critically ill patients.

Table 1.

TOTAL POPULATION (N=100)		SURVIVORS (N=50)	NOT-SURVIVORS (N=50)	p
DEMOGRAPHIC FEATURES				
AGE (YEARS)	66 ± 17	58,3	69,6	0,00241
MALES (N, %)	80, 80%	36	44	n.s.
BMI (KG/M²)	28,5 ± 4,6	27,7	29,1	n.s.
COMORBIDITIES (N, %)				
OBESITY KG/M^2	38, 38%	22	16	n.s.
HYPERTENSION (N, %)	56, 56%	26	30	n.s.
DIABETES (N, %)	20, 20%	8	12	n.s.
COPD (N, %)	11, 11%	4	7	n.s.
PREVIOUS AMI(N, %)(N, %)	11, 11%	2	9	n.s
ICU ADMISSION				
SOFA SCORE	5,9 ± 2,5	5,3	3,6	0,00012
SAPSII SCORE	36,4 ± 9,3	37,2	27,7	0,00011
SYMPTOMS' ONSET TO ICU ADMISSION DELAY (DAYS)	10 ± 6,7	10,2	10	n.s
PAO2/FIO2	135 ± 75	148	131	n.s.
ICU STAY				
ICU STAY (DAYS)	13 ± 11	7	19	n.s.
MECHANICAL VENTILATION (N, %)	64	19	45	0,0013
THROMBOEMBOLIC COMPLICATIONS (N, %)	20, 20%	11	9	n.s.
LABORATORY DATA AT ICU ADMISSION				
WBC (10^9/L)	10,2 ± 5,2	8,96	12,52	n.s.
PTL (10^9/L)	246 ± 107	225,7	267,1	0,01441
DD HG/ML	5088 ± 1688	4282	5988	0,00854
PT%	70 ± 15,2	68,8	71,5	n.s.
APTT SEC	30,3 ± 8,5	30,1	29,8	n.s.
FIBRINOGEN MG/DL	640 ± 112	616,6	664,3	n.s.
ROTEM PARAMETERS AT ICU ADMISSION				
CT INTEM SEC	177,4 ± 28	176,9	177,8	n.s.
CFT INTEM SEC	59,1 ± 22,8	56,1	50,2	n.s.
MCF INTEM MM	69,8 ± 6	69,5	70	n.s.
ML INTEM %	8,9 ± 4,2	7,9	10	0,01467
CT EXTEM SEC	81,9 ± 29	85,8	77,8	n.s.
CFT EXTEM SEC	54,2 ± 21,6	54,9	49,6	n.s.
MCF EXTEM MM	72 ± 5,8	71,6	72,4	n.s.
ML EXTEM %	9,3 ± 4	8,7	10,5	0,05554
MCE FIBTEM MM	29,8 ± 8,3	29,7	29,9	n.s.

OC054

RETROSPECTIVE SINGLE-CENTER ANALYSIS OF THE EFFECTS OF HEPARIN PROPHYLAXIS IN COVID-19 PATIENTS ADMITTED TO MEDICAL WARDS: ANTI-THROMBOTIC OR ANTI-VIRAL?

A. D'Angelo, L. Galli, P. Della Valle, C. Tresoldi, M. Spessot, D. Palumbo, F. De Cobelli, D. Baccellieri, L. Apruzzi, M. Locatelli, M. Tresoldi, S. Calvisi, P. Scarpellini, M. Ripa, G. Martino, E. Bosi, L. Dagna, A. Castagna, P. Rovere-Querini, F. Ciceri, on behalf of COVID-BioB Study Group

Scientific Institute Ospedale San Raffaele and Vita e Salute University, Milano, Italy

Background and Aims: Mechanisms whereby therapeutic-dose heparin appears beneficial in non-critically ill, but detrimental in critically ill covid-19 patients are unclear. We retrospectively evaluated the effect of LMWH prophylaxis on the composite endpoint of symptomatic thromboembolism, transfer to ICU, major and NMCR bleeding, and 28-day mortality of covid-19 patients hospitalized in medical wards of the San Raffaele Hospital during the first wave of the pandemic. Secondary outcomes were the individual contributors to the composite endpoint. **Methods**: Of 519 patients with laboratory confirmed respiratory syndrome coronavirus 2 (SARS-CoV-2) infection admitted to the medical wards of the hospital from March 1st to April 30th 2020, 56 were on therapeutic anticoagulation before infection, 243 received prophylactic anticoagulation, and 220 patients did not. A multivariable Cox proportional hazard model was used to assess the association between demographic and other clinical or laboratory factors and the risk of ≥1 clinical event; the stabilized inverse probability weighting, calculated on propensity score for heparin prescription, was considered in the model. The hospital's EC approved the study, registered on ClinicalTrials.gov (NCT04318366). **Results**: Incidence rates of the composite endpoint (per 100 patient-days of follow up) were 3.71 (2.54-5.41) for patients on therapeutic anticoagulation, 3.30 (2.59-4.20) for those not receiving prophylaxis, and 1.99 (1.56-2.49) for those on prophylactic anticoagulation (p=0.003). In addition to latin ethnicity (HR 5.15), O_2 saturation (HR per 1% higher 0.93), lymphocyte count (HR per $1x10^9$/L higher 0.61), 4-items SOFA score (HR per 1 point higher 1.32) and steroid use (any type and dosage, HR 0.54, all p values ≤0.03), all of the above at hospital admission, heparin prophylaxis – considered as a time-varying covariate (HR 0.38, 0.21-0.71, p=0.002) was predictor of the 28-day risk of the composite endpoint's occurrence. The incidence rate of 28-day mortality was the only individual contributor to the endpoint significantly different between patients receiving prophylaxis or not (0.49 *vs* 1.33 per 100 patient-days of follow up, p=0.0001). Notably, the incidence of major and CRNM bleeding, although numerically higher in

patients receiving prophylaxis, was not significantly different among the three groups. The results persisted in several sensitivity analyses. **Conclusions**: Similar to additional reports, in this retrospective analysis of the early stage of the covid-19 pandemic, heparin prophylaxis reduced the 28-day mortality rate, but not the rate of symptomatic thromboembolic events. The SARS-CoV-2-specific anti-viral effect of heparin may explain why the covid-19 infection appears to be the first clinical setting showing a survival effect of heparin prophylaxis.

OC055

EFFECTS OF GASTROINTESTINAL RESECTION AND OSTOMY SURGERY ON THE PLASMA CONCENTRATION OF APIXABAN: AN OBSERVATIONAL PROSPECTIVE STUDY

R. Talerico[1], R. Caprari[1], A. Porfidia[1], E. Fondi[1], E. De Candia[2], R. Pola[1]

[1]Dipartimento di Medicina Interna, Fondazione Policlinico Universitario AGemelli IRCCS, Università Cattolica del Sacro Cuore, Roma, Italy; [2]Dipartimento di Diagnostica per Immagini, Radioterapia Oncologica ed Ematologia, Fondazione Policlinico Universitario AGemelli IRCCS, Università Cattolica del Sacro Cuore, Roma, Italy

Background and Aims: Patients who underwent gastrointestinal resection or ostomy surgery may have altered absorption of orally administered drugs. This could be an important clinical problem in subjects taking direct oral anticoagulants (DOACs), for whom an evaluation of effective anticoagulation is not warranted. In this study, we evaluated whether therapeutic plasmatic concentrations of Apixaban can be found in this category of patients. **Methods**: We designed an observational prospective study and included patients with medical history of gastrointestinal resection or ostomy surgery who were on oral anticoagulation with Apixaban for the treatment or prophylaxis of venous thromboembolism (VTE) and/or for the prevention of cardioembolic stroke due to nonvalvular atrial fibrillation (NVAF). We measured the peak and the trough (2-3 and 12 hours after drug intake respectively) plasma concentrations of Apixaban using a chromatographic assay and compared the levels obtained with the expected levels, as established by the European Medicines Agency. We included in the study patients who had undergone gastric, small and/or large intestine resection up to the descending colon and/or ostomy surgery. We excluded resection of sigmoid colon and/or rectum due to the poor absorption properties of these bowel tracts. **Results**: 26 patients were enrolled, of whom 12 (46%) were taking Apixaban 5 mg BID for the treatment of VTE, 4 (15%) were taking Apixaban 5 mg BID for the prevention of cardioembolism in NVAF, and 10 (39%) were taking Apixaban 2.5 mg BID for the prevention of VTE recurrence. The mean age was 65±11.7 years, the mean BMI was 24.8±9.6, and the mean creatinine clearance was 78.9±32.9 ml/min (according to Cockroft–Gault). Regarding surgical interventions, 10 (38%) patients had undergone ileostomy, 4 (15%) subtotal gastrectomy, 5 (19%) small bowel resection (mainly ileum), and 7 (27%) colon resection. Two patients had undergone both gastric resection and small bowel resection, while 2 others both ileostomy and colectomy. The peak and trough plasma concentrations of patients on Apixaban 5 BID for VTE treatment were respectively 174.4±92.1 ng/mL and 72.25±41.3 ng/mL, on Apixaban 2.5 mg BID for VTE secondary prevention was 107.3±38.8 ng/mL and 50.4±14.2 ng/mL, and on Apixaban 5 mg BID for NVAF was 304.7±183.1 ng/mL and 238.0±213.3 ng/mL [Figure 1]. We observed that 2/26 (8%) patients, both receiving Apixaban 5 mg BID for the treatment of VTE, were below the trough reference range. 1/26 (4%) patient, on therapy with Apixaban 5 mg BID for the treatment of VTE, was below the peak reference range. **Conclusions**: These results show that almost all patients (92% for trough and 96% for peak plasma concentrations, respectively) who had undergone resections of the gastrointestinal tract, during Apixaban therapy achieved plasma drug concentrations within the reference range. Despite the limitations of our study, undoubtedly due to the small sample size and variety of surgeries, these results are encouraging for further studies.

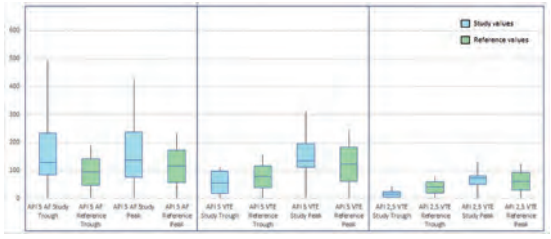


Figure 1.

OC056

PREVALENCE AND RISK FACTORS FOR PULMONARY EMBOLISM IN PATIENTS HOSPITALIZED IN INTERNAL MEDICINE UNITS FOR ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A PROSPECTIVE MULTI-CENTER FADOI STUDY

I. Giarretta[1], F. Dentali[1,2], P. Gnerre[3], F. Pomero[4], R. Re[5], N. Mumoli[6], F. Pieralli[7], P. Di Micco[8], M. Bigossi[9], A. Mazzone[6], F. Colombo[10], D. Manfellotto[11]

[1]Medicina Generale 1, Medical Center, Ospedale di Circolo e Fondazione Macchi, ASST Sette Laghi, Varese, Italy; [2]Department of Medicine and Surgery, Insubria University, Varese, Italy; [3]Internal Medicine, "San

Paolo" Hospital, Savona, Italy; [4]Division of Internal Medicine, Michele and Pietro Ferrero Hospital, Verduno, Italy; [5]Presidio Ospedaliero SAndrea, ASL VC, Vercelli, Italy; [6]Department of Internal Medicine, ASST Ovest Milanese, Magenta (MI), Italy; [7]Intermediate Care Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [8]Department of Internal Medicine and Emergency Room, Ospedale Buon Consiglio Fatebenefratelli, Naples, Italy; [9]Section of Internal Medicine and Thromboembolic Diseases, Department of Medicine, Fondazione Policlinico Universitario AGemelli IRCCS, Rome, Italyand Division of Population Health & Genomics, Pat McPherson Centre for Pharmacogenetics & Pharmacogenomics, School of Medicine, Ninewells Hospital & Medical School, University of Dundee, Dundee, United Kingdom; [10]Internal Medicine Ward, Ospedale Niguarda Ca' Granda, Milan, Italy; [11]Department of Internal Medicine, Fatebenefratelli Hospital, Rome, Italy

Background and Aims: The differential diagnosis between acute exacerbation of Chronic obstructive pulmonary disease (ECOPD) and pulmonary embolism (PE) in patients admitted to Internal Medicine Units is often challenging. Furthermore, PE may itself be the trigger or may complicate the clinical course of ECOPD. The aim of this study was to evaluate the PE prevalence and the associated risk factors in patients affected by COPD, hospitalized for acute worsening of respiratory symptoms. **Materials and Methods**: This was a multicenter prospective observational study, promoted by the Italian Federation of Associations of Hospital Doctors on Internal Medicine (FADOI). The primary outcome of the study was the incidence of PE among patients admitted for ECOPD within 90 days from hospitalization; the secondary outcome was to identify potential risk factors associated with PE. From January 2015 to November 2016, 521 consecutive patients with ECOPD were enrolled in 30 Italian centers and evaluated for PE according to clinical suspicion. **Results**: The estimated PE incidence was 9.8% (95% CI, 7.25-12.35%). Patients with PE did not differ from those without PE in terms of age, sex, or comorbidities. The multivariate analysis demonstrated that clinical signs of deep vein thrombosis (DVT) was associated with a 20-fold increased risk of PE (OR 20.60; 95% IC, 6.92-61.35). Recent prolonged bed rest (OR 2.39; 95% IC 1.10-5.20), arterial systolic blood pressure (ASBP) <130 mmHg (OR 2.11; 95% IC 1.08-4.14), and hypocapnia (OR 2.88; 95% IC, 1.50-5.51) were also associated with PE (Figure 1). The survival analysis showed no influence of PE on hospital stay and mortality. The prevalence of PE in patients with 0, 1, 2, 3 or 4 of the identified risk factors progressively increases from 3.1 % in patients without risk factors to 50.0% in subjects with 4 factors. **Conclusions**: This study demonstrated a high prevalence of PE in patient hospitalized for ECOPD which was associated with prolonged bedrest, clinical signs of DVT, SAP<130 mmHg and hypocapnia. Further studies are needed to test the reliability and the efficacy of a rule out approach including these variables in COPD patients admitted for suspected acute exacerbation.

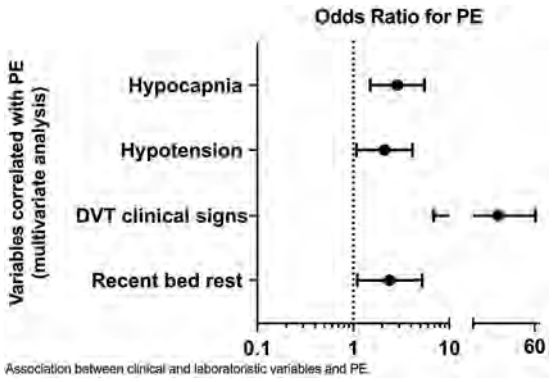


Figure 1.

OC057

PREDICTION OF EARLY MAJOR BLEEDING IN ACUTE PULMONARY EMBOLISM PATIENTS: EXTERNAL VALIDATION OF PE-SARD SCORE

L.A. Cimini, M.C. Vedovati, P. Quadalti, A. Vinci, C. Becattini

Internal, Vascular, and Emergency Medicine - Stroke Unit, University of Perugia, Perugia, Italy

Background and Aims: In the acute phase of pulmonary embolism (PE), anticoagulant treatment is necessary to reduce mortality. The most worrying consequence is the occurrence of major bleeding. Recently, the PE-Sard score (Syncope, Anemia, Renal Dysfunction) was derived and internally validated to predict major bleeding events in patients with acute PE. We validated the proposed PE-SARD model, regardless of the severity of PE and the anticoagulant treatment. **Methods**: From an observational cohort including consecutive patients with acute PE, we performed a preliminary post hoc analysis. The primary outcome was the occurrence of major bleedings at 30 days. **Results**: Overall, 376 patients were admitted with acute pulmonary embolism in our hospital. Of those, 12 were excluded due to the lack of baseline data. 364 patients were included in the analysis and followed for 30 days after PE diagnosis. The mean age was 69.7 (±15.7) and 51.6% were female. During the follow-up period, 18 major bleedings occurred (5%), of those 2 were fatal (0.6%). Among the PE-SARD items, only anemia resulted as a major bleeding predictor (p=0.004). 43.9% of patients were classified as at the low risk (160 patients), 43.9% as at intermediate risk (160 patients), and 10.4% (39 patients). Observed bleeding rates increased with the risk group, from 2.5% in the low-risk group to 7.7% in the high-risk group. C-statistic (AUC) was 0.622 (95% CI 0.497-0.746). **Conclusions**: In a real-life cohort of patients with acute PE, the predictive value of the PE-SARD bleeding risk score is fairly good in identifying patients at intermediate and high risk of bleeding in the acute phase of PE. The role of the PE-SARD score should be assessed in management studies.

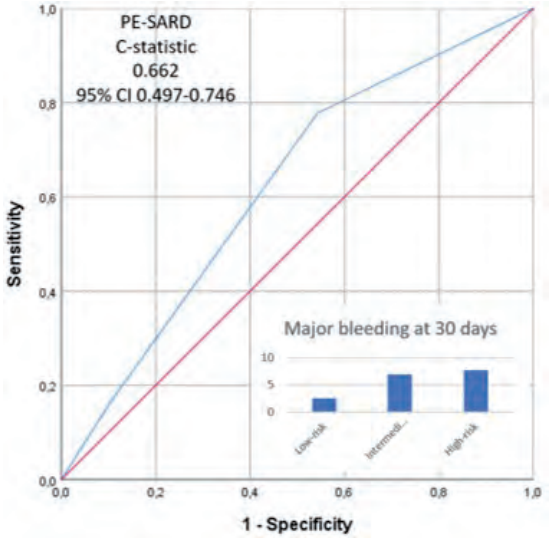


Figure 1.

OC058

START2-POST VTE REGISTRY: OUTCOMES AFTER TWO YEARS OF FOLLOW-UP

E. Antonucci[1], L. Migliaccio[1], B. Cosmi[2], E. Bucherini[3], S. Birocchi[4], V. Fregoni[5], T. Lerede[6], E. Montevecchi[7], R. Pancani[8], R. Parisi[9], A. Serrao[10], G.M. Podda[4,11], S. Testa[12], D. Poli[13], G. Palareti[1]

[1]Fondazione Arianna Anticoagulazione, Bologna, Italy; [2]UO Angiologia e Malattie della coagulazione, Dipartimento di specialità diagnostiche e Medicina sperimentale, IRCCS AOU S.Orsola-Malpighi, Bologna, Italy; [3]SS Aziendale di Angiologia Faenza AUSL Romagna, Ravenna, Italy; [4]Unità di Medicina 2, ASST Santi Paolo e Carlo, Milan, Italy; [5]UOC Medicina Generale, ASST Valtellina e Alto Lario, Sondalo (SO), Italy; [6]USC SIMT, Centro Emostasi e Trombosi, Ospedale Papa Giovanni XXIII, Bergamo, Italy; [7]UOC Angiologia Ospedale San Giovanni Apostolo, Castelfranco Veneto, Treviso, Italy; [8]UO Pneumologia, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; [9]UOSD Ipertensione e Patologie Endocrine Metaboliche Angiologiche, Ospedale SSGiovanni e Paolo, Venezia, Italy; [10]Sezione Ematologia, Dipartimento di Biotecnologie Cellulari ed Ematologia Azienda Ospedaliero Universitaria Policlinico Umberto I, Roma, Italy; [11]Dipartimento di Scienze della Salute, Università Degli Studi di Milano, Milan, Italy; [12]Dipartimento di Medicina di Laboratorio, Centro Emostasi e trombosi, ASST Cremona, Cremona, Italy; [13]SOD Malattie Aterotrombotiche, Azienda Ospedaliero Universitaria-Careggi, Firenze, Italy

Background and Aim: Patients with acute venous thromboembolism (VTE) need immediate active anticoagulant treatment (AT), currently performed using different drugs, for at least 3 to 6 months of treatment. Patients with VTE should receive a decision on the duration of AT that is often not easy to make. START2 POST-VTE register study is aimed to investigate how Italian physicians deal with this issue; in particular, when they take a decision on duration of AT after a recent VTE episode, which decision is taken and why, and what happens during follow-up of the patients. The present analysis examined thromboembolic or bleeding complications, and deaths due to VTE occurring during two years of follow-up in the patients who had already received a decision on AT. **Methods**: The START POST-VTE register includes 1100 Italian patients with a recent VTE event recruited in 16 clinical centers. Adverse events occurring during follow up were recorded. **Results**: In the present analysis we describe the 472 patients who had already received a decision on duration of AT and have completed the 2 years of follow-up. In 59.3% of the cases the treatment was stopped and extended in 40.7%. Patients who extended anticoagulation were followed for 398 patients/years (pt-yrs); during this period, overall 10 adverse events were recorded: 2 major bleeding (rate 0.5 pt-yrs) (1 gastrointestinal and 1 cerebral); none was fatal; 2 CRNMB, 5 minor bleeding; 1 VTE recurrence (rate 0.5 pt-yrs). One patient died for Covid disease; 2 patients are on indefinite treatment for onset of atrial fibrillation. One patient was lost at follow up. In patients who discontinued anticoagulation 40 recurrent events were recorded: 38 VTE (13%) [16/38 proximal DVT (42,1%)]; 2 events involved the cerebral arteries. 1 gastrointestinal bleeding (non fatal) was recorded in a patient who developed gastric cancer. Mean time of events from discontinuation of treatment was 279 ±203 (38 – 695) days; 57% of patients with recurrence were males; median age 68 years. **Conclusions**: In patient who continued treatment major bleeding rate was lower than those recorded in clinical trials. None event was fatal. The rate of recurrent VTE in patients who stopped anticoagulation was more frequent in male sex, what confirms available data on VTE recurrence.

OC059

SHORT AND LONG-TERM RISK OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH ATRIAL FIBRILLATION. A SYSTEMATIC REVIEW AND METANALYSIS

T. Bucci[1], G. Franchino[2], G. Galardo[1], P.R.J. Ames[3,4], D. Menichelli[2], A. Farcomeni[5], P. Pignatelli[2], D. Pastori[2]

[1]Department of General Surgery, Surgical Specialties and Organ Transplantation "Paride Stefanini," Sapienza University of Rome, Rome, Italy; [2]Department of Clinical, Internal Medicine, Anaesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy; [3]Department of Haematology, Dumfries Royal Infirmary, Cargenbridge, Dumfries, UK;

[4]Department of Clinical, Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy; [5]Department of Economics and Finance, University of Rome "Tor Vergata", Rome, Italy

Background: Atrial fibrillation (AF) carries an increased risk of thromboembolism. While the relationship between AF and arterial thromboembolism is well known, its association with venous thromboembolism (VTE) is still controversial. **Methods**: We performed a systematic review of PubMed and EMBASE databases and metanalysis of observational study. The studies were divided according to the time of VTE onset after the AF diagnosis as follows: 1) up to 3 months, 2) up to 6 months, 3) >6 months. Pooled hazard ratio (HR) was used as measure of association. **Results**: We included 11 observational studies: 6 retrospective and 5 prospective. A total of 689,092 AF patients were considered for the analysis. In the up to 3 months group, AF was associated with a higher risk of VTE (HR:5.92, 95% CI: 2.36-14.85), DVT (HR:5.35, 95%CI:1.73-16.49) and PE (HR:4.92, 95%IC:1.76-13.8). When we analysed the up to 6 months group, AF was still associated with a higher risk of VTE (HR:3.76, 95%CI: 1.65-8.55), DVT (HR:1.75, 95%CI:1.43-2.14) and PE (HR:3.4, 95%CI:1.82-6.37). However, after 6 months from the onset, AF was not associated with a higher risk for VTE (HR:1.02, 95%CI:0.97-1.08), DVT (HR:1.2, 95%CI:0.99-1.45) and PE (HR:1.13, 95%CI:1.01-1.26). **Conclusions**: An increased risk of VTE is evident in the first 3-6 months after AF diagnosis. The early initiation of anticoagulation in AF patients may reduce not only the risk of stroke but also the risk of VTE.

OC060

LOW DOSE RIVAROXABAN TO PREVENT RECURRENCES OF VENOUS THROMBOEMBOLISM IN CANCER PATIENTS: A REAL-WORLD EXPERIENCE

P. Santini[1], C. Mosoni[1], E. Porceddu[1], A. Porfidia[1], A. Lupascu[1], P. Tondi[2], R. Pola[1]

[1]Medicina Interna e Patologie Tromboemboliche, Fondazione Policlinico Universitario AGemelli IRCCS Roma; [2]Servizio di Angiologia, Fondazione Policlinico Universitario AGemelli IRCCS Roma, Italy

Background and Aims: Patients with active cancer are a high-risk population for venous thromboembolism (VTE) and its recurrences. For this reason, international guidelines suggest long-term anticoagulation for secondary prophylaxis beyond 6 months in cancer patients. However, this is a conditional recommendation with low level of evidence since no data are available on the optimal type and dose of anticoagulant that should be used. Studies such as DalteCan, TiCAT, Hokusai Cancer and Select-D have evaluated extended treatment with various anticoagulants beyond six months from a VTE index episode in cancer patients, with a VTE recurrence rate of 4.0%, 1.1%, 2.0% and 4,0%, respectively. A clinical trial, named APICAT, which is currently ongoing, is aimed to evaluate efficacy and safety of extended anticoagulation with full- and low-dose apixaban in cancer patients who had a previous VTE. In the present study, we provide real-world data on the efficacy and safety of extended anticoagulation with low-dose rivaroxaban in cancer patients, after completion of a full anticoagulation regimen of at least 6 months for the treatment of acute VTE. **Methods**: This is a prospective observational study performed at the "Section of Internal Medicine and Thromboembolic Diseases" of the A. Gemelli University Hospital of Rome, Italy. Cancer patients who received rivaroxaban 10 mg once daily (od) for the secondary prevention of VTE were enrolled. All patients had previously completed a cycle of at least six months of full-dose anticoagulation for the treatment of a VTE qualifying event. For VTE qualifying event, we intended either deep vein thrombosis (DVT) of the lower limbs (both proximal and distal), pulmonary embolism (PE), or VTE in unusual sites, including splanchnic/abdominal veins, cerebral veins, jugular veins, and deep veins of the upper limbs, either in the presence or the absence of a central venous catheter (CVC). The primary efficacy endpoint was any type of objectively verified recurrent VTE. Primary safety endpoints were major bleedings or clinically relevant non-major bleedings (CRNMB), defined according to the criteria of the International Society of Thrombosis and Haemostasis (ISTH). **Results**: To date (17 May 2022) the study has included 106 patients, whose baseline characteristics and information about the qualifying VTE event are shown in the attached Table 1.

Table 1.

Baseline characteristics	Total (N=106)	VTE in usual site (N=60)	VTE in unusual site (N=46)
Sex - no. (%)			
Female	100 (94.3)	56 (93.3)	44 (95.7)
Male	6 (5.7)	4 (6.7)	2 (4.3)
Median age (IQR) - yrs	60 (50-69)	63 (56-72)	56 (47-64)
Primary cancer site - no. (%)			
Breast	31 (29.2)	15 (25.0)	16 (34.8)
Ovarian	43 (40.6)	28 (46.7)	15 (32.6)
Endometrial	17 (16.0)	7 (11.7)	10 (21.7)
Cervical	7 (6.6)	3 (5.0)	4 (8.7)
Colorectal	5 (4.7)	3 (5.0)	2 (4.3)
Others	7 (6.6)	7 (11.7)	0 (0.0)
Synchronous	4 (3.8)	3 (5.0)	1 (2.2)
Cancer stage - no. (%)			
Localized	25 (23.6)	12 (20.0)	13 (28.3)
Metastatic (without evidence of brain metastasis)	81 (76.4)	48 (80.0)	33 (71.7)
Metastatic (with brain metastasis)	6 (5.7)	4 (6.7)	2 (4.4)
Cancer activity - no. (%)			
Active cancer	94 (88.7)	53 (88.3)	41 (89.1)
History of cancer	12 (11.3)	7 (11.7)	5 (10.9)
Venous Thromboembolism Index Event - no. (%)			
Typical	60 (56.6)		
Atypical	46 (43.4)		
Venous Thromboembolism Index Event site - no. (%)			
Pulmonary Embolism	39 (36.8)	39 (65.0)	0 (0.0)
Proximal Deep Vein Thrombosis	28 (26.4)	28 (46.7)	0 (0.0)
Distal Deep Vein Thrombosis	13 (12.3)	13 (21.7)	0 (0.0)
Upper-extremity Deep Vein Thrombosis non catether-related	3 (2.8)	1 (1.7)	2 (4.4)
Upper-extremity Deep Vein Thrombosis catether-related	45 (42.5)	2 (3.3)	43 (93.5)
Atrial thrombosis catether-related	1 (0.9)	0 (0.0)	1 (2.2)
Splanchnic Thrombosis	1 (0.9)	1 (1.7)	0 (0.0)
Outcomes			
Efficacy			
Venous Thromboembolism recurrence rate - no. per 100 person-years	4.0	7.5	0.0
Venous Thromboembolism recurrence site - no. (%)			
Pulmonary embolism	0 (0.0)	0 (0.0)	0 (0.0)
Proximal Deep Vein Thrombosis	4 (100.0)	4 (100.0)	0 (0.0)
All-cause mortality rate - no. per 100 person-years	11.0	13.2	8.5
Safety			
All Bleeding Events incidence - %	27.4	28.3	26.1
Major Bleeding incidence - %	0.0	0.0	0.0
Clinically relevant non-major bleeding incidence - %	2.8	3.3	2.2
Minor bleeding incidence - %	24.5	25.0	23.9

Upon completion of a therapeutic regimen of full anticoagulation for at least 6 months, all patients have initiated treatment with rivaroxaban 10 mg od. To date, they have been followed for a median time of 333 days (IQR 156-484). So far, we have observed 4 objectively verified VTE recurrences, with a rate of 3.8%. Interestingly, all 4 recurrences were proximal DVTs of the lower limbs in patients whose qualifying previous event was a proximal DVT of the lower limbs. In 2 patients, the VTE recurrence developed in the presence of extrinsic vascular compression. In terms of safety, we have observed no major bleedings (0.0%) and 3 CRNMB (2.8%). **Conclusions**: The interim results of this ongoing study provide preliminary evidence that extended treatment with rivaroxaban 10 mg may be a valid and safe therapeutic strategy to prevent CTE recurrences in cancer patients. Large-scale studies are needed to confirm these data.

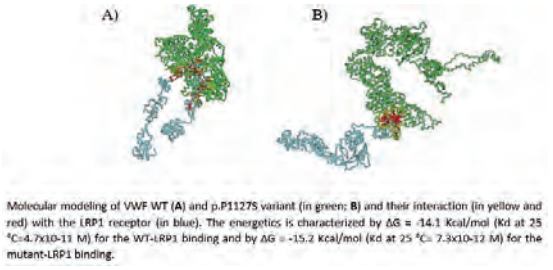
OC061

THE P.P1127S PATHOGENIC VARIANT LOWERS VON WILLEBRAND FACTOR (VWF) LEVELS THROUGH HIGHER AFFINITY FOR THE MACROPHAGIC SCAVENGER RECEPTOR LRP1: CLINICAL PHENOTYPE AND PATHOGENIC MECHANISMS

M. Tardugno, M. Sacco[1], S. Lancellotti[2], A. Branchini[3], F. Bernardi[3], F.M. Testa[3], B. Lunghi[3], M. Pinotti[3], B. Giusti[4], G. Castaman[5], R. De Cristofaro[1,2]

[1]Catholic University of Sacred Heart; [2]Fondazione Policlinico Universitario "AGemelli", Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS); [3]University of Ferrara; [4]University of Florence; [5]Careggi University Hospital, Hemorrhagic and Coagulation Disease, Department of Oncology, Italy

Background and Aims: Type 1 von Willebrand Disease (VWD) is extremely heterogeneous and variably reduced VWF levels could depend on different molecular mechanisms, such as an alteration of secretion, intracellular retention and accelerated plasmatic clearance. Moreover, a variety of cell surface receptors, such as the low-density-lipoprotein receptor-related protein 1 (LRP1) and the asialoglycoprotein receptor (ASGPR), binds with high affinity the FVIII/VWF complex, modulating its half-life in circulation. Thus, studies on rare missense variants could contribute to better characterize VWD type 1 phenotypes. On this background, this study aims to genetically and biochemically characterize the molecular pathogenesis of a mild haemorrhagic tendency in a 21-year-old Italian woman with low levels of both VWF (VWF:Ag=34.3 IU/dL; VWF:GpIbR=32.8 IU/dL) and Factor VIII (FVIII:C=55.3 IU/dL). The index case inherited from her mother a new VWF heterozygous missense mutation c.C3379>T in exon 25, causing the p.P1127S substitution in the D'D3 domain. **Methods**: VWF:Ag and VWF:GpIbR were measured by chemiluminescence assays, FVIII:C by chromogenic assay; the VWF-FVIII binding (VWF:VIIIB) and pro-peptide levels (VWF:pp) by ELISA assays; ADAMTS-13:activity by FRET-based-assay; the VWF multimeric pattern (mVWF) by SDS-agarose-gel electrophoresis; ristocetin-induced platelet aggregation (RIPA) by Born assay. Molecular modeling was performed by using I-TASSER software. *In vitro* expression of VWF WT and p.P1127S variants was obtained by HEK-293 cells, while their interaction with LRP1 was analyzed by fluorescence assay. The single-nucleotide variant (SNV) of ASGR2 subunit was detected by PCR. **Results**: The p.P1127S variant was clinically associated with a mild VWD type 1 phenotype. Although desmopressin infusion normalized the patient's VWF levels, the protein clearance resulted enhanced (t1/2=6.7h) than in normal subjects (t1/2=12±0.7h). The VWF:pp/VWF:Ag ratio was slightly increased (1.3), reaching 1.52, after desmopressin infusion. The VWF:VIIIB and ADAMTS-13 activity were normal, as well as the RIPA assay and the mVWF pattern. The p.P1127S variant was normally synthesized and secreted by HEK-293 cells. Molecular modeling predicted the molecular structure of the VWF sequence 764-2191 for both the WT and p.P1127S variant. The presence of a Ser residue could perturb the non-covalent interactions between VWF monomers, causing a local disruption of the correct folding in the Gly1115-Arg1133 region. The loss of the hairpin-like structure produces long-range allosteric effects, which propagate to the Glu950-Ser1028 loop, globally generating a more opened conformation of the mature mutated VWF. These conformational changes were further investigated to predict their effects on the VWF and LRP1 interaction. Compared to the WT, the molecular modeling of the p.P1127S variant showed an increased binding affinity for the LRP1, confirmed by *in vitro* studies. Thus, the energetics, defined by ΔG, were -14.1Kcal/mol (Kd at 25°C=4.7x10-11M) and -15.2Kcal/mol (Kd at 25°C=7.3x10-12M) for the WT and p.P1127S variant, respectively (Figure 1). The index case was also homozygous for the rs2289645 T>C in the ASGR2, potentially modulating VWF clearance. **Conclusions**: Type 1 VWD mild phenotype was induced by VWF p.P1127S mutation that causes functional changes in VWF conformation, increasing the VWF affinity for the scavenger receptor LRP1, thus accelerating the VWF clearance.



Molecular modeling of VWF WT (A) and p.P1127S variant (in green; B) and their interaction (in yellow and red) with the LRP1 receptor (in blue). The energetics is characterized by ΔG = -14.1 Kcal/mol (Kd at 25 °C=4.7x10-11 M) for the WT-LRP1 binding and by ΔG = -15.2 Kcal/mol (Kd at 25 °C= 7.3x10-12 M) for the mutant-LRP1 binding.

Figure 1.

OC062

CLINICAL AND PHENOTYPIC PRESENTATION OF PATIENTS WITH LOW VWF IN THE MILAN CENTER

O. Seidizadeh[1,2], A. Ciavarella[1], L. Baronciani[1], M.T. Pagliari[1], G. Cozzi[1], P. Colpani[1], S.M. Siboni[1], E. Biguzzi[1], F. Peyvandi[1,2]

[1]Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; [2]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy

Background: Low von Willebrand factor (VWF) refers to patients with VWF levels of 30-50IU/dL. Managing patients with low-VWF levels is a clinical challenge. **Aim**: To determine the clinical and phenotypic features of patients with low-VWF. **Methods**: We included 235 well-characterized patients with low-VWF levels (VWF antigen and/or VWF activity 30-50 IU/dL with a VWF activity/VWF antigen ratio>0.6). The ISTH bleeding assessment tool was used to assess the severity and frequency of clinical symptoms. The VWF propeptide (VWFpp) assay was performed to determine VWF clearance, with results compared to 120 healthy controls. The Mann-Whitney U test was used to compare medians between two independent groups. The abnormal bleeding score (BS) was defined as≥4 in adult males, ≥6 in adult females, and≥3 in children. **Result**: The median (range) of VWF antigen and activity was 52 IU/dL(30-72) and 40 IU/dL(30-55) respectively, with a VWF activity/VWF antigen ratio of 0.77. The median BS was 4 (n=160, range 0-17) and 33% of patients had bleeding that required treatment. Abnormal bleeding was seen in 33% of children, 43% of females and 51% of males. Epistaxis, bruising, surgery, menorrhagia and bleeding from minor wounds were the most common clinical manifestations (Figure 1).

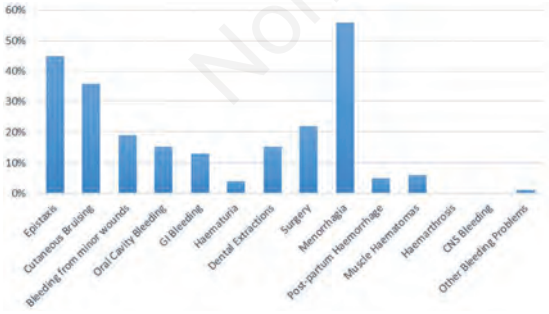


Figure 1.

Nevertheless, severe bleeding symptoms as GI bleeding and haemarthrosis were also observed. Patients VWFpp/VWF antigen ratio was 1.4 (n=130, range 0.5-2.4), significantly higher than that of controls (median 0.98, range 0.6-1.6, P<.002). An increased clearance was observed in 35% of cases (n=46) evaluated for VWFpp. No difference was found for the BS between cases with and without enhanced clearance. **Conclusions**: Low-VWF levels can be associated with significant bleeding and >42% of patients had an abnormal BS. Therefore, these patients should be considered to have a mild bleeding disorder rather than a risk factor for bleeding. We further demonstrated the important role of VWF increased clearance as a pathogenic mechanism.

OC063

FINAL ANALYSIS FROM THE PIVOTAL PHASE 3 HOPE-B GENE THERAPY TRIAL: STABLE STEADY-STATE EFFICACY AND SAFETY OF ETRANACOGENE DEZAPARVOVEC IN ADULTS WITH SEVERE OR MODERATELY SEVERE HEMOPHILIA B

W. Miesbach[1], F.W.G. Leebeek[2], M. Recht[3], N.S. Key[4], S. Lattimore[3], G. Castaman[5], M. Coppens[6], D. Cooper[7], S. Slawka[7], S. Verweij[7], R. Gut[7], R. Dolmetsch[7], Y. Li[8], P.E. Monahan[8], S.W. Pipe[9], HOPE-B Investigators

[1]University Hospital Frankfurt, Frankfurt, Germany; [2]Erasmus MC, University Medical Center Rotterdam, Netherlands; [3]Oregon Health & Science University, Portland, OR, United States; [4]University of North Carolina, Chapel Hill, NC, United States; [5]Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence, Italy; [6]Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands; [7]uniQure BV, Amsterdam, Netherlands/ uniQure IncLexington, MA, USA; [8]CSL Behring, King of Prussia, PA, United States; [9]University of Michigan, Ann Arbor, MI, United States

Introduction: Etranacogene dezaparvovec (formerly AMT-061), an investigational gene therapy for hemophilia B (HB), comprises an adeno-associated virus serotype 5 (AAV5) vector, containing a codon-optimized Padua variant human factor IX (FIX) transgene under the control of a liver-specific promoter. **Methods**: Adult male participants (n=54) with severe or moderately severe HB (FIX ≤2%), with (n=33) or without (n=21) pre-existing AAV5 neutralizing antibodies (NAbs), were treated in the Phase 3, open-label, single-dose, single-arm, HOPE-B trial (NCT03569891) with 2x1013 gc/kg etranacogene dezaparvovec, following a ≥6-month lead-in period of FIX prophylaxis. FIX activity, annualized bleed rate (ABR), and FIX infusions were assessed over a uniform ≥52-week period after achieving stable FIX expression, designated as Months 7–18 after vector infusion to serve as the basis for regulatory approval. Adverse events (AEs) were recorded. **Results**: Mean FIX activity was 39.0 IU/dL (±18.7; 8.2, 97.1) (standard deviation; min, max) at Month 6 and 36.9 IU/dL (±21.4; 4.5, 122.9) at Month 18. Individual participant FIX activity and titer of pre-existing AAV5 NAbs (present in 21 participants) did not correlate up to a titer of 678; one participant with NAbs=3212 did not express FIX Padua.

Compared with the ≥6-month lead-in period (ABR 4.19), 52-week adjusted ABR for all bleeds during Months 7–18 (ABR 1.51) was reduced by 64% (p=0.0002), demonstrating statistical superiority over FIX prophylaxis. FIX-treated bleeds were reduced by 77% (from ABR 3.65 to 0.83; p<0.0001). 52/53 (98%) participants who received a full dose discontinued prophylaxis, with an overall 97% reduction in mean unadjusted annualized FIX consumption (from lead-in period 257338.8 to Months 13–18 8486.6 IU/yr/participant). After dosing, 37 participants experienced 92 treatment-related AEs, of which 74 (80.4%) were mild. A serious AE of hepatocellular carcinoma was determined by independent molecular tumor characterization and vector integration analysis to be unrelated to vector. **Conclusions**: Following a single dose of etranacogene dezaparvovec, participants experienced a stable and durable increase in mean FIX activity into the near-normal range at 18 months and hemostatic protection, achieving the HOPE-B primary efficacy endpoint.

OC064

ENGINEERED SUPPRESSOR TRNAS AS A NOVEL CORRECTION APPROACH FOR RECURRENT HEMOPHILIA A-CAUSING NONSENSE MUTATIONS

M.F. Testa[1], J.D. Lueck[2], A. Di Fiore[1], F. Bernardi[1], M. Pinotti[1], A. Branchini[1]

[1]Department of Life Sciences and Biotechnology and LTTA Center, University of Ferrara, Ferrara, Italy; [2]Department of Physiology and Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, USA

Background and Aims: *F8* nonsense mutations, relatively frequent cause (>10%) of Haemophilia A (HA), lead to the synthesis of truncated/loss-of-function factor VIII (FVIII) variants by inserting premature termination codons (PTCs), and are commonly considered "null mutations". Anticodon Engineered transfer RNAs (ACE-tRNA) are small molecules tailored to accurately drive the insertion of the original amino at PTC during protein synthesis, thus restoring production of the wild-type molecule. In this view, ACE-tRNAs might represent a novel correction approach to specifically suppress disease-causing PTCs. In this pioneer project we challenged ACE-tRNA to productively suppress highly frequent *F8* PTCs and restore FVIII biosynthesis and secretion of functional FVIII, with potential therapeutic implications. **Methods**: Transient expression studies in HEK293T cells through an optimized expression platform taking advantage of a fusion protein (FVIII-GL) joining FVIII and the naturally-secreted Gaussia luciferase (GL). Treatment of cells with the ACE-tRNA engineered to introduce arginine (ACE-tRNAArg) on TGA *F8* PTCs. Luciferase assays (FVIII-GL) and ELISA (FVIII) on cell media to detect FVIII secreted levels. **Results**: Expression studies with the FVIII-GL fusion protein bearing the recurrent *F8* PTCs (n=12, patient number from 10 to 57) arising from the highly frequent CGA(arginine)>TGA change (p.R15X, p.R355X, p.R446X, p.R602X, p.R814X, p.R1715X, R1960X, p.R1985X, p.R2135X, p.R2166X, p.R2228X and p.R2326X) were conducted in HEK293T cell lines to detect luciferase activity, directly related to the amount of secreted full-length FVIII levels. Noticeably, the selected mutations are representative of 475/1053 (45%) HA patients with nonsense mutations. All expressed *F8* variants showed FVIII traces due to spontaneous suppression ("readthrough"), predicted to produce full-length proteins by inserting amino acid subsets including the original residue at low frequency. Nevertheless, higher degree of suppression (range 8-77% of wild-type FVIII-GL) was observed after ACE-tRNAArg treatment, which restored secreted levels of wild-type FVIII-GL. In particular, the differential degree of correction allowed to stratify *F8* PTCs in three groups, namely i) low (p.R602X; 0-10%), ii) intermediate (p.R15X, p.R446X, p.R814X, p.R1715X, p.R1985X, p.R2135X, p.R2166X; 10-50%, and iii) high (p.R355X, p.R1960X, p.R2228X, p.R2326X; >50% WT) responders to ACE-tRNAArg-mediated suppression. Noticeably, comparison between groups showed significantly higher secreted luciferase activity (p=0.0002) for the ACE-tRNA treated group. Intrigued by these promising results in a system taking advantage of a reporter gene, the efficiency of ACE-tRNAArg was further challenged by inserting *F8* PTCs in the proper FVIII-coding context. Preliminary results on the FVIII variants revealed higher secreted FVIII levels after ACE-tRNAArg treatment, supporting the restored biosynthesis and secretion of wild-type FVIII. Functional assays to assess activity levels are in progress. **Conclusions**: Our data propose a novel correction approach for nonsense mutations, to faithfully introduce the original amino acid and restore biosynthesis and secretion of functional wild-type FVIII. This experimental evidence, provided for the first time in the coagulation field, opens the way for an innovative therapeutic approach which might be translated to other diseases caused by nonsense mutations.

OC065

OSTEOCLAST ROLE IN HAEMOPHILIC BONE DISEASE

M. Sacco[1], S. Lancellotti[2], G. Battafarano[3], M. Tardugno[1], L. Di Gennaro[2], R. De Cristofaro[1,2], A. Del Fattore[3]

[1]Catholic University, Translational Medicine and Surgery Department Rome; [2]Fondazione Policlinico Universitario AGemelli, IRCCS, Center for Hemorrhagic and Thrombotic Diseases and Haemophilia Center, Rome; [3]Bone Physiopathology Group, Multifactorial Disease and Complex Phenotype Research Area, Bambino Gesù Children's Hospital, Roma, Italy

Background and Aims: Bone disease is a relevant complication of haemophilia A (HA), but its pathogenesis still remains unknown. In people with hemophilia (PWH), an association between hemophilia and low bone mineral density (BMD) has been found. Indeed, 43% of HA patients show low bone density and 27% develop osteoporosis. Over the last few years, clinical and experimental evidences indicated that the deficiency of FVIII leads to decreased BMD independently of hemarthrosis and medical comorbidities, suggesting a novel effect for FVIII outside of the coagulation system. The reduction of BMD seems to be caused by perturbations of the Receptor Activator of Nuclear factor-B RANK Ligand (RANKL) and osteoprotegerin (OPG) pathways. In haemophilic alterations of bone remodeling, osteoclasts seem to play an important role. Osteoclasts are multinucleated cells which derived from the CD14+ monocyte/macrophage lineage. Until recently, the identity of osteoclast progenitors has not been well defined, but evidences report that CD16−CD14+ rather than CD16+CD14+ monocytes were prone to differentiate into osteoclasts. The purpose of this study is to better understand how the osteoclastogenesis could be influenced by the FVIII, von Willebrand Factor (VWF) and thrombin and to assess the osteoclastogenic potential from two different HA patients. **Methods**: Osteoclastogenesis of healthy donors-derived PBMC (Peripheral Blood Mononuclear Cells) were assessed in the presence of plasma derived VWF/FVIII complex, rVWF, rFVIII and thrombin. Moreover, in order to study cell alteration in HA patients, osteoclastogenesis was performed with PBMC isolated from two HA patients and compared with healthy controls. Moreover, FACS analysis was performed in order to study circulating osteoclast precursors, of adult haemophilic patient. **Results**: The treatment of PBMC with coagulation factors reduced osteoclastogenesis even at levels comparable to that induced by osteoprotegerin. In particular, VWF showed a key role in regulation of osteoclasts differentiation process, inhibiting ~43% of the osteoclastogenesis while, when VWF was complexed with FVIII, the 50% of inhibition was observed. On the other hand, thrombin inhibits osteoclast differentiation (up to 95% of inhibition). PBMC isolated from both severe/pediatric and mild/adult haemophilic patients, showed an increased ability to produce mature osteoclasts in comparison to those obtained from healthy donor. Osteoclast precursors (CD16−CD14+CD11b+) are significantly higher in adult/mild HA patient than age and sex matched controls (~33%), whereas the percentage of CD16+ cells was reduced. According to these data, gene expression analysis on RNA extracted from patient's and control's derived mature osteoclasts, revealed high-levels of mRNA levels of RANK, TRAF6, CATHEPSIN-K and TCIRG1 compared to matched controls. **Conclusions**: All these data support that bone loss observed in haemophilic patients could be related to increased osteoclast formation and activity and that coagulation factors directly impact on bone cells. Further studies are needed in order to translate the results into benefits for clinical practice of haemophilia patients suffering from bone disease.

OC066

NEW GENOME EDITING APPROACHES: BASE AND PRIME EDITING TO REVERT HEMOPHILIA A-CAUSING POINT MUTATIONS

E. Tonetto[1], S. Pignani[2], G. Roman[2], A. Follenzi[2], F. Bernardi[1], D. Liu[3], M. Pinotti[1], D. Balestra[1]

[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Department of Health Sciences, University of Piemonte Orientale, Novara, Italy; [3]Howard Hughes Medical Institute, Harvard University, Cambridge, USA

Background and Aims: Hemophilia A (HA), the most common coagulation bleeding disorder (1:5000 males), is an X-linked hereditary disease caused by *F8* gene mutation leading to deficiency of factor VIII (FVIII). Current HA therapies, albeit significantly improving HA treatment, are mainly aimed at replacing the missing factor and do not provide a definitive cure. On the other hand, gene therapy, albeit providing promising results, still have significant drawbacks and is far from being translated into the clinic. Genome editing approaches based on homologous recombination (HSR) have been also explored but are not optimized yet, with still safety concerns to be addressed. Recently, new genome editing approaches non-relying on HSR, namely Base Editing (BE) and Prime Editing (PE) strategies, have been developed and showed their ability to efficiently and cleanly install or revert point mutations which, in the HA field, are the most represented ones when excluded the IVS22 inversion. The BE and PE approaches, based on engineered Cas proteins fused with nucleotide deaminase or reverse transcriptase domains, respectively, are able to efficiently and selectively modify the target nucleotide without inducing any double strand breaks or recombination events. The aim of this study is to develop BE and PE tools to revert a panel of frequent *F8* missense and nonsense variants associated with severe HA phenotypes and thus rescue FVIII expression. **Methods**: Design and cloning of BE/PE components as well as of FVIII cassettes to create expression vectors; Creation of cellular models by transient and stable expression of recombinant FVIII variants; Delivery of genome editing actors by lipofection and evaluation of rescue at DNA (DNA sequencing) and protein (antigen by ELISA and activity by aPTT assay) levels. **Results**: c.6046 C>T (p.R2016W), c.6496 C>T (p.R2166*), c.6545 G>A (p.R2182H), c.6682 C>T (p.R2228*), c.6683 G>A (p.R2228Q), through transient and stable expression studies, are associated with reduced secreted FVIII levels (p.R2166* 0%; p.R2228Q 13%; p.R2016W 39%; p.R2182H 65% of WT counterpart). On the other hand, the p.R2228* change resulted in appreciable FVIII protein levels (84% of WT) likely attributable to the secretion of a truncated FVIII isoform. Studies on FVIII activity revealed that p.R2228Q maintains the same specific activity of the wt counterpart, indicating in the secretion defect the major cause of patient phenotype. We identi-

fied, through a screening process, a panel of BE/PE able to rescued FVIII secretion for the p.R2166*, p.R2182H and p.R2228Q variants. Additional studies on stable clones expressing the p.R2166* and p.R2228Q mutations demonstrated that the BE and PE tools were able to revert the mutations at DNA levels and resulted in significant rescue (up to 20% of FVIII-WT) of secreted FVIII protein and activity levels. **Conclusions**: Overall, for the first time we applied BE and PE to frequent FVIII point mutations leading to severe Haemophilia A. Experimental data provided the proof-of-principle of efficacy in cellular models, which are currently under validation in HA blood-outgrowth endothelial cells (BOECs) and planned in mouse models.

OC067

RESCUE OF A HEMOPHILIA A-CAUSING FVIII SPLICING VARIANT VIA ENGINEERED U1SNRNAS

L. Peretto[1], R. Tarantino[1], A. Borchiellini[2], F. Bernardi[1], A. Follenzi[3], M. Pinotti[1], D. Balestra[1]

[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Citta della salute e della scienza, Torino, Italy; [3]Department of Health Sciences, University of Piemonte Orientale, Novara, Italy

Background and Aims: Hemophilia A (HA), the most common coagulation bleeding disorder (1:5000 males), is an X-linked hereditary disease caused by F8 gene mutations leading to deficiency of factor VIII (FVIII). Current HA therapies, albeit significantly improving HA treatment, are mainly aimed at replacing the missing factor and do not provide a definitive cure. Among all HA-causing mutations, those affecting splicing are relatively frequent, particularly in the severest forms. These mutations are generally associated with exon skipping and can be potentially rescued by RNA therapeutics. Among these, variants of the key U1snRNA spliceosomal component have been shown to efficiently rescue exon inclusion impaired by mutations located at the 3'splice site (ss), 5'ss or within the defective exon, in both cellular and animal models of human disease. This study aims to dissect the molecular mechanism underlying the moderate HA (FVIII:C<5%) phenotype identified in two brothers carrying the F8 c.1752+5G>C variant and to develop RNA therapeutics based on engineered U1snRNAs to restore proper exon 11 definition. **Methods**: Creation of expression vectors for the wild-type (pIVS11wt) and mutant (pIVS11+5G>C) F8 minigenes and for the engineered U1 snRNAs designed to base pairs to the mutated 5'ss (compensatory U1snRNA) or to less-conserved downstream intronic sequences (Exon Specific U1snRNA). Transient expression of F8 minigenes, either wild-type (pIVS11wt) or harbouring the variant c.1752+5G>C (pIVS11+5G>C), in different hepatoma cell lines (Huh7, HepG2, Hepa1-6) as well as HEK293T, followed by splicing pattern analysis. Rescue of exon 11 definition by co-transfection of mutant minigene with compensatory (n=1) or Exon Specific U1snRNA (n=3) followed by splicing pattern analysis with plasmid-specific amplicons. **Results**: Bioinformatic analysis did not predict aberrant splicing due to the F8 c.1752+5G>C mutation. However, splicing assays in different hepatoma cells demonstrated that exon 11 is well defined, as confirmed by the complete exon inclusion in the pIVS11wt context, and that the c.1752+5G>C change induces exon 11 skipping, to an extent that depends on the transfected cell lines. In the worst scenario, the mutation is associated with low levels of correctly spliced transcripts (~10%). Notably, co-transfection of different engineered U1snRNA significantly improves FVIII exon 11 definitions and thus inclusion, with the compensatory U1snRNA associated with exon 11 inclusion up to 92%. **Conclusions**: Overall, we provide experimental evidence that the F8 c.1752+5G>C change leads to exon skipping by impairing proper exon 11 definition and is associated with trace levels of correctly spliced transcripts, in accordance with the HA patients' phenotype. Moreover, the splicing outcome is cell-dependent, and further studies aimed at identifying the involved splicing factor will be conducted. Notably, F8 exon 11 inclusion can be efficiently restored by RNA therapeutics based on engineered U1snRNAs, which are currently under investigation through lentiviral-mediated delivery in Blood Outgrowth Endothelial Cells (BOEC) isolated from HA patients.

OC068

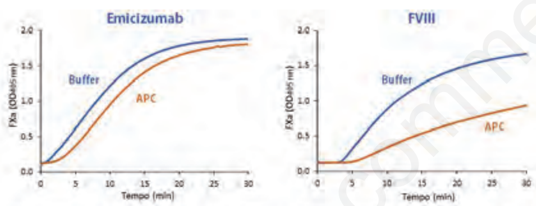
INFLUENCE OF EMICIZUMAB OF PROTEIN C-MEDIATED CLOTTING REGULATION

F. Mancazzo, L. Dirienzo, A. Vitulli, C.T. Ammollo, F. Semeraro, M. Colucci

Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Bari "Aldo Moro", Italy

Background and Aims: Emicizumab is a bispecific antibody which functions as FVIII-mimetic by simultaneously binding FIXa and FX. At variance with plasma FVIII, emicizumab is insensitive to degradation by activated protein C (APC) and may thus tilt the coagulation balance towards a prothrombotic state. We investigated the effect of emicizumab on PC-mediated inhibition of coagulation under *in vitro* conditions mimicking physiological and pathological clotting activation. **Methods**: Hemophilic plasma (<1% FVIII) was supplemented with emicizumab (50 or 100 µg/ml) or recombinant FVIII (Kovaltry, 1 IU/ml). Thrombin generation was assessed by CAT assay using as clotting trigger tissue factor (TF, 1 pM) or an intrinsic pathway activator (1/50 diluted aPTT reagent or 20 pM FXIa). The effect of the PC system was assessed by adding APC (0.2-0.8 µg/ml) or by activating plasma PC by thrombomodulin (TM, 4 nM) or endothelial cells (EA.hy926, 50,000/well). In some experiments, FXa generation was assessed by a chro-

mogenic substrate (S-2732). **Results**: In TF-triggered coagulation (physiologic activation), emicizumab-plasma displayed a good response to added APC as well as to endogenous PC activation by TM or endothelial cells addition, with a clear-cut inhibition of thrombin generation, which was comparable to that observed in FVIII-plasma. When coagulation was triggered through the intrinsic pathway (pathologic activation), the prolongation of lag-time by increasing concentrations of APC was less pronounced in emicizumab-plasma than in FVIII-plasma, whereas the reduction of thrombin peak and ETP was similar. Using the latter condition, we found that the generation of FXa, which persisted after prothrombin consumption, was significantly greater in emicizumab-plasma and, contrary to FVIII-plasma, barely affected by APC (Figure 1). Finally, when purified prothrombin was added to (defibrinated) plasma after consumption of endogenous prothrombin, the second wave of thrombin generation in emicizumab-plasma was greater and markedly less affected by APC as compared to FVIII-plasma, likely because of the higher concentration of FXa in the former. **Conclusions**: *In vitro*, in a "closed" system, thrombin generation in emicizumab-plasma was effectively inhibited by the PC system (with the only exception of a weaker lag-time prolongation in contact-phase activated plasma), suggesting a major role of FVa inactivation by APC. However, the greater generation of FXa in emicizumab-plasma in the presence of APC might translate *in vivo* ("open" system) in a greater and sustained thrombin generation thanks to the continuous supply of prothrombin.



FXa generation induced by FXIa (20 pM) in hemophilic plasma supplemented with emicizumab (50 µg/ml, left panel) or FVIII (1 IU/ml, right panel), in the absence and in the presence of APC (0.4 µg/ml). FXa formation was monitored by the chromogenic substrate S-2732. Results denote optical density (OD405nm). A representative experiment is shown.

Figure 1.

OC069

SURGICAL PROCEDURES IN SUBJECTS WITH SEVERE HEMOPHILIA A TREATED WITH EMICIZUMAB: A SINGLE CENTRE EXPERIENCE

S. Linari, L. Pieri, F. Demartis, R. Fjerza, M.C. Di Stefano, G. Castaman

Centre for Bleeding Disorders and Coagulation, Department of Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

Background and Aims: The management of surgery in persons with severe haemophilia A (PWSHA) requires intensive treatment with factor VIII (FVIII) and postoperative close monitoring of circulating FVIII to maintain protective levels for bleeding, while avoiding excessive levels with inherent thrombotic risk. The recent availability of the bi-specific monoclonal antibody emicizumab for long-term prophylaxis in PWSHAhas opened a new scenario in planning antihemorrhagic perioperative treatment with FVIII with its potential risks. The aim of the present study is to report our experience in management of surgical procedures in PWSHA on emicizumab. **Methods**: Between January 2018 and April 2022, 17 PWHA underwent 34 surgical procedures (5 major surgeries, 29 minor surgeries).All major surgeries were managed under FVIIIcoverage, with factor activity levels of at least 50 IU/dL maintained for 7-14 days. Adjunctive intravenous tranexamic acid (TA) 1 g every 12 hours for 7 days was also administered. Thromboprophylaxis was not prescribed. Minor surgeries were managed with a rFVIII preoperative bolus (25-50 IU/kg), except for tooth extractions, dental procedures, and cataract, performed without rFVIII coverage. In dental surgeries TA has been used as mouthwash. When clinically appropriate, FVIII activity levels were monitored by using a bovine chromogenic assay. **Results**: No thromboembolic events have occurred. No transfusional support was needed. Bleeding was as expected depending on the type of surgery (Table 1). **Conclusions**: Major surgery with a regimen of emicizumab and rFVIII in PWSHA has been safely and efficaciously performed. Minor surgeries can be safely performed without prophylaxis with rFVIII. A multidisciplinary team and strict monitoring are recommended for the management of surgical procedures in PWSHA treated with emicizumab.

Table 1.

Major surgery	N	Age of patient (yrs)	rFVIII use	TA use	Post-surgical bleeding	Days of hospitalization
Total knee replacement with forced extension on post-operative day 9	1	28	SHL-rFVIII 70 IU/kg: 43 IU/kg every 12 hours x 3 days; 43 IU/kg every 24 hours x 5 days; 43 IU/kg every 12 hours x 1 day; 28 IU/kg every 12 hours x 3 days; 14 IU/kg every 12 hours x 2 days; 14 IU/kg every 24 hours x 7 days; 28 IU/kg before physiotherapy	yes	no	15
Muscle biopsy (thigh pseudotumor)	1	63	SHL-rFVIII 66 IU/kg: 50 IU/kg every 24 hours x 3 days; 50 IU/kg every 48 hours x 4 days	yes	no	8
Thigh pseudotumor excision	1	63	SHL-rFVIII 85 IU/kg: 50 IU/kg every 12 hours x 1 day; 30 IU/kg every 12 hours x 6 days; 50 IU/kg every 24 hours x 7 days	yes	no	7
Splenectomy	1	47	SHL-rFVIII 70 IU/kg: 35 IU/kg every 12 hours x 3 days; 23 IU/kg every 12 hours x 4 days; 35 IU/kg every 24 hours x 7 days	yes	no	11
Cleft palate correction (2nd operation)	1	5	SHL-rFVIII 55 IU/kg: 55 IU/kg every 12 hours x 1 day; 55 IU/kg every 24 hours x 6 days; 55 IU/kg every 48 hours x 7 days	yes	no	7
Turbinate reduction	1	19	EHL-rFVIII 25 IU/kg: 25 IU/kg every 24 hours x 2 days	yes	no	2
Minor surgery						
Port removal	2	2/2	SHL-rFVIII 50 IU/kg	no	no	1
Synoviorthesis with rifampicin	2	47/54	SHL-rFVIII 25 IU/kg	no	no	0
Gastro-duodenal endoscopy with biopsy	2	47/52	SHL-rFVIII 45 IU/kg	no	no	5*/0
Colonoscopy	1	52	SHL-rFVIII 30 IU/kg	no	no	0
Cistoscopy	1	52	SHL-rFVIII 45 IU/kg	no	no	0
Phimosis	1	39	EHL-rFVIII 25 IU/kg: 25 IU/kg every 24 hours x 1 day	yes	no	0
Skin biopsy	1	65	SHL-rFVIII 50 IU/kg	no	no	0
Dental extraction and implant	4	62/63	SHL-rFVIII 50 IU/kg	yes	no	0
Dental extraction	2	47/54	no	yes	no	0
Dental procedure	12	18/42/56/57/60/63	no	no	no	0
Cataract	1	65	no	no	no	0

*Performed for hematemesis

OC070

ANTI-INTEGRIN GPIIBIIIA IMMUNE THROMBOCYTOPENIA AUTOANTIBODIES IMPAIR MEGAKARYOCYTE MIGRATION, POLARIZATION AND PROPLATELET FORMATION

E. Giglio, E. Petito, L. Bury, A. Mezzasoma, P. Gresele

Section of Internal and Cardiovascular Medicine, Department of Medicine, University of Perugia, Perugia, Italy

Background and Aims: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia due to antiplatelet autoantibodies against platelet surface glycoproteins (αIIbβ3-GPIIb/IIIa, α2β1-GPIa/IIa, GPIb/IX/V) leading to increased platelet clearance. Recent data however show that they bind surface glycoproteins also on megakaryocytes (MKs) possibly impairing platelet production. Both MK migration from the osteoblastic to the vascular niche and MK polarization are crucial steps in thrombopoiesis and their defect, with consequent ectopic platelet production in the bone marrow, has been reported to contribute to thrombocytopenia in some congenital thrombocytopenias. Defective MK adhesion, spreading and proplatelet formation (PPF) induced by anti-αIIbβ3 and anti-αIIbβ3+anti-α2β1 autoantibodies were previously reported (Grodzielski *et al.* Br J Haematol. 2018;183:319-323) but no studies on MK migration and polarization were carried out. Our aim was to evaluate the effects of anti-αIIbβ3 antiplatelet autoantibodies detected in serum of a series of ITP patients on MKs, focusing on their ability to modulate some crucial steps of thrombocytopoiesis, like MK migration, polarization, spreading and PPF. **Methods**: Anti-αIIbβ3 autoantibodies were detected by the MAIPA assay in serum from 10 ITP patients. Control serum was obtained from 10 healthy subjects. Human MKs were differentiated from peripheral blood-derived CD34+ cells from healthy subjects. MK migration towards SDF-1α was assessed by a transwell system, MK polarization on fibrinogen by fluorescence microscopy upon staining with anti-CD42b MoAb and Hoechst for nuclei, MK spreading and PPF on fibrinogen and on type I collagen by fluorescence microscopy upon staining with anti-β1-tubulin antibody for microtubules, rhodamine-phalloidin for actin and Hoechst for nuclei. All data are reported as mean±SEM. **Results**: The area of the bottom face of the transwell filter covered by MKs was significantly reduced (32%; $p<0.01$) upon coincubation with anti-αIIbβ3 ITP serum compared to control serum showing impaired MK migration. The percentage of polarized MKs on fibrinogen was significantly reduced upon coincubation with anti-αIIbβ3 ITP serum compared with control serum (15.6±1.3% *vs* 36.2±2%; $p<0.01$). The percentage of spread MKs in the presence of anti-αIIbβ3 ITP serum compared to control serum was not impaired, either on fibrinogen or on type I collagen. However, anti-αIIbβ3 autoantibodies significantly reduced the percentage of PPF on fibrinogen (27.7±2.8%) compared to control serum (41.9±4.2%; $p<0.05$) while no differences were found on collagen type I. **Conclusions**: The role of αIIbβ3 in MK spreading is well known, indeed αIIbβ3 antagonists block spreading on fibrinogen. The role of αIIbβ3 in PPF is instead debated (Balduini *et al.* J Thromb Haemost. 2008;6:1900-7; Bury *et al.* PLoS One. 2012;7:e34449). We show that anti-αIIbβ3 autoantibodies not only impair PPF formation by MKs but also prevent MK polarization and migration, two crucial steps for the release of platelets in the blood stream. Our results therefore unravel a new pathogenic mechanism of anti-αIIbβ3 autoantibodies possibly explaining thrombocytopenia that characterizes a subgroup of ITP patients, suggesting that ectopic platelet release, besides impaired PPF, may participate in thrombocytopenia induced by anti-αIIbβ3 autoantibodies.

OC071

THE ITALIAN REGISTRY ON ACTIVE ADULT ITP: UPDATE AND PRELIMINARY RESULTS ON QUALITY OF LIFE

L. Ghiotto[1], N. Vianelli[2], G. Carli[3], G. Auteri[2], M. Carpenedo[4], A. Ricco[5], E. Rossi[6], V. De Stefano[6], V. Carrai[7], F. Morelli[7], W. Barcellini[8], B. Fattizzo[8], A. Chistolini[9], C. Santoro[9], S. Cantoni[10], A. Borchiellini[11], F. Valeri[11], U. Consoli[12], V. Innao[12], G. Gaidano[13], A. Patriarca[13], E. Rivolti[14], V. Pavone[15], S. Sibilla[15], F. Pane[16], F. Chiurazzi[16], S. Siragusa[17], M. Napolitano[17], F. Zaja[18], E. Lucchini[18], A. Poloni[19], D. Lame[19], C. Bosi[20], D. Lazzarotto[21], O. Mulas[22], L. Pontalto[1], E. Crea[23], P. Fazi[23], F. Rodeghiero[1]

[1]Fondazione Progetto Ematologia, Vicenza, Italy; [2]Istituto di Ematologia "L. e A. Seragnoli" - Policlinico "S. Orsola Malpighi", Università degli Studi di Bologna, Bologna, Italy; [3]Divisione Ematologia, Ospedale "S. Bortolo", Vicenza, Italy; [4]Unità di Ematologia con Trapianto, ASST "S. Gerardo", Monza, Italy; [5]UO Ematologia con Trapianto, Policlinico Universitario "Giovanni XXIII", Università degli Studi di Bari "Aldo Moro", Bari, Italy; [6]Istituto di Ematologia, Università Cattolica del Sacro Cuore, Policlinico "A. Gemelli", Roma, Italy; [7]SOD Ematologia, AOU Careggi, Firenze, Italy; [8]UOC Oncoematologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; [9]Divisione di Ematologia, Policlinico Universitario "Umberto I", Università degli Studi "Sapienza", Dip. Biotecnologie Cellulari ed Ematologia, Roma, Italy; [10]Dipartimento di Ematologia e Oncologia, Niguarda Cancer Center, Azienda Socio Sanitaria Territoriale Ospedale Niguarda, Milan, Italy; [11]Divisione di Ematologia dell'Università degli Studi di Torino, "Città della Salute e della Scienza di Torino", Torino, Italy; [12]UOC Ematologia, PO Nesima, ARNAS "Garibaldi", Catania, Italy; [13]Ematologia, AOU Maggiore della Carità, Dipartimento di Scienze Mediche, Università del

Piemonte Orientale "Amedeo Avogadro", Novara, Italy; [14]Dipartimento di Ematologia, Azienda Unità Sanitaria Locale (AUSL), IRCCS Reggio Emilia, Italy; [15]UOC Ematologia e TMO, AO "Card. G. Panico", Tricase (LE), Italy; [16]Dipartimento di Ematologia e Trapianto del Midollo Osseo, Azienda Ospedaliera Universitaria "Federico II", Napoli, Italy; [17]UO di Ematologia con Trapianto, Dipartimento Biomedico di Medicina Interna, Azienda Ospedaliera Universitaria "Paolo Giaccone", Palermo, Italy; [18]Ematologia, Azienda Sanitaria Universitaria Integrata, Trieste, Italy; [19]Clinica di Ematologia, Università Politecnica delle Marche e AOU Ospedali Riuniti di Ancona Italy; [20]Dipartimento di Oncologia-Ematologia, Azienda USL Ospedale di Piacenza, Italy; [21]Ematologia e Trapianto di Cellule Staminali, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy; [22]SC Ematologia e CTMO, Ospedale Businco, ARNAS "G. Brotzu", Cagliari, Italy; [23]Fondazione GIMEMA Onlus, Roma, Italy

Background and Aims: Registries are essential for clinical and patient-report data in rare diseases. In Europe, 3 population-based and 12 clinical cohorts of ITP patients, including the Italian ITP registry, have been identified by the European Research Consortium on ITP (www.ercigroup.org) (Moulis *et al.* BJHaem 2021). The Italian registry, sponsored by GIMEMA Foundation (Rome) and managed by the Hematology Project Foundation (Vicenza), started in late 2018 and is aimed at producing a dynamic picture of adult ITP natural history as modified by management by collecting and analyzing standardized data. Variables, including comorbidities, safety and efficacy of treatments and QoL will be considered. **Methods**: Multicenter observational (prospective and retrospective) study, including a 3-year enrollment period with a subsequent follow up of at least 2 years, recruiting patients on active treatment. Patient conditions at entry were classified as: initiating a first-line (group A); requiring modification of previous treatment (group B); being on treatment (group C). Historical data are collected at entry, while prospective data at annual visits. Data quality is monitored and assured by electronic queries and interactions between a central team and recruiting centers. To produce a dynamic individual picture, each patient is tracked as it moves through different disease statuses (complete response, response, no response, toxicity, not on treatment) according to the various treatment lines. **Results**: As to May 10, 2022, 676 patients were sequentially recruited (195 more than the previous report of May 2020) by 21 active centers. 576 cases were fully evaluable regarding the variables of interest. Main patients' characteristics and data on bleeding, thrombosis and QoL at entry are reported in the Table 1. Among patients with at least a grade 1 bleeding using the **S**kin **M**ucosal **O**rgan **G**rading system (SMOG) (Rodeghiero *et al.* Blood 2013) at entry or in the previous month, 47 patients had only skin, 5 had only mucosal, 16 had both skin and mucosal, 2 had both skin and organ and 4 had only organ bleeding. Most S≥2 (N=16) were petechiae. Most M≥1 (N=21) were in oral cavity. O≥1 (N=6) were 2 menorrhagia, 1 severe menorrhagia, 2 gastrointestinal bleeding, 1 intracranial bleeding. Thromboembolic events occurred in 47 patients (8%). By a preliminary analysis, QoL and Fatigue scores appear lower than in normals. In multivariate analysis considering patients without missing data, the absence of general risk factors for thrombosis was significantly associated with fatigue and both SF-12 scores. Sport practice was significantly associated with fatigue and MCS-12. Female gender was significantly associated with fatigue and PCS-12. No statistically significant association was found between HRQoL measures and patient condition at entry. SMOG index and splenectomy status also did not influence HRQoL and fatigue. Absence of association between platelet count at enrollment and both fatigue and HRQoL measures was also observed. **Conclusions**: Our data confirm that patients seen in current clinical practice have an increased incidence of thromboembolic events and that most patients show minor bleeding symptoms with a moderate negative impact on QoL. The enrollment seems to have slowed down with the advent of the COVID-19 pandemic with the number of new diagnoses probably underestimated. A linkage with the existing European ITP registries is planned.

Table 1. Main characteristics of the 576 evaluable patients.

Condition at entry n/N (%)	
Group A	61/576 (11%)
Group B+C	515/576 (89%)
Gender	Male 244 (42%) Female 332 (58%)
Age yrs, media (range)	At diagnosis 50.5 (2- 92) At entry 59 (18-93)
Time from diagnosis to entry yrs, median (interquartile)	4.7 (1.1-11.6)
Platelet count (x 10^9/L) at diagnosis, median (interquartile)	20 (8-42)
Platelet count (x 10^9/L) at entry, median (interquartile)	
Group A	28 (15-105)
Group B+C	106 (53-156)
Splenectomized patients n/N (%)	76/576 (13%)
Age at splenectomy yrs, media (range)	39 (5.5-79.5)
Worst past patients' reported SKIN bleeding manifestation from initial diagnosis (575 evaluable) n (%)	
Petechiae	136 (23.7%)
Ecchymoses	110 (19.1%)
Subcutaneous Hematomas	39 (6.8%)
Bleeding from Minor Wounds	4 (0.7%)
None or minimal	286 (49.7%)
Worst past patients' reported MUCOSAE bleeding manifestation from initial diagnosis (574 evaluable) n (%)	
Epistaxis	58 (10.1%)
Gum bleeding	69 (12.0%)
Hemorrhagic bullae or Blisters	18 (3.1%)
Bleeding after bites to lip & tongue or after deciduous teeth loss	4 (0.7%)
Subconjunctival hemorrhage (not due to conjunctival disease)	5 (0.9%)
None or minimal	420 (73.2%)
Worst past patients' reported ORGAN bleeding manifestation from initial diagnosis (576 evaluable) n (%)	
Gastrointestinal bleeding	25 (4.3%)
Lung Bleeding	1 (0.2%)
Hematuria	4 (0.7%)
Menorrhagia (in woman, compared with pre-ITP)	18 (11%)
Intramuscular Hematomas	1 (0.2%)
Ocular bleeding	4 (0.7%)
Intracranial bleeding	9 (1.6%)
Bleeding after surgery or invasive procedures	4 (0.7%)
None	510 (88.5%)
SMOG index at entry and during the previous month * n	
No bleeding (almost all in Groups B+C) S=0 M=0 O=O	492/566 (87%)
Skin (only) S(1:3) 47	Mucosal (only) M=3 5 Organ (only) O=2 3 O=4 1
Skin plus Mucosal S=(1:3) M=(1:4) 16	Skin plus Organ S=1 O=2 1 S=1 O=4 1
Past thromboembolic events at entry n (%)	
Total events[§]: 50 in 47/576 patients (8%)	
Group A Arterial 0/61	Venous 2/61 (3.3%)
Group B+C[£] Arterial 27/515 (5.2%)	Venous 21/515 (4.1%)
FACIT-Fatigue[$] subscale score, media (range)	
Group A 41.6 (12-52)	Group B+C 40.1 (5-52) Overall 40.3 (5-52)
SF-12[%] indices, media (range)	
PCS-12[%] Group A 48.2 (25.6-60.6)	Group B+C 45.3 (17.2-64.7) Overall 45.6 (17.2-64.7)
MCS-12[%] Group A 47 (26.7-62.4)	Group B+C 46.6 (14.7-66.3) Overall 46.6 (14.7-66.3)

n: number of patients fitting each specific entry of the table; N: total number of evaluable patients for each specific entry of the table

* According to IWG Classification (Rodeghiero et al, Blood 2013)

£ 3 patients had both venous and arterial thromboembolic events

$ Functional Assessment of Chronic Illness Therapy - Fatigue Scale (FACIT-Fatigue); General population norms for the FACIT-Fatigue subscale mean score: 43.5 ± 8.3 (Montan et al, Value Health 2018)

% 12-Item Short Form Heath Survey (SF-12); Physical Component Summary (PCS-12); Mental Component Summary (MCS-12); PCS-12 standardized mean: 51.2; MCS-12 standardized mean: 49 (data from the Italian survey conducted by the Italian National Institute of Statistics [ISTAT] on equitable and sustainable well-being (Benessere Equo e Sostenibile, BES: https://www.istat.it/it/files//2014/06/01_Salute-Bes2014.pdf)

OC072

ANTI-ADAMTS13 ANTIBODIES IN CONGENITAL TTP PATIENTS

I. Mancini[1], M. Biganzoli[2], A. Cairo[2], M. Capecchi[2,3], A. Artoni[2], B. Ferrari[2], S.M. Trisolini[4], L. Facchini[5], G. Giuffrida[6], A. De Fanti[7], P. Agosti[1], F. Peyvandi[1,2]

[1]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, and Fondazione Luigi Villa, Milan, Italy; [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; [3]Università degli Studi di Milano, Department of Biomedical Sciences for Health, Milan, Italy; [4]Hematology, Department of Translational and Precision Medicine, "Sapienza" University of Rome, Rome, Italy; [5]Hematology Unit, Azienda Unità Sanitaria Locale, IRCCS di Reggio Emilia, Reggio Emilia, Italy; [6]Hematology Division, Department of Clinical and Molecular Biomedicine, University of Catania, Catania, Italy; [7]Departmental Simple Unit of Pediatric Rheumatology, AUSL-IRCSS Reggio Emilia, Reggio Emilia, Italy

Background: Congenital thrombotic thrombocytopenic purpura (cTTP) is an autosomal recessive disease due to mutations in the ADAMTS13 gene. cTTP manifests as acute episodes of thrombotic microangiopathy characterized by ADAMTS13 activity <10 IU/dl and absence of anti-ADAMTS13 antibodies, which are treated by plasma infusion to replenish the deficient protease. cTTP patients with anti-ADAMTS13 antibodies were rarely described. **Aims**: To investigate anti-ADAMTS13 IgG in our cTTP cohort. **Methods**: We performed a cross-sectional study of cTTP patients with homozygous or compound-heterozygous ADAMTS13 mutations, enrolled in the Milan TTP registry from 2002 to 2018. Plasma samples collected during the acute or remission phase were tested for anti-ADAMTS13 IgG using an in-house western blot or ELISA based assay or the Technozym ADAMTS13 Inhibitor ELISA kit (Technoclone). Neutralizing activity of anti-ADAMTS13 antibodies was measured using a FRET-based Bethesda-like assay. **Results**: Ninety-one samples from 27 cTTP patients (52% females, median age at onset 6.5 years [interquartile range 1.2–20.1]) were tested for anti-ADAMTS13 IgG (samples per patient, median [range]: 2 [1-13]). Four acute phase samples from four cTTP patients were positive by at least two anti ADAMTS13 IgG assays. In one case, non-neutralizing anti-ADAMTS13 IgG (83 U/ml) developed during ticlopidine treatment, considered the trigger of the acute event. Two patients presented non-neutralizing anti-ADAMTS13 IgG (33 and 19 U/ml) after infusion of four units of fresh frozen plasma over 33 and 59 days, respectively. The last case presented extremely high titers of anti-ADAMTS13 IgG (8800 U/ml) with neutralizing activity (295 BU/ml) after being misdiagnosed with acquired TTP due to baseline low titer anti-ADAMTS13 IgGs (17 U/ml) and treated with plasma-exchange every other day for nine months, rituximab, vincristine, cyclophosphamide and splenectomy. **Conclusions**: Neutralizing anti-ADAMTS13 IgG could develop very rarely in cTTP patients after prolonged exposure to plasma, exogenous source of ADAMTS13. Monitoring of anti-ADAMTS13 antibodies in cTTP patients with a reduced therapeutic response is recommended.

OC073

RISK FACTORS FOR SARS-COV-2 INFECTION AND IMPACT OF DISEASE IN A MONOCENTRIC COHORT OF PATIENTS WITH ACTIVE IMMUNE THROMBOCYTOPENIA (ITP)

G. Carli, G. Marchesini, I. Nichele, A. Tosetto

Hemophilia and Thrombosis Center, Hematology Department, SBortolo Hospital, Vicenza, Italy

Background: After the outbreak of COVID-19, several cases of *de novo* ITP secondary to SARS-CoV2 infection or COVID-19 vaccination have been reported. Little is known about the impact of SARS-CoV-2 infection in patients with active ITP. **Aim**: We aimed to assess risk factors for SARS-CoV-2 infection and the effect of COVID-19 infection on ITP clinical course treatment in a monocentric cohort of ITP patients with active disease. **Materials and Methods**: We included all patients with active ITP (either newly diagnosed, persistent, or chronic ITP, with ongoing therapy and platelet counts <100 x109/L) on regular follow-up from February 2020 to May 2022. Either antigenic or molecular nasal swab confirmed SARS-CoV-2 infection. We retrieved data on a previous splenectomy, exposure to rituximab up to one year before the pandemic, or ongoing steroid or immunosuppressant therapy. In pts with SARS-CoV-2 infection, we recorded the severity of infection (WHO classification), need for hospitalization, specific treatment for COVID-19, need to modify ITP treatment, bleeding, and thrombotic events. **Results**: Between Feb 2020 and May 2022, 88 pts were regularly followed up in our center for active ITP. 29 pts (32.9%) developed SARS-CoV-2 infection (Table 1). Patients with a history of exposure to rituximab were more frequently infected in our cohort (p=0.002). Patients with SARS-CoV-2 infection showed a mild disease in 90% of cases (26 pts), while three patients (10%) needed to be hospitalized for severe pneumonia (one with ARDS and need for intubation). Two hospitalized patients were treated with hyperimmune plasma because of recent exposure to rituximab. One of them experienced after one year a SARS-CoV-2 reinfection requiring treatment with monoclonal antibodies as an outpatient. No patient died from COVID-19. Three patients at home have been treated with antiretroviral therapy (nirmatrelvir/ritonavir) and two patients with monoclonal antibodies (bamlavinimab/etesevimab)

with no side effects and rapid resolution of symptoms. No patients experienced significant bleeding events, and the great majority (27 patients, 93%) maintained the same therapy for ITP (TPOra in 40% of cases). One hospitalized patient needed to acutely start treatment (steroid plus immunoglobulins) for a significant drop in platelet count with subsequent partial response. Another outpatient has stopped therapy with rituximab (after three doses) and has been treated with antiretroviral therapy, maintaining a complete response on the platelet count. No thrombotic adverse events have been recorded. **Conclusions**: Previous exposure to rituximab appears to be an important risk factor for SARS-CoV-2 infection in patients with active ITP. In most cases, COVID-19 had a benign course without consequences on the ITP course, but patients with an immunodeficient status (*e.g.*, after Rituximab exposure) can be prone to more severe disease.

Table 1.

	PATIENTS COVID+ N (%)	PATIENTS COVID- N (%)
Number	29 (32.9)	59 (67.1)
Median Age	50	64
Female	18 (62)	40 (67)
Previous splenectomy	5 (17.2)	4 (6.7)
Rituximab exposure	6 (20.6)	0
Steroid exposure	16 (55)	26 (44)
Other immunosuppressive therapy exposure	0	2 (3.3)

OC074

RISK OF ITP EXACERBATION IN CHRONIC ITP PATIENTS AFTER COVID19 INFECTION AND VACCINATION: A SINGLE-CENTER OBSERVATIONAL STUDY

C. Dainese[1], F. Valeri[1], M. Bardetta[1], C. Sella[1], B. Bruno[2], A. Borchiellini[1]

[1]Centro Riferimento Regionale per Malattie trombotiche ed Emorragiche dell'adulto, Ematologia Universitaria, AOU Città della Salute e della Scienza di Torino; [2]Ematologia Universitaria, Dipartimento di Oncologia, AOU Città della Salute e della Scienza di Torino, Italy

Background and Aims: Current Sars-Cov 2 disease (COVID 19) pandemic is characterized by severe pneumonia, multi-organ disease and dysregulation of immunity. Vaccination is one of the most effective weapons to control the disease. Both SARS-CoV-2 infection and vaccination have raised concern in immune mediated diseases, including immune thrombocytopenic purpura (ITP) considering the risk of *de novo* ITP development and ITP recurrence. Here, we report data from a single Center retrospective-prospective collection aiming to evaluate platelet (plt) dynamics in patients (pts) with chronic ITP after COVID-19 infection and vaccination. **Methods**: All pts currently followed for chronic ITP who experienced COVD19 infection and/or who completed first and second dose vaccination with an available plt count before and after such events were included in the analysis. Demographic features, ITP duration, number and type of previous and current treatment; reduction in plt count of any grade, described as absolute and percentage reduction were recorded. Finally, ITP exacerbations, defined >50% decline in plt count compared with baseline, >20% decline in plt count compared with baseline and a plt nadir <30.000/ul or need for rescue medication) were also included. Proportion comparisons were carried out with Chi square test. **Results**: 64 chronic ITP cases were included in the analysis. Study population characteristics are resumed in Table 1.

Table 1.

Study Population features		Range
Age (years), mean	62,4	1,41 – 36,78
Number of previous ITP treatments, median	2	0 – 5
Previous splenectomy (%)	15 (23,4)	
On therapy at infection/vaccination time (%)	44 (68,75)	
• Steroids	1 (1,56)	
• Steroids and TPO-RA	3 (4,68)	
• TPO-RA	37 (57,81)	
• Azathioprine	2 (3,12)	
• Azathioprine and TPO-RA	1 (1,56)	
Off Therapy at infection/vaccination time (%)	20 (31,25)	
First Dose AntiSarsCov2 Vaccination	61	
• Comirnaty , Pfizer Biontech (%)	52(85,24)	
• Spikevax, Moderna (%)	8 (13,10)	
• Vaxzevria, Astrazeneca (%)	1 (1,64)	
Second Dose AntiSarsCOV2 Vaccination	58	
• Comirnaty , Pfizer Biontech (%)	50 (86,20)	
• Spikevax, Moderna (%)	8 (13,79)	

Table1 Study Population Features. ITP Immune Thrombocytopenic Purpura. TPO-RA TPO Receptor Agonist.

Before any vaccination, 11 pts got COVID19 infection. A reduction in plt count was observed in 6 patients (54,5%) with median plt count after infection of 4.000/ul (IQR1 2000- IQR3 46500/ul), mean absolute plt reduction of 122.166/ul (IQR1 58.000 – IQR3 157.750/ul) and mean percentage reduction of 92% (IQR1 67.37 - 97.50%). ITP exacerbation was observed in 5 pts (45,5%), all cases requiring rescue therapy. First vaccine dose was received by 61 pts; an available plt count after first dose was available for 41 pts after a mean time of 11,4 days (2 – 15 days). A reduction in plt count was observed in 17 pts (41.4%) with a mean plt count after first dose of 147.736/ul (IQR1 72250 - IQR3 220750/ul), a mean absolute reduction of 53058/ul (IQR1 9000 - IQR3 70500/ul) and a mean percentage reduction of 30% (IQR1 5.2 - IQR3 49.75%). An ITP exacerbation after first vaccine dose was observed in 6 pts (14.63%) with 3 pts (7.31%) requiring rescue treatment. 58 pts received second vaccine dose; an available plt count after second dose was available for 36 pts after a mean time of 16.8 days (8 –21 days). A reduction in plt count was observed in 16 pts (44.4%) with a mean plts count after first dose of 147.615,38/ul (IQR1 77.000/ul – IQR3 201.750/ul), a mean absolute reduction of 77.437/ul (IQR1 14.500 - IQR3 66.750/ul) and a mean percentage reduction of 28% (IQR1 6.75 - 40.25%). An ITP exacerbation after second vaccine dose was observed in 5 pts (13.88%), with 2 pts (5,5%) requiring rescue treatment. **Conclusions**: Beyond the

indubitable protective effect against severe infection, our data support anti SarsCOV2 vaccination in chronic ITP patients considering the higher rate of ITP exacerbation observed after COVID19 infection (45,5%) than after first (14,63%, p=0,07) and second (13.88%, p=0,06) vaccine dose together with a higher rate of rescue therapy necessity in case of COVID 19 infection (45,5% *vs* 7,3% and 5,5%, p=0,008 and p=0,005).

OC075

IMMUNE THROMBOCYTOPENIA *DE NOVO* OR EXACERBATED AFTER SARS-COV-2 VACCINATION: A MONOCENTER EXPERIENCE

S. Ceglie, F. Ramundo, P. Arcuri, A. Ciminello, T. Za, S. Betti, F. Bartolomei, F. Di Landro, E. Rossi, V. De Stefano

Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica, Fondazione Policlinico AGemelli IRCCS, Roma, Italy

Background and Aims: ITP onset without accompanying thrombosis was reported after SARS-CoV-2 vaccination, with an incidence of 0.8-2 and 8-11.3 per million doses of mRNA and ChAdOx1 vaccines. Moreover, pre-existing ITP can flare up after SARS-CoV-2 vaccination in about 20% of cases. **Methods**: From March 2021 to March 2022, 48 ITP pts newly diagnosed were referred to our Center; 15 of them had *de novo* ITP after SARS-Cov2 vaccination; moreover, 12 vaccine-related ITP exacerbations were recorded among 200 pts on active follow up. **Results**: Fifteen pts (M/F=9/6, median age 55 years, range 30-84) developed *de novo* ITP following SARS-CoV-2 vaccination. Eight pts (53.3%) received an adenoviral (Ad) vector-based vaccine and seven (46.7%) an mRNA-based vaccine (Table 1). The platelet count (PC) decreased after a median of 15 days (range 4-32), without a difference between vaccines. Eleven pts (73.3%) had at diagnosis a PC <30x10^9/L; the median PC was 6x10^9/L (range 1-38) and 17.5x10^9/L (range 1-32) in pts having received Ad vector-based vaccines and mRNA-vaccines, respectively (p=0.66). Thirteen pts (86.7%) had bleeding manifestations, mainly mucocutaneous (N=12, grade ≤2) and genitourinary (N=2, grade 2 and 3). Fourteen pts required a treatment: high-dose dexamethasone (HD-DEX) (N=12) (median 2.9 cycles) or prednisone (N=2). Four cases (26.7%) received rescue therapy with high-dose intravenous immunoglobulin (HD-IVIG). The median time to reach a PC >30x10^9/L was 4 days (range 2-11), and all pts achieved a complete response >100x10^9/L. The median follow-up was 35 weeks (range 3-59). Five pts (36%) relapsed after a median of 42 days (range 36-55), requiring a 2nd-line treatment; afterward, 3 of them received a 3rd-line treatment. Twelve ITP pts (M/F=3/9), median age 58.5, range 21-79) were referred for exacerbation after Ad vector-based vaccines (N=1) or mRNA vaccines (N=11). Nine pts were off-treatment, in 2 cases after splenectomy (5 after one line, 4 after 2 or more lines), 2 were on eltrombopag (EPG) (after more than 2nd line), and 1 was receiving the 2nd HD-DEX cycle as 1st-line treatment. The PC decreased after a median of 15 days (range 1-29). Seven pts (58.3%) had a relapse a PC <30x10^9/L; the median PC was 20x10^9/L (range 2-93). Two pts (24%) had mucocutaneous bleeding (grade ≤2), associated with rectal bleeding and metrorrhagia in 1 of them (grade 3). Among the pts off-treatment, six started HD-DEX, in 1 case with HD-IVIG; among the pts on-treatment, 2 continued EPG (N=1) or the HD-DEX cycles (N=1), and 1 added HD-DEX to EPG. Among the 9 pts requiring treatment, the median time to reach a PC >30x10^9/L was 4 days (range 2-6), and all pts achieved a complete response >100x10^9/L. The median follow-up was 24 weeks (range 6-52). One patient (11.1%) relapsed after 2 months and started EPG. **Conclusions**: In the last year, one-third of ITP pts newly diagnosed at our Center was SARS-CoV-2 vaccination-related (15/48, 31.2%), and 6% of ITP pts in active follow-up experienced exacerbation after SARS-CoV-2 vaccination. However, both clinical entities had a benign course; 14/27 (51.8%) had bleeding manifestations <grade 2, and only 2 pts experienced a bleeding grade 3. Twenty-three pts required treatment, and all of them reached a safe PC >30x^{10}L within 11 days. The response was maintained in 16 of them (69.6%). The risk of ITP onset after vaccination is not predictable, but its management seems not to differ from primary ITP.

Table 1.

Types of vaccines and doses associated with de novo or exacerbated ITP

	De novo ITP (n=15)	Exacerbation of pre-existing ITP (n=12)
Type of vaccine		
Astrazeneca	7	1
Johnson&Johnson	1	0
Pfizer/BionTech	6	9
Moderna	1	2
Number of doses		
First dose	7 (6 Ad-vax)	3 (2 mRNA-vax)
Second dose	8 (6 mRNA-vax)	6 (6 mRNA-vax)
Third dose	0	3 (3 mRNA-vax)

OC076

IMMUNE THROMBOCYTOPENIA (ITP) AFTER SARS-COV-2 VACCINATION: EXPERIENCE OF A SINGLE CENTER

F. Viola, G. Maestrini, E. Baldacci, A. Ferretti, R. Mormile, A. Chistolini, C. Santoro

Università La Sapienza, Roma, Italy

Background: Since the start of COVID-19 pandemic there has been the urgency to develop specific vaccines. ITP cases have been described after SARS-CoV-2 vaccination. Aim of this study is to describe a monocentric experience regarding the occurrence of either *de novo*

or relapsing ITP after SARS-CoV-2 vaccination. **Methods**: Retrospective, monocentric study. Relapse definition: reduction of ≥30% in platelet (plt) count. **Results**: A total of 17 patients (pts) experienced either *de novo* (8) or relapsing (9) ITP, following SARS-CoV-2 vaccination. *De novo* ITP. 8 cases, 4 females, 4 males, median age 66.8 years (26.2-84.1). 2 pts received AstraZeneca, 1 Moderna, 5 Pfizer-BioNTech vaccine. 4 subjects were diagnosed after the 1st and 4 after the 2nd dose [median time 21.5 days (7-45)]. Median plt count at diagnosis: $9x10^9$/L (1-60); median follow up: 7.1 months (1.5-10.9). 6 pts presented muco-cutaneous bleeding symptoms. 6/8 subjects received 1st line therapy: 5 IvIg+corticosteroids, 1 corticosteroids. All 6 pts obtained a response [median plt count $120x10^9$/L (105-140); median time 7 days (5-39)]. Only 3/8 pts received vaccine booster after ITP development: 1 (1st dose AstraZeneca) received 2 doses of Moderna without performing a blood count; 2 (ITP developed after Pfizer 2nd dose) received the 3rd Pfizer dose without plt count reduction. 5/8 pts did not receive booster for their decision. Relapsed ITP. 9 pts experienced an exacerbation of ITP after either 1 or 2 vaccine doses: 5 females, 4 males, median age 48.9 years (37.5-63.6). Median number of therapies before vaccination, 1 (0-3): corticosteroids, IvIg, TPO-RA, splenectomy. Median follow up 6.96 years (1.13-30.75). All pts received Pfizer-BioNTech vaccine. Median plt count before any of the doses: $86x10^9$/L (30-197); median plt count at relapse after any of the doses: $26x10^9$/L (4-48); median time at relapse 7 days (1-35). At the time of 1st dose, 6 pts were off therapy, 2 on treatment with TPO-RA, 1 with corticosteroids: 2/9 relapsed (plt count 4 and $5x10^9$/L). 1 patient presented cutaneous petechiae and was treated with IvIg+corticosteroids; the other one was asymptomatic: corticosteroid was added to TPO-RA therapy. These pts obtained a response: plt count 56 and $120x10^9$/L, after 5 and 33 days, respectively. All 9 pts received a 2nd vaccine dose: 5 were off therapy, 3 on TPO-RA, 1 on corticosteroids: 6/9 relapsed (median plt count $31x10^9$/L; 14-$48x10^9$/L). 3 pts showed muco-cutaneous bleeding symptoms and needed therapy (median plt count $21x10^9$/L;14-35): 1 received corticosteroids, 1 corticosteroids+TPO-RA, 1 increased corticosteroids dose. They all obtained a response: median plt count $160x10^9$/L (42-284), median time at response 13 days (7-34). 5/9 patients received a 3rd vaccine dose: 3/5 were on therapy, 2 with TPO-RA, and 1 with corticosteroids. 3/5 cases relapsed (median plt count $35x10^9$/L; 21-45), without bleeding symptoms. Just in 1 case therapy (corticosteroids) was necessary because of low plt count ($21x10^9$/L). The patient obtained a response (plt count $180x10^9$/L), after 4 days. 4 pts did not receive a third dose: 2 for patient choice, 1 for medical decision, 1 for previous COVID-19 infection. **Conclusions**: In our experience, few *de novo* or relapsed ITP occurred after SARS-COV-2 vaccination, with absent or mild/moderate symptoms and resolved with conventional therapies or, in few cases, without any treatment. These observations confirm the favorable balance towards vaccination either in pts with previous ITP.

OC077

COMBINATION OF CLIA AND ELISA ASSAYS FOR DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

B. Montaruli, P. Valesella, M. Papandrea, A. Insana, D. Cosseddu

SC Laboratory Analysis AO Ordine Mauriziano Torino, Italy

Background and Aims: Heparin induced thrombocytopenia (HIT) is a serious adverse effect of heparin treatment. Prompt evaluation of suspected cases is essential for clinical decisions. Aim of our study was to evaluate the HemosIL Acustar HIT IgG CliA cut-off for anti-platelet factor 4 (PF4)/heparin antibodies of <0.13 U/ml suggested by Marchetti *et al.* in their bayesian diagnosis of HIT. **Methods**: We determined in a cohort of 52 consecutive patients investigated for suspected HIT with a 4T pre test probability score greater than or equal to 2 anti PF4-heparin IgG antibodies with CliA HemosIL acustar HIT IgG (Werfen, USA) assay and Asserachrom anti PF4 IgG (Stago, France) ELISA assay. Patients with CliA assay <0.13 U/ml and ELISA assay OD <0.194 antiPF4 heparin antibodies were considered as negative. Confirmation Platelet Aggregation Test (PAT) and HITALERT flow cytometer assays were performed in order to confirm suspected HIT in all CliA and/or ELISA positive patients. **Results**: 14/52 patients were found to be positive with both CliA and ELISA assays. 9/14 of these patients were confirmed HIT for both PAT and HITALERT functional assays. 1/52 patient was found to be positive only to CliA and 3/52 only to ELISA anti-PF4-heparin IgG immunoassay but negative with both HIT functional assays. 34/52 (63%) of the patients investigated for suspected HIT with a 4T score=o >2 were found to be negative for antiPF4-heparin antibodies by CliA assays by using literature suggested cut-off of <0.13 U/ml. 3 of these patients showed anti-PF4 heparin antibodies ELISA positivity but were not confirmed as HIT by functional assays. **Conclusions**: We confirmed that the CLIA cut-off <0.13U/ml for anti-PF4.-heparin IgG antibodies is a reasonable limit to exclude HIT in patients with a 4t score ≥2 or a strong HIT clinical suspicious in emergency settings. The combination of rapid CliA assay and ELISA assay allowed us not to miss any functional confirmed suspected HIT (see Figure 1).

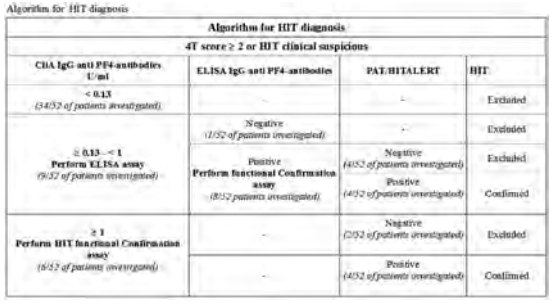


Figure 1.

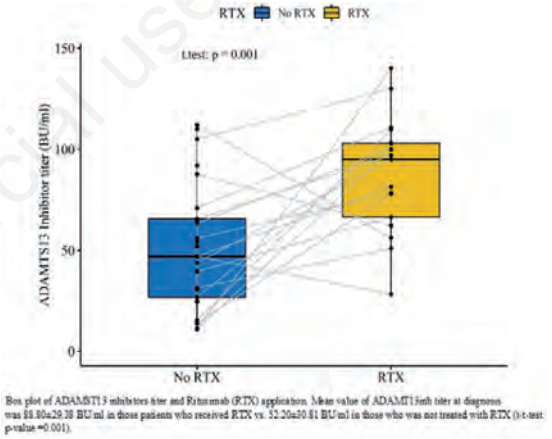
OC078

ADAMTS13 AUTOANTIBODIES AND BURDEN OF CARE IN IMMUNE THROMBOTIC THROMBOCYTOPENIC PURPURA: EVIDENCE AND FUTURE IMPLICATIONS

C. Dainese[1], F. Valeri[1], A. Valpreda[2], P. Sivera[3], B. Montaruli[3], M. Massaia[4], B. Bruno[5], A. Borchiellini[1]

[1]Centro Riferimento Regionale per Malattie trombotiche ed Emorragiche dell'adulto, Ematologia Universitaria, AOU Città della Salute e della Scienza di Torino, Italy; [2]Centro Riferimento Regionale per Malattie trombotiche ed Emorragiche dell'adulto, Laboratorio Centrale, AOU Città della Salute e della Scienza di Torino, Italy; [3]Ematologia, Ospedale Ordine Mauriziano di Torino, Italy; [4]Divisione di Ematologia, Ospedale Santa Croce e Carle di Cuneo, Italy; [5]Ematologia Universitaria, Dipartimento di Oncologia, AOU Città della Salute e della Scienza di Torino, Italy

Background: Immune thrombotic thrombocytopenic purpura (iTTP) is a rare, life-threatening thrombotic microangiopathy. The introduction of a novel treatment option in the acute management of iTTP, Caplacizumab, has raised different questions, considering its cost-efficacy and the optimal immunosuppressive treatment (IST) to associate. Considering Caplacizumab phase II and III trials results, in this study we looked for variables associated with a higher burden of care and necessity of an implemented IST (with early Rituximab (RTX) rescue) in order to optimize resource utilization. **Methods**: A retrospective multicenter collection of all first iTTP treated in three different hematology units from 2007 to 2020 was conducted. Data collected included demographic data, signs and symptoms at hospital admission, laboratory data, ADAMTS13 activity and inhibitors titer, number of total PEX sessions (PEXtot) and needed to achieve clinical response (PEXtoCR), days of hospitalization (DoH), IST and RTX use, refractory and relapse. The Mann U-Whitney test was used to assess the median differences for independent samples. Pearson correlation coefficient (r) was applied to evaluate the correlation among continuous variables and number of total PEX, PEXtoCR and DoH. **Results**: A total of 42 first iTTP episodes were identified. Clinical variable resulting significantly correlated with both PEXtot and PEXtoCR were age (r=- 0.31, p=0.040 and r=-0.35, p=0.030), platelet count (r=-0.30, p=0.050 and r=-0.30, p=0.014), LDH (r=0.44, p=0.006 and r=0.41, p=0.012), total bilirubin (r=0.54, p=0.001 and r=0.35, p=0.04) and ADAMTS13 Inhibitors (r=0.46, p=0.002 and r=0.48, p=0.025). ADAMTS13 inhibitors resulted the only variable associated with DoH (r=0.44, p=0.005). Considering dichotomic variables, no difference in terms of median number of PEXtot and PEXtoCR and DoH was observed considering gender, Charlson comorbididty index score or types of symptoms at hospital admission. The Mann Whitney rank sum test was also performed to evaluate a difference in RTX rescue use: no significant differences were detected considering age, median platelet count or LDH level at hospital admission, while a statistically significant difference was observed in terms of median ADAMTS13 inhibitor titer at diagnosis (96.00 *vs* 47.00 BU/ml, p=0.015) activity. ADAMTS13 activity did not correlated with PEXtot, PEXtoCR nor DoH. **Conclusions**: From our analysis, ADAMTS13 inhibitor titer at diagnosis emerges as marker of iTTP burden of care, associated with higher total number of PEX sessions, PEX needed to achieve clinical response, days of hospitalization, and a higher probability of requiring RTX rescue to achieve clinical response. Younger age, lower platelet count, higher LDH and bilirubin level at hospital admission also show a significant correlation with total number of PEX sessions and PEX to achieve clinical response. Despite the need for further validation, ADAMTS13 inhibitors titer could be a useful tool for guiding clinician defining first line treatment of new iTTP cases. Finally, such variable could be included in future Caplacizumab cost-efficacy analysis to better optimize iTTP cases stratification.



Box plot of ADAMTS13 inhibitors titer and Rituximab (RTX) application. Mean value of ADAMTS13nh titer at diagnosis was 88.80±29.38 BU/ml in those patients who received RTX vs. 52.20±30.81 BU/ml in those who was not treated with RTX (t-test p-value =0.001).

Figure 1.

OC079

THROMBOTIC EVENTS WITH OR WITHOUT THROMBOCYTOPENIA AFTER COVID-19 VACCINATION IN ITALY: RESULTS FROM THE SISET VAX COVID-19 DATABASE

M.P. Donadini[1], E. Tarasconi[1], L. Bertù[1], E. Antonucci[2], L. Contino[3], V. De Stefano[4], L. Russo[5], G. Tiscia[6], A. Tripodi[7], P. Gresele[8]

[1]Centro di ricerche per le malattie tromboemboliche e le terapie antitrombotiche, Dipartimento di Medicina e Chirurgia, Università dell'Insubria Varese; [2]Fondazione Arianna Anticoagulazione, Bologna; [3]SSD Haemostasis and Thrombosis Center, Internal Medicine Department, "SS Antonio e Biagio" Hospital, Alessandria; [4]Sezione di Ematologia, Università Cattolica, Fondazione

Policlinico AGemelli IRCCS, Roma; [5]Division of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo; [6]U.O.SDipartimentale e di Ricerca Emostasi e Trombosi; Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo; [7]IRCCS Ca' Granda Maggiore Hospital, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano, Italy; [8]University of Perugia, Department of Medicine and Surgery – Section of Internal and Cardiovascular Medicine, Italy

Background and Aims: Since vaccine induced immune thrombotic thrombocytopenia (VITT) became evident after administration of adenoviral vector-based COVID-19 vaccines in early 2021, research has focused on characterizing this new pathological entity. Moreover, even in absence of previously described link between vaccines and thrombosis in the pre-COVID era, data were urgently needed on the association, if any, between all COVID-19 vaccines and thrombosis, that epidemiological data have only recently excluded. Aim of the study was to collect relevant information on all cases of thrombosis occurring after COVID-19 vaccination. **Methods**: SISET VAX COVID-19 is an ongoing national, multicenter, prospective registry that was set up by the Italian Society on Thrombosis and Haemostasis, enrolling consecutive adult patients objectively diagnosed with any thrombotic event (TE) occurring within 30 days after receiving any COVID-19 vaccine dose, with or without thrombocytopenia. Primary objective was the characterization of TEs, in terms of type and extension (venous, arterial, microvascular), diagnosis, treatment and adverse events (thrombosis progression/early recurrence, major bleeding and death) during 30-day follow-up. The study was approved by the national ethics committee for studies on COVID-19 and by the participating centers. Fondazione Arianna Anticoagulazione provided support for web-based dataset implementation, data management and approval procedures. **Results**: From June 3rd, 2021, to March 20th, 2022, 237 patients from 41 centers were included in the registry. The mean age was 57 years (standard deviation (SD) 16.8) and female/male proportion was 45%/55%. TEs occurred after a mean of 14.5 days (SD 9.2) after the latest vaccine dose (72.2% after 1st or unique dose, 33.8% after 2nd dose, 1.7% after 3rd dose), that was represented by ChAdOx1 nCOV-19 (Astrazeneca) in 28.7% of patients, Ad26.COV2.S (Janssen) in 1.3%, BNT162b2 (Pfizer/BioNTech) in 54.4% and mRNA-1273 (Moderna) in 14.2%. 152 patients (64.1%) had at least one comorbidity, 47 patients (19.8%) reported history of thrombosis and 37 (17.3%) had known thrombophilia; 27 patients (11.4%) reported previous SARS-CoV-2 infection. TEs involved one or more veins in 214 patients (90.3%) and arteries in 37 (15.6%). Among venous TE events, 170/214 (79.4%) were represented by deep vein thrombosis and/or pulmonary embolism, whereas 42 (19.6%) by unusual site thrombosis. In 199 patients (84%) the TE was not associated with thrombocytopenia. Among 38 patients (16.9%) with thrombocytopenia, 22 were diagnosed with VITT, all after receiving Astrazeneca or Janssen vaccines. After excluding them (described in another report), no differences were found in terms of thrombosis location between patients receiving mRNA and adenoviral vector-based vaccines (Table 1). Overall, during 30-day follow-up, 13 patients (5.5%) had thrombosis progression, 10 patients (4.2%) major bleeding and 10 patients (4.2%) died. **Conclusions**: Except for the very rare and peculiar cases of VITT, reported only with adenoviral vector-based vaccines, the clinical characteristics and outcomes of the remaining TEs occurring after any type of COVID-19 reflect known literature data on thrombosis and do not differ between adenoviral vector-based and mRNA vaccines. These findings seem to support recent epidemiological evidence of a temporal only association, instead of a causal relationship, between vaccines and thrombotic events.

Table 1.

	Astrazeneca or Janssen latest vaccine dose	Pfizer or Moderna latest vaccine dose	P value
Patients, n	72	164	
Age, years, mean (SD)	58.0 (14.7)	56.6 (17.8)	0.39
Males/Females, n (%)	35(48.6)/37(51.4)	95(57.9)/69(42.1)	0.18
Time interval between vaccine and thrombosis, days, mean (SD)	14.3 (8.1)	14.5 (9.7)	0.89
1 st dose / subsequent dose, n (%)	65(90.3)/7 (9.7)	102 (62.2)/62 (37.8)	<0.0001
Patients with VITT, n (%)	22 (30.5)	0 (0.0)	<0.0001
- Unusual site venous thrombosis	16 (72.7)	0 (0.0)	<0.0001
Thrombosis site in patients without VITT, n (%)*			
- lower or upper limb DVT and/or PE	41 (82)	130 (79.2)	0.84
- unusual site venous thrombosis	5 (10)	20 (12.2)	0.80
- arterial thrombosis	6 (12)	18 (10.9)	0.80
Thrombosis progression, n (%)	7 (9.6)	6 (3.7)	0.12
Death, n (%)	5 (7.4)	5 (3.5)	0.29

*Thrombosis sites can be multiple
DVT deep vein thrombosis; PE pulmonary embolism

OC080

VACCINE-INDUCED THROMBOTIC THROMBOCYTOPENIA (VITT): EVALUATION OF IMMUNOLOGIC AND FUNCTIONAL TESTS ON AN ITALIAN CASE SERIES

A. Rogolino, F. Cesari, A.M. Gori, B. Giusti, B. Bandinelli, E. Sticchi, A. Bertelli, M. Giannini, D. Poli, E. Lotti, L. Mannini, R. Marcucci

Department of Experimental and Clinical Medicine, University of Florence, Atherothrombotic Disease Unit, Azienda Ospedaliera Universitaria Careggi, Italy

Background and Aims: Several cases of unusual thrombotic events and thrombocytopenia, called VITT (vaccine-induced thrombotic thrombocytopenia), have been developed after vaccination with adenoviral vectors encoding the spike protein antigen of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Vaxzevrya, AstraZeneca; Johnson&Johnson). **Methods**: We assessed the clinical and laboratory features of 8 patients in Italy in whom thrombosis and thrombocytopenia developed after vaccination with adenoviral vectors-vaccines. We used three different immunologic tests to detect platelet factor 4 (PF4)–heparin antibodies: two standard enzyme-linked immunoadsorbent assays

(ELISA) (Lifecode PF4-IgG, Immucor; Zymutest HIA IgG, Hyphen), latex immunoturbidimetric assay (LIA) (Hemosil HIT-Ab PF4, Werfen, Italy) and chemiluminescence immunoassay (CLIA) (Acustar IgG, Werfen, Italy). In order to detect the functional ability of the antibodies in inducing platelet activation, we also performed two different functional tests: Heparin-induced platelet Activation-HIPA test modified by the addition of PF4 on washed platelets and flow cytometry (FC) on platelet rich plasma. **Results**: All patients [2M/6F; median age: 66 (41-78)] had a confirmed diagnosis of VITT based on clinical and laboratory features. Blood samples were obtained, except cases #1,#2,#6,#8 before i.v. Immunoglobulin (IG) or anticoagulant administration. As shown in the Table 1, there was a significant correlation between the two different ELISA tests (rho=0.683, p=0.042). According to the ELISA cut-off values (>0.4 for Immucor and >0.3 for Hyphen) all patients were positive for the presence of anti-PF4 antibodies. None of the patients had LIA and CLIA values above the cut-off (>1 U/mL). By performing functional tests, all VITT patients resulted positive to the functional modified HIPA test, except for VITT #6 patient, who resulted negative and VITT #2 patients who resulted weakly positive, probably due to the concomitant IG therapy. By performing FC functional test, VITT #1, #2, #6, #8, with the concomitant IG therapy showed a significant lower percentage of activated platelets (mean fluorescence intensity (MFI) of CD62P platelets) with respect to the VITT patients without IG therapy [MFI: 4458 (4320-4629) *vs* 11170 (6716-17492), p=0.03]. Patients #4F, #5F, showed a great platelet activation with VITT serum and buffer, and a decreased CD62P expression with low dose of unfractionated heparin(UFH) (0.3 IU/mL), by showing a typical positive VITT pattern. In patients #7, we found an enhanced CD62P expression in the tube with unfractioned heparin 0.3 IU/mL, with a typical HIT pattern. **Conclusions**: Vaccination with adenoviral vectors-vaccines encoding the spike protein antigen of SARS-CoV-2 can result in the rare development of VITT. Our data indicate that the ELISA test in association with functional test assay are able to detect the presence of platelet activating antibodies against PF4 in VITT. HIPA test remains the gold standard to detect functional ability of antibodies anti PF4 to activate platelets. In addiction the FC seems a sensitive and reliable method with respect to HIPA, however the evaluation in sera of patients treated with IG might be associated with false negative results and in some cases it results less sensitive with respect to HIPA test in detecting platelet activating antibodies.

Table 1.

VITT case	Lifecode PF4-IgG (OD)	Zymutest HIA IgG (OD)	Hemosil Acustar IgG (U/mL)	Hemosil HIT-Ab PF4 (U/mL)	HIPA	Flow Cytometry
VITT #1	2.47	1.94	0.04	0.4	POSITIVE	NEGATIVE
VITT #2	3.13	2.26	0.06	0.1	WEAKLY POS	NEGATIVE
VITT #3	2.89	3.32	0.03	0	POSITIVE	WEAKLY POS
VITT #4	3.34	3.74	0.01	0.2	POSITIVE	POSITIVE
VITT #5	1.47	3.63	0.15	0	POSITIVE	POSITIVE
VITT #6	1.91	0.24	0.15	0.3	NEGATIVE	NEGATIVE
VITT #7	3.26	3.69	0.51	0	POSITIVE	HIT PATTERN
VITT #8	3.02	2.54	0.19	0	POSITIVE	WEAKLY POS

OC081

ANTI-SARS-COV-2 ADENOVIRAL VECTOR VACCINES TRIGGER SUBCLINICAL ANTIPLATELET AUTOIMMUNITY AND *IN VIVO* PLATELET ACTIVATION

E. Petito[1], E. Colonna[1], E. Falcinelli[1], A.M. Mezzasoma[1], E. Cesari[1], E. Giglio[1], T. Fiordi[2], F. Almerigogna[3], A. Villa[4], P. Gresele[1]

[1]Section of Internal and Cardiovascular Medicine, and [2]Section of Occupational Medicine and Toxicology, University of Perugia, Perugia, Italy; [3]Unit of Allergology and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy; [4]Central Clinical Chemistry Laboratory, S.Mdella Misericordia Hospital, Perugia, Italy

Background and Aims: To slow down the COVID-19 pandemic, an unequalled vaccination campaign against SARS-CoV-2 has been started all around the world (*PMID: 35491495*). While a few studies have evaluated the incidence of anti-PF4 positivity and platelet activation in anti-SARS-CoV-2 vaccine recipients (*PMID: 33988688*, *PMID: 34246010*, *PMID: 34887867*), no studies have assessed whether an immunological response involving platelets develops in healthy subjects (HS) after anti-SARS-CoV-2 vaccination and if this associates with platelet activation. We carried out a prospective study in HS who received the first dose of adenoviral-vector vaccines (ChAdOx1 or Ad26.COV2.S) or mRNA vaccine BNT162b2 to evaluate platelet-specific immune response and *in vivo* platelet activation. **Methods**: 72 HS undergoing first dose ChAdOx1 (n=32), Ad26.COV2.S (n=20) or BNT162b2 (n=20) administration were enrolled and studied at two time points: on the day of vaccination (T0), just before vaccine administration, and after 13.9±0.4 days (T1). Peripheral venous blood was collected in trisodium citrate 3.2% (0.109 M, 1/10 v/v), 0.18% K_3EDTA and non-anticoagulated glass tubes. Anti-PF4/heparin antibodies were searched in serum by two different ELISA; their ability to induce platelet activation was assessed by flow-cytometry; antiplatelet auto- and allo-antibodies were searched in serum by ELISA; platelet-derived microparticles (PMPs) were measured in platelet-free plasma by flow cytometry; plasma levels of sP-sel were measured by ELISA.**Results**: Anti-PF4/heparin antibody positivity after vaccination was relatively infrequent and comparable in the three groups, and when present it was at low titer and not associated with thrombocytopenia or with platelet activation. On the other hand, adenoviral vector vaccine-elicited antiplatelet auto- (34.3% in ChAdOx1 and 25% in Ad26.COV2.S) and allo-antibody positivity (18.7% in ChAdOx1 and 30% in Ad26.COV2.S). Antiplatelet auto- or allo-antibody positivity pattern was not specific, but rather an unspecific immune response against platelet antigens. In the ChAdOx1 group, circulating PMPs and s-Psel increased significantly at T1

compared with T0 (Figure 1). Moreover, subjects who became positive for antiplatelet auto- or allo-antibodies after ChAdOx1 vaccination displayed a significantly higher number of circulating PMPs compared to those who remained negative. **Conclusions**: Our study shows that an immunological reaction involving platelets is not uncommon in subjects receiving anti-SARS-CoV-2 vaccination, especially after ChAdOx1 and, although less commonly, with Ad26.COV2.S, and that it associates with *in vivo* platelet activation, suggesting that a deregulated immune response involving platelets following adenoviral-based vector vaccination occurring in some subjects triggers platelet activation. Given the absence of vaccine complications in enrolled subjects, our data show that these alterations do not have a predictive value for VITT, but it is conceivable that this response may represent a priming trigger which, in association with some yet unknown rare individual predisposition, may lead to full blow VITT in a few subjects.

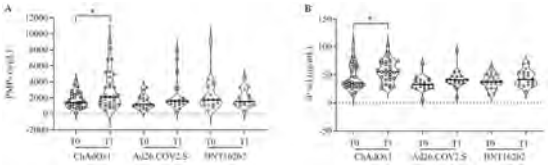


Figure 1. Platelet activation. (A) Plasma levels of PMPs and (B) Soluble platelet P-selectin in subjects undergoing first dose of ChAdOx1 or BNT162b2, or unique dose of Ad26.COV2.S on the day of vaccination (T0) and after 13.9±0.4 days (T1).

OC082

IMPACT OF SARS-COV2 VACCINATION IN THROMBOTIC THROMBOCYTOPENIC PURPURA PATIENTS

A. Artoni, M. Capecchi, P. De Leo, M. Biganzoli, I. Mancini, P. Agosti, B. Ferrari, R. Gualtierotti, F. Peyvandi, A. Bianchi Bonomi

Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

Background: Thrombotic thrombocytopenic purpura (TTP) has occasionally been described after vaccination. Since the availability of anti-SARS-CoV-2 vaccines, 12 cases have been described on a possible association with TTP onset. **Aims**: This study aims to evaluate the relapse rates in patients affected by TTP undergoing anti-SARS-CoV-2 vaccination. **Methods**: All consecutive TTP patients undergoing anti-SARS-CoV-2 vaccination from March to May 2021 were enrolled. Blood samples were collected before vaccination (T0), 2 weeks after the first (T1) and the second dose (T2) to evaluate ADAMTS13 activity and anti-ADAMTS13 antibody titer. **Results**: A total of 49 TTP patients were enrolled (48 acquired and 1 congenital), all vaccinated with an mRNA vaccine. No patients had a clinical TTP relapse, with an ADAMTS13 relapse rate of 1.36% per month. Mean levels of ADAMTS13 activity were stable among the three timepoints (Figure 1). In only two patients a significant drop in ADAMTS13 levels occurred after the first dose (from 28% to <3% and from 101% to 82%), and both remained stable after the second dose, with negative anti-ADAMTS13 antibodies. Due to a stable undetectable ADAMTS13, the first patient was treated with 4 doses of weekly 375 mg/m2 rituximab with a rapid ADAMTS13 response. One patient had positive basal anti-ADAMTS13 antibodies with a titer remaining stable after the two vaccine doses, while in another patient anti-ADAMTS13 antibodies became detectable after the first dose, with no corresponding drop in ADAMTS13 levels and a stable titer after the second dose. **Conclusions**: The result of our study prospectively evaluating the effect of anti-SARS-CoV-2 vaccination on the risk of relapse in a large cohort of patients with TTP in Milan showed a lower than reported relapse rate (1.36% *vs* 2.6%) with an observed to expected incidence rate ratio of 0.52, confirming the safety of mRNA-based anti-SARS-CoV-2 vaccination in TTP patients.

OC083

VACCINE INDUCED THROMBOTIC THROMBOCYTOPENIA: EVALUATION OF GENETIC SUSCEPTIBILITY THROUGHT WHOLE EXOME SEQUENCING

T. Capezzuoli[1], R. De Cario[1], E. Sticchi[2], M. Giannini[1], R. Orsi[1], L. Squillantini[1], S. Suraci[1], A.M. Gori[2], R. Marcucci[2], B. Giusti[2]

[1]University of Florence, Department of Experimental and Clinical Medicine, Florence; [2]University of Florence, Department of Experimental and Clinical Medicine, Atherothrombotic Diseases Center, Careggi Hospital, Florence, Italy

Background: In late February 2021, a prothrombotic syndrome was observed in a small number of individuals who received the adenoviral vector-based vaccine Vaxzevria (AstraZeneca). Similar pictures were observed in some individuals who received the Janssen (Johnson & Johnson) vaccine. This syndrome has been named vaccine-induced thrombotic thrombocytopenia (VITT) and is characterized by multiple venous/arterial thrombosis, with atypical venous thrombosis of cerebral and splanchnic districts, associated with thrombocytopenia. Hemorrhagic manifestations and sometimes disseminated intravascular coagulation (DIC) are present. Evidences suggest that this rare syndrome is caused by platelet-activating antibodies directed against platelet factor 4 (PF4). Aim of this work is to apply a Whole Exome Sequencing (WES) analysis approach, for the identification of possible genetic predisposition profiles underlying VITT. **Methods**: Due to the referral role of the Center for Atherothrombotic Diseases (University of Florence/AOU Careggi) for the management of COVID-19 patients and diagnosis of VITT, fifty patients were

examined. Ten out of fifty patients were diagnosed with VITT. In our analysis we used Next Generation Sequencing techniques, by a WES approach, with Illumina NextSeq500 platform and SureSelect XT HS enrichment kit (Agilent Technologies), to analyse six out ten VITT patients. **Results**: VITT patients analysed were 6 females (mean age 64,2±13,8), who received an adenoviral vector-based vaccine. WES analysis revealed a total of 140,563 variants. Rare variants (MAF <1%) identified range from 1,619 to 1,774 and their distribution by type (frameshift, missense, splicing, nonsense, UTR, samesense, intronic) is similar in the six patients. In this work we decided to focus on rare variants involved in different biological processes underlying VITT. We found a total of 89 rare variants in genes involved in integrin signaling pathways (*ITGA2B, ITGAD, ITGB4, GP6, FGA, FGB*), in thrombocytopenia (*MASTL, PDIA6, FYB, MYH9*), and other genes inducing/inhibiting platelet aggregation/activation processes. Interestingly, the two patients (VITT05 and VITT18), with most severe clinical complications, showed a higher number of rare variants identified in such pathways (21 and 27 variants, respectively). Among the abovementioned pathways, 15 variants with putative functional effect have been identified in genes encoding for integrins or integrin ligands, as well as in molecules involved in pathways triggered by these molecules. Interestingly, both VITT05 and VITT18, patients with a more severe phenotype, carried variants in *GP6* gene, encoding a collagen receptor involved in collagen-induced platelet adhesion and activation. **Conclusions**: WES analysis exhibit a considerable number of variants in molecular pathways involved in integrin signaling, thrombocytopenia and platelet aggregation/activation processes The two patients with the worst clinical outcome presented a significantly higher number of suggestive rare variants with respect to other patients investigated; consequently, it is not possible to exclude the possible contribution of a greater number of rare suggestive variants in the modulation of the phenotype of patients with worse clinical course. Further investigation on other mechanisms (inflammation, immunity, viral response) and functional assays are needed for more clarity with respect to the impact of genetic background on VITT susceptibility.

OC084

THE RISK OF DEVELOPING THROMBOTIC EVENTS IN INDIVIDUALS WITH ANTITHROMBIN DEFICIENCY UNDERGOING COVID VACCINATION: A FAMILY COHORT STUDY

D. Tormene, E. Bicego, N. Perin, S. Gavasso, P. Simioni

Thrombotic and Haemorrhagic Diseases Unit, Department of Medicine, University of Padua Medical School, Padua, Italy

Background and Aims: After SARS-CoV-2 vaccination campaign initiation, rare, unusual, and severe thrombotic events, and other autoimmune adverse reactions have been reported in connection with some of the SARS-CoV-2 vaccines, and have caused a lot of worry and a severe confusion within the population and the medical community. Antithrombin deficiency is recognized as the most severe form of inherited thrombophilia. We assessed the risk of thrombotic events in carriers of antithombin deficiency after SARS-CoV-2 vaccination. **Methods**: We estimated the absolute risk of thrombosis within 30 days of each single dose of 3 SARS-CoV-2 vaccinations [ChAdOx1-S (AstraZeneca), COVID-19 Vaccine Moderna mRNA - 1273 (Spikevax), Pfizer mRNABNT162b2 (Comirnaty)] in carriers of antithrombin deficiency, compared with non carriers. **Results**: In this prospective family cohort study, on the basis of the pedigree analysis, we enrolled a total of 141 subjects (47% men; median age 57 years), belonging to 21 different families with inherited deficiency of antithrombin. Out of 141 subjects, 81 (57%; 47% men) were carriers of antithrombin deficiency and 60 (43%; 48% men) were not. In the group of defect carriers 68 received three vaccine doses, 6 two doses and 2 received 1 dose; 3 carriers refused vaccination. In the non-carrier group 55 received three vaccine doses, and one two doses; 4 non-carriers refused vaccination. A total of 220 (172 Comirnaty, 32 Spikevax, 16 ChAdOx1-S) and 167 (109 Comirnaty,48 Spikevax, 10 ChAdOx1-S) vaccine doses were administered in carriers and non-carriers, respectively. Thirty-five carriers of the defect were taking anticoagulant therapy for previous thrombotic events. Only one thrombotic event occurred in a 44-year-old woman with the defect two weeks after receiving the second dose of the vaccine. The relative risk was of 0.75 (95% CI 0.05 to 11.8); even when anticoagulated patients were excluded the relative risk was of 1.3 (95% CI 0.08 to 20). Six carriers developed covid disease (one before vaccination, one after the first dose, 2 after the second, 2 after the third) *versus* two in the non-carrier group (after the third dose) : RR 2.14 (95% CI 0.5 to 10.2). **Conclusions**: The data of our study show that even in patients genetically predisposed to an increased thrombotic risk, vaccination against Covid does not seem to increase this risk.

OC085

HEMOSTATIC BIOMARKERS AND SEROLOGICAL RESPONSE IN SUBJECTS RECEIVING ANTI-COVID-19 VACCINATION: A PROSPECTIVE COHORT STUDY

F. Schieppati[1], S. Gamba[1], E. Galimberti[1], L. Russo[1], C. Giaccherini[1], S. Bolognini[1], C.J. Tartari[1], C. Ticozzi[1], A.M. Palladino[1], O.C. Cretu[1], L. Barcella[1], M. Marchetti[1], A. Falanga[1,2]

[1]Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo; [2]Medicine and Surgery, University of Milano Bicocca, Milano, Italy

Background and Aims: SARS-COV2 infection is associated with inflammation, hypercoagulability and endothelial damage. Anti-SARS-COV2 vaccines have radically changed the course of the pandemic, although reports on rare thrombotic adverse events have raise concern in the scientific and general communities. We prospectively enrolled a cohort of adult subjects undergoing mRNA or adenovirus vector vaccination, with the aim to longitudinally evaluate: 1. changes in hemostatic biomarkers (*i.e.* clotting activation, endothelium and fibrinolysis); 2. the serological response; 3. the occurrence of manifest thrombotic complications. **Methods**: Peripheral blood samples were collected at enrollment (day 0, D0) before the 1st vaccine dose, and on 15 (D15), 60 (D60), 90 (D90) and 180 (D180) days after the 1st dose. At each time point, hemostatic markers (*i.e.*, fibrinogen, D-dimer, FVIII, von Willebrand Factor [vWF] antigen and activity, F1+2, thrombomodulin, protein C, protein S, FXIII, tPA, and PAI-1), and anti-Spike receptor-binding-domain protein (anti-S/RBD) IgG were measured. **Results**: Fifty-three subjects (57% males, median age 50 years [range 23-86]) were enrolled into the study and followed-up for 180 days: 36 (68%) received BNT162b2, 6 (11%) mRNA-1273, and 8 (15%) ChAdOx1 nCoV-19 vaccines, in 2 doses over 21, 30 and 77 days, respectively; 3 (6%) subjects received Ad26.COV2.S as single shot. A previous history of COVID-19 was reported by 20 individuals (38%), with a mean of 10 months (4-18) before vaccination; only 1 required hospitalization. Nine subjects presented cardiovascular risk factors and 4 a prior, non-active, cancer; 3 were on anticoagulation for atrial fibrillation. The evaluation of the hemostatic biomarkers at the different time points showed variations in some of the parameters evaluated, with median values remaining within normal range levels. Specifically, compared to baseline, we observed a significant increase in thrombomodulin at D90 (p=0.001) and D180 (p=0.03), and a significant decrease in fibrinogen (D60), vWF-Ag (D60 and D180), FVIII (D60, D90 and D180), and TPA (D60 and D90) levels. The reduction of these biomarkers was particularly evident in individuals with a history of COVID-19. Of interest, this group of subjects was also characterized by significantly lower levels of PAI-1 both at baseline (7.18 ng/mL *vs* 17.53 ng/mL; p<0.0001), and at other time points (p<0.0001), and by an increase in F1+2 at D90 (p=0.02). The association between lower baseline PAI-1 levels with history of COVID-19 was confirmed by linear regression analysis (B=-10.351, p=0.013), and was independent by the time of infection resolution. Notably, no differences were observed in the hemostatic biomarkers according to vaccine types. All subjects positively responded to vaccination with a significant increase in anti-S/RBD IgG from baseline (D0) to each time point, especially COVID-19 subjects (D15, D60, and D90:p<0.0001; D180:p=0.031). No thrombotic or cardiovascular events occurred during follow-up. **Conclusions**: We found no hypercoagulable state elicited by COVID-19 vaccination, contrarily we detected an overall persistent reduction of clotting activation over time. Subjects with previous SARS-COV2 infection had persistently low levels of PAI-1, supporting enhanced fibrinolysis activation. Compared with recent studies, our results provide a longer observation follow-up with all vaccine types and reassure on the safety of anti-SARS-COV2 vaccination.

OC086

NATURAL HISTORY OH ANTI PFA ANTIBODIES IN PATIENTS WITH VITT

E. Lotti, F. Vannini, A. Rogolino, F. Cesari, A. Bertelli, L. Mannini, D. Poli, B. Giusti, R. Marcucci

Department of Experimental and Clinical Medicine, University of Florence; Atherothrombotic center, AOU Careggi, Italy

Background and Aims: Vaccine-induced Immune Thrombocytopenia and Thrombosis (VITT) is a new clinical syndrome discovered during the first half of 2021, when very rare cases of thrombocytopenia combined with unusual thrombosis (venous and/or arterial), have been reported one to two week after vaccination with Adenovirus-based vaccines against SARS-CoV-2. VITT seems to share the same pathogenetic mechanisms of autoimmune heparin-induced thrombocytopenia (aHIT); specifically, both VITT- and aHIT-patients develop an high serum titer of IgG antibodies directed against platelet-factor-4 (PF4) even in the absence of heparin exposure. Initial data on the natural history of antiPF4 antibodies in large VITT-patients cohorts have been recently published suggesting a longer antiPF4 titer durability compared to what has been previously described in HIT patients. Here we present the results of an ongoing study on a VITT-patients cohort followed with serial determinations of quantitative serum antiPF4 antibodies titer and functional HIPA testing for a median follow-up duration of 6 months. **Methods**: We included nine patients (seven females and three males) with a diagnosis of VITT confirmed between March and June 2021. The median age at the time of diagnosis was 63,5 years. All patients developed thrombocytopenia with a median platelet count at the nadir of 23.500/mmc (10.000-95.000). All developed thrombotic manifestations with a median D-Dimer level at the presentation of 28330 ng/ml (5.151-74.124). The overall mortality rate in this cohort was 20% (2/9), whereas another 20% (2/9) of patients survived developing moderate to severe disability. To detect platelet factor 4 (PF4)–heparin antibodies we used a standard enzyme-linked immunoadsorbent assay (ELISA) (Lifecode PF4-IgG test, Immucor, Itay) and a chemiluminescence immunoassay (CLIA) (Acustar IgG test, Werfen, Italy). A functional test Heparin-induced platelet Activation (HIPA) test modified by the addition of PF4 in order to detect the functional ability of the antibodies in inducing platelet activation was performed (Figure 1). **Results**: At the time of diagnosis the median antiPF4 antibodies quantitative titer was extremely high (2472 OD units, range), associated in all cases with a positive functional test. In the remanining

survivors cohort (7 patients), 3 patients remained positive at the quantitative assay after a median follow-up duration of 6 months. In one of these three patients we registered the maximum durability of positive antiPF4 of 9 months, with a value of 1176 OD units with a persistent functional positivity. Interestingly in another patient we found a still detectable functional positivity after almost 1 year from diagnosis, despite seroconversion to negative quantitative assay occurred after 4 months. None of the surviving patients included in this study showed clinical manifestations of relapse. **Conclusions**: For the first time in a real life study we have documented in patients with diagnosis of VITT the natural history of anti-PFA IgG antibodies at long follow up intervals (up to 12months) both by immunological and functional assays.

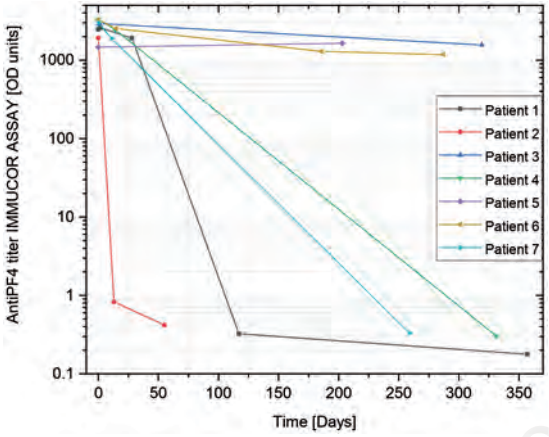


Figure 1.

OC087

SENSITIVITY AND SPECIFICITY OF DIAGNOSTIC TESTS FOR THROMBOSIS WITH THROMBOCYTOPENIA SYNDROME - A SYSTEMATIC REVIEW OF CASE REPORTS AND CASE SERIES

B. Clerici[1], M. Scavone[1], S. Birocchi[1], C. Aloise[1], B. Peroni[1], A. Negrini[1], G. Casazza[2], G.M. Podda[1]

[1]Divisione di Medicina Generale II, ASST Santi Paolo e Carlo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy; [2]Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", Università degli Studi di Milano, Milan, Italy

Background and Aims: Diagnostic accuracy measures of the available diagnostic tests for Thrombosis with Thrombocytopenia Syndrome (TTS) are presently lacking. The aim of the study was to estimate sensitivity and specificity of PF4-ELISAs, Platelet Activation Assays (PAAs) and PF4-enhanced PAAs (PF4-PAAs). **Methods**: A systematic search of the literature was performed by 2 researchers with a pre-defined research string and a snowball strategy. We evaluated for inclusion (i) observational studies on TTS providing results of the performed laboratory diagnostic tests, (ii) studies investigating the prevalence of anti-PF4 antibodies in vaccinees with an adenoviral vector vaccine with no signs or symptoms of TTS, and (iii) laboratory studies investigating novel diagnostic tests and/or comparing different available diagnostic tests.

Table 1.

Panel A.

Test	Positive tests/total tests (n)	Test sensitivity (95% CI)
SCREENING TESTS		
IgG-specific ELISAs		
Asserachrom HPIA IgG	42/49	85.71% (72.76-94.06%)
Zymutest HIA IgG	59/65	90.77% (80.98-96.54%)
Lifecodes PF4 IgG	90/93	96.77% (90.86-99.33%)
AESKULISA HIT II	21/29	72.41% (52.78-87.27%)
Total	212/236	89.83% (85.25-93.37%)
Polyspecific ELISAs		
Asserachrom HPIA	26/27	96.30% (81.03-99.91%)
Lifecodes PF4 Enhanced	40/42	95.24% (83.84-99.42%)
Total	66/69	95.65% (87.82-99.09%)
Unspecified ELISAs		
	338/347	97.41% (95.13-98.81%)
All ELISAs		
	616/652	94.48% (92.44-96.10%)
CONFIRMATION TESTS		
Platelet Activation Assays		
HIPA	39/61	63.93% (50.63-75.84%)
SRA	14/26	53.85% (33.37-73.41%)
HIMEA	6/7	85.71% (41.13-99.64%)
HIT Alert KIT	5/7	71.43% (29.04-96.33%)
PAT by LTA	5/5	100.00% (47.82-100.00%)
Total	69/106	65.09% (55.22-74.10%)
PF4-Platelet Activation Assays		
HIPA	54/57	94.74% (85.38-98.90%)
SRA	17/17	100.00% (80.49-100.00%)
PIFPA	16/16	100.00% (79.41-100.00%)
Total	87/90	96.67% (90.57-99.31%)

Abbreviations: CI, Confidence Intervals; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; PF4, Platelet Factor 4; HIPA, heparin-induced platelet activation; SRA, serotonin release assay; HIMEA, heparin-induced multiple electrode aggregometry; HIT, heparin-induced thrombocytopenia; PAT, platelet aggregation test; LTA, light transmission aggregometry; PIFPA, PF4-induced flow cytometry-based platelet activation.
95% Confidence Intervals were calculated using the exact method.
Tests that were performed less than 5 times were excluded from this analysis.

Panel B.

Test	Negative tests/total tests (n)	Test Specificity (95% CI)
IgG-specific ELISAs		
Lifecodes PF4 IgG	486/492	98.78% (97.36-99.55%)
Zymutest HIA IgG	37/41	90.24% (76.87-97.28%)
Unspecified ELISAs		
	137/158	86.71% (80.40-91.58%)
All ELISAs		
	660/691	95.51% (93.69-96.93%)
Platelet Activation Assays		
HIMEA	6/6	100.00% (54.07-100.00%)
PF4-Platelet Activation Assays		
HIPA	30/30	100.00% (88.43-100.00%)
PIFPA	20/20	100.00% (83.16-100.00%)
Total	50/50	100.00% (92.89-100.00%)

Abbreviations: CI, Confidence Intervals; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; PF4, Platelet Factor 4; HIMEA, heparin-induced multiple electrode aggregometry; HIPA, heparin-induced platelet activation; PIFPA, PF4-induced flow cytometry-based platelet activation.
95% Confidence Intervals were calculated using the exact method.
Tests that were performed less than 5 times were excluded from this analysis.

Patients were considered affected by TTS according to

the definition of the authors or guidelines. **Results**: Fifty-seven studies describing diagnostic test results of 508 TTS patients and 691 vaccinees with adenoviral vector vaccines without signs or symptoms of TTS were included in the analysis. The summary sensitivity estimates of PF4-ELISAs, PAAs and PF4-PAAs were 94.5% (95%CI, 92.4-96.1%), 65.1% (95%CI, 55.2-74.1%), and 96.7% (95%CI, 90.6-99.3%), respectively (Table 1, Panel A). Summary estimates of specificity were 95.5% (95%CI 93.7-96.9%) for PF4-ELISAs and 100% (95%CI, 92.9-100.0%) for PF4-PAAs; data for specificity estimation of PAAs were scarce (Table 1, Panel B). **Conclusions**: Our results suggest that PF4-ELISAs, due to high sensitivity and high specificity, constitute appropriate tests to rule in and rule out TTS. PF4-PAAs, highly sensitive and specific tests limited by their low availability and technical complexity, can be used in selected cases to confirm or exclude TTS.

OC088

MAJOR BLEEDINGS IN MECHANICAL PROSTHETIC HEART VALVES PATIENTS ON VITAMIN K ANTAGONIST TREATMENT. DATA FROM THE PLECTRUM STUDY

D. Poli[1], E. Antonucci[2], R. Facchinetti[3], P. Falco[4], G. Serricchio[5], T. Lerede[6], L. Masciocco[7], P. Gresele[8], G. Palareti[2], S. Testa[9] on the behalf of the Italian Federation of Anticoagulation Clinics

[1]Center of Atherothrombotic Disease, Azienda Ospedaliero-Universitaria Careggi, Firenze; [2]Fondazione Arianna Anticoagulazione, Bologna; [3]Laboratorio Analisi, Azienda Ospedaliera Universitaria Integrata (AOUI) Ospedale Civile Maggiore Di Borgo Trento; Verona; [4]Poliambulatorio Specialistico MEDICAL PONTINO, Latina [5]Lab analisi Ospedale Sant'Anna San Fermo della Battaglia, ASST Lariana Como; [6]Centro Trombosi e Emostasi Immunoematologia e Medicina Trasfusionale ASST Papa Giovanni XXIII Bergamo; [7]UOC Medicina Interna, Ospedale Lastaria, Lucera (FG); [8]Department of Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia; [9]Department of Laboratory Medicine, Haemostasis and Thrombosis Centre, ASST Cremona, Cremona, Italy

Background and Aims: Patients with mechanical prosthetic heart valves (MHV) need vitamin K antagonist (VKA) treatment, due to the high thrombotic risk. The need to evaluate the bleeding risk of these patients is of great clinical relevance. **Methods**: Observational retrospective multicenter study among Centers affiliated to the Italian Federation of Anticoagulation Clinics (FCSA) on MHV patients, with the aim to evaluate the risk of major bleeding (MB) and associated risk factors. **Results**: 2357 patients with MHV were included in the study, 246 patients had Major Bleeding (MB) (rate1.0 x100 pt-yrs), 54 were intracranial hemorrhage (rate 0.22 x100 pt-yrs). Patients with MB were significantly older, more affected by peripheral obstructive arterial disease (POAD) and atrial fibrillation (AF), and presented a history of previous MB, with respect to patients who did not bleed. Patients with MB showed a trend for lower time in therapeutic range (TTR), and a significant number of patients had a TTR in the lower quartile. Patients with MB had a higher mortality rate with respect to patients who did not bleed (p=0.001). The history of previous bleeding, the presence of POAD or of AF, and a TTR in the lowest quartile, were significantly associated with MB. **Conclusions**: MHV patients treated with VKAs followed by Anticoagulation Clinics, showed a low bleeding risk. Risk factors associated with major bleeding are older age, the presence of POAD or AF, the history of previous bleeding, and poor quality of anticoagulation. Patients who experienced MB during anticoagulation are at high risk of death.

OC089

DOACS USE IN EXTREME BODY WEIGHTED PATIENTS: RESULTS FROM THE PROSPECTIVE START-REGISTER

M. Guarascio[1], L. Bertù[2], M.P. Donadini[2,3], E. Antonucci[4], G. Palareti[4], W. Ageno[2,3]

[1]U.OPneumologia 2Covid, "Vito Fazzi" Hospital, Lecce, Italy; [2]Research Center on Thromboembolic diseases and Antithrombotic Therapies, University of Insubria, Varese, Italy; [3]Emergency Medicine and Thrombosis and Haemostasis Center, ASST Sette Laghi, Varese, Italy; [4]Arianna Anticoagulazione Foundation, Bologna, Italy

Background and Aims: Direct oral anticoagulants (DOACs) are widely used for the treatment of venous thromboembolism (VTE) and for stroke prevention in atrial fibrillation (AF). However, evidence in obese and underweight patients is limited. We assessed the safety and effectiveness of DOACs and vitamin K antagonists (VKAs) in patients >120 Kg or <50 Kg enrolled in an observational prospective cohort study, the START-Register. **Methods**: Adult patients started on anticoagulant therapy were followed up for a median of 1.5 years (IQR 0.6-2.8). Primary efficacy outcome was the occurrence of VTE recurrence, stroke and systemic embolism. Primary safety outcome was major bleeding (MB) **Results**: 10,080 AF and VTE patients were enrolled between March 2011 and June 2021, 295 patients weighted <50 Kg and 82 patients >120 Kg. Obese patients were significantly younger than underweight patients. Rates of thrombotic events were low and similar between DOACs and VKAs in underweight patients (1 event on DOACs therapy [0.9% 95%CI 0.11-5.39] and 2 on VKAs [1.1% 95%CI 0.01-47.68]) and in overweight patients (0 events on DOACs, 1 on VKAs [1.6%, 95%CI 0.11-5.79]. Two MB events occurred on DOACs (1.9%, 95%CI 0.38-6.00) and 3 on VKAs (1.6%, 95%CI 0.04-22.06) in the underweight group; 1 MB on DOACs

(5.3% 95%CI 0.33-16.68) and 2 on VKAs (3.3%, 95%CI 0.02-130.77) in the overweight group. (Table 1). **Conclusions**: DOACs seem to be effective and safe also for the treatment of patients with extreme body weights, both underweight and overweight. Further prospective studies are needed to support these findings.

Table 1.

	DOAC			VKA		
Weight (Kg)	**<=50**	**51-119**	**>=120**	**<=50**	**51-119**	**>=120**
	N (%) 95% CI	*N (%) 95% CI*	*N (%) 95% CI*	*N (%) 95% CI*	*N (%) 95% CI*	*N (%) 95% CI*
Thrombotic events	1(0.9) 0.11-5.39	89 (2.2) 1.18-1.79	0(0.0)	2(1.1) 0.01-47.68	52(0.9) 0.26-0.77	1(1.6) 0.11-5.79
MB +CRNMB	3(2.7) 0.72-6.98	183(4.5) 2.61-3.50	2(10.0) 1.18-18.79	11(6.0) 0.30-37.56	368(6.5) 2.35-4.49	2 (3.2) 0.06-47.41
MB	2(1.9) 0.38-6.00	93(2.4) 1.30-1.94	1(5.3) 0.33-16.68	3(1.6) 0.04-22.06	139(2.5) 0.77-1.92	2(3.3) 0.02-130.77
CRNMB	1(0.9) 0.11-5.33	85(2.2) 1.17-1.77	1(5.3)	8(4.3) 0.04-139.53	228(4.1) 1.29-3.22	0(0.0)
Death	(6.4) 2.50-10.99	201(4.9) 2.79-3.69	0(0.0)	28(15.1) 1.73-39.98	501(8.9) 3.14-5.75	4(6.5) 1.22-8.69

OC090

MANAGEMENT OF MAJOR BLEEDING IN PATIENTS TREATED WITH DIRECT ORAL ANTICOAGULANTS: UPDATE DATA FROM START EVENTS REGISTRY

E. Antonucci[1], O. Paoletti[2], W. Ageno[3], M. Righini[4], P. Verhamme[5], G. Colombo[6], P. Sivera[7], M.S. Cotelli[8], M. Tala[2], R. Morandini[2], C. Dalla Noce[2], D. Poli[9], G. Palareti[1], S. Testa[2]

[1]Fondazione Arianna Anticoagulazione, Bologna, Italy; [2]Dipartimento di Medicina di Laboratorio, Centro Emostasi e trombosi, ASST Cremona, Cremona, Italy; [3]Dipartimento di Medicina e Chirurgia, Università degli Studi dell'Insubria, Varese, Italy; [4]Division of Angiology and Hemostasis, Department of Medical Specialties, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland; [5]Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium; [6]DB Internistica e Centro Trombosi ed Emostasi, AO di Circolo, ASST dei Sette Laghi, Varese, Italy; [7]SCDU Ematologia E Terapie Cellulari Ospedale Mauriziano Umberto I di Torino, Torino, Italy; [8] SSD Neurologia ASST Valcamonica Esine, Brescia, Italy; [9] SOD Malattie Aterotrombotiche, Azienda Ospedaliero Universitaria-Careggi, Firenze, Italy

Background and Aims: The management of major bleeding in patients treated with direct oral anticoagulants (DOACs) is a serious medical concern. START-Events, prospective, observational, multicenter, international studyand a branch of the START registry (Survey on anTicoagulatedpAtientsRegisTer) (NCT02219984), aims to describe the management of bleeding or recurrent thrombotic events in routine clinical practice. We here present the results of the management of bleeding patients. **Methods**: Clinical characteristics of patients, laboratory data at admission and during follow-up, site of bleeding, therapeutic strategies and outcomes at the time of hospital discharge and after 6 months were recorded on a web-based case report form. To define the major bleeding we adopted the criteria of the International Society on Thrombosis and Haemostasis. **Results**: Between January 2015 and December 2021, 224 patients with major bleeding events were enrolled. Non-valvular atrial fbrillation (NVAF) was the indication for treatment in 86% of patients; 59% were males; median age 80 years; 31.7% of patients were on apixaban; 21.9% on dabigatran,7.1% on edoxaban; 39.3% on rivaroxaban. An inappropriate dose of DOAC were administered in 41 patients (18%). Concomitant antiplatelet treatment was present in 14% of enrolled patients; 111 patients (49.5%) had intracranial bleeding (ICH), 11 of them were fatal, 83 (37.1%) patients had gastrointestinal bleedin (GIB), 6 were fatal; 30 (13.4%) patients had major bleeding in other site, none was fatal. Therapeutic interventions for the management of bleeding were performed in about 90% of patients. Only in 10% of patients a conservative approach was recorded, expecially in patients enrolled within 2015 and 2018.Therapeutic strategies with/without surgery or invasive procedures included: fluid replacement or red blood cells transfusion, prothrombin complex concentrates (3 or 4 factors), antifbrinolytic drugs, and the administration of specific reversal strategies. In particular idarucizumab was administered in 10 patients (6 ICH and 4 GIB); andexanet alpha in 2 patients with ICH. Creatinine, blood cell count, and PT/aPTT were the most frequent tests requested (85%), while specific DOAC measurements were performed only in 30% of patients. At hospital discharge, 63% of all patients had complete resolution of bleeding signs.When we consider ICH patients only, we observed complete resolution of symptoms in about 58% of patients and residual disability in 46/111patients (41%). During hospitalization the mortality rate was 11%. At 6 months, the complete resolution was recorded in 78% of patients. Overall mortality rate of the study was 14.3%. **Conclusions**: Our data show that the therapeutic strategies for management of bleeding are mainly symptomatic treatment of blood loss, treatment of the anatomical cause of bleeding and the use of specific reversal agent as suggested by current guidelines. In a very low number of patient we registered a conservative approach. Nearly one-quarter of patients received an inappropriate dose of DOAC. A low rate of specifc drug measurements is still reported, despite the current guidelines for management of DOAC associated bleeding are being updated to perform the measurement in this specifc clinical conditions.

OC091

GENDER DIFFERENCE AND RISK OF MAJOR BLEEDING IN PATIENTS ON ANTI-THROMBOTIC TREATMENT FOR MECHANICAL HEART VALVE PROSTHESIS. ANALYSIS CONDUCTED AT THE SALAM CENTRE FOR CARDIAC SURGERY - EMERGENCY NGO – KHARTOUM, SUDAN

N. Erba[1,2,3], A. Mocini[2], A. Sawi[2], F. Hamuda[2], M. Langer[1], G. Portella[1], F. Masini[2], S. Testa[3], D. Poli[3]

[1]Emergency NGO ONLUS, Milan, Italy; [2]SALAM Centre for Cardiac Surgery, Khartoum, Sudan; [3]Federazione Centri per la Diagnosi Della Trombosi e la Sorveglianza delle Terapie Antitrombotiche (FCSA), Milan, Italy

Background and Aims: Anticoagulant therapy with Warfarin exposes the patient to the risk of major bleeding, and consequently, to life-threatening risks, temporary or permanent disabilities and thrombotic risk related to temporary withdrawal of anticoagulant therapy. The study was aimed to analyze gender-related risk of major bleeding and to investigate the gender-related differences in patients on Warfarin for mechanical heart valve (MHV) prostheses operated at the Salam Centre for Cardiac surgery in Khartoum. **Methods**: Between August 1, 2018 and September 30, 2019 all patients followed by the Anticoagulation Clinic of the Salam Centre for Cardiac surgery were included in a prospective observational study to collect data on the adherence and quality of the anticoagulant treatment. Bleeding events occurred during follow-up were recorded, Major Bleedings were defined according to ISTH criteria. Patients with less than three months of observation were excluded from the observation. Patients with intervals between controls longer than 3 months were defined "incompliant". Patients who omit the check INR for 6 months or more, despite follow-up calls, were classified "lost" at follow-up. **Results**: We studied 3647 patients (median age 25.7 years; females 53.9%) who were on anticoagulant treatment with warfarin after MHV implantation. Patients aged <14 y were 19% of the included males and 16.1% of the included females (p=0.02). Aortic valves were implanted in 23.8% of males and 7.2% of females (p value <0.001); mitral valves were implanted in 40.5% of males and 68.4% of females (p value <0.001); mitral-aortic valves were implanted in 35.7% of males and 24.4% of females (p value <0.001). 80% of patients received also aspirin (75 or 100 mg OD) with not significant differences between males and females (respectively 79.4 *vs* 81.2, p=0.2). Females showed worse Time in Therapeutic Range (median F 50% *vs* M 56%, p value <0.001), instead they had better compliance to therapy (uncompliant F 6.8% *vs* M 10.2%, p value <0.001) and less of them were lost at follow-up (F 2.0 *vs* M 3.4, p value=0.01). During the observational period we recorded 85 major bleeding episodes (2.16 % p-y, n. 9 fatal): 16 were cerebral, 19 were gastrointestinal, and 32 were menometrorrhagia. At univariate analysis, female gender and aspirin treatment were significantly associated with bleeding risk (OR 2.2, 95%CI 1.4-3.4; p value <0.001 and OR 1.8, 95%CI 1.0-3.4; p value=0.05, respectively). When gynaecological bleedings were excluded, women were not at a higher risk of bleeding respect to men (OR 1.2, 95%CI 0.7-1.9; p value=0.5). **Conclusions**: Women of childbearing age on anticoagulant therapy are particularly exposed to the risk of major predominantly gynaecological bleedings. The use of associated aspirin treatment should be carefully evaluated, especially in women. Specific gynaecological programs should be implemented for anticoagulated women.

OC092

PREDICTIVE ABILITY OF THE HAS-BLED SCORE OF THE NEWLY PUBLISHED SWISS-AF SCORE IN ATRIAL FIBRILLATION PATIENTS ON ANTICOAGULANT TREATMENT WITH VITAMIN K ANTAGONITS AND DIRECT ORAL ANTICOAGULANTS. PRELIMINARY RESULTS OF THE START2 AF REGISTER

D. Poli[1], L. Sechi[2], E. Antonucci[3], F. Crudele[2], M. Berteotti[2],G. Colombo[4], L. Masciocco[5], T. Lerede[6], A. Toma[7], P. Pignatelli[8], D. Pastori[8], S. Testa[9], R. Marcucci[2], G. Palareti[3]

[1]SOD Malattie Aterotrombotiche, Azienda Ospedaliero Universitaria-Careggi, Firenze; [2]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze; [3]Fondazione Arianna Anticoagulazione, Bologna; [4]DB Internistica e Centro Trombosi ed Emostasi, AO di Circolo, ASST dei Sette Laghi, Varese; [5]UO Medicina Interna, Presidio Ospedaliero Francesco Lastaria di Lucera, ASL Foggia, Lucera (FG); [6]USC SIMT, Centro Emostasi e Trombosi, Ospedale Papa Giovanni XXIII, Bergamo; [7]UO Laboratorio Analisi Distretto Ovest, ULSS 8 Berica, Arzignano (VI); [8]Department of Clinical, Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Roma; [9]Dipartimento di Medicina di Laboratorio, Centro Emostasi e trombosi, ASST Cremona, Cremona, Italy

Background and Aims: Oral anticoagulant therapy (OAT) is recommended in patients with atrial fibrillation (AF) due to the associated risk of ischemic stroke. However, the stratification of the bleeding risk associated with OAT is still not optimal. The HAS-BLED is the most widely used bleeding score, but it demonstrate a low predictive ability. The SWISS-AF score to predict bleeding risk has been published. The score is easy to calculate and includes 4 items (age≥75 years, hypertension, previuos major bledings, cancer).The purpose of the study is to compare the predictive ability HAS-BLED and of the SWISS-AF score in AF patients enrolled in the observational, prospective START2-Register – Survey on anTicoagulated pAtients RegisTer. **Methods**: We investigated the clinical characteristics of AF patients receiving included in the Register and we apply the different scores. Thrombotic events, major and clinically significant relevant not major bleeding events and death occurred in the follow-up were recorded. Major, clinically relevant non-major bleedings and thrombotic events were recorded. Predictive ability for bleeding was calculated for both scores among patients on VKAs and patients on DOACs. **Results**: We studied 6165 patients (54% males), median age 75.6 years, 34.1% of whom were on direct oral anticoagulants (DOACs). During follow-up 179 major bleedings were recorded (rate 1.46 x100 patient years; rate 1.28 and 1.89 for patients on VKAs and on DOACs respectively. At multivariate analysis the history of previous bleeding, heart failure,

the history of previous stroke/TIA, the concomitant use of antiplatelet therapy, the presence of active cancer were significantly associated with bleedings. The predictive ability calculated with the c statistic (95% CI) for major haemorrhage for HASBLED was: 0.56 (0.51-0.62), p=0.03 when patients on VKA were considered; 0.59 (0.52-0.66), p=0.01 when patients on DOAC were considered. For the Swiss-AF score the c statistic (95% CI) was: 0.55 (0.5-0.6), p=0.03 when VKAs were considered and 0.60 (0.52-0.67), p=0.007 when DOACs patients were considered. **Conclusions**: The main result of the study is that the predictive ability for bleeding of SWISS-AF score and of HAS-BLED score are similarly modest, both either in VKAs patients or in DOACs patients.

OC093

INTRACRANIAL HEMORRHAGE IN PATIENTS WITH PRIMARY OR METASTATIC BRAIN CANCER TREATED OR NOT WITH ANTICOAGULANTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

M. Giustozzi[1], G. Proietti[2], C. Becattini, F. Roila[3], G. Agnelli, M. Mandalà[3]

[1]Internal, Vascular and Emergency Medicine - Stroke Unit, University of Perugia, Perugia, Italy; [2]Medical student, University of Perugia, Perugia, Italy; [3]Unit of Medical Oncology, University of Perugia, Perugia, Italy

Introduction: Anticoagulant treatment in patients with primary and metastatic brain cancer is a concern due to risk of intracranial hemorrhage (ICH). We performed a systematic review and meta-analysis to evaluate the risk of ICH in patients with primary or metastatic brain cancer treated or not with anticoagulants. **Methods**: Articles on ICH in patients with primary or metastatic brain cancer treated or not with anticoagulants published up to September 2021 were identified by searching PUBMED, EMBASE and CENTRAL.The primary outcome of this analysis was ICH. **Results**: Thirty studies were included. Rate of ICH was 13.0% in 1,009 patients with metastatic and 6.4% in 2,353 patients with primary brain cancer [Relative risk (RR) 3.26, 95% CI 2.69-3.94; I2 92.8%). In patients with primary brain cancer, ICH occurred in 12.5% and 4.4% of patients treated or not treated with anticoagulants, respectively [11 studies, 659 treated and 1,346 not treated patients, RR 2.63, 95% CI 1.48-4.67, I2 49.6%]. In patients with metastatic brain cancer, ICH occurred in 14.7% and 15.4%, respectively (5 studies, 265 treated and 301 not treated patients, RR 0.92, 95% CI 0.43-1.93, I2 0%). ICH occurred in 8.3% of 172 treated with direct oral anticoagulant (DOAC) and in 11.7% of 278 treated with low-molecular weight heparin (LMWH) (5 studies, RR 0.44, 95% CI 0.25-0.79, I2 0%). **Conclusions**: Patients with metastatic brain cancer have a particularly high risk of ICH. Patients with primary brain cancer have an increased risk of ICH during anticoagulation. DOACs are associated with a lower risk of ICH than LMWH.

OC094

MANAGEMENT OF PATIENTS WITH THROMBOTIC EVENTS DURING TREATMENT WITH DIRECT ORAL ANTICOAGULANTS

G. Cammà[1], E. Antonucci[3], D. Poli[4], M. Tala[5], O. Paoletti[5], G. Palareti[3], S. Testa[5], W. Ageno[2]

[1]Department of Internal Medicine, Fondazione Policlinico Universitario AGemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Department of Emergency Medicine, Fondazione Macchi, Università dell'Insubria, Varese, Italy; [3]Fondazione Arianna, Bologna, Italy; [4]Azienda Ospedaliera Careggi, Florence, Italy; [5]Azienda Ospedaliera di Cremona, Cremona, Italy

Background and Aims: Direct oral anticoagulants (DOACs) are effective in the treatment of venous thromboembolism (VTE) and in the prevention of cardioembolic events in patients with atrial fibrillation (AF). Little evidence exists on the management of patients who present with acute thrombotic events during treatment with DOACs. **Methods**: We conducted a prospective observational study on patients started on anticoagulant treatment at Italian Thrombosis Clinics (START-Register). Patients diagnosed with an arterial or venous thrombotic event during treatment with DOACs were identified. Information on management strategies and outcomes was collected. **Results**: We enrolled 110 patients with a median age of 71 years, 65 (60%) were males, 70 (64%) had AF, 40 (36%) had VTE. Of these patients, 53 were receiving low dose DOACs, only 19 according to the label. Median time from starting DOAC to thrombotic events was 230 days (IQR 72-585); 266 days (IQR 122-710) in AF group and 117 days (IQR 35-389) in the VTE group. In the AF group the majority of patients had ischemic stroke (n:46), in the VTE group recurrent VTE was the most common event (n:32). Overall, there were 64 arterial thrombotic events: 49 strokes, 6 transient ischemic attacks (TIA), 6 peripheral emboli, and 3 acute myocardial infarctions (AMI). DOACs therapy was stopped in 40 patients (34 strokes, 5 TIA and 1 AMI). Low molecular weight heparin (LMWH) was started in 17 strokes, 1 peripheral embolism and 1 AMI. LMWH in addition to aspirin was used in 14 strokes and 1 TIA; aspirin alone in 8 strokes and 2 TIA. Nine patients (5 stroke and 4 peripheral emboli) received thrombolysis or thrombectomy. At discharge, 18 patients (10 strokes, 4 TIA, 2 peripheral embolism and 2 AMI) continued with the same anticoagulant regimen used before the event; 7 patients (6 strokes and 1 TIA) added aspirin to the DOAC. DOAC dose was increased, while maintaining the same molecule, in 6 strokes and 1 TIA. A different DOAC was prescribed to 15 patients (13 strokes and 2 peripheral embolism). A different DOAC plus aspirin to 4 strokes and 1 peripheral embolism. Vitamin K antagonists (VKAs) were given to 8 patients (6 strokes, 1 peripheral embolism and 1 AMI). Four patients died, all

after stroke, 24 stroke patients had residual disability. Among the 46 venous thrombotic events, there were 21 deep vein thrombosis (DVT), 9 DVT plus pulmonary embolism (PE),11 isolated PE, 4 superficial vein thrombosis (SVT) and 1 retinal vein occlusion. All patients stopped DOACs therapy. LMWH therapy was started in 41 cases (20 DVT, 6 DVT, 11 isolated PE, 4 SVT) and fondaparinux in 3 (1 DVT and 2 DVT+PE). At discharge, LMWH was continued in 7 patients (2 DVT, 3 DVT+PE, 1 PE, 1 SVT). The same DOAC was confirmed in 19 patients (11 DVT, 2 DVT+PE, 6 PE); the dose was increased in two patients. A different DOAC was prescribed to 5 DVT, 1 DVT+PE and 2 PE; 9 patients were switched to VKAs (2 DVT, 3 DVT+PE, 1 PE, 2 SVT). No patient died.
DOACs activity at baseline was measured in 35 patients. It was less than 50 ng / ml in 12 patients: 8 strokes and 4 VTE; less than 30 ng / mL in 3 patients, 2 were receiving low dose DOACs inappropriately. **Conclusions**: The management of thrombotic events during DOACs therapy is heterogeneous and varies between arterial and venous events. A substantial proportion of patients were discharged with the same drug and dosage. DOACs activity at the time of the event was measured in only one third of patients.

OC095

FINDINGS FROM THE ORTHO-START REGISTRY: PERIOPERATIVE MANAGEMENT OF ANTICOAGULANT THERAPIES IN ELDERLY PATIENTS UNDERGOING MAJOR ORTHOPEDIC SURGERY

E. Grandone, E. Antonucci, D. Colaizzo, A. De Laurenzo, C. Rostagno, F. Marongiu, F. Gorgoglione, G. Maccagnano, G. Di Rienzo, L. Mirabella, M. Senzolo, A. Ostuni, G. Palareti

Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; The First IM Sechenov Moscow State Medical University, Obstetrics & Gynecology, Moscow, Russian Federation; University of Foggia; Fondazione Arianna, Bologna, Italy; University of Florence, Italy; Hospital F. Perinei, Bari, Italy; University of Cagliari, Italy; Padua University Hospital, Italy; A.O.U. Policlinico di Bari, Transfusion Medicine, Bari, Italy

Background: Peri-operative management of oral anticoagulant therapy in the context of emergency orthopedic surgery for femur fracture or elective major orthopedic surgery for hip/knee replacement in elderly patients, is challenging, because of the presence of multiple co-morbidities and medical treatments. For this reason in elderly patients, interruption and re-initiation of the Oral Anticoagulant (OAC) and antiplatelets therapy require a multidisciplinary team. In the context of peri-operative clinical management, OAC therapy raises the issue of bleeding risk, which is higher in these patients. There is no certain evidence regarding the peri-operative management of elderly patients taking OAC- vitamin K antagonists (VKAs) or direct oral anticoagulants (DOACs) and antiplatelets- that require major orthopedic surgery. To date, there is no consensus on the timing and period of OAC/antièlatelets interruption and re-initiation of therapy in these patients. Aims of the ORTHO-START registry are: 1) To collect data on the peri-operative management of anticoagulation therapies in elderly patients who need emergency or elective orthopedic surgery; 2)To investigate transfusion practices in the context of emergency or elective orthopedic surgery in elderly patients, with the objective to collect information useful to set up a patient blood management program. **Methods**: The ORTHO-START is a multicentre, prospective, observational study investigating management of patients taking OAC and / or antiplatelet agents requiring elective intervention of hip replacement or knee or urgent surgery for hip fracture. Data collection is web-based. Patients not taking OAC or antiplatelets and undergoing the above-listed procedures have been simultaneously recruited with the objective to compare clinical outcomes and laboratory characteristics to those of patients taking OAC and/or antiplatelets. Six Centres have recruited patients (July 2018- March 2022). **Results**: Of 1050 patients recruited (median age 81, IQR 73-87; BMI: 25.7, range 15-46)], 755 (72%) were women. 635 (60.5%) had chronic hypertension, 194 diabetes (171 of them suffered from hypertension and diabetes), 266 (25.3%) suffered from cardiovascular disease. At admission, hemoglobin (Hb) median values were 12.2 g/dl (IQR 5-17), 794 (80.1%) patients showed values <10.5 g/dl. Non-0 group was recorded in 641 (65.3%) patients. Regarding surgical procedure, we have completed information on 871 patients, 356 (40.9%) of whom underwent an urgent surgery. Furthermore, 326 (37.4%) patients were taking antiplatelets and 247 (28.4%) OAC (185 of them for atrial fibrillation). The attached Table 1 summarizes use of peri-operative thrombo-prophylaxis, transfusion rate and follow-up on the entire cohort.

Table 1.

Perioperative antithrombotic prophylaxis

Perioperative prophylaxis	n= 831
LMWH (%)	764 (91.9)
AVK (%)	26 (3.1)
DOACs (%)	28 (3.4)
Antiplatelets (%)	13 (1.6)

Follow-up at 3 months

Type of event	n=978
Deaths	12^ (5 in patients on OAC, 1 in ASA)
Complications	60 (59 in OAC or antiplt)
VTE	3 (2 PE, 1 DVT)
TIA	2
Ischemic stroke	5
Major Hemorrhage	7

^ 3 Miocardial Infarction

Transfusion rate°

	Transfused n (%)
Transfused pts/ N of surgeries	339/1050 (32.3%)
Patients on OAC (n=326)	70/ 326 (21.5%)
Patients on antiplatelets (n=247)	23/247 (9.3%)

° PERI-OPERATIVE TRANEXAMIC ACID WAS USED IN 62 (7.5%) PATIENTS

Mortality rate was not significantly higher in those taking *vs* "not taking" OAC/antiplatelets (1.0 *vs* 1.3), whereas overall complications were significantly higher in patients taking OAC/antiplatelets (p<0.001). Transfusion rate was not dependent on chronic OAC or antiplatelets therapy, as among the 339 transfused, only 93 (27.4%) were taking OAC/antiplatelets. **Conclusions**: This registry gives a snapshot on peri-

operative management of Italian patients undergoing major orthopedic surgery. This preliminary analysis shows that mortality rate, number of complications and transfusion rate in patients taking OAC or antiplatelets are not higher that those observed in other patients.

OC096

MANAGEMENT AND OUTCOMES OF PATIENTS WITH DOAC-RELATED GASTROINTESTINAL BLEEDING: FINDINGS FROM PROSPECTIVE COHORT STUDY

M. Verso[1], O. Morelli[2], D. Castellani[2], G. Bassotti[2], A. Vinci[1], C. Becattini[1]

[1]Emergency and Vascular Medicine-Stroke Unit, University of Perugia; [2]Gastroenterology Division, University of Perugia, Italy

Background and Aims: Direct oral anticoagulants (DOACs) have been associated with an increased risk of gastrointestinal bleeding (GIB). The aim of the study was to prospectively collect data on real-life management and outcomes of DOACs-related GIB. **Methods**: A prospective, cohort study with a 6-month follow-up (FUP) was performed. **Results**: From November 2013 to March 2022, 208 patients (121 male) with DOACs-related GIB were included. At entry, the mean patient age was 83±4 years. 63 (30.1%%) patients presented GIB on dabigatran (low dose in 45), 73 (35.1%) on rivaroxaban (low dose in 35) 48 (23.1%) on apixaban (low dose in 15) and 24 (11.5%) on edoxaban (low dose in10). 9.6% received inappropriate doses of DOACs. The indication for DOAC treatment was atrial fibrillation (AF) in 87.5% of patients, venous thromboembolism in 9.1% and both indication in 3.4% of patients. In AF patients, median CHA2DS2-VASC was 4.5 and median HAS-BLED was 2.6. The mean number of concomitant drugs was 6. According to the ISTH criteria, GIB was classified as major in 152 patients and as clinically relevant non-major bleeding in 56 patients. The bleeding site was in upper GI tract for 79 patients (42.5%), in lower gastrointestinal tract in 65 patients (34.9%), in both upper and lower tract in 42 patients (22.6%). In 22 patients (10.6%), the endoscopic procedures were not performed or were not able to detect the source of bleeding. All patients discontinued the DOAC administration, except one. 143 patients were transfused with packed red cells, with a median number of blood transfusions of 2.4. A reversal strategy was adopted in 13.5% of patients. The endoscopic procedures were performed within 24 hours after admission in 52.0% of cases. Fifty-four patients received an endoscopic haemostasis. The anticoagulant treatment was discontinued in 50 patients. During the 6-month FUP, 12 patients had a bleeding recurrence and 8 patients had thrombotic complications. Six patients died during hospitalization and 45 during 6-month FUP. **Conclusions**: Patients with DOAC-related GIBs were old and comorbid patients. General supportive measures seem to be effective in the most of cases. The rate of death was clinically relevant.

OC097

PROSPECTIVE EVALUATION OF CANCER PATIENTS AT HIGH THROMBOTIC RISK DURING ANTICANCER TREATMENT WITH KHORANA AND VIENNA-CATS NOMOGRAM SCORES

C. Verzeroli[1], M. Marchetti[1], C. Giaccherini[1], L. Russo[1], S. Gamba[1], C.J. Tartari[1], S. Bolognini[1], C. Ticozzi[1], A. Santoro[2], F. De Braud[3], G. Gasparini[4], F. Petrelli[5], F. Giuliani[6], A. D'Alessio[7], M. Minelli[8], R. Labianca[9], C. Morlotti[10], P. Malighetti[10], D. Spinelli[11], A. Falanga[1,12] on behalf of the HYPERCAN Investigators*

[1]Immunohematology and Transfusion Medicine, ASST Papa Giovanni XXIII, Bergamo, Italy; [2]Oncology Unit, IRCCS Humanitas Institute, Rozzano, Italy; [3]Oncology Unit, IRCCS National Cancer Institute, Milan, Italy; [4]Oncology Unit, Hospital San Filippo Neri, Rome, Italy [5]Oncology Unit, Hospital Treviglio-Caravaggio, Treviglio, Italy; [6]Oncology Unit, IRCCS Cancer Institute Giovanni Paolo II, Bari, Italy; [7]Medical Oncology and Internal Medicine, Policlinico San Marco, Zingonia-Bergamo, Italy; [8]Oncology Unit, Hospital San Giovanni Addolorata, Rome, Italy; [9]DIPO, ASST Papa Giovanni XXIII, Bergamo, Italy; [10]Università degli Studi di Bergamo, Bergamo, Italy; [11]Università degli Studi Milano-Bicocca, Milan, Italy; [12]University of Milan Bicocca, School of Medicine, Italy

Background and Aims: Identifying cancer outpatients at high risk of venous thromboembolism (VTE), in whom anticoagulant thromboprophylaxis is justified, is an unmet clinical need. The use of validated risk assessment models (RAMs) involving clinical and biological parameters can be a relevant approach to hit this goal. Among several proposed RAMs, the Khorana risk score (KRS) and the Vienna-CATS nomogram score have been developed and externally validated in newly diagnosed ambulatory cancer patients and currently appear to be the most promising ones. In a large prospective cohort of 1,286 outpatients with metastatic non-small cell lung, colorectal, gastric, or breast cancers undergoing chemotherapy [the HYPERCAN study], we aimed to describe the incidences of objectively verified VTE and compare the discriminatory performance of the two validated RAMs (KRS and the Vienna-CATS nomogram) for the identification of high VTE risk subjects. **Methods**: KRS and Vienna-CATS nomogram score were applied to the HYPERCAN cohort using pre-chemotherapy patient variables (leucocyte and platelet counts, hemoglobin level, and BMI for KRS and D-dimer levels for Vienna-CATS nomogram). Only VTE events within 6 months, confirmed and validated by the Independent Central Adjudication Committee, were included in the analysis. The actual VTE rate according to KRS and Vienne-

CATS nomogram score was estimated by the Kaplan-Meier method considering death as a competing event. **Results**: One hundred-twenty objectively confirmed VTE (10.3%) were recorded, including isolated deep vein thrombosis (DVT, 45.8%), pulmonary embolism (PE, 42.5%), and PE+DVT (11.7%). By ROC analysis, KRS provided a non-relevant AUC of 0.39 for 6-months VTE. Cumulative VTE incidence was 6.0 % (95% CI 3.8-9.7) for KRS<2 and 12.3% (95% CI 9.9-15.3) for KRS≥2 (p=ns). Differently, Vienna-CATS nomogram score provided an AUC of 0.63 for 6-months VTE. Cumulative VTE incidence was 7.2% (95% CI 5.6-9.2) and 17% (95% CI 13.5-21.3) in low- and high-risk patients, respectively (SHR=2.4, p<0.001). **Conclusions**: In the prospective cohort of metastatic cancer patients of the HYPERCAN study, the KRS showed a low performance. Differently, the Vienna-CATS nomogram score significantly categorized patients at low VTE risk, in which the increased risk of bleeding due to thromboprophylaxis would outweigh benefits, and high VTE risk, which might benefit from thromboprophylaxis. Therefore, the inclusion of D-dimer in RAM, as in the Vienna-CATS nomogram, seems to be a promising approach toward reliable and clinically applicable RAMs for VTE prediction in these widespread cancers.

Funded by Italian Association for Cancer Research (AIRC) "5xMILLE" n.12237 grant.

OC098

CONSUMPTIVE COAGULOPATHY AFTER TREATMENT WITH CAR-T CELLS CORRELATES WITH MARKERS OF ENDOTHELIAL ACTIVATION

E. Galli[1,2], F. Sorà[1,2], F. Autore[2], E. Metafuni[2], I. Innocenti[2], A. Fresa[1], M.A. Limongiello[2], S. Giammarco[2], L. Laurenti[1,2], A. Bacigalupo[1,2], P. Chiusolo[1,2], V. De Stefano[1,2], S. Sica[1,2]

[1]Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica del Sacro Cuore, Roma; [2]Area Ematologica, Dipartimento di Diagnostica per Immagini, Radioterapia Oncologica ed Ematologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy

Background and Aims: Cytokine release syndrome (CRS) and classical consumptive coagulopathy are associated in up to 56% of patients treated with chimeric antigen T (CAR) cells. In this report, we describe coagulative patterns and laboratory parameters of endothelial activation following CAR-T cells reinfusion. **Methods**: Here we report 27 consecutive patients with aggressive lymphoproliferative diseases treated with CAR-T cells in our center from September 2019 to April 2022. The main diagnoses were diffuse large B cell lymphoma and primary mediastinal B cell lymphoma. In the setting of hematopoietic stem cell transplantation, endothelial activation has been correlated to an easy bedside score, the Endothelial Activation and Stress Index (EASIX). Recently, Pennisi and colleagues reported that a modified version of EASIX (mEASIX)- combining platelets, CRP, and LDH - correlated with CRS and oncological outcomes. Firstly, we measured a baseline coagulative asset on day 0 (D0) before CAR-T reinfusion, and we compared it with a coagulative pattern sampled on day 4 (D4). We then calculated mEASIX for every other day following CAR-T cells reinfusion, we graded daily CRS and ICANS and we analyzed correspondent coagulation parameters. **Results**: Between D0 and D4, we found a significant decrease in antithrombin and platelets, and a concurrent increase of aPTT, INR, and D-dimer (paired t-test, p<0.001, <0.001, 0.001, <0.001, and 0.01 respectively) (Figure 1A). Fibrinogen did not significantly modify between D0 and D4 (median 390 *vs* 427 mg/Dl, p=0.36); nevertheless, fibrinogen is highly specific in predicting consumptive coagulopathy but shows low sensitivity in highly inflammatory conditions. We hypothesized that fibrinogen remained stable due to opposite trends originating from activated consumptive coagulopathy and acute inflammatory state. To corroborate this hypothesis, we verified that fibrinogen and CRP were positively correlated even in the post-infusional setting of CAR-T cells treatment (p<0.001). On D4, 5/20 (25%) evaluable patients presented an ISTH score of 5, consistent with disseminated intravascular coagulation (DIC). This score takes into account platelet count, fibrinogen, PT, and D-dimer; however, low platelet count may also be due to the previous fludarabine-based lymphodepletion in this setting, and DIC scoring systems may not be performant. Greater mEASIX correlated with higher aPTT, D-dimer, fibrinogen, and von Willebrand factor, and with lower antithrombin (Figure 1B). CRS graded more than 1 was associated with higher aPTT, fibrinogen, factor VIII and von Willebrand factor. Occurrence of neurotoxicity (ICANS) correlated with higher D-dimer and showed a trend with higher aPTT. In our cohort, no patients experienced major bleedings, and in one case, we observed a catheter-related deep vein thrombosis (DVT) with pulmonary embolism (PE).

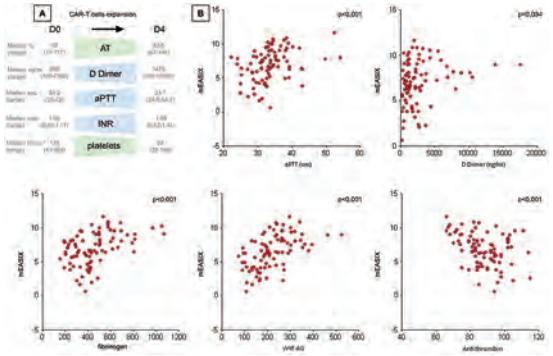


Figure 1.

Conclusions: Overall, we observed a laboratory consumptive coagulopathy immediately after CAR-T cells reinfusion, lacking a fibrinogen decrease possibly due to the high inflammatory burden. This altered coagulative asset figures as a laboratory – and not clinical- condition. Endothelial hyperactivation may play a key role,

enforced by the close connection between mEASIX, coagulopathy, and the increase of von Willebrand factor. The endothelial hyperactivation needs to be framed in the scenario of sustained moderate-to-severe CRS.

OC099

EFFECTIVENESS AND SAFETY OF THE ANTI-P2Y12 TICLOPIDINE AND CLOPIDOGREL AGENTS IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN COMPARISON WITH ASPIRIN

F. Ramundo, S. Ceglie, S. Betti, T. Za, A. Ciminello, F. Bartolomei, F. Di Landro, P. Arcuri, P. Chiusolo, E. Rossi, V. De Stefano

Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica, Fondazione Policlinico AGemelli IRCCS, Roma, Italy

Background and Aims: Aspirin (ASA) is widely employed in patients with Philadelphia-negative myeloproliferative neoplasms (MPN) for antithrombotic prophylaxis. Its efficacy has been firmly demonstrated in polycythemia vera (PV) by the ECLAP randomized clinical trial (RCT); however, ASA is also employed in pts with essential thrombocythemia (ET) and myelofibrosis (MF). Nevertheless, about 2% of the general population suffers from ASA intolerance, and about 5% of ASA users complain of gastric discomfort and gastrointestinal bleeding. Thus, many MPN pts are treated with anti-P2Y12 agents, mostly ticlopidine (TKL) and clopidogrel (CPG). Monotherapy with CPG has been reported to be more effective and safe than ASA for antithrombotic secondary prophylaxis. No comparative data are available for MPN pts. **Methods**: We examined the medical records of 1278 MPN pts diagnosed at our Center from 1978 to 2021; 1222 were genotyped for JAK2V617F, CALR, and MPL mutations. We recorded arterial and venous thrombosis and major bleedings. We computed the observation pt-years under continued monotherapy with ASA 100 mg once daily (od) or TKL/CPG. We excluded pts not receiving antithrombotic prophylaxis (n=38), without complete data (n=68), receiving oral anticoagulants (VKA or DOACs) (n=119), or receiving ASA <100 mg od or other antiplatelet drugs or dual antiplatelet treatment (n=19). Moreover, we excluded the pts receiving >2 lines of antithrombotic treatment where the time estimate under ASA or TKL/CPG was fragmented (n=241). In patients having received only ASA or TKL/CPG during their clinical history, we estimated the thrombosis- and bleeding-free survival according to the Kaplan Meier method; pts were censored at the time of death, thrombosis, bleeding, or the last visit. The incidence rate (IR) per 100 pt-years was also estimated, dividing the number of thrombotic events for the total observation years. **Results**: We included in the study 793 pts (M/F 339/454, median age 59, range 16-93). The diagnosis was PV in 273 pts. ET in 406, pre-MF in 53, MF in 57, and unclassifiable MPN in 4; 710 and 83 pts received ASA and TKL/CPG as unique antithrombotic treatments during 4406 and 531 observation years, respectively. Thromboses were 84 (arterial/venous 20/64) in the ASA group and 19 (A/V 8/11) in the TKL/CPG group. The IR of thrombosis per 100 pt-years was 1.91 and 3.58 under ASA and TKL/CPG, respectively (p=0.01). The hazard ratio for thrombosis in the TKL/CPG *versus* ASA pts was 2.36 (95%CI 1.25-4.45, p=0.007) (Figure 1). There was a significant difference between ASA and TKL/CPG in the IR per 100 pt-years of arterial thrombosis (0.45 *vs.* 1.51, p=0.002), but not for venous thromboses (1.45 *vs.* 2.1, p=0.3). In ET pts, the IR per 100 pt-years of thrombosis was 1.48 and 3.7 under ASA and TKL/CPG, respectively (p=0.02). In contrast, we detected no significant difference in the PV and pre-MF/MF cohorts (p=0.3 and p=0.8). We recorded 63 major bleedings in the ASA group and 3 in the TKL/CPG group (IR per 100 pt-years 1.43 *vs.* 0.56, p=0.1). **Conclusions**: In MPN pts, the anti-P2Y12 antiplatelet agents TKL and CPG are less effective than ASA in preventing thrombosis; this finding is more evident for arterial thrombosis and ET patients. No difference was found in the IR of major bleeding events. Therefore, special attention should be paid to antithrombotic prophylaxis administered to MPN patients with contraindications to ASA, and ad-hoc studies are needed.

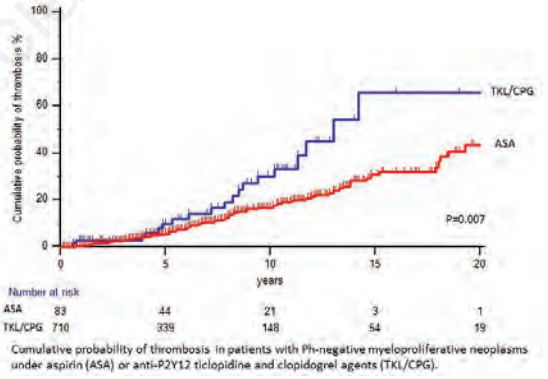


Figure 1.

OC100

ARTERIAL AND VENOUS THROMBOSIS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A SINGLE-CENTER RETROSPECTIVE ANALYSIS OF 577 CONSECUTIVE PATIENTS

E. Morsia[1], E. Torre[1], F. Marrara[1], I. Battilà[1], G. Tarantino[2], G. Svegliati Baroni[2], A. Olivieri[1], S. Rupoli[1]

[1]Clinica di Ematologia, Ospedali Riuniti di Ancona; [2]Clinica di Gastroenterologia, Ospedali Riuniti di Ancona, Italy

Background and Aims: Arterial thrombotic events and venous thromboembolism (VTE) are the most common causes of morbidity and mortality in the myeloproliferative neoplasms (MPN). MPN are the leading systemic cause of non-provoked VTE in unusual site, including splanchnic vein thrombosis (SVT) and cerebral vein thrombosis (CVT). This subgroup of patients has been showed to have distinct characteristics compared to MPN patients with VTE in usual site, including deep vein thrombosis and pulmonary embolism. The aim of this study is analyzed clinical features, molecular signature and outcome data in a real-life single-center cohort of MPN patients who have experienced thrombosis, with a particular focus on unusual site VTE. **Materials and Methods**: In our study, we retrospectively analyzed a cohort of 577 consecutive patients with MPN who referred to our institute between 2009 and 2020. JMP® Pro 14.0.0 software from SAS Institute, Cary, NC, USA, was used for all statistical analysis. **Results**: Among all the MPN population, 19.58% of patients (n=113) experienced a vascular event during a median follow up of 94.3 months (range, 2.4-416.0). According to WHO 2016 criteria, diagnosis of Polycythemia Vera, Essential Thrombocythemia, Primary Myelofibrosis, and MPN-unclassified was made in 26 patients, 54 patients, 28 patients, and 5 patients, respectively. In our cohort, only 29 patients presented arterial thrombotic events, including myocardial infarction (45%), stroke (41%), and peripheral arterial disease (14%). Usual and unusual site venous thrombosis occurred in 41% and 34% in MPN patients with thrombosis, respectively. From the 38 MPN patients with unusual site VTE, 27 patients had an SVT and 11 a CVT. In this setting of patients, the driven mutation was JAK2V617F in 79% with a median allelic burden of 20% using NGS, followed by CALR in 13% of patients. Compared to those with usual site VTE, MPN patients with SVT and CVT are younger (ORR 0.87 (0.82-0.93), pValue <0.0001), with higher splenomegaly rate (ORR 0.97 (0.90-0.99), pValue=0.0003), and with higher PLT count at diagnosis (ORR 0.06 (0.01-0.38), pValue=0.0085). Unlike usual site VTE, unusual site VTE seems to presented as the first symptom of disease in about half of cases, revealing an underlying MPN (pV<0.001). Long-term oral anticoagulation with vitamin K-antagonists (VKA) was adopted in 71% of SVT and CVT patients due to the MPN-related permanent prothrombotic state, but only 32% of MPN patients with usual site VTE was treated with VKA. Survival data in our population reported an overall survival (OS) of 238 months (95% I.C. 177-336), poorer in patients with arteriosus events compared to VTE (usual and unusual sites; pValue=0.017). (Figure 1) Older age (HR 1.06 (1.02-1.84), pValue=0.042) and level of hemoglobin at time of thrombosis (HR 0.47 (0.39-0.76), pValue=0.004) significantly influence survival in MPN patients with unusual site VTE. Finally, during the follow-up only few patients experienced recurrence thrombosis with a recurrence thrombosis free survival rate at 5 years of 85%. **Conclusions**: Our results confirm the thrombotic rate in MPN patients already reported in literature and highlight the role of unusual site thrombosis as clue to look for an underlying MPN, particularly in young patients with splenomegaly and high platelet level.

OC101

IDIOPATHIC ERYTHROCYTOSIS WITH THROMBOTIC COMPLICATIONS HAS AN ALTERED BIOMOLECULAR PROFILE

A. Benetti, I. Bertozzi, E. Cosi, G. Biagetti, M.L. Randi

First Clinical Medic, Department of Medicine - DIMED, University of Padova, Italy

Background and Aims: Polycythemia vera (PV), a myeloproliferative neoplasm, is characterized by a consistent risk of hemorrhagic and thrombotic complications, considered mainly due to rheological alterations induced by the excess of red blood cells (RBC). A diagnosis of idiopathic erythrocytosis (IE) is ruled out when all the known causes of erythrocytosis are excluded after a complete and accurate diagnostic process. Surprisingly, in a recent paper, the estimated prevalence of thrombotic complications in IE resulted about half than in PV patients, in spite of the high hematocrit (HT) levels. The availability of next generation sequences (NGS) and of a specific genes panel to search molecular new alterations in patients with unexplained erythrocytoses prompted us in comparing patients' clinical data with their molecular genotype. **Methods**: A complete biomolecular study was available in 122 patients (102 males and 20 females, mean age 53.5±17.2 years). DNA was extracted by granulocytes of patients' blood samples using EuroGold Blood DNA Mini Kit Plus (EuroClone) according to the manufacturer's instructions and stored at -20°C. DNA concentration was determined by Quibit 4 Fluorometer with QubitTM 1X dsDNA HS Assay Kits (Thermo Fisher Scientific). The coding sequencing of genes (EGLN1, EPAS1, VHL, EPOR) well known to be involved in erythrocytosis were studied with NGS. The panel is an "On Demand AmpliSeq Panel" designed from the Illumina Design Studio platform and validated *in silico* by Illumina. **Results**: At the time of the study, in our 122 patients, RBC were 5.67±0.3 x 1012/L, hemoglobin (Hb) 172.5±10.5 g/L and HT 50.75±2.56%. No patients had altered white blood cells (WBC) or platelet (Plts) number. Mean serum erythropoietin (EPO) levels was 10.7±7 U/L. In 22 (18%) patients EGNL1, 3 (2.4%) VHL, 10 (8.2%) germinal JAK2 and 7 (5.7%) EPAS1 mutations were found. Only in 4 cases (3.3%) a major thrombotic event was reported and the Table 1 contains their main data. **Conclusions**: The prevalence of thrombosis observed in our cohort is lower than the once reported in the literature (Am. J. Hematol. 2017;92:E639-E641). Even if few patients in this cohort had thrombotic complications, half of them carry mutations in genes involved in erythrocytosis suggesting that the presence of bio-molecular alterations may have a correlation with the thrombotic risk. One patient out of the four with VHL mutations had a myocardial infarction (MI); VHL mutations

are known to be associated with cerebral-vascular events and tumor developing while, in contrast, our patient had a MI. The germline mutations in JAK2 gene has been reported to precede the acquisition of PV, JAK2V617F typical somatic mutation, and thrombotic complications may precede PV overt diagnosis: we will strictly follow this patient in the suspicion of development of PV. Larger studies are needed to confirm this observation.

Table 1.

Sex/age	RBC	Hb	HT	Plts	WBC	EPO	genotype	thrombosis
M/60	5.89	188	53.1	180	17	6.5	VHL Pro25Leu + EGLN1 Cys127Ser	MI
M/82	5.64	184	54.4	210	7.6	4.4	JAK Asn1107Leu	Stroke
M/58	5.52	181	53	320	11.8	15		MI
F/83	5.62	177	51	234	7.59	5.1		Stroke

OC102

HEMOSTATIC BIOMARKERS MAY PREDICT AN INCREASED RISK OF CANCER OCCURRENCE IN HEALTHY SUBJECTS: RESULTS FROM THE HYPERCAN STUDY

S. Gamba[1], M. Marchetti[1], L. Russo[1], C. Giaccherini[1], S. Bolognini[1], C.J. Tartari[1], C. Verzeroli[1], C. Ticozzi[1], A. Vignoli[1], F. Schieppati[1], G. Sampietro[2], P. Malighetti[3], D. Spinelli[3], A. Falanga[1,4]

[1]Department of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo, Italy; [2]Unit of Epidemiology and Statistics, ATS Bergamo, Italy; [3]Department of Management, Information and Production Engineering, University of Bergamo, Bergamo, Italy; [4]Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

Background and Aims: Malignancy induces a hypercoagulable state in the host, that may represent the initial manifestation of occult cancer. The aim of this study is to establish whether, in a large cohort of healthy blood donors from the Bergamo area enrolled in the HYPERCAN study, a hypercoagulable state or its persistence may predict early cancer diagnosis. **Methods**: The HYPERCAN study is a prospective Italian, multicenter observational study specifically designed to evaluate whether hemostatic biomarkers may be an innovative tool for risk assessment, early diagnosis, and prognosis in cancer. From 2012 to date, 10,294 healthy blood donors (72% males; median age: 47 years) have been prospectively enrolled in the study and followed up for at least 5 years for cancer occurrence. Blood samples were collected at enrollment (T0) and after 6-18 months (T1), together with clinical, hematological, and lifestyle questionnaires. Search for malignant tumors was conducted every 6 months. Plasma D-dimer, FVIII, fibrinogen, tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) were measured. **Results**: During a median follow-up of 5.9 years, 217 blood donors developed cancer, 190 (122M/68F, 54 years) of them received a cancer diagnosis after at least 6 months from enrollment (cases). Incident cancer cases were ascertained by direct linkage with the hospital discharge form. The most frequent tumor site was breast (45%) in females and prostate (25%) in males, followed by non-melanoma skin (11%) and colon-rectum (8%) cancers. From the same HYPERCAN cohort, we extracted a group of 286 randomly cancer-free subjects (154M/132F, 53 years) to perform a case-control study. Hemoglobin, hematocrit, tPA, and FVIII levels at T0 were significantly higher in cases compared to controls. Logistic regression analysis corrected for gender showed that high tPA levels at T0 were significantly associated with cancer occurrence (OR=1.052, p=0.039). By ROC curve analysis (AUC=0.563) a cut-off of 5.62 ng/mL of tPA was established to predict cancer occurrence. Logistic regression analysis corrected for age, smoke habits, alcohol intake, BMI, and hypertension revealed that a tPA level >cut-off was an independent risk factor for cancer occurrence (OR 1.70; 95% CI:1.04-2.77; p=0.034). In the same cohort of case-control donors, we also compared the variation in the hemostatic biomarker levels between T0 and T1 in relation to cancer risk. We found that only D-Dimer levels were significantly (p=0.023) different between the two-time points. An increase of one or more quartiles in D-dimer levels from T0 to T1 was significantly associated with cancer occurrence in a model of logistic regression analysis corrected for age, smoke habits, alcohol intake, BMI, hypertension (OR 1.80; 95% CI:1.05-3.01; p=0.032). **Conclusions**: Our study shows that among the different hemostatic parameters analyzed, tPA and D-dimer seem to be potential biomarkers to identify subjects at increased cancer risk. In particular, higher levels of tPA, measured at enrollment, are significantly associated with cancer occurrence. In addition, the predictive capacity of higher D-dimer levels is linked to the possibility to measure its variation over time.

OC103

VENOUS THROMBOEMBOLISM (VTE) IN PATIENTS WITH HODGKIN LYMPHOMA: MONOCENTRIC STUDY OF THROLY AND KHORANA RISK SCORES

G.M. Assanto, A. Serrao, S. Pontecorvo, F. Malfona, G.M. D'Elia, G. Annechini, A. Pulsoni, I. Del Giudice, A. Chistolini

Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy

Background and Aims: Venous Thromboembolism (VTE) affects the morbidity of Hodgkin Lymphoma (HL) patients. Thrombosis Lymphoma (ThroLy) and Khorana scores, established for lymphomas and cancer patients, can be applied to HL to assess the basal risk of VTE, due to heterogeneous thrombotic risk; currently there is no indication to perform thrombosis prophylaxis. The study's aim was to validate prognostic scores and to assess clinical factors, which could improve their predic-

tive power. **Methods**: Retrospective monocentric cohort study, we evaluated patients with newly diagnosed HL (from 2018 to 2020). A total of 134 patients were included to validate these scores and evaluate other potential risk factors in terms of thrombotic event rate and thrombotic-free survival. The median age at diagnosis was 42 years (range 18-85), and median follow-up was 23 months (range 6-49), thrombotic scores were validated in univariate and multivariate analysis for categorical and continuous variables as an association to primary endpoints. **Results**: According to Khorana criteria, intermediate-risk (IR) were 80.2% (n=105), high-risk (HR) were 19.8% (n=26). Regarding ThroLy criteria, low-risk (LR) patients were 16.7% (n=22), IR were 57.6% (n=76) and HR were 25.8% (n=34). All patients received first-line therapy, and 19 (14.4%) subsequently experienced relapse/progression. Central venous catheter (CVC) was implanted in 42.9% (n=57). No patient had previous thrombotic events. A total of 22 thrombotic events were observed after a median follow-up of 20 months (range 1-45), 10 were CVC-related. The presence of CVC was related to a higher incidence of thrombotic events, 23% against 10.8% thrombotic rate in patients without (p 0.049, OR 1.6 0.90-2.79). Khorana HR group showed an increased rate of thrombotic events compared to IR, 28% (7/25) *vs* 11.7% (12/103) respectively (p 0.045). A robust association was found between ThroLy risk categories and thrombotic risk: among HR patients, VTE was observed in 32.4% (11/34), against 10.5% (8/76) and 4.55% (1/22), for IR and LR respectively, with an estimated thrombotic EFS of 30 months for HR *vs* 44 for LR (p 0.002 Figure IA). Prophylaxis with enoxaparin abrogated the thrombotic risk in HR patients (p 0.56). Hypertension (HTA) showed a significant association to thrombotic events, other factors did not show a significant association. Among hypertensive patients, a higher rate of VTE was observed with 29.6% (8/27) as opposed to 12.6% (13/103) in non-hypertensive patients (p 0.038). HTA (HR 2.44, p 0.04 CI1.01-6) and HR ThroLy (HR 2.81, p 0.009 CI1.2-6) were confirmed on multivariate analysis. We then defined a new risk category which included HR ThroLy patients with HTA, which showed inferior estimated EFS *vs* other HR patients (21 *vs* 35 months) with an incidence of 50% of VTE events (6/12) *vs* 22.8% of conventional HR (5/22) (p 0.001 Figure IB). **Conclusions**: ThroLy score proved its utility in VTE-risk assessment, HTA could be integrated to augment ThroLy predictive power improving the management of VTE in HL, thrombotic prophylaxis should be addressed to high-risk categories.

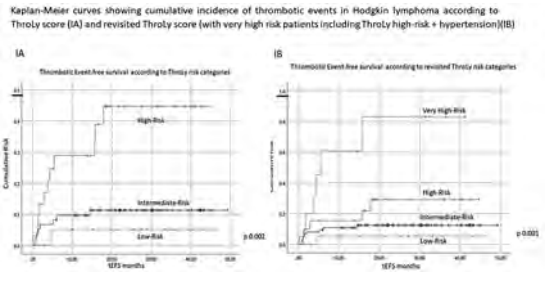


Figure 1.

OC104

THE IMPACT OF HEMATOCRIT ON POSTOPERATIVE BLEEDING RISK IN PATIENTS UNDERGOING ROBOTIC RADICAL PROSTATECTOMY

S. Arcudi[1], A. Artoni[1], E. De Lorenzis[2], C. Silvani[2], F. Spanu[3], S. Villa[3], M. Capecchi[1], E. Montanari[2], F. Peyvandi[1]

[1]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy; [2]Urology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [3]Clinical Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Background and Aims: Robot-assisted radical prostatectomy (RARP) represents the first-choice surgical procedure for the treatment of localized prostate cancer. The effects of blood parameters and blood group on the bleeding risk had never been investigated. Given this background, our study aims to investigate whether blood parameters and/or blood group may influence the bleeding risk in patients undergoing RARP. **Methods**: We conducted a prospective cohort study enrolling consecutive patients undergoing RARP in our hospital, from January 2017 to December 2020. Patients were evaluated with full blood count before and after surgery. Demographic and clinical data were collected with particular attention to other known risk-factors for RARP-associated bleeding (prostate volume, BMI, smoking status, nerve sparing technique). Bleeding was assessed both as direct quantification of blood loss during surgery and as the difference in hemoglobin values pre- and post-surgery. Linear regression analyses were performed, corrected for confounders and known risk factors for RARP-associated bleeding, and risk estimates were evaluated with the method of Woolf. **Results**: In the study period 191 consecutive patients were enrolled; mean age was 67.8 years. Mean blood loss was 366.2 mL and mean hematocrit pre-surgery was 42.9%. Table 1 shows the pre-surgical clinical characteristics of the 191 patients enrolled. Continuous variables are expressed as mean, range and standard deviation (SD). When analyzing the basal hematocrit with linear regression, a strong negative correlation was seen with the difference in hemoglobin pre- and post-surgery ($p<0.001$). The relationship remained strong after correction for age, smoke, BMI, prostate volume and nerve sparing ($p<0.001$). Pre-surgery hemoglobin levels were less associated with an increased bleeding risk (p=0.07), while PT, aPTT and platelet count did not influence surgical losses. Individuals carrying the blood group O were not at increased risk for higher blood loss (OR=0.76; 95%CI 0.4-1.4; p=0.4). **Conclusions**: A lower basal hematocrit is associated with an increased bleeding during RARP. It can be hypothesized that patients with higher hematocrit may be more hemostatic

competent since platelets are radially pushed near the vessel's wall, as previously described in other settings.

Table 1.

	Age	Blood loss (ml)	Hb (g/dL)	Htc (%)	PLT ($x10^9$/mL)	PT	aPTT
Mean	67.8	366.2	14.7	42.9	227.4	1.0	1.0
Max	79	1,400.0	18.6	52.7	721	1.25	1.84
Min	48	36.5	9.6	28.0	88	0.84	0.77
SD	6.11	236.7	1.32	3.65	64.4	0.66	0.11

OC105

PHARMACOLOGICAL THROMBOPROPHYLAXIS TO PREVENT VENOUS THROMBOEMBOLISM IN AMBULATORY PATIENTS WITH CANCER UNDERGOING ANTINEOPLASTIC DRUGS IN REAL WORLD CLINICAL PRACTICE: A COHORT STUDY

M.P. Donadini[1], A. Squizzato[2], P. Ferrazzi[3], C. Secco[3], S. Maggioni[4], C. Lodigiani[5]

[1]Department of Medicine and Surgery, University of Insubria and ASST Sette Laghi, Varese, Italy; [2]Research Center on Thromboembolic Disorders and Antithrombotic Therapies and ASST Lariana, University of Insubria, Como, Italy; [3]IRCCS Humanitas Research Hospital, Rozzano, Italy; [4]School of Medicine, University of Insubria, Varese, Italy; [5]Center for Thrombosis and Hemorrhagic Diseases, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Background and Aims: Venous thromboembolism (VTE) is the second leading cause of death in cancer patients. Clinical prediction models can identify ambulatory patients undergoing antineoplastic drugs who may benefit from primary pharmacological thromboprophylaxis. Evidence is scant on determinants of thromboprophylaxis and outcomes in clinical practice. Aim of the study was to estimate the incidence of VTE in a cohort of cancer ambulatory patients undergoing antineoplastic drugs, who were systematically assessed for VTE pharmacological thromboprophylaxis. **Methods**: In a retrospective, observational cohort study, based on a standardized protocol being active at the Humanitas Research Hospital from 2012, consecutive adult ambulatory patients starting antineoplastic drugs were evaluated for VTE thromboprophylaxis (low molecular weight heparin 80 UI/Kg) based on Khorana score and clinical judgment. The primary outcome was symptomatic VTE and asymptomatic deep vein thrombosis at ultrasound performed at 6 months. The study was approved by the local institutional review board. **Results**: 443 patients were enrolled up to November 2019. Baseline characteristics are described in Table 1. Among 206 patients (46.5%) who were given thromboprophylaxis, 112 (54.4%) had Khorana score ≥2, as compared to 30/227 (13.2%) who did not receive thromboprophylaxis (p <0.01). Indications for thromboprophylaxis in the remaining 94 patients who had Khorana score <2 were: previous VTE (6 patients), other VTE risk factors (31), cancer-related risk factors (34), thrombophilia (5), multiple indications (18). Patients receiving thromboprophylaxis had a worse performance status (p 0.03). At 6 months, VTE occurred in 32/443 (7.2%) patients, of whom 12/206 (5.9%) on thromboprophylaxis and 20/227 (8.9%) not receiving thromboprophylaxis (p 0.27). The same comparison for major bleeding was 3.9% and 2.6% (p 0.45) and for death 4.4% and 0.4% (p 0.01). **Conclusions**: In real-world clinical practice setting, pharmacological thromboprophylaxis choice was based on Khorana score or other clinical determinants. This strategy was associated with similar VTE incidence as compared to clinical trials.

Table 1. Baseline characteristics.

	Study population (433)	Thrombo-prophylaxis (206)	No thrombo-prophylaxis (227)	P value
Age, years - mean (SD)	61.2 (12.5)	62.1 (12.2)	60.4 (12.7)	ns
Males, n (%)	246 (56.8)	115 (55.8)	131 (57.7)	ns
Cancer type, n (%)				
Solid	425 (98.2)	203 (98.5)	222 (97.8)	ns
Haematologic	8 (1.9)	3 (1.5)	5 (2.2)	
Cancer site, n				
Brain	90 (20.8)	43 (20.9)	47 (20.7)	ns
Lung/Pleura	51 (11.8)	26 (12.6)	25 (11)	ns
Stomach	32 (7.4)	24 (11.7)	8 (3.6)	0.001
Esophagus	3 (0.7)	3 (1.5)	0 (0)	ns
Gallbladder/biliary tract	12 (2.8)	10 (4.9)	2 (0.9)	0.01
Liver	2 (0.5)	2 (1)	0 (0)	ns
Pancreas	59 (13.7)	48 (23.3)	11 (4.9)	<0.0001
Colon/rectum	142 (33)	26 (12.6)	116 (51.6)	<0.0001
Kidney	1 (0.2)	0 (0)	1 (0.4)	ns
Bladder	3 (0.7)	2 (1)	1 (0.4)	ns
Prostate	3 (0.7)	3 (1.5)	0 (0)	ns
Uterus	3 (0.7)	2 (1)	1 (0.4)	ns
Ovary	4 (0.9)	2 (1)	2 (0.9)	ns
Breast	1 (0.2)	0 (0)	1 (0.4)	ns
Testicle	1 (0.2)	1 (0.5)	0 (0)	ns
Sarcoma	9 (2.1)	6 (2.9)	3 (1.3)	ns
Other	15 (3.5)	8 (3.9)	7 (3.1)	ns
Stage, n (%)				
I	4 (0.9)	1 (0.5)	3 (1.4)	
II	37 (8.6)	12 (5.9)	25 (11)	0.09
III	122 (28.5)	55 (26.8)	67 (30)	
IV	265 (61.9)	137 (66.8)	128 (57.4)	
Metastases, n (%)	191 (44.3)	99 (48.1)	92 (40.9)	ns
ECOG performance status, n (%)				
0	228 (53)	94 (45.9)	134 (59.6)	
1	162 (37.7)	89 (43.4)	73 (32.4)	
2	33 (7.7)	18 (8.8)	15 (6.7)	0.03
3	6 (1.4)	4 (2)	2 (0.9)	
4	1 (0.2)	0 (0)	1 (0.4)	

SD standard deviation; ns not significant; ECOG Eastern Cooperative Oncology Group

OC106

ACUTE ISCHEMIC STROKE: TRANSCRIPTOMICS AS AN ALLURING APPROACH TO UNDERSTANDING THE PATHOPHYSIOLOGY AND CLINICAL OUTCOMES

G. Cassioli[1], A. Kura[1], A. Sodero[2], E. Sticchi[1], A. Magi[1], S. Suraci[1], R. De Cario[1], A. Consoli[3], A. Rosi[3], S. Nappini[3], L. Renieri[3], N. Limbucci[3], B. Piccardi[2], F. Arba[2], C. Sarti[2], D. Inzitari[2], S. Mangiafico[3], R. Marcucci[1], A.M. Gori[1], B. Giusti[1]

[1]University of Florence, Department of Experimental and Clinical Medicine, Florence; [2]AOU Careggi, Stroke Unit, Florence; [3]AOU Careggi, Interventional Neuroradiology, Florence, Italy

Background and Aims: Acute ischemic stroke (AIS) is a complex multifactorial disease characterized by a high rate of morbidity and mortality and represents one of the leading causes of death and disability worldwide. Current treatments are focused on recanalization of the occluded cerebral vessel via intravenous thrombolysis with rt-PA and/or endovascular mechanical thrombectomy (MT). The latter approach created a golden ticket from a research perspective, supplying new study material that enabled histopathological analysis of cerebral thrombi (CT), which not only provided important evidence on their cellular composition, but also represents a key pillar for the creation of virtual predictive models of different gene expression. The aim of the study is therefore to investigate global gene expression profiles of both CT and peripheral venous blood (PB), not only highlighting and exploring whether PB profiles mirrored CT profiles, but also focusing on the identification of potential markers of different pathophysiological mechanisms of AIS and/or determinants of clinical outcomes, such as haemorrhagic transformation, 24h edema, modified 3 months Rankin scale-mRS, death. **Methods**: We performed gene expression profiles of RNA samples obtained from 40 CT and 37 PB of 52 patients. The CT obtained during MT were stored in RNA later, while PB, collected before and 24 hours after MT, in tubes containing a reagent that protects RNA from degradation and minimizes *ex vivo* changes in gene expression. RNA was then extracted by PAX gene blood miRNA kit, the global gene expression profile was assessed by Affymetrix technology using GeneChip Human Transcriptome Array 2.0, allowing the analysis of 44,699 genes, with more than 285,000 full-length transcripts coverage. Data analysis was then performed in R environment with dedicated pipelines. **Results**: Data processing and the application of appropriate filtering criteria showed an average of analyzable probe sets of 440,085 in CT and 602,874 in PB. In the two different type of specimens 20,341 were found to be common features, whereas 3 and 562 symbols were unique in CT and PB, respectively. The Gene Ontology (GO) enrichment analysis allowed the identification of the biological processes, common and peculiar, in CT and PB, indicating that peripheral and local mechanisms of damage and response to damage are present in both. The significance analysis of microarrays, according to different outcomes and GO analysis, brought into focus 221 significant biological processes associated with poor outcome according to mRS in CT, and 27 terms associated with 24h edema in PB. Among significant terms in CT, those associated with regulation of neutrophil mediated immunity and activation play a crucial role. Concerning PB, particularly significant enriched terms were associated with regulation and activation of transcriptomes of cells. **Conclusions**: Our results provided interesting insights into the mechanisms underlying the AIS and the response to treatments. In particular, the analysis of CT and PB gene expression profiles, differentially expressed probe sets and their biological processes alterations according to stroke outcomes, has not only confirmed and extended several known pathophysiological mechanisms, but also suggested novel pathways to be explored that may provide an important starting point for expanding knowledge on ischemic stroke disease.

OC107

CIRCULATING MIRNAS RELEASE PREDICTS SUBOPTIMAL RESPONSE TO ASPIRIN IN PATIENTS AT HIGH CARDIOVASCULAR RISK WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

R. Liani[1,2], P.G. Simeone[1,2], S. Ciotti, R. Tripaldi[1,2], A. Boccatonda[2,3], D. D'Ardes[2,3], A. Recchiuti[4], M. Romano[4], F. Cipollone[1,2,3], F. Santilli[1,2,3]

[1]Center for Advanced Studies and Technology (CAST), Chieti University, Chieti, Italy; [2]Haemostasis and Thrombosis Unit CAST, Chieti University, Chieti, Italy; [3]Internal Medicine, Chieti University, Chieti, Italy; [4]Department of Medical and Oral Sciences, Italy

Background: The recovery rate of platelet COX-1 activity during the 12 to 24h dosing interval of aspirin administration, in aspirin-treated subjects, is characterized by substantial interindividual variability. Circulating myeloid-related protein (MRP)-8/14 is an inflammatory protein associated with residual thromboxane (TX)-dependent platelet activation in aspirin-treated patients with acute coronary syndrome. **Aims**: To identify any circulating miRNAs associated with a suboptimal ASA response in patients at high cardiovascular (CV) risk. **Methods**: Two-hundred high CV risk patients (100 with type 2 diabetes mellitus (T2DM)) in chronic treatment with ASA (100 mg/day), for cardiovascular prevention, were enrolled. Blood sampling was performed at 10 (T10) and 24 hours (T24) after a witnessed ASA administration. Patients were stratified in tertiles according to serum TXB_2 slope. First *vs.* third tertile were compared. Circulating miRNAs custom array cards were applied to assay the expression levels of 14 miRNAs in plasma. We also measured plasma myeloid-related protein (MRP)-8/14 as an inflammatory index and predictor of cardiovascular events. **Results**: miRNA-21-5p (p=.017), 22-5p (p=.026), 24-3p (p=.020), 150-5p (p=.026), 155-5p (p=.007), 181b-5p (p=.011), 223-5p (p=.021) were significantly lower in first *vs.* third tertiles at 24 hours after ASA administration in all patients (Figure 1). MRP-8/14 were higher in third *vs.* first $sTXB_2$ slope tertile in all patient. MRP-8/14 was directly correlated with miRNA-21-5p (rho=.279, p=.008), 22-5p (rho=.264, p=.012), 24-3p (rho=.239, p=.023), 150-5p (rho=.236, p=.025), 155-5p (rho=.270, p=.011), 181b-5p (rho=.240, p=.023) and 223-5p (rho=.244, p=.030) in all patients (data not shown). **Conclusions**: MRP 8/14 may contribute to circulating miRNA release and response variability to ASA. *Vice versa,* shorter duration of aspirin effect at 24 hours in third $sTXB_2$ slope tertile patients translates into higher degree of TX-dependent platelet activation, possibly promoting the release of both circulating MRP8/14 and miRNAs.

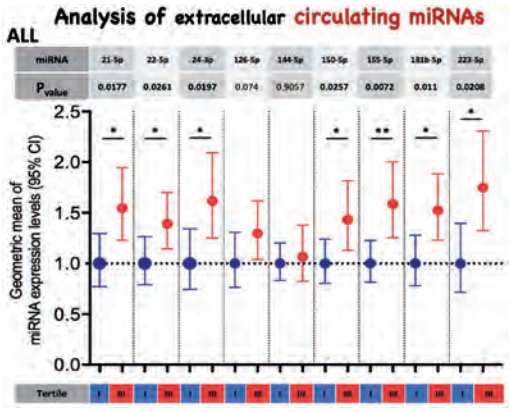


Figure 1.

OC108

GENETIC COMPONENTS IN FACTOR VIII RECEPTORS FOR INDIVIDUAL AND GENOTYPE-MODELED HEMOPHILIA A TREATMENT

B. Lunghi[1], M. Morfini[2], N. Martinelli[3], A. Branchini[1], S. Linari[4], G. Castaman[4], F. Bernardi[1]

[1]Department of Life Sciences and Biotechnology, University of Ferrara; [2]Italian Association Hemophilia Centers (AICE), Naples; [3]Department of Medicine, University of Verona; [4]Center for Bleeding Disorders, Department of Oncology, Careggi University Hospital, Florence, Italy

Background and Aims: Age, von Willebrand factor (VWF) levels and the ABO blood group all together explain only 30% of the large inter-patient variability of factor VIII (FVIII) pharmacokinetics (PK) in hemophilia A (HA) patients. Gene variation in the numerous scavenging receptors for circulating FVIII/VWF is candidate to explain an additional portion of variability. We have recently reported genetic components of PK variability with standard half-life (SHL) FVIII, most of them independent from VWF antigen levels, in i) the low-density lipoprotein receptor (LDLR) and in the C-type lectin domain family, particularly in ii) the asialoglycoprotein receptor minor subunit gene (ASGR2) and iii) the family 4 member M (CLEC4M). The LDLR c.81 CC homozygotes and the ABO O/CLEC4M rs868875 G-carriers showed fast FVIII clearance, and the ASGR2 c.-95 TC heterozygotes short mean residence time (MRT). Based on differential cell distribution (macrophages and liver sinusoid endothelial cells) and protein/glycan recognition of receptors, we compared the influence of these genetic components. **Methods**: Linear regression models of SHL FVIII two-compartment model PK parameters in 31 HA patients (FVIII coagulant activity ≤2 IU/dL). Main parameters (Alpha and Beta HL, Clearance, MRT) were evaluated in relation to the LDLR, ASGR2, CLEC4M, F8, and ABO blood-group genotypes, and to VWF antigen levels. **Results**: α-HL - Both the LDLR c.1773 TT (Beta-coefficient 0.329, p=0.047) and ASGR2 c.-95 TT (Beta-coefficient -0.432, p=0.013) were found to contribute to α-HL, with the ASGR2 association predicted independently from ABO genotypes. These data point out genetic components increasing (ASGR2 TT, 3.60h±0.94SEM) or decreasing (LDLR TT, 0.42h±0.16SEM) α-HL through FVIII/VWF-receptors interactions occurring in the initial FVIII distribution phase. These information may be relevant for FVIII concentrate dosing in on-demand treatment of bleeding. CLEARANCE - No genetic component remained a significant and independent contributor of Clearance, which may point out multiple interactions partially competing each other. Since infrequent homozygous conditions appear to contribute a large proportion of variance and unfavorable clearance (CLEC4M rs868875 GG, 4.30 ml/h/kg ±1.70SEM), further investigation is needed in larger studies. β-HL - The ASGR2 c.-95 T>C genotypes were the most significant contributors (TT *vs* TC, Beta-coefficient -0.548, p=0.009), independently from LDLR, CLEC4M and ABO genotypes. This model includes a significant prediction by ABO genotypes (Beta-coefficient 0.428, p=0.049). MRT - The ASGR2 c.-95T>C genotypes were significant contributors (Beta-coefficient -0.493 p=0.023) independently from LDLR, CLEC4M and ABO genotypes. Since also ABO genotypes were MRT modifier as a trend (Beta-coefficient 0.407, p=0.075), this analysis points out a specific glycan-dependent association. Further, the noticeable differences between ASGR2 genotypes values (c.-95TT, 40% longer than in c.-95TC) deserve to be explored for individual and genotype-based modulation of prophylaxis regimens. **Conclusions**: With the limitation of the small number of HA patients, these novel observations may reflect different FVIII-scavenging properties of receptors "*in vivo*" and provide valuable information for individual and genotype-based modulation of on demand/prophylaxis regimens. This analysis will also favor comparison of receptor function in prophylaxis with extended half-life FVIII concentrates.

OC109

A MULTI-TRAIT ASSOCIATION ANALYSIS OF PLATELET TRAITS AND BRAIN DISORDERS IDENTIFIES NOVEL SUSCEPTIBILITY LOCI FOR MAJOR DEPRESSION, ALZHEIMER AND PARKINSON DISEASE

A. Tirozzi[1], M. Shasa Quiccione[1], C. Cerletti[1], M.B. Donati[1], G. de Gaetano[1], L. Iacoviello[1,2], A. Gialluisi[1,2]

[1]Department of Epidemiology and Prevention, IRCCS NEUROMED, Pozzilli, Italy [2]EPIMED Research Center, Department of Medicine and Surgery, University of Insubria, Varese, Italy

Background: Among candidate neurodegenerative/neuropsychiatric risk-predictive biomarkers, platelet count (Plt), mean volume (MPV) and distribution width (PDW) have been associated with the risk of Major Depressive Disorder (MDD), Alzheimer's (AD) and Parkinson's disease (PD), both through epidemiological and through genomic studies, suggesting partial co-heritability. **Methods**: We exploited these relationships for a multi-trait association analysis (MTAG), using GWAS associations of three different platelet traits – Plt, MPV and PDW – and three neurodegenerative/neuropsychiatric disorders, AD, PD and MDD. Gene-based enrichment tests were carried out through MAGMA v1.08, while a network analysis of significantly enriched genes was implemented in dbSTRING v11.5. **Results**: We analyzed 4,540,326 Single Nucleotide Polymorphisms (SNPs) shared among the analyzed GWAS, observing 149 genome-wide significant multi-trait Linkage Disequilibrium independent associations ($p<5\times10^{-8}$) for AD, 70 for PD and 139 for MDD. Among these, 27 novel associations were detected for AD, 34 for PD and 40 for MDD. Out of 18,781 genes with annotated variants within ±10 kb, 62 genes were enriched for associations with AD, 70 with PD, and 125 with MDD ($p<2.7\times10^{-6}$). Of these, 7 genes were novel for AD (*EPPK1*, *TTLL1*, *PACSIN2*, *TPM4*, *PIF1*, *ZNF689*, *AZGP1P1*), 2 for PD (*SLC26A1*, *EFNA3*) and 1 for MDD (*HSPH1*). The resulting network showed a significant excess of interactions (enrichment $p=1.0\times10^{-16}$) (Figure 1).

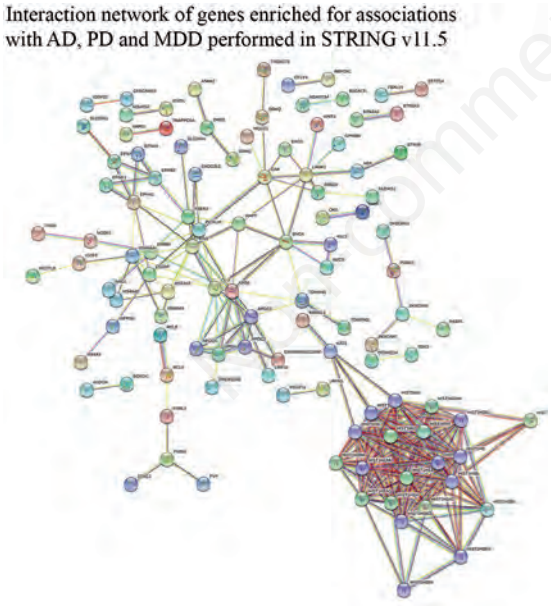


Figure 1.

Discussion: We identified novel genes involved in the organization of cytoskeletal architecture (*EPPK1*, *TTLL1*, *PACSIN2*, *TPM4*), in telomere shortening (*PIF1*), regulation of cellular aging (*ZNF689*, *AZGP1P1*) and in neurodevelopment (*EFNA3*), gaining novel insights into the underlying biology of neurodegenerative/neuropsychiatric disorders.

OC110

GENETIC DELETION OF PRENYLCYSTEINEOXIDASE 1 (PCYOX1) IMPAIRS ARTERIAL THROMBOSIS IN MICE

P. Amadio[1], C. Banfi[2], M. Zarà[1], M. Brioschi[2], S. Ghilardi[2], L. Sandrini[1], S.S. Barbieri[1]

[1]Unit of Brain-Heart Axis, Centro Cardiologico Monzino IRCCS, 20138Milano, Italy; [2]Unit of Functional Proteomics, Metabolomics and Network Analysis, Centro Cardiologico Monzino IRCCS, 20138Milano, Italy

Prenylcysteine Oxidase 1 (PCYOX1), an enzyme involved in the degradation of prenylated proteins, is expressed in different tissues, including vascular and blood cells. The secretome from *Pcyox1*-silenced cells reduced platelet adhesion to both fibrinogen and endothelial cells, suggesting its potential contribution to thrombosis. In this study, we analysed the contribution of PCYOX1 in arterial thrombosis by using a murine model. All procedures were performed on Pcyox1 knock-out (*Pcyox1*KO) mice and compared to littermate wild type (WT) mice. Ferric Chloride (FeCl3) arterial injury and pulmonary thromboembolism models have been used to induce thrombosis. The phenotype and the function of platelets were studied by flow cytometry and functional tests. The presence of PCYOX1 in platelets was assessed by mass spectrometry analysis. Thrombus formation after FeCl3 injury of the carotid artery was delayed in *Pcyox1*KO mice, which were also protected from collagen-epinephrine induced thromboembolism. *Pcyox1*KO mice displayed physiologic blood cell count, vascular pro-coagulant activity, and plasma fibrinogen levels but reduced platelet/leukocyte aggregates in whole blood, as well as platelet aggregation, alpha granules release and the αIIbβ3 integrin activation in platelet-rich plasma in response to ADP or TRAP. PCYOX1 was greatly expressed in washed platelets isolated from WT mice but phosphorylation pathway activation, adhesion ability and aggregation were similar to those observed in platelets from *Pcyox1*KO. The presence of *Pcyox1*KO mice-derived plasma, impaired agonists-induced aggregation of WT mice-derived platelets. Our findings, showing platelets hyporeactivity and impaired arterial thrombosis in the absence of PCYOX1, suggest it as a potential novel target for antithrombotic therapy.

OC111

FAMILIAL HYPERCHOLESTEROLEMIA: INSIGHT INTO GENETIC PREDISPOSITION PROFILE

E. Sticchi[1], A. Kura[2], R. De Cario[2], G. Barbieri[2], A. Magi[3], S. Suraci[2], A.M. Gori[1], F. Cesari[4], G.M. Scaturro[5], E. Lotti[4], R. Marcucci[1], B. Giusti[1]

[1]University of Florence, Department of Experimental and Clinical Medicine, Atherothrombotic Diseases Center, Careggi Hospital, Florence; [2]University of Florence, Department of Experimental and Clinical Medicine, Florence; [3]University of Florence, Department of Information Engineering, Florence; [4]Atherothrombotic Diseases Center, Careggi Hospital, Florence; [5]Metabolic Disease Unit, AOU Meyer, Italy

Background and Aims: Familial hypercholesterolemia (FH) represents a heritable disorder associated with pathogenic variants in LDLR or APOB or PCSK9 (dominant form), and in LDLRAP1 (recessive form) with increased risk of premature coronary artery disease. Moreover, previous data showed that in about 60% of patients who are mutation-negative the clinical phenotype can be associated with an accumulation of common small-effect LDL-C-raising alleles using a 12- Single nucleotide polymorphisms (SNPs) score (Talmud *et al.* 2013). In the present study we genetically characterize a cohort of adult and paediatric FH patients in order to provide further insight into the genetic predisposition profile. **Methods**: We analysed 90 FH patients [adults with clinically possible/probable or definite FH using the most common diagnostic algorithm, Dutch Lipid Clinic Network Score (DLCN)]. Genetic analysis was perfomed through a targeted high-throughput (HTS) panel of 57 genes, including loci involved in lipid metabolism, genes supposed to be involved in dyslipidemia, pharmacogenetics of statins, genes related to higher susceptibility for the polygenic forms of FH, HDL and triglycerides related diseases, and regions interested by polymorphisms of the Talmud genetic score. DNA libraries were prepared using Agilent HaloplexHS enrichment system, and sequencing was assessed by Illumina MiSeq platform. **Results**: Among 90 patients analyzed, 41 carried a rare variant in LDLR gene, whereas 49 patients were LDLR-negative. Talmud score evaluation in both groups showed a higher mean value in patients without LDLR mutations, with respect to the LDLR-positive group (0.972±0.207 *vs* 0.941±0.175). HTS analysis revealed that 14 LDLR mutation-positive patients also carried likely pathogenetic/uncertain significance mutations in APOB or LDLRAP1 genes. In patients without LDLR mutations, at least 2 rare variants were identified in 24 patients (49%), and at least 3 rare variants were identified in 18 patients (37%). In these patients, a total of 117 rare variants with uncertain significance/conflicting interpretation of pathogenicity have been identified in 44 different genes (APOB, PCSK9, LDLRAP1, ABCB1, ABCG2, ABCG5, ABCG8, ANGPTL3, APOA4, CELSR2, CETP, CREB3L3, DAB2, GCKR, GHR, HFE, ITIH4, LCAT, LIPC, LIPI, LMF1, LPA, LPL, LRP1, MTTP, NPC1, NYNRIN, PON1, PPP1R17, SCARB1, SLCO1B1, SLC12A4, SREBF1, SREBF2, SLC22A1, EPHX2, GPD1, OSBPL5, STAP1, ABCA1, DGAT1, INSIG2, NPC1L1, APOA5). Among patients analyzed, 29 were younger than 18 yrs. In the adult population, LDL cholesterol levels were comparable between LDLR positive and LDLR negative group, whereas in subjects younger than 18 yrs significantly higher LDL cholesterol levels in the LDLR positive group were observed. Moreover, as concerns DLCN score, performed in the adult population, significantly higher values in subjects carrying LDLR mutation were found. **Conclusions**: Present data support the involvement of other multiple loci, beyond LDLR gene, in the modulation of lipid profile, as well as cardiovascular risk. Nevertheless, further expansion of genetic analysis to a larger cohort might allow a better comprehension of the role of further major/modifier genes, as well as of accumulation of common small-effect LDL-C raising alleles in determining LDL-C levels and cardiovascular events.

OC112

DATA MINING FOR PROFILING THE MUTATIONAL LANDSCAPE OF VON WILLEBRAND DISEASE

O. Seidizadeh[1,2], A. Cairo[1], L. Baronciani[1], M.T. Pagliari[1], S.M. Siboni[1], E. Biguzzi[1], F. Peyvandi[1,2]

[1]Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; [2]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy

Background: Von Willebrand disease (VWD) with a population prevalence of 1% is caused by mutations in the von Willebrand factor (VWF). However, its exact prevalence is not yet established. More than 900 distinct mutations have been found in the VWF so far. **Aims**: To explore the VWF mutational burden and to estimate VWD prevalence in the general population using the genome Aggregation Database (gnomAD). **Methods**: VWF variants were obtained from the gnomAD (v2.1.1; 141,456 subjects) and were compared to the Human-Gene-Mutation-Database (HGMD) and Leiden-Open-Variation-Database (LOVD v.2.0 and v.3.0). The following variants were considered pathogenic: nonsense and frame-shift; splicing (+2/-2); splicing (+8/-8) and missense predicted deleterious by 3/3 and 7/7 tools respectively or being associated with VWD in HGMD and LOVD2/3. All identified pathogenic variants were used to evaluate the VWF mutational burden and VWD prevalence. **Results**: Using gnomAD, we identified 505 distinct VWF pathogenic variants (244 unique): 287 not reported (novel) and 218 already reported. 138 of 218 gnomAD variants were not predicted as pathogenic by all in-silico tools but were reported to be associated with VWD in HGMD and/or LOVD databases. Thirteen variants had a minor-allele-frequency (MAF) >0.01 in at least one population. In particular, 3 variants (p.Arg2185Gln; p.Met740Ile; p.His817Gln) were very frequent in Africans/Africans American (MAF: 0.11-0.18). Due to these variants, the African population was excluded from the calculation of VWD prevalence in the general population and was analyzed standalone. This analysis revealed a heterozygote frequency of 0.13 among all populations with a

prevalence of 0.45 and 13.4 for recessively-inherited and autosomal-dominant, respectively among 100 individuals. **Conclusions**: Through our systematic analysis of>140,000 individual exome/genome data from the gnomAD database, we found 287 novel VWF variants predicted as pathogenic. Our results demonstrated that the "true" worldwide prevalence of VWD is more than 10 times higher than that reported so far.

OC113

HYPOPLASMINOGENEMIA: AN INTERNATIONAL RETROSPECTIVE AND PROSPECTIVE COHORT STUDY (HISTORY): THE INTERNATIONAL REGISTRY OF PATIENTS WITH PLASMINOGEN DEFICIENCY

M. Menegatti[1], J. Bowen[2], M. Boscarino[1], C. Nakar[2], R. Palla[3], S. Siboni[1], M. Torres[4], M. Batur[5], R. Natesirinilkul[6], C. Roberson[2], H. McDaniel[7], S. Al[8], N. Rodriguez[9], T. Celkan[10], J. Ceresetto[11], N. Thukral[2], B. Hardesty[2], F. Peyvandi[1,3], A. Shapiro[2]

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; [2]Indiana Hemophilia & Thrombosis Center, Indianapolis, United States; [3]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy; [4]Cook Children's, Hematology/Oncology, Fort Worth, United States; [5]Van Yuzuncu Yil University, Ophthalmology Department, Van, Turkey; [6]Chiang Mai University, Department of Pediatrics, Chiang Mai, Thailand; [7]Vanderbilt Children's Hematology-Oncology, Nashville, United States; [8]Dokuz Eylül University Research Clinical Immunology, Izmir, Turkey; [9]The University of Texas Health Science Center at Houston, Houston, United States; [10]Istanbul University Cerrahpasa, Cerrahpasa Medical Faculty Pediatric Hematology, Istanbul, Turkey; [11]Hospital Britanico Buenos Aires, Buenos Aires, Argentina

Background and Aims: Plasminogen (PLG) deficiency (PLGD) is a rare inherited chronic disorder affecting ~1.6 per million population. Uncontrolled growth of fibrin-rich pseudomembranes on mucous membranes carries potential mortality risk and may affect eyes, oral cavity and middle ears, central nervous system and skin as well as respiratory, female genitourinary, gastrointestinal and renal tracts and is associated with morbidity and mortality. Ligneous conjunctivitis is the most common manifestation (~80%) and may lead to sight loss. Data regarding management of patients with PLGD is scarce and based on case reports/series and small clinical trials. In addition, even though in 2021, the FDA approved the first PLG concentrate for such patients, specific replacement therapies (PLG ophthalmologic and systemic concentrates) are not currently commercially available. With this as background, the first comprehensive retrospective/prospective international registry was created to document and evaluate PLG deficiency unmet needs with the goal of define its natural history. **Methods**: Up to 100 probands with their 1st degree non-affected family members will be enrolled in the 4 -year study. Clinical, genetic, and laboratory data are collected at baseline and at semestral visits; unscheduled visits are also included (new lesions or pregnancy). Specimens are also collected to perform measurements of PLG activity (PLG:C) and antigen (PLG:Ag) and to confirm diagnosis by molecular characterization. Therapeutic interventions are recorded along with efficacy and safety observations. **Results**: To date, 11 centers (6 countries) are actively enrolling with 115 subjects entered. Analysis was performed on 27 subjects (21 probands, 6 siblings) with complete data available; Table 1 includes demographic data and most frequently affected areas. Mean PLG:C and PLG:Ag were 15.3% (nr 80 – 132) and 15.8 ug/ml (normal range 70 – 215), respectively. Eyes and ear lesions were reported early in life, 1 and 3.5 years (median), respectively. **Conclusions**: HISTORY is the first comprehensive retrospective/prospective international registry to document and evaluate PLGD current knowledge gaps. This first analysis demonstrates a significant decrease in PLG:C and PLG:Ag levels in affected patients, and that severe symptoms including eye and ear lesions occur early in life. Interestingly, the study has not yet recorded a subject of African descent.

Table 1.

	Siblings (N=6)	Probands (N=21)	Overall (N=27)
Sex			
F	4 (66.7%)	12 (57.1%)	16 (59.3%)
M	2 (33.3%)	9 (42.9%)	11 (40.7%)
Age (years)			
Mean	20	23.5	22.7
Median [min, max]	15.5 (7 – 44)	13 (5 – 78)	14 (5 – 78)
Ethnicity			
Caucasian	5 (83.3%)	14 (66.7%)	19 (70.4%)
Spanish	1 (167%)	-	1 (3.7%)
Hispanic	-	2 (9.5%)	2 (7.4%)
Hispanic/latino	-	1 (4.8%)	1 (3.7%)
Non-Hispanic	-	1 (4.8%)	1 (3.7%)
Thai	-	1 (4.8%)	1 (3.7%)
White	-	1 (4.8%)	1 (3.7%)
missing	-	1 (4.8%)	1 (3.7%)
Plasminogen activity	14.8%	15.5%	15.3%
Plasminogen antigen	14.1 µg/ml	16.3 µg/ml	15.8 µg/ml
Affected areas			
Eyes	1 (16.7%)	21 (100%)	22 (81.5%)
Reproductive tract	2 (33.3%)	7 (33.3%)	9 (33.3%)
Gums	-	7 (33.3%)	7 (25.9%)

OC114

IMPACT ON PLATELET FUNCTION OF A NOVEL VARIANT OF THE BETA ISOFORM OF THE TBXA2R GENE ASSOCIATED WITH A HETEROGENEOUS BLEEDING PHENOTYPE

L. Bury, E. Falcinelli, A.M. Mezzasoma, M. Borghi, E. Cesari, P. Gresele

Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Perugia, Italy

Background and Aims: The thromboxane A_2 receptor (TP) plays an essential role in hemostasis by binding thromboxane A_2 (TXA_2), a potent platelet agonist and a vasoconstrictor. There are two isoforms of the receptor, TPα and TPβ, generated by alternative splicing from the TBXA2R gene, which show similar ligand-binding activity. While the expression and function of TPα in platelets is well established, that of TPβ is debated. TXA_2 binds to the TP receptor leading to the activation of Gq/11 and G12/13, but also of Gh, Gαs and Gαi. Different coupling to G-proteins of the two isoforms has been suggested but actually very few studies have analyzed this aspect. TP-receptor deficiency is an autosomal dominant platelet disorder characterized by mucocutaneous hemorrhages caused by variants in *TBXA2R*. Only 6 variants in few families all involving TPα have been described. Moreover, the severity of bleeding differs among patients with the same gene variant for unclear reasons. We recently found a novel *TBXA2R* variant (g.A16755T) affecting the alternative splice site of TPβ in a woman with a lifelong bleeding diathesis and in her father who did not report bleeding symptoms. Aim of our study was to shed light on the impact of this novel *TBXA2R* variant on platelet function and to elucidate the function of TPβ in platelets. **Methods**: TP isoforms expression was assessed by real time PCR and Western blotting (WB) using antibodies selective for the two TP isoforms. Platelet function was studied by light transmission aggregometry (LTA), flow cytometry and lumi-aggregometry. Ca^{2+} mobilization induced by various agonists was assessed by flow cytometry using the FLUO 3-AM dye. cAMP production was assessed using the cAMP Enzymeimmunoassay Biotrak (EIA) System dual range. Genetic variants affecting genes involved in platelet function were assessed by next generation sequencing, performing targeted sequencing of 89 genes. **Results**: We show that ultrapurified platelets express the TPβ isoform mRNA and protein. Platelet LTA was defective in response to arachidonic acid, U46619, U44069 and secondary wave to ADP and epinephrine in the patient and in her father. Real time PCR showed a 50% reduction of the mRNA coding for TPβ accompanied by a reduction of the TPβ protein by WB in patient platelets. ADP- and U46619-induced Ca^{2+} mobilization was impaired, suggesting reduced activation of Gq/11 and Gh. Stimulation with U46619 decreased cAMP in platelets pre-treated with Iloprost to a lower extent than in control platelets (20±2% *vs* 47±15% of controls), suggesting impaired activation of Gαi. We did not identify by targeted sequencing rare variants in other platelet genes, but found that the patient and not the father carries the common rs5758 in *TBXA2R*, previously associated with reduced platelet activation in response to U46619. **Conclusions**: Our study characterizes the first reported inherited platelet function defect due to a *TBXA2R* variant regulating the expression of the TPβ isoform associated with a mucocutaneous bleeding diathesis. We also show that the TPβ defect was associated with impairment of Gq/11, Gh and Gαi activation, suggesting that TPβ is involved in the regulation of all these pathways similar to TPα. These findings definitely suggest that TPβ has a role in platelet function. However, defective TPβ requires for a full phenotypic expression that other genetic variant(s), like the common rs5758 polymorphism of *TBXA2R*, are simultaneously present.

Poster

PO001

LONG TERM COVID-ASSOCIATED COAGULOPATHY

A. Milia[1], S. Neroni[2], B. Giusti[2], A.M. Gori[2], A. Rogolino[2], F. Cesari[2], E. Sticchi[2], A. Bertelli[2], D. Poli[2], R. Marcucci[2]

[1]Internal Medicine and Intermediate Care Unit, AOU Careggi, Florence; [2]Department of Experimental and Clinical Medicine, University of Florence; Atherothrombotic Center, AOU Careggi, Florence, Italy

Background and Aims: Covid associated coagulopathy (CAC) in SarsCoV2 infected patients is a recognized marker of disease's severity, however the usefulness of its monitoring after hospital discharge is not defined and poorly studied. We analyzed the prevalence of CAC and other hemostatic alterations in the follow up period of patient with previous hospitalization for COVID related pneumonia to investigate their role as post discharge thrombotic risk factors. **Methods**: This is a retrospective, monocentric observational study. We collect data from electronic archives of Careggi Hospital, in Florence. We collect blood exams performed at hospital admission and at the follow up evaluation, the intensity of medical care required and incidence of thrombotic events (deep vein thrombosis and pulmonary embolism) during hospitalization and after discharge. CAC was defined according to the presence of almost two of the following criteria: (1) decrease in platelet count (less than 150 × 109/L); (2) increase in D-dimer (more than two times the upper limit of normal); (3) >1 s prolonged prothrombin time or INR >1.2. **Results**: 430 patients were identified, 249 man (57.9%) and 181 woman (42.1%), with median age of 65 years (IQR 55-74). 109 patients with an intensive or sub-intensive treatment during hospitalization, 43 (7%) with no oxygen treatment during hospitalization, 30 patients (7%) had thrombotic events during hospitalization. At the admission, 79 patients had CAC criteria and those patients required more often an increase of intensity of medical care (33.6% *vs* 22.3%; p 0.019) and had more thrombotic events (10% *vs* 5.8% p=0.14). Laboratory parameters associated with future thrombotic events and need of increase intensity of medical care during the hospitalization were higher platelets, D-dimer, fibrinogen, C-reactive protein, ferritin and LDH. At follow up evaluation (median 5.08 months, IQR 3.8-6.4) CAC was still present in 7 patients (1.4%); none of them required increase of intensity medical care nor developed thrombotic complication during the hospitalization. 3 (0.6 %) patients had thrombotic events after discharge; none of them had CAC at the follow up evaluation. Patients who had developed thrombotic events during the hospitalization still have high platelets level at follow up (244 *vs* 233 x 109/L; p=0.038). Prothrombin time (PT) was more prolonged in both groups compared to controls. Patients with more severe previous pneumonia and thrombotic complications were more often on anticoagulant treatment and PT didn't significantly differ between mild and severe cases and between previous thrombotic events or not grouping the patients for anticoagulant treatment. **Conclusions**: In our population the presence of CAC at hospital admission was associated with more severe illness and development of thrombotic complication. Otherwise the presence of CAC during the follow up seems have no value as a marker of previous disease severity or thrombotic risk after discharge. Our results also shows that patients who developed thrombotic complication during the hospitalization had higher platelets levels on admission and maintains this association at follow up. PT prolongation at follow up seems reflecting anticoagulant treatment instead of previous disease severe or thrombotic risk. In conclusion our results do not support the need of monitoring of coagulation parameters after the discharge form the hospital and shows that post discharge thrombotic risk was very low.

PO002

IDENTIFICATION OF RARE COAGULATION VARIANTS AND SCREENING OF THROMBOPHILIC POLYMORPHISMS (F2 RS1799963, F5 RS6025) IN COVID-19 PATIENTS

M. Giannini, E. Sticchi, T. Capezzuoli, R. De Cario, S. Suraci, G. Cassioli, S. Neroni, A.A. Rogolino, M. Berteotti, A.M. Gori, R. Marcucci, B. Giusti

Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze; Atherothrombotic Diseases Center, AOU Careggi, Firenze, Italy

Background and Aims: Severe SARS-CoV-2 infections are characterized by perturbation of physiological coagulation mechanisms. COVID-19 is associated with a high incidence of thrombotic complications, such as venous thromboembolism or arterial thrombotic events (myocardial infarction and cerebral events). An association between thrombophilia and the most severe clinical course of COVID-19 has been suggested. Thrombophilia is a condition of altered haemostasis, characterized by increased blood clotting, which predisposes to adverse thrombotic events. This condition may be due to hereditary factors, acquired changes or, in the prevalence of cases, an association of genetic and acquired factors. Among the hereditary thrombophilia factors, the most frequent causes are factor V Leiden polymorphism (rs6025) in F5 gene and the G20210A polymorphism (rs1799963) in F2 gene. Aim of the study was to identify genetic variants and/or genetic profiles associated with severity of the disease and thrombotic events susceptibility. **Methods**: Starting from these considerations, an NGS analysis was conducted on a cohort of n=40 patients with COVID-19; genetic analysis included a sequencing panel of 11 genes (PROC, PROS1, FGA, FGB, FGG, SERPINC1, F2, F5, F10, PLAT, PLG) known to be involved in the coagulation process. Moreover, a genotyping analysis of rs6025 and rs1799963 polymorphisms has been conducted through Real Time PCR on the whole cohort of n=994 patients hospitalized at the AOU Careggi with COVID-19. **Results**: As regards NGS analysis, 29 rare variants (MAF≤1%) have been identified at the heterozygous state in 24 of the 40 patients studied: 7 missense variants (on the F10, F2, PLAT, SERPINC1, F5 and FGB genes), 13 synonymous variants (on FGB, F2, PLAT, PLG, PROC and F5 genes), 4 variants concerning zone 3'-5' UTR/downstream (on F10, PLAT, PROC and F5 genes) and 5 non-deep intronic variants (on F5, PROC, F10 and PLG genes). In particular, there are five rare variants which were identified in two different patients each. A higher prevalence of rare missense variants with potential pathogenic prediction in ICU or death patients (26.7%) was observed than in ordinary ward patients (8%). Concerning common genetic thrombophilia, in the whole n=994 patients cohort, n=45 were heterozygous for the rs1799963 polymorphism and n=31 were heterozygous for rs6025 polymorphism. MAF for F2 G20210A was 0.023, higher than that reported in the literature for the population of Tuscany (0.016), while for the FV Leiden was 0.016, comparable to that observed in the tuscan population (0.020). Among a subgroup of n=324 patients, for which information concerning the clinical outcome was available, emerged that in patients who developed a thromboembolic event (5.9%) there was a higher allelic frequency, but not statistically significant, of FVL polymorphism, compared with patients who did not develop such an event (0.026 *vs.* 0.018, p=0.519); no differences were observed for F2 G20210A polymorphism. **Conclusions**: The presence of common genetic factors of hereditary thrombophilia does not seem to indicate a significant contribution in modulating the risk of developing thromboembolic complications in SARS-CoV-2 patients; on the other hand, NGS results show that genetic variability, due to rare variants, might modulate clinical severity of COVID-19 disease in patients.

PO003

THE DIAGNOSTICS OF HEPARIN-INDUCED THROMBOCYTOPENIA IN ITALY AND THE IMPACT ON IT OF THE COMING TO LIGHT OF VITT

E. Falcinelli[1], R. Marcucci[2], P. Gresele[1]

[1]Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy; [2]Center for Atherothrombotic disease, AOU Careggi, Department of Experimental and Clinical Medicine, University of Florence, Italy

Background and Aims: Although rare, heparin-induced thrombocytopenia (HIT) is a potentially ominous side effect of heparin treatment and must be promptly recognized to prevent serious consequences. The diagnosis is suspected clinically, by taking into account several factors, among which the 4T pre test probability score, and requires laboratory confirmation by the detection of antibodies against PF4/heparin. Tests include immunological assays, such as ELISAs (enzyme linked immunosorbent assays) and others, as well as a range of functional test exploring if circulating antibodies can activate platelets. Most of these are available only in specialized centers. In the wake of the large-scale SARS-CoV-2 vaccine roll-out, a rare but potentially fatal complication of adenoviral-vector vaccines, called vaccine-induced immune thrombotic thrombocytopenia (VITT), has emerged with a vast negative impact on mainstream media. Similar to HIT, also this novel catastrophic thrombotic syndrome requires the detection of high levels of anti-PF4 antibodies, either by ELISA (possibly with two or more different assays) or by a functional assay. Our study aimed to assess how VITT emergence has influenced the request for these tests in an acute care setting of Italian hospitals. **Methods**: A 10-item self-administered questionnaire was sent by email to all specialized laboratories performing anti-PF4 testing in Italy. Data collection included: methods for anti PFA/heparin identification,

request for the 4Tscore, total and median number of requests per year and per month, rate of positive tests. A direct comparison between the period following the emergence of VITT (from 01 March 2021 to 30 January 2022) and the preceding 3 years (from 01 January 2018 to 31 December 2020) was made. **Results**: A total of 28 laboratories (40% of the invited centers), from almost all regions of Italy, responded to the survey. Only 39% of respondents ask for the results of the clinical pre test probability score (4Tscore). The most commonly used laboratory assays for HIT antibodies are immunological (68%), with only five laboratories using two different immunological assays at the same time, while functional tests are performed only in a minority of laboratories (32%). Among immunological tests the most used were ELISA (n=17) and chemiluminescence (n=10) followed by a range of less used assays (n=3 latex test and n=2 rapid assay). The main commercial sources of reagents are Immucor (46%) and Werfen (43%), followed by Stago (14%). Total number of tests/year was significantly increased comparing the post VITT period to the preceding 3 years (n=138, 95%CI: 31.3-302 *vs* 58.5, 95%CI: 9.4-115; +138% p<0.05) (Figure 1A). The number of tests/month was also increased from n=6.04 (95%CI: 1.1-10.5) to n=10.41 (95%CI: 2.3-22.8) (+66%) (Figure 1B). No difference in the incidence rate of positive tests was observed in the two periods (8%, 95%CI: 3.8-11 *vs* 11%, 95% CI: 5-20;p=ns). **Conclusions**: The results of this survey, which included responses from 28 laboratories representing 14 regions along Italy, showed important differences in laboratory practice for HIT diagnosis and an important impact of the coming to light of the VITT complication in Italy. The use of HIT assays markedly increased after VITT identification, although this complication is very rare. The major cause behind this increase is likely related to the hyper vigilance among emergency physicians raised by this unprecedented complication of vaccination.

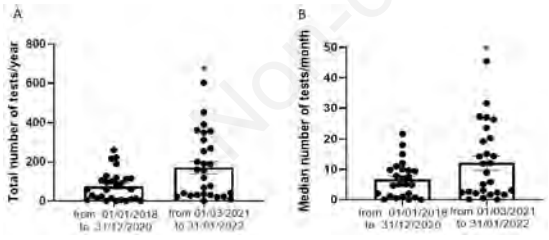


Figure 1.

PO004

THROMBOEMBOLIC COMPLICATIONS IN PATIENTS WITH COVID-19 HOSPITALIZED IN ITALIAN ORDINARY WARDS: DATA FROM THE MULTICENTRE OBSERVATIONAL START-COVID REGISTER

E. Antonucci[1], D. Poli[2], W. Ageno[3], S. Galliazzo[4], A. Milia[5], A Pizzini[6], M. Di Nisio[7], B. Zalunardo[8], P. Preti[9], L. Sarti[10], A. Trovati[10], E. Lotti[1], G. Palareti[1], R. Marcucci[2,11]

[1]Fondazione Arianna Anticoagulazione, Bologna; [2]SOD Malattie Aterotrombotiche, Azienda Ospedaliero Universitaria-Careggi, Firenze; [3]Dipartimento Di Medicina e Chirurgia, Università Degli Studi Dell'Insubria, Varese; [4]UO Medicina Generale Ospedale SValentino, Montebelluna, TV; [5]UO Medicina Interna ad Alta Intensità Azienda Ospedaliero-Universitaria Careggi Firenze; [6]Dipartimento di Medicina Interna, Ospedale Maggiore "Carlo Alberto Pizzardi", Bologna; [7]Dipartimento di Medicina e Scienza dell'Invecchiamento, Università GD'Annunzio, Chieti; [8]U.O.CAngiologia, Ospedale Castelfranco Veneto, Azienda ULSS 8-Regione Veneto, Castelfranco Veneto; [9]Thrombosis and Haemostasis Centre, Department of Internal Medicine, IRCCS SMatteo Foundation, University of Pavia, Pavia; [10]UOC Medicina Interna d'Urgenza e Area Critica, Ospedale di Baggiovara, Azienda Ospedaliero Universitaria Modena; [11]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze, Italy

Background and Aim: COVID-19 infection causes acute respiratory pathology insufficiency with severe interstitial pneumonia and extra-pulmonary complications; in particular, it may predispose to thromboembolic disease. The reported incidence of thromboembolic complications varies from 5% to 30% of cases. **Methods**: We conducted a multicentre, Italian, retrospective, observational study on COVID-19 patients admitted to ordinary wards, to describe clinical characteristic of patients at admission, bleeding and thrombotic events occurring during hospital stay. **Results**: 1135 patients hospitalized were included in the START-COVID-19 Register, 1091 patients hospitalized in ordinary wards were included in the study, 653 males (59.9%), median age 71 years (IQR 59-82 years). During the observation, 2 (0.2%) patients had acute coronary syndrome episodes, 1 patients (0.1%) had a stroke; no other arterial thrombotic events were recorded. Fifty-nine patients had symptomatic VTE (5.4%), 18 (30.5%) patients had deep vein thrombosis (DVT), 39 (66.1%) patients had pulmonary embolism (PE), and 2 (3.4%) patients had DVT + PE. Among patients with DVT, 8 (44.4%) were isolated distal DVT, and 2 cases were jugular thrombosis. Among patients with PE, 7 (17.9%) events were limited to sub-segmental arteries. No fatal PE were recorded. Major bleedings occurred in 9 (1.2%) patients, and clinically relevant non- major bleeding in 9 (1.2%) patients. All bleedings occurred among patients treated with antithrombotic drugs receiving thromboprophylaxis, more frequently when treated with sub-therapeutic/therapeutic dosages. **Conclusions**: We confirmed that patients admitted to ordinary wards for COVID-19 infection are at high risk for thromboembolic events. VTE recorded among these patients are mainly isolated PE, suggesting a peculiar characteristic of VTE in these patients.

PO005

VITT OR NOT VITT? 61-YEARS WOMAN WITH THROMBOCYTOPENIA AFTER SOMMINISTRATION OF AD26.COV2.S VACCINE

A. Bertelli, A.A. Rogolino, F. Cesari, B. Giusti, E. Sticchi, B. Bandinelli, E. Lotti, A.M. Gori, R. Marcucci

Department of Experimental and Clinical Medicine, University of Florence; Atherothrombotic Diseases Center, AOU Careggi, Florence, Italy

Background: Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare but severe immunological reaction to the non-replicable adenoviral vector-based COVID-19 vaccines (AstraZeneca vaccine and Johnson & Johnson). VITT is caused by antibodies that recognize platelet factor 4 (PF4) bound to platelets. These antibodies are immunoglobulin G (IgG) molecules that activate platelets via low-affinity platelet FcγIIa receptor. Extreme activation of platelet and the coagulation system leads to clinically significant thromboembolic complications. **Case Report**: We describe the case of a 61-years woman, who presented in June 2021 to the emergency department for bruising and conjunctival hemorrhagic manifestations. Patient had been administered Ad26.COV2.S vaccine (J&J) 20 days earlier. Her medical history showed hypothyroidism and hypertension. Blood tests showed thrombocytopenia with platelet count of $38x10^9$/L. In the days following hospitalization, the patient was administered with corticosteroids and intravenous immunoglobulin (IVIG) (400 mg/kg). Then platelet count dropped to $23x10^9$/L, and the patient was admitted for further investigations. The temporal relationship between the onset of symptoms and vaccine administration raised the suspicion of VITT. Subsequently patient was administered with corticosteroids and intravenous immunoglobulin (IVIG) (400 mg/kg). Samples for platelet factor 4 (PF4) antibody analysis were sent to the laboratory of the Atherothrombotic Centre of Florence to confirm the diagnosis. The titre of antibodies anti-PF4 was assessed by using two enzyme-linked immunosorbent assay (ELISA), PF4 IgG test, Immucor-Lifecodes and Zymutest HIA IgG, Hyphen BioMed-, and a chemiluminescent assay (Acustar Werfen). Patient's sample was negative with all immunoassays: 0.125 optical density (OD) for Immucor, 0.109 OD for Zymutest and 0.10 U/mL for Acustar. To evaluate the capability of anti-PF4 antibodies in inducing platelet activation, a functional test (HIPA Test) was also performed. Unexpectedly, at the HIPA test we observed a strong capacity of patient's serum in inducing platelet activation both in the presence of only buffer and in the presence of PF4 (10 µg/mL). The discordance between immunoassays and the HIPA test suggested a further evaluation at 30 days. In the meantime, the platelet count increased to $166x10^9$/L and there was no evidence of thrombosis. The test was repeated at 30 days and confirmed the negativity of both ELISA and the chemiluminescence test; instead the HIPA test confirmed positivity (4/4 buffer, 4/4 PF4 10 µg/mL, and 4/4 heparin 0.2 U/mL). The patient was re-evaluated after further 30 days and the results of test were negative both at immunological (ELISA test and chemiluminescence test) and functional tests. **Conclusions**: This is one of the rare cases of discordance between the immunoassay and the functional test. It occurs in HIT in about 0.2% of cases and can be ascribed to the presence of antibodies directed against other than PF4/Heparin complex such as Nap2- and IL8-Heparin complexes. In fact, these complexes were not recognized by the classical immunoassay anti-PF4, which are specific for the PF4/Heparin polyanion complexes. Therefore, the discordance between immunological and functional tests may be explained by the presence of these antibodies in our patient, in whom the timely pharmacological intervention likely stopped the progression of the disease.

PO006

INDIVIDUAL SUSCEPTIBILITY TO SARS-COV-2 INFECTION: ROLE OF THE HOST'S VIRUS ENTRY MACHINERY GENETIC PROFILES

R. De Cario[1], E. Sticchi[1], A. Kura[1], T. Capezzuoli[1], M. Giannini[1], G. Cassioli[1], G. Barbieri[1], S. Suraci[1], A.M. Gori[1], M. Berteotti[2], A.A. Rogolino[1], R. Marcucci[1], B. Giusti[1]

[1]Department of Experimental and Clinical Medicine, University of Florence; Atherothrombotic Diseases Center, AOU Careggi, Florence, Italy; [2]Cardiomyopathy Unit, Cardiothoracic and Vascular Department and Cardiomyopathy Unit, Careggi University Hospital, Florence, Italy

Background: Since the 2019 SARS-CoV-2 pandemic outbreak, hundreds of studies reported evidence of a host genetic background's role, alongside age, comorbidities, and gender, in influencing both susceptibility to virus infection and severity of the clinical pictures. Genes-regulated biological pathways of COronaVIrus Disease 2019 (COVID-19) suggested to be mainly implicated in the different clinical outcomes are represented by virus entry, immunity, and inflammation. Concerning the first, several factors are involved both at the cell surface and intracellular level. Several genetic variants (mutations/polymorphisms) were found to be associated with the severity of the disease in a detrimental or beneficial way, their identification potentially representing useful prognostic/therapeutic tool. In this study, genetic characterization of 40 Covid19 patients referred to the Advanced Molecular Genetics Laboratory, Atherothrombotic Diseases Center, Careggi Hospital-University of Florence, was made by Next Generation Sequencing (NGS) to identify genetic profiles potentially representing prognostic factors modulating the susceptibility to virus infection. **Methods**: NGS was performed by Illumina MiSeq and Haloplex HS protocol targeting 7

virus entry-related genes (*ACE2*, *TMPRSS2*, *CTSL*, *CTSB*, *HSPA5*, *IL6*, *FURIN*). **Results**: 18 heterozygous rare variants [Minor Allele Frequency (MAF)<0.01] were identified in 16/40 patients involving all 7 genes included in the panel. Potentially functional mutations (10/18) involved *ACE2*, *HSPA5*, *CTSL* with *TMPRSS2* being the most affected gene, in accordance with literature data which identified this gene as promising therapeutic target. Some of those rare variants were interpreted as protective due to their potential role in diminishing the activity of the virus-entry machinery (*i.e. ACE2*), others may instead increase the susceptibility to infection (*i.e. TMPRSS2*). With regards to the disease progression in terms of death/hospitalization in intensive care unit (ICU, 15 subjects, with 3 variants) or early discharge (25 subjects, carrying 7 variants), no statistically significant difference was observed among the 2 rare variants carriers' groups (20% *vs* 28%, showing a slight tendency towards a protective effect of rare variants). About the polymorphic burden, the most affected genes were *FURIN*, *CTSB*, and *TMPRSS2*, with an average number of variants of 1.4, 4.1 and 3.3 per patient, respectively. Considering the total number of polymorphisms per gene, the only slight difference among the abovementioned subgroups was observed regarding the *FURIN* gene as 37 polymorphisms affected the early discharge group, while 18 (67% *vs* 83%) were carried by the death/ICU group. **Conclusions**: Our data suggest how the identified genetic variants involving the host's virus entry machinery are not likely to exert a significant effect in modulating the severity of the COVID-19 progression when considering the prognosis parameters of death, ICU hospitalization and early discharge from the hospital. According to our data, the contribution of other factors (genetic or not), i.e comorbidities and individual thrombophilic state, are more likely to contribute to the disease severity and the potential complications at different levels (coagulation processes, cardiac or neurological involvement, extension of the pulmonary injury) along with inflammation, and immunity process, that may be, at least in part, influenced by alterations at the virus-entry level.

PO007

CLINICAL IMPLICATIONS OF FIBRINOLYTIC SYSTEM ALTERATIONS IN PATIENTS WITH COVID-19 RELATED PNEUMONIA

L. Spiezia, M. Isola, C. Simion, E. Campello, F. Dalla Valle, P. Simioni

Thrombotic and Hemorrhagic Diseases Unit, Padova University Hospital, Padova, Italy

Background and Aims: SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) infection that causes COVID-19 (Coronavirus Disease-2019) is a global pandemic. The clinical manifestations of SARS-CoV-2 infection are extremely varied, most patients (80%) develop a mild upper respiratory tract infection, but 15% of patients develop, instead, a serious disease that can evolve into acute respiratory distress syndrome (ARDS), shock, macro and microvascular thrombosis, multiorgan dysfunction with poor prognosis. It has been shown that patients with COVID-19 exhibit a hypercoagulable profile characterized by an increase in D-dimer, fibrinogen, factor VIII and von Willebrand factor (vWF) plasma levels and an increased whole blood coagulation capability as identified by viscoelastic tests (thromboelastometry/graphy). The first aim of our study was to evaluate the possible presence of a hypofibrinolysis condition in subjects with acute COVID-19 pneumonia. Secondly, we evaluated the possible role of these alterations in determining a state of hypercoagulability which could contribute to increase the thrombotic risk in these patients. **Methods**: We performed a case-control study that included 63 patients, admitted to the Internal Medicine Ward of University of Padova between November 6th, 2020 and April 26th, 2021 with a diagnosis of SARS-CoV-2 infection, as defined by WHO. In each patient we studied the coagulation profile with particular reference to the fibrinolytic system, by means of traditional coagulation tests and by rotational thromboelastometry conducted on whole blood using the ROTEM® method. The data obtained in this group of patients (cases) were compared with those obtained in a group of healthy subjects (controls) sex and age matched with cases. **Results**: In patients with COVID-19 related pneumonia, we found a significant increase in ferritin, CRP, procalcitonin and IL-6 plasma levels. The traditional coagulative profile, characterized by a significant increase of D-dimer, fibrinogen, FVIII and vWF, demonstrated a state of hypercoagulability. We also found a significant increase of both tPA and PAI plasma levels in cases than controls. Finally, according to thromboelastographic tests we found a statistically significant reduction of Clot Formation Time (CFT) and Maximum Lysis (ML), both in the INTEM and EXTEM tests, and a statistically significant increase of Maximum Clot Firmness (MCF) in INTEM, EXTEM and FIBTEM assays. **Conclusions**: The results of our study allowed us to confirm that patients with acute COVID-19 related infection have a state of hypercoagulability. Furthermore, the study of the fibrinolytic parameters revealed a picture of hypofibrinolysis. Larger studies are needed to identify a possible correlation between our findings and the development of thrombotic complications.

PO008

POST-DISCHARGE THROMBOPROHYLAXIS IN PATIENTS WITH COVID-19: A SINGLE-CENTER EXPERIENCE

C. Simion, E. Campello, D. Tormene, L. Spiezia, F. Dalla Valle, P. Simioni

General Medicine and Thrombotic and Hemorrhagic Diseases Unit, Department of Medicine – DIMED, University-Hospital of Padova, Italy

Background and Aims: COVID-19 is associated with an increased risk of venous thromboembolism (VTE). However, data regarding rates of VTE after discharge for patients hospitalized for COVID-19 are limited and extended out-of-hospital thromboprophylaxis is not routinely recommended because the risk-benefit remains uncertain. **Methods**: We enrolled all consecutive adults admitted to our General Internal Medicine Unit for confirmed COVID-19 between November 2020 and May 2021. We collected demographics, comorbidities, laboratory findings, risk factors for VTE and medications, including in-hospital and post-discharge thromboprophylaxis. Any VTE, bleeding event, re-hospitalization and all causes of mortality within 30 days after discharge were considered as outcomes. **Results**: Out of 388 enrolled adults (male 51.0%, median age 77 years), 70 (18%) were excluded because already receiving therapeutic anticoagulation before the hospitalization, mostly (72.9%) for atrial fibrillation (AF). Forty-five subjects (11.6%) died during hospitalization, mainly because of respiratory or multiorgan failure and septic shock. Eight patients (2.9%) did not receive thromboprophylaxis during the hospitalization (2 died within the first 24 hours for respiratory failure, 6 had a major bleeding at admission). During hospitalization, full anticoagulation treatment was required in 26.0%: 19 cases (7.0%) for AF onset and 49 (17.9%) for VTE occurrence, mainly distal or catheter-related deep venous thrombosis (DVT); three patients developed both AF and VTE. Out of 202 (74.0%) patients discharged without full anticoagulation, 25 (12.4%) did not received thromboprophylaxis, while 177 (87.6%) received extended prophylaxis with enoxaparin 4000 IU subcutaneous daily (6000 IU if obese). Particularly, 25 patients (12.4%) received less than 7 days of prophylaxis, 99 (49%) up to 7-10 days, 4 (2.0%) up to 15-21 days, 1 (0.5%) 30 days because of recent hip fracture; for 48 subjects (23.7%) the duration of thromboprophylaxis was not specified (until complete mobilization/negativization/full recovery). Among the 177 patients who received post-discharge prophylaxis, 2 (1.1%) VTE events occurred during the follow-up: a proximal DVT of the leg occurred in one patient (male, 56 years, heterozygous FV Leiden, with a past history of distal DVT, who had severe COVID-19) soon after stopping a 7 day-prophylaxis; a 71 years old female patient was re-admitted 42 days after for a stroke due to paradoxical embolism from proximal DVT; she had a history of previous stroke and received a 7 day-thromboprophylaxis after discharge, but did not recover full mobilization. Five (2.8%) patients were re-hospitalized within 30 days, not relating to VTE or bleeding; 4 patients (2.3%) died within 16 days (1 multiorgan failure, 1 worsening of general condition, 2 unknown reason) and 1 (0.6%, cancer) 32 days post discharge. Among the 25 patients discharged without prophylaxis, neither VTE nor bleeding were recorded, there were no re-hospitalization but 2 patients (8.0%) died within 15 days (p *vs* extended prophylaxis 0.12) (Table 1). **Conclusions**: According to our findings, extended post-discharge thromboprophylaxis up to 7 days appears to be safe in patients with COVID-19. A longer course of prophylaxis up to 14-20 days may be more effective in preventing any VTE. Post-discharge mortality was non-significantly higher in patients without extended thromboprophylaxis.

Table 1.

Baseline characteristics					
Overall population, n°	388				
Age, years	77 (66-84)				
Gender male (%)	198 (51.0)				
Anticoagulation therapy before admission (%)	70 (18.0)				
New anticoagulation during hospitalization (%)	71 (26.0)				
In-hospital mortality (%)	45 (14.5)				
Clinical outcomes:					
		30d- mortality	30d- re-hospitalization	30d- VTE	30d- bleeding
Eligible population, n	202	7 (3.5)	2 (1.0)	1 (0.5)	-
No thromboprophylaxis at discharge (%)	25 (12.4)	2 (1.0)*	-	-	-
Extended thromboprophylaxis at discharge (%)	177 (87.6)	5 (2.5)**	1 (0.5)*	1 (0.5)*	-
- 1-6 days	25 (12.4)	-	-	-	
- 7-10 days	99 (49.0)	2 (1.0)	1 (0.5)	1 (0.5)	
- 15-21 days	4 (2.0)	-	-	-	
- 30 days	1 (0.5)*	-	-	-	
- not specified/until full recovery or mobilization or negativization	48 (23.7)	3 (1.5)	-	-	

Baseline characteristics and clinical outcomes. Variables are expressed as median and interquartile range (IQR) or number (%). VTE: venous thromboembolism. *For hip fracture, *1 for ab ingestis pneumonitise, 1 unknown. **3 for cancer, 1 for multiorgan failure in elderly, 1 for worsening of general condition, 2 unknown. ^Deep vein thrombosis of the leg.

PO009

HYPERINFLAMMATORY STATE AND DEATH IN PATIENTS AFFECTED BY COVID-19: ROLE OF VACCINES

L. Marchini, M.C. Vedovati, C. Becattini

Internal, Vascular and Emergency Medicine – Stroke Unit, University of Perugia, Perugia, Italy

Background: Systemic hyperinflammation in patients with COVID-19 is associated with a higher probability of severe disease and poor prognosis. The COVID-19 vaccines are expected to be associated with a lower risk of moderate-severe disease. However, data on the effects of vaccination on the inflammatory state and adverse outcomes in patients affected by COVID-19 are lacking. **Aims**: To evaluate the association of hyperinflammation state and adverse outcome among vaccinated and unvaccinated patients for SARS-CoV-2 infection. **Methods**: We performed an observational study on COVID-19 patients admitted to a non-ICU ward at Perugia Hospital from August to December 2021. The inclusion criteria were: age ≥18 years, hospitalization due to respiratory failure, and SARS-CoV-2 infection confirmed by nasopharyngeal RT-PCR swab. A patient was defined as vaccinated after a full cycle. Study outcomes were all-cause-death or symptomatic venous thromboembolism (VTE) and hyperinflammation state (defined as at least four values among CRP, LDH, ferritin, CPK, D-dimer above the threshold). **Results**: Overall, 182 patients were included (mean age 68 years, range 18-98). All-cause death occurred in 16 patients (8.8%). Vaccinated patients were older (76 *vs* 61 years); they had a higher rate of comorbidities and a lower rate of NIV/HFNC requirements than unvaccinated patients. After age adjustment, the hyperinflammation state was significantly more frequent in unvaccinated compared to vaccinated patients (65 *vs* 37%, p=0.004). Lack of vaccination was an independent predictor of in-hospital all-cause-death (HR 2.71, 95% CI 1.05-7.00, p=0.040) and all-cause-death or symptomatic VTE (HR 3.10, 95% CI 1.31-7.35, p=0.010). The risk of symptomatic VTE was

not significantly higher in unvaccinated compared to vaccinated patients (5 *vs* 1%, HR 5.66, 95% CI 0.63-50.85). **Conclusions**: Covid-19 vaccination is associated with a lower hyperinflammation state and lower risk of death compared to lack of vaccination. These findings should be confirmed in a larger population.

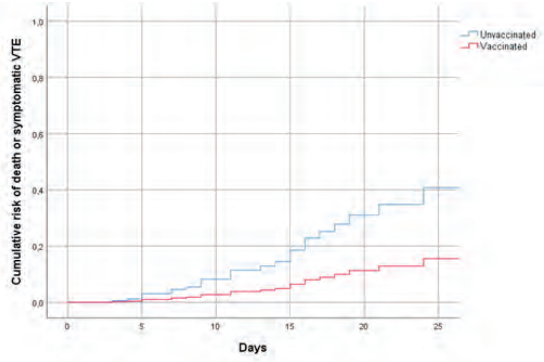


Figure 1.

PO010

INCIDENCE OF VENOUS THROMBOEMBOLISM IN HOSPITALIZED PATIENTS WITH COVID-19 AND ANTICOAGULATION WITH HEPARIN. A SINGLE-CENTER COHORT STUDY

D. Tormene, E. Bicego, N. Perin, E. Campello, M. Sartori, C. Simion, P. Simioni

Thrombotic and Haemorrhagic Diseases Unit, Department of Medicine, University of Padua Medical School, Padua, Italy

Background and Aims: Coagulopatthy and venous thromboembolism are common findings in patients with Coronavirus disease. The aim of the study was to investigate the incidence of objectively confirmed thrombotic events (both venous and arterial) in hospitalized patients with COVID-19. **Methods**: Single-center cohort study of 284 hospitalized patients with COVID-19. **Results**: Two hundred and eighty-four patients were admitted to our internal medicine ward: 136 (48%) were male and the mean age was 77 years. One hundred thirteen patients (40%) had cardiovascular disease, 48 (17%) metabolic syndrome, 20 (7%) active cancer and 53 (19%) previous cancer. Prophylactic doses of heparin were used in 202 patients, sub-therapeutic or therapeutic doses in 47 and in 35 no heparin prophylaxis was performed. Fifteen thrombotic events [6 DVT (4 distal), 4 myocardial infarcts, 5 cerebral ischemic events] occurred in the group of patients on prophylactic heparin, *versus* three thrombotic events [1 distal DVT, 2 myocardial infarcts] in the group of patients on heparin at therapeutic or sub-therapeutic doses: RR 1.16 (95% CI 0.32 to 4.2). Three thrombotic events [1TVP and 2 myocardial infarcts] occurred in the 35 patients without prophylaxis : RR 1.32 (95% CI 0.28 to 6.2). Twenty-seven patients in the prophylaxis group died *versus* 3 in the therapy group and 6 in the non-prophylaxis group: RR 1.97 (95%ci 0.62 to 6.2) and 2.44 (0.65 to 9.1) respectively. Major bleeding occurred in 11 % of the patients receiving therapeutic-dose anticoagulation and in 5 % of those receiving thromboprophylaxis. 81 patients were transferred to the ICU. Of these 30 developed a thrombotic event: 24 thrombotic events [1 proximal DVT] occurred in the group of 64 patients on prophylactic heparin, *versus* 4 thrombotic events [1 proximal DVT] in the group of 8 patients on heparin at therapeutic or sub-therapeutic doses: RR 0.82 (95% CI 0.34 to 1.95). Two thrombotic events [distal DVT] occurred in the 9 patients without prophylaxis : RR 0.54 (95% CI 0.12 to 2.4). Fifteen patients in the prophylaxis group died *versus* 1 in the therapy group and 4 in the non-prophylaxis group: RR 1.71 (95%ci 0.25 to11.5) and 2.77(0.37 to 21) respectively. **Conclusions**: The risk of developing thrombotic events in patients with Covid 19 admitted to an internal medicine ward in which heparin prophylaxis is performed does not appear significantly increased. There appears to be an increased risk of death in patients receiving prophylactic heparin doses compared to those receiving therapeutic or sub-therapeutic doses. This employer is less evident in those transferred to the ICU. An adequate assessment of the patient's haemorrhagic risk will be useful in the most appropriate treatment choice.

PO011

INFIAMMAZIONE VASCOLARE CORONARICA STIMATA MEDIANTE L'ATTENUAZIONE PERI-VASCOLARE CORONARICA ALLA TOMOGRAFIA COMPUTERIZZATA DEL TORACE NEI PAZIENTI RICOVERATI PER COVID-19 E MORTALITÀ INTRAOSPEDALIERA.

D. Tuttolomondo[1-2], C. Martini[1-3], M. De Filippo[1-3], N. Gaibazzi[2]

[1]Università degli studi di Parma; [2]Azienda Ospedaliero-Universitaria Parma, UOC Cardiologia, Parma, Italia; [3]Azienda Ospedaliero-Universitaria Parma, UOC Radiologia, Parma, Italy

Background and Aims: SARS-CoV-2 acute respiratory disease (COVID-19) can present with different clinical scenarios ranging from flu-like symptoms to respiratory distress syndrome. Up to 45.3% of patients hospitalized for COVID-19 have documented myocardial damage (increased cardiac troponin); this determines a worsening of the prognosis with an increase of in-hospital mortality. Cardiac involvement in these patients may be multifactorial but the prevailing hypothesis seems to be the thrombotic destabilization of the atherosclerotic plaque secondary to the "cytokine storm". In this scenario, the estimate of vascular inflammation should be contextualized by means of coronary peri-vascular attenuation (PVAT)

with chest computed tomography (CT). This parameter can be obtained ex-post (offline) and does not require further exams or additional costs. The prognostic power of PVAT is known but has not yet been tested in COVID-19 patients. **Methods**: Single-center retrospective study conducted on 800 patients hospitalized for COVID-19 from 01.03.2020 to 30.04.2020 and from 01.03.2021 to 30.04.2021 who performed chest CT according to clinical judgment. The objective of the study is to evaluate the predictive capacity of PVAT respect to the primary end-point of all-cause-death and the composite end-point of: cardiovascular death, acute myocardial infarction, stroke/transient ischemic attack, major cardiac arrhythmias, non-invasive ventilation, invasive ventilation and hospitalization in intensive care units. **Results**: 765 patients out of 850 patients were enrolled (study in progress) and PVAT analysis on chest CT was performed in 643 cases. A preliminary statistical analysis was performed regarding the patients who had hospitalized for COVID-19 from 01.03.2020 to 30.04.2020 (399 patients). PVAT mean in dead patients (n=175) calculated on the anterior interventricular artery (IVA) is -68.89 UH and on the right coronary artery (RCA) is -69.9 UH; in surviving patients (n=224) the mean PVAT was -73.75 UH (IVA) and -72.66 (RCA) both $p < 0.01$. **Conclusions**: Preliminary results show that the degree of coronary inflammation on chest CT in hospitalized COVID-19 patients could be a useful parameter in prognostic stratification.

PO012

DEATHS AFTER ANTI SARS-COV-2 (COVID-19) VACCINE ADMINISTRATION IN ITALY: AUTOPSY REPORT OF ELEVEN CASES

C. Franzetti[1], L. Tajana[1], F. Piovella[2]

[1]Unità di Medicina Legale e Scienze Forensi "AFornari", Dipartimento di Sanità Pubblica, Medicina Sperimentale e Forense, Università di Pavia, Pavia; [2]Angioematologia, Casa di Cura Lecco "Beato Luigi Talamoni", Lecco, Italy

Background: As of may 12, 2022, 516.922.683 acute respiratory infections and 6.259.495 deaths from coronavirus SARS-CoV-2 (COVID-19) have been reported worldwide. Venous thrombotic complications have been associated with up to 60% of these deaths. As a consequence, we witnessed unprecedented rapid clinical development of vaccines. As of may 10, 2022, 11.655.356.423 doses have been delivered. Four vaccines have been approved in Italy. Two are vaccines based on incapsulated mRNA in lipid nanoparticles (COMIRNATY, Pfizer/BioNTech and SPIKEVAX, Moderna), two use a non-replicating recombinant chimpanzee adenovirus to deliver genetic material (VAXZEVRIA, Oxford–AstraZeneca and JCOVDEN, Johnson & Johnson/Janssen). Vaxzevria vaccine campaign started in U.K. on January 4, 2021. In march 2021, concerns developed regarding an increased risk of thrombosis associated with thrombocytopenia: a new syndrome of vaccine-induced immune thrombotic thrombocytopenia (VITT) has emerged as a rare side-effect of vaccination against COVID-19. Reports described a small number of cerebral venous thrombosis among tens of millions of Vaxzevria vaccinated individuals. In january 2022 the U.S. Vaccine Adverse Events Reporting System (VAERS) identified 54 cases of thrombosis with thrombocytopenia among over 14 million recipients of Jcovden vaccine. Of these, one third had cerebral venous sinus thrombosis. The remaining cases have affected the splanchnic system, heart, lungs or limbs. In Italy, 2 anaphylaxis, 1 acute or subacute neuropathy (Guillain-Barrè syndrome), and 1 atypical intracranial thrombotic event associated with thrombocytopenia (VITT) can be counted per million doses administered. With a prevalence of young women. **Case Reports**: We describe 11 fatal cases of suspected VITT, 7 females and 4 males aged 18-74, occurred in northern Italy between february and july 2021. We studied all the cases on assignment from the Public Prosecutor Office at the Court. Cases were studied at autopsy, and relationship to the vaccine was considered according to the following criteria: age, sex, vaccine given, interval, thrombosis, thrombocytopenia, PF4 antibody testing. For every case, 4 evaluation steps (Eligibility, Checklist, Algorithm, Classification) according to the WHO Causality Assessment recommendation were applied. Four autopsies (2 Vaxzevria and 2 Comirnaty vaccines) revealed myocardial infarctions, classified as "indeterminate". Five autopsies (all Comirnaty vaccines) revealed two myocardial infarctions, one venous thromboembolism, one subarachnoideal hemorrage, one necrotizing pancreatitis, classified as "inconsistent with causal association to immunization". Two autopsies of young women (2 Vaxzevria vaccines) revealed cerebral hemorrhage associated with thrombosis of the dural venous sinuses and presence of anti-PF4 antibodies, classified as "consistent with causal association to immunization, vaccine product-related reaction as per published literature". **Conclusions**: Our results confirm the likely association of VITT and Vaxzevria vaccine in two cases. In april, 2021 AIFA recommended a preferential use of Vaxzevria and Jcovden in subjects older than 60. On may 5, 2022 FDA has limited the use of the Jcovden vaccine to individuals 18 years of age and older. The trend of adverse reactions to vaccination against COVID-19 has been judged as acceptable from the clinical and epidemiological point of view and the benefits of the vaccine continue to outweigh the risks.

PO013

LONG TERM FOLLOW-UP OF A MULTICENTRE COHORT OF COVID-19 PATIENTS WITH PULMONARY EMBOLISM: ANTICOAGULATION MANAGEMENT AND OUTCOMES

L. Filippi[1], M. Milan[2], G. Turcato[1], S. Barbar[3],

D. Tonello[1], D. Milazzo[1], A. Zaboli[4], L. Panepinto[5], E. Miozzo[6], S. Cuppini[2], M. Marchetti[1]

[1]Internal Medicine Alto Vicentino Hospital Santorso; [2]Internal Medicine Rovigo Hospital "Santa Maria della Misericordia"; [3]Internal Medicine Cittadella Hospital; [4]Emergency department Merano Hospital "Franz Tappeiner"; [5]Internal Medicine University of Verona; [6]Internal Medicine University of Padua, Italy

Background and Aims: Pulmonary embolism (PE) is a frequent complication in COVID-19 hospitalized patients. Inflammatory storm and endothelial dysfunction due to the virus seem to be the two major risk factors for PE. Consequently, PE related to COVID-19 could be consider as triggered by a transient inflammatory acute phase and treated for no longer than 3 months. However, few data are available on management of anticoagulation and risk of venous thromboembolic (VTE) recurrences in these patients and guidelines are still undefined. Aim of the present study is to evaluate the long-term follow-up of a cohort of covid-19 patients with PE. **Methods**: We conducted a retrospective multicenter study in four Italian hospitals between March 1 st, 2020, and May 31st, 2021 in patients who experienced a PE during hospitalization for a COVID-19 pneumonia excluding patients who died during hospitalization. Basal characteristics were collected and patients were grouped according to duration of anticoagulant treatment (≤3 months or >3 months). Primary outcome was incidence of VTE relapses, secondary outcome was the composite of deaths, major hemorrhages and VTE relapses during follow-up. **Results**: 106 patients with PE were discharged, of these 95 (89.6%) had a follow up longer than 3 months (seven patients were lost to follow up and four died within three months). The median follow-up was of 13 months (IQR 1-19). Overall the 23.2% of subjects (22/95) was treated up to three months or less whereas the 76.8% of them (73/95) received anticoagulation for more than three months. Basal characteristics are shown in Table 1.

Duration of anticoagulant therapy	Short (≤ 3 months)	Long (> 3 months)	p
Patients, n (%)	22 (23.2)	73 (76.8)	
Median age (IQR)	65 (56-76)	68 (56-75)	0.870
Sex, n (%)			0.021
Female	3 (13.6)	31 (42.5)	
Male	19 (86.4)	42 (57.5)	
Chronic renal failure, n (%)	1 (4.5)	7 (9.7)	0.405
Obesity, n (%)	4 (18.2)	14 (19.2)	0.594
Diabetes, n (%)	4 (18.2)	17 (23.3)	0.773
Hypertension, n (%)	10 (45.5)	47 (64.4)	0.139
Previous VTE, n (%)	2 (9.1)	6 (8.2)	1.000
Active cancer, n (%)	0 (0.0)	9 (12.3)	0.083
COPD, n (%)	1 (4.5)	8 (11.0)	0.267
Coronary artery disease, n (%)	0 (0.0)	5 (6.8)	0.587
Peripheral artery disease, n (%)	2 (9.1)	13 (17.8)	0.508
Persistent immobility, n (%)	5 (22.7)	15 (20.5)	0.775
Concomitant deep venous thrombosis, n (%)	2 (9.1)	16 (21.9)	0.150
Anticoagulation before PE, n (%)	1 (4.5)	4 (5.5)	1.000
PE extention, n (%)			0.164
Sub-segmentary	9 (40.9)	18 (24.7)	
Segmentary	6 (27.3)	36 (49.3)	
Lobar or principal	7 (31.8)	19 (26)	
Anticoagulant therapy at discharge, n (%)			0.001
Heparin	10 (45.5)	8 (11)	
Oral anticoagulant	12 (54.5)	65 (89)	

Table 1. Basal characteristics.

The 4.5% of patients in the short treatment group died in comparison to the 5.5% of patients in the long term group (p=NS); no difference was also shown in risk of VTE relapses (0% *vs* 4.1%, p=ns), major bleeding (4.5% *vs* 4.1%, p=NS) and in composite outcome (9.1% *vs* 11%, p=ns). No difference was found between the two treatment groups for the composite outcome at the Kaplan-Meier analysis (Log Rank Test p=0.387). **Conclusions**: In our retrospective multi-center cohort prolongation of duration of anticoagulation seems not to affect risk of VTE recurrences, deaths and bleeding after a PE related to COVID-19.

PO014

SOME PARAMETERS OF PLATELET FUNCTION IN COVID-19 PATIENTS

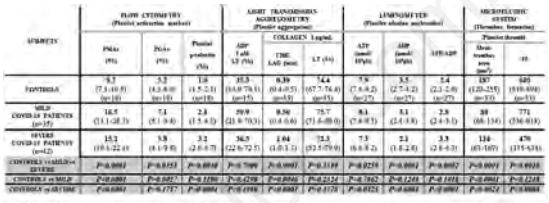
M. Scavone[1], C. Ghali[1], M. Calogiuri[1], M. Sala[2], E. Bossi[1], T. Mencarini[3], S. Bozzi[3], B. Clerici[1], S. Birocchi[1], N. Maugeri[4], G. Marchetti[2], M. Cattaneo[1], G. Podda[1]

[1]Divisione di Medicina Generale II, ASST Santi Paolo e Carlo, DISS, Università degli Studi di Milano; [2]Dipartimento di Malattie Infettive, ASST Santi Paolo e Carlo, Università degli Studi di Milano, Milan, Italy; [3]DEIB, Politecnico di Milano; [4]Division of Immunology, Transplantation & Infectious Diseases, IRCCS San Raffaele Institute, Milan, Italy

Background and Aims: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) causes COVID-19, an uncommon viral pneumonia commonly associated with a broad spectrum of presentations, including thrombotic and bleeding complications. The aim of this study was to characterize some parameters of the platelet function in patients with COVID-19 without (mild) and with pneumonia (severe). **Methods**: We studied 35 non-hospitalized patients with mild COVID-19 (54% men; median age 67 (23-86) years), 42 hospitalized patients with severe COVID-19 (62% men, median age 64 (25-88) years), and 15 healthy controls (HC) (61% men, median age 42 (29- 65) years). The following parameters were measured: 1) percentages of platelet/monocyte (PMAs) and platelet/granulocyte (PGAs) aggregates and of platelets expressing p-selectin on their membrane: these platelet activation markers were measured by flow cytometry in whole blood; 2) platelet aggregation (PA) induced by ADP 1 µM and Collagen 1 µg/mL in citrated platelet-rich plasma by light transmission aggregometry (LTA); 3) platelet adenine nucleotides (ATP and ADP) content, by luminometer; 4) capacity of platelets to form thrombi on collagen (10 µg/mL)-coated microfluidic chambers perfused by whole blood at a shear rate of 300/s; D-dimer plasma levels, which were measured only in patients with severe COVID-19. **Results**: Median values of markers of *in vivo* platelet activation were increased in mild and severe COVID-19 patients, compared to HC: PMAs, 16.5% *vs* 15.2% *vs* 8.2%, *P*<0.0001; PGAs, 7.1% *vs* 5.8% *vs* 5.2%,

P=0.0355; p-selectin expression, 2.1% *vs* 3.2% *vs* 1.8%, *P*=0.0030. In patients with severe COVID-19, plasma D-dimer levels were increased and positively correlated with PMAs (r=0.4433, *P*=0.0026) and PGAs (r=0.5310,*P*=0.0002). The extent of PA induced by exogenous agonists was heterogeneous in patients with mild and severe COVID-19; its median values were not significantly different from that of HC (ADP: 59.9% *vs* 36.5% *vs* 35.3%, *P*=0.7000; collagen: 75.7% *vs* 72.3% *vs* 74.4%, *P*=0.3599). However, the lag time of collagen-induced platelet aggregation was significantly longer in mild and severe COVID-19 than in HC. Platelets from patients with mild or severe COVID-19 showed a reduced capacity to form thrombi on collagen-coated microfluidic chambers, compared to HC (Table 1). Compared to HC, severe COVID-19 patients had lower platelet ADP and ATP contents (ADP: 1.8 *vs* 3.5 nmol/10^8 platelets, *P*<0.0001; ATP: 6.7 *vs* 7.9 nmol/10^8 platelets, *P*=0.0023) and higher ATP/ADP ratios (4.1 *vs* 2.4, *P*<0.0001). There was a statistically significant inverse correlation between plasma D-dimer levels and the platelet ADP content (r=-0.3616, *P*=0.0279). **Conclusions**: *In vivo* platelet activation markers were increased in COVID-19 patients, independently of the severity of the disease. The median platelet ADP content was low in severe COVID-19 patients, likely as a consequence of *in vivo* secretion by activated platelets. COVID-19 patients displayed impaired collagen-induced PA by LTA and formation of platelet thrombi on collagen-coated microfluidic chamber under controlled blood flow conditions.

Table 1. Platelet parameters in patients with mild or severe COVID-19.



PMAs= platelet/monocyte aggregates, PGAs= platelets/granulocyte aggregates; LT= Light transmission, FI= fluorescence intensity. Data are expressed as medians with interquartile ranges (25%-75%). One-way Analysis of Variance (ANOVA) test or t-tests were used when normal distribution was satisfied. Statistical significance was assumed for p-values <0.05.

PO015

PORTAL VEIN THROMBOSIS IN LIVER CIRRHOSIS PATIENTS CARRYING THE HOMOZYGOUS MTHFR C667T GENOTYPE DEVELOPS 9 YEARS EARLIER THAN IN WILD TYPE

P.R.J. Ames[1,2], G. D'Andrea[3], A. Arcaro[4], V. Marottoli[5], L. Iannaccone[5], F. Gentile[4], M. Maraglione[3]

[1]Immune Response & Vascular Disease Unit, NOVA Medical School, Universidade NOVA de Lisboa, Lisboa, Portugal; [2]Dumfries & Galloway Royal Infirmary, Dumfries, UK; [3]Medical Genetics, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; [5]Multimedica Srl, Naples, Italy; [4]Department of Medicine and Health Sciences 'VTiberio', University of Molise, Campobasso, Italy

Background and Aims: The role of methylentetrahydrofolate reductase (MTHFR) C667T genotype, plasma homocysteine (HC) and natural anticoagulants in portal vein thrombosis (PVT) with liver cirrhosis (LC) derives from case-control studies comparing LC patients with and without PVT. We compared age at PVT in a cohort of LC patients genotyped for MTHFR C677T and identified predictors of age at PVT. **Methods**: We recruited 128 LC patients with a PVT genotyped for MTHFR; we excluded 20 patients with other thrombotic risk factors and investigated 108 LC participants (36 MTHFR CC, 53 MTHFR CT and 19 MTHFR TT); age, sex, age at PVT, plasma HC and natural anticoagulant concentrations was available for all participants. **Results**: Age at PVT was lower and plasma HC was higher in MTHFR TT; antithrombin, protein C and S were lower in the MTHFR TT group (Table 1). MTHFR TT, male gender and protein C predicted age at PVT (p-0.02, p=0.04 and p=0.08 respectively); MTHFR TT and Child-Pugh score positively predicted plasma protein C (p<0.0001 and p=0.0002 respectively) while plasma HC negatively predicted protein C (p<0.0001). Age at PVT of the MTHFR TT group was greater than that that of 19 patients with idiopathic PVT carrying the same genotype (56±13 *vs* 31.3±8.5, p<0.001) (cohort presented in separate abstract). **Conclusions**: MTHFR TT anticipates age at PVT in LC by an average of 9 years compared to MTHFR CC. HC adversely affects protein C that plays a part in the premature PVT presentation in the MTHFR TT setting.

Table 1.

MTHFR	CC	CT	TT	P value
No	36	53	19	
M/F	21/15	39/14	12/7	
Age years (x̄ ± σ)	82±11	76±12	76±13	
Age at PVT, years (x̄ ± σ)	64±9	57±13	56±13	0.03
Child-Pugh score (x̄ ± σ)	7.9±2.2	8.7±2.3	11.6±3.1	0.001
PT G20210A	3 | 8.3%	2 | 3.7%	3 | 15.7%	
INR (x̄ ± σ)	1.2±0.13	1.4±0.27	1.6±0.25	0.001
aPTT (x̄ ± σ)	1.12±0.10	1.13±0.14	1.40±0.16	0.001
AT IU/dl (x̄ ± σ)	86±17	82±20	55±11	0.001
PC IU/dl (x̄ ± σ)	60±10	52±11	41±9	0.001
PS IU/dl (x̄ ± σ)	70±12	67±12	60±7	0.02
HC µmol/L (x̄ ± σ)	11±3	12±7	24±27	0.001
HC >12.5 µmol/L	14 | 38.8%	27 | 50.9%	13 | 68.4%	

PO016

CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: THE ONCO-VTE START2 – REGISTRY

L. Barcella[1], E. Antonucci[2], L. Russo[1], A. Chistolini[3], A. Serrao[3], G. Elmi[4], P. Sivera[5], F. Schieppati[1], A. Tufano[6], S. Pedrini[7], G. Colombo[8], W. Ageno[8,9], A. Falanga[1,10], G. Palareti[2]

[1]UOC Immunoematologia e Medicina Trasfusionale - Centro Emostasi e Trombosi, ASST Papa Giovanni

XXIII, Bergamo; [2]Fondazione Arianna Anticoagulazione Bologna; [3]Sezione Ematologia, Dipartimento di Biotecnologie Cellulari ed Ematologia Azienda Ospedaliero Universitaria Policlinico Umberto I, Roma; [4]Dipartimento di Medicina Interna, Ospedale Maggiore Bologna; [5]SCDU Ematologia e Terapie cellulari AO Ordine Mauriziano di Torino; [6]Centro di Riferimento Regionale per le coagulopatie, Dipartimento di Medicina e Chirurgia, AOU Federico II Napoli; [7]UO Laboratorio Analisi, Dipartimenti di Medicina di Laboratorio, Fondazione Poliambulanza Istituto Ospedaliero, Brescia; [8]DB Internistica e Centro Trombosi ed Emostasi - AO di Circolo - ASST dei Sette Laghi, Varese; [9]Dipartimento Di Medicina e Chirurgia, Università Degli Studi Dell'Insubria, Varese; [10]Dipartimento di Medicina e Chirurgia, Università Milano-Bicocca, Monza, Italy

Background and Aim: Cancer-associated thrombosis (CAT) is a major cause of morbidity and mortality in patients with malignancy. Anticoagulant treatment is challenging because of the increased risk of recurrent venous thromboembolism (VTE) and bleeding. Information about the management of patients with CAT in real life are still scanty. The purposes of the "VTE in the cancer patient (Onco-VTE) START2 (Survey on anTicoagulated pAtients RegisTer 2) register are: 1) to prospectively collect data about CAT patients, in particular the type of anticoagulant administered and the duration of treatment; 2) to collect information about the management of these patients in real life: the choice of anticoagulant drug and the duration in relation to type of tumor, stage of disease, comorbidities, associated therapies; 3) to evaluate the incidence of thromboembolic and haemorrhagic complications of the anticoagulant treatment. **Methods**: The START2 is a multicenter, observational, independent ongoing register which prospectively collect clinical information about patients on anticoagulant treatment in "everyday clinical practice". Data are collected by researchers of the participating centres by means of an on-line electronic case report form. Onco-VTE is a branch of the START2 register that includes additional information on the natural history of CAT and associated oncological disease. **Results**: Presently, in Onco-VTE START2 register 122 CAT patient were enrolled; 45% males; median age (range) 66 (24;92) years; 48% with hematological cancer. In 52% of patients CAT occurred at the onset of cancer disease and in 33% of patients CAT has been diagnosed incidentally. VTE events (79% deep venous thrombosis with or without pulmonary embolism; 21% isolated pulmonary embolism) were treated in 52% of patients with direct oral anticoagulant (DOAC); 4% of patients were treated with Vitamin K Antagonist (VKA); 44% with low molecular weight heparin (LMWH) or fondaparinux. Concomitant use of antiplatelet drugs was recorded in 10% of patients. **Conclusions**: CAT patients are very heterogeneous. Several factors can influence the choice, duration, efficacy and safety of treatment of CAT. Randomized clinical trials necessarily investigated a sample of CAT patients and evidence for the treatment of CAT is still limited. "Real life" data are needed to monitor current clinical practice, to identify variables associated to efficacy and safety of treatment and to obtain suggestions for improving clinical practice and promote research.

The Foundation has received an unrestricted research grant from LEO Pharma S.p.A., specifically dedicated to the realization of this study

PO017

INFERIOR VENA CAVA FILTER PLACEMENT IN PATIENTS WITH ACUTE VENOUS THROMBOEMBOLISM: ANALYSIS OF CLINICAL INDICATIONS, ANTICOAGULATION MANAGEMENT AND IN-HOSPITAL MORTALITY

D. Santagata[1], G. Cammà[2], A. Porfidia[2], E. Porceddu[2], F. Piacentino[3], F. Fontana[3], M. Venturini[3], W. Ageno[1], R. Pola[2], M.P. Donadini[1]

[1]Department of Medicine and Surgery, Research Center on Thromboembolic Disorders and Antithrombotic Therapies, University of Insubria, Varese, Italy; [2]Department of Internal Medicine, Fondazione Policlinico Universitario AGemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; [3]Department of Diagnostic and Interventional Radiology, Ospedale di Circolo e Fondazione Macchi, University of Insubria, Varese, Italy

Background: Inferior vena cava filters (IVCF) are currently the most commonly used mechanical method to prevent embolization in patients with acute venous thromboembolism (VTE) when an absolute contraindication to anticoagulation (AC) exists. However, the population requiring IVFC placement is highly heterogenous and still poorly characterized. Therefore, there are no specific indications on how to restart AC therapy after IVFC placement. Therefore, we aimed to collect data on patients with acute VTE undergoing IVCF placement, in order to better characterize them and provide information on their current in-hospital management. **Methods**: A retrospective cohort study was conducted including consecutive patients objectively diagnosed with acute lower limb deep vein thrombosis (DVT) and/or pulmonary embolism (PE) who underwent IVCF placement by interventional radiology service at Ospedale di Circolo, ASST Sette Laghi (Varese) between 2015 and 2019, and at IRCCS Fondazione Policlinico Universitario A. Gemelli (Rome) between 2010 and 2019. The objectives were to assess the characteristics of the population, including baseline characteristics, type of VTE events and indication for IVFC placement, and to evaluate anticoagulation management and in-hospital mortality. Baseline characteristics and data on IVCF placement, AC management and in-hospital mortality were collected from hospital databases. **Results**: We included 317 patients undergoing IVCF placement: 143 women

(45.1%) and 174 men (54.9%) with a mean age of 67,0 years (SD 15.7, range 14-99) (Table 1). Active cancer was present in 56.5% of patients and at least one comorbidity, in addition to acute VTE, was present in all patients, including 47.6% with hypertension, 16.4% with diabetes mellitus and 12.6% with a history of VTE. The main reported indication for IVFC placement was recent bleeding (58.7%), followed by non-deferrable surgery (40.7%), high bleeding risk lesion (21.5%), floating thrombus (3.8%) and anticoagulation failure (3.2%). Thirty-five patients (11,0%) died during hospitalization after a mean of 31.7 days (SD 40) from IVFC placement. The main cause of death was sepsis (34.3%) followed by bleeding (11.4%) and VTE (5.7%). At discharge, 218/282 patients (77.6%) were receiving AC (20.6% parenteral at prophylactic dose, 62.4% parenteral at intermediate/therapeutic dose, 7.8% DOACs and 4.2% VKAs). No significant association was found between filter indications and in-hospital mortality or AC initiation at discharge. **Conclusions**: This study confirms that patients selected for IVCF implantation are highly comorbid and show significant short-term mortality. AC was started in-hospital with parenteral drugs in the great majority of cases, most of them at therapeutic doses.

Table 1.

IVCF indications, in-hospital mortality and AC therapy prescribed at discharge

	Overall population	In-hospital mortality	AC at discharge
	317	35/317 (11,0%)	218/282 (77,3%*)
Recent Bleeding	186 (58,7%)	19/186 (10,2%)	123/167 (73,7%*)
Non-deferrable surgery	129 (40,7%)	16/129 (12,4%)	91/113 (80,5%*)
Floating thrombus	12 (3,8%)	3/12 (25,0%)	9/9 (100%*)
Anticoagulation failure	10 (3,2%)	1/10 (10%)	7/9 (77,8%*)
High-risk bleeding lesion	68 (21,5%)	12/68 (17,7%)	42/56 (75,0%*)

*: Calculated on the population alive at hospital discharge; AC: Anticoagulation

PO018

ENDOVASCULAR TREATMENT OF DEEP VEIN THROMBOSYS SECONDARY TO ILIAC VEIN COMPRESSION (MAY THURNER SYNDROME): A MONOCENTRIC CASE SERIES

G. Marchesini[1], G. Carli[1], I. Nichele[1], L. Boi[2], A. Tosetto[1]

[1]Hemophilia and Thrombosis Center, Hematology Department, SBortolo Hospital, Vicenza; [2]Complex Operational Unit of Radiology, SBassiano Hospital, Bassano del Grappa, Italy

Background: May-Thurner syndrome (MTS) is a rare clinical entity featuring venous obstruction of the left lower leg that can cause deep vein thrombosis (DVT) resistant to anticoagulant therapy with a high risk of severe post-thrombotic syndrome (PTS). There are no standard guidelines to treat DVT secondary to MTS or its long-term sequelae, but endovascular treatment appears to be a promising option in selected cases. **Aim**: We aimed to describe a case series of patients with MTS presenting with proximal DVT and treated with an endovascular procedure and long-term anticoagulation. **Materials and Methods**: This study included patients with a DVT secondary to MTS diagnosed from March 2017 to May 2022 and treated with an endovascular procedure. In all cases, MTS was diagnosed with CT venography or endovascular ultrasound scan. We collected clinical and radiological data: sex, age, thrombophilic status, the pattern of DVT, endovascular procedure time and type, adverse events, and status at last follow-up. **Results**: From March 2017 to May 2022, seven patients presented a DVT secondary to an MTS. Patients characteristics are summarized in Table 1. Six patients were female, and one was male. The median age was 38 years old. All patients presented a left leg proximal DVT with involvement of the proximal iliac axis; in one case, there was also an extension to inferior vena cava (IVC). In one patient, the diagnosis of MTS was made at the second relapse of DVT. Three patients (42%) presented a major thrombophilic status: one protein S deficiency, one protein S deficiency + factor V Leiden, and one with combined factor V Leiden and factor II G20210A heterozygosity. One patient showed heterozygosity for factor II G20210A mutation; the other three patients had no abnormalities. All patients underwent an endovascular procedure. Two patients were treated in an acute phase (within 30 days of DVT diagnosis), while five patients were treated after median 17 months from the acute event. The procedure consisted of manual aspiration thrombectomy using a guide catheter, followed by balloon angioplasty and venous stent placement. Only in one case, there was acute restenosis after two days, which required reoperation and placement of a new stent. Other two patients presented anemia treated with blood transfusion in the first seven days after the procedure. No significant bleeding events have been recorded. All patients were treated with heparin for one month, followed by a long-term anticoagulant therapy with DOAC (apixaban in five patients, rivaroxaban and edoxaban each in one patient). After a median follow-up of 18 months, no patients experienced a relapse of DVT or stent restenosis with a significant improvement of the PTS. **Conclusions**: It is important to suspect MTS in cases of DVT in the left lower limb with involvement of the iliac axis and resistant to conventional anticoagulant therapy. In these cases, the endovascular approach represents a viable therapeutic option with high success rates, especially in mitigating the severity of the PTS.

Table 1. Patients' characteristics (DVT=deep vein thrombosys, PTS= post thrombotic syndrome).

PO019

OUTCOMES DURING FOLLOW-UP IN VENOUS THROMBOEMBOLISM PATIENTS INCLUDED IN THE INTERNATIONAL, PROSPECTIVE, OBSERVATIONAL WHITE STUDY

M. Cini[1], T. Urbanek[2], Y.J. Li[3], J. Madaric[4], K. Bouslama[5], G.Y. Sokurenko[6], G.M. Andreozzi[7], J. Matuška[8], A. Mansilha[9], V. Barinov[10], G. Palareti[1], for the WHITE study group (Fondazione Arianna Anticoagulazione)

[1]Fondazione Arianna Anticoagulazione, Bologna, Italy; [2]Katowice, Poland; [3]Peking Union Medical College, Beijing, China; [4]Comenius University, Bratislava, Slovakia; [5]Faculty of Medicine of Tunis, Tunisia; [6]StPetersburg, Russia; [7]University of Padua, Italy; [8]Clinical Trial Centre, Hodonin, Czechia; [9]University of Porto, Portugal; [10]Clinical hospital N1 "Volynskaya", Moscow, Russia

Background and Aims: How to prevent recurrencies after a first venous thromboembolism (VTE) may be influenced by many factors. The WHITE study was an international, prospective, observational, investigators-initiated, no profit study (ClinicalTrials.gov Identifier: NCT04646993) promoted by Fondazione Arianna Anticoagulazione (Bologna, Italy), that aimed at analyzing a) the treatment decisions taken on this issue in clinical practice in various countries, different for socio-economic conditions and healthcare systems, and b) the clinical outcomes recorded during one year follow-up (FU) after the treatment decisions were taken (which is the aim of this presentation). **Methods**: 1004 patients (510 females), with a prior first VTE event, were included in the study in 62 clinical centers in 7 countries from April 2018 to December 2020 [China (n. 258), Czechia (n. 67), Poland (n. 128), Portugal (n. 33), Russia (n. 415), Slovakia (n. 23), and Tunisia (n. 80)], and followed-up for one year after receiving a decision about treatment beyond the initial and maintenance phases. **Results**: The index event was unprovoked in 57.9% of patients and provoked in the remaining. During FU, 53.4% of patients continued anticoagulation, 27.4% continued with antiplatelets, 19.2% stopped all treatments. When anticoagulation was continued, a direct oral anticoagulant (DOAC) was used in 88.6%, vitamin K antagonist (VKA) in 8.4% and low molecular weight heparin (LMWH) in 3.0% of cases ($p<0.001$). Anticoagulant treatment continued in 53.0% of patients with unprovoked and in 53.9% with provoked events ($p=0.78$). Anticoagulation was continued in: 59.0% of patients with proximal (±distal) deep vein thrombosis (DVT), 39.0% of isolated distal DVT, 65.0% of cases with pulmonary embolism (PE) with or without DVT ($p<0.001$). Primary outcomes (recurrent VTE, and major bleed-MB) during FU occurred in: 2.9% pt/y in patients who extended the anticoagulation with DOAC (47.3% of the total), 2.2% pt/y when all treatments were stopped (19.2%), 3.5% pt/y in patients shifted to sulodexide (20.1%), and 4.9% pt/y (including one MB) in those shifted to antiplatelets (7.3%) ($p=0.23$). **Conclusions**: The provoked/unprovoked nature of the index event was not the prevalent criterion for duration of treatment. Anticoagulation was continued in more than half of cases, whereas treatment was shifted to different antithrombotic agents in 27.4% of patients and the recommendation to stop any specific treatment was given to about 20% of all patients. Almost all patients in whom anticoagulation was extended were treated with DOAC. Only in Tunisia DOACs were not available when the study was realized. During follow up, one MB occurred among patients treated with antiplatelet drugs, while the rate of VTE recurrences was in general low in all patients. The rate of primary events was the highest in patients treated with antiplatelets.

PO020

CATHETER-RELATED RIGHT ATRIAL THROMBOSIS IN CANCER PATIENTS: A RETROSPECTIVE ANALYSIS

E. Porceddu[2], E. del Sordo[1], A. D'Errico[1], A. Porfidia[2], P. Tondi[2], A. Gasbarrini[1], R. Pola[2]

[1]Department of Internal Medicine, Fondazione Policlinico Universitario AGemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Department of Geriatrics, Neurosciences and Orthopedics, Fondazione Policlinico Universitario AGemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

Background and Aims: Literature data on catheter-related right atrial thrombosis (CRAT) are scarce and based only on case reports or case series1-2. Data mostly concern dialysis patients while reports on oncological patients are poor2. In this study we investigated characteristics of cancer patients with CRAT to provide a better characterisation and to improve the knowledge of this disease. **Methods**: We retrospectively analysed data of 21 consecutive cancer outpatients with CRAT that referred to our Internal Medicine and Thromboembolic Pathology Unit over a period of 3 years. Of these patients we collected epidemiological and clinical data and evaluated the efficacy (resolution of the thrombotic process and recurrences) and safety (bleedings) of the different anticoagulant treatments used. **Results**: In our study, the median age was 57 years. There were 4 (19%) males and 17 (81%) females. All patients had active neoplasia: 8 (38%) ovarian, 5 (24%) uterine, 5 (24%) colorectal, and 3 (14%) breast cancer. Of them, 15 (71%) were metastatic. In 5 (24%) cases, thrombosis was present at other sites, in addiction to the right atrium, *i.e.*, pulmonary embolism (PE) and deep veins of the upper limbs. In 2 cases, the diagnosis of CRAT was made by echocardiography, while in the other 19 cases by CT-angiography, with further confirmation by echocardiography in 15 patients. The median follow-up was 10

months. Nineteen (90%) patients received anticoagulant treatment for 3-6 months: 17 with low molecular weight heparin ad 2 with direct oral anticoagulants (DOACs). After completion of the treatment period, 9 (43%) patients extended treatment with low dose anticoagulation. In 12 (57%) patients there was complete resolution of CRAT, in 5 (24%) a partial resolution, and in 3 (14%) the thrombus remained stable. Data are not available in 1 (5%) patient. No major bleedings occurred, nor clinically relevant non major bleedings. There were only 4 minor bleedings: 2 epistaxis and 2 genital bleedings. One pulmonary embolism occurred during anticoagulant treatment. **Conclusions**: With the increase of patients with central venous catheters, CRAT is becoming less uncommon than previously thought. Early treatment may lead to complete thrombosis resolution in more than half of patients. Anticoagulants are safe with low risk of bleeding. The risk of recurrences is similar to the risk displayed by thrombosis in usual sites in cancer patients3-4.

References:

1. Aristeidis Stavroulopoulos, Vasiliki Aresti *et al.* Right atrial thrombi complicating haemodialysis catheters. A meta-analysis of reported cases and a proposal of a management algorithm Nephrol Dial Transplant (2012) 27: 2936–2944
2. Minh-Ha Tran1,2, Tanya Wilcox *et al.* Catheter-related right atrial thrombosis. The Journal of Vascular Access 2020, Vol. 21(3) 300–307
3. Prandoni P, Lensing AW, Piccioli A, *et al.* Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. Blood 2002;100:3484-8
4. T.F. Wang, A. Li, *et al.* Managing thrombosis in cancer patients. Res Pract Thromb Haemost, 2 (3) (2018), pp. 429-438

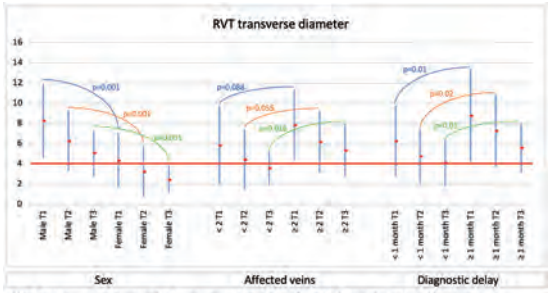
PO021

RESIDUAL VEIN THROMBOSIS AND PREDICTIVE FACTORS OF RECANALIZATION UNDER DIRECT ORAL ANTICOAGULANTS. A SINGLE CENTER PROSPECTIVE STUDY

M. Bardetta[1], A. Pizzuto[2], M. Simoncini[3], P. Martinelli[1], C. Sella[1], C. Dainese[1], F. Valeri[1], B. Bruno[4], A. Borchiellini[1]

[1]University Division of Hematology, Regional Center for Hemorrhagic and Thrombotic Diseases, City of Health and Science University Hospital of Molinette, Turin, Italy; [2]University Division of Internal Medicine [2]"GMontrucchio", City of Health and Science University Hospital of Molinette, Turin, Italy; [3]Emergency Medicine Division, City of Health and Science University Hospital of Molinette, Turin, Italy; [4]University Division of Hematology, City of Health and Science University Hospital of Molinette, Turin, Italy

Background and Aims: The optimal management strategy for prevention of recurrent deep vein thrombosis (DVT) is still uncertain. Clinical and laboratory assays and imaging tests such us residual vein thrombosis (RVT) have been proposed as risk factors for recurrence. Though, which patients have a higher risk of RVT is still unclear. Some categories have been identified, such as the male gender, obese and cancer patients or the extensive thromboses. Furthermore, most of data are from vitamin K antagonists (VKA) treatment and experience on direct oral anticoagulants (DOACs) is scanty. Therefore, we designed a prospective study to evaluate which clinical features are more predictive of pathological RVT during DOACs treatment. **Methods**: The local Ethics Committee approved our study, that we called READ ("residual veins thrombosis under DOACs"), in November 2019. All the eligible patients signed an informed consent based on the Declaration of Helsinki. As from literature, RVT was defined as persistent thrombotic material in a diameter of at least 4 mm, identified by compression ultrasonography (CUS) at common femoral or popliteal veins. Patients have been evaluated at 6 weeks (T1), 3 months (T2) and 6 months (T3) through clinical and demographic variables. The study is still ongoing and we have so far enrolled 80 patients. We are here presenting the interim results on a total of 50 patients who completed a 6-month follow up in which they received a direct oral anticoagulant (DOAC). **Results**: In our cohort of patients, composed of 66% and 34% of respectively male and female patients, 54% was treated with Apixaban, 40% with Rivaroxaban, 4% with Edoxaban and 2% with Dabigatran. RVT rate was 77.1% at six weeks, 58% at three months and 43.5% at six months. Male gender was independently associated with RVT at six weeks (OR 4.75, CI 1.13 – 19.68, p=0.036), three months (OR 6.4, CI 1.75 – 23.35, p=0.006) and six months (OR 7.71, CI 1.48 – 40.24, p=0.011). In the univariate model, DVT of at least two segments was associated at higher RVT rate at six weeks (OR 5.29, CI 1.00 – 27.93, p=0.045) and three months (OR 3.54, CI 1.07 – 11.78, p=0.047) and to greater RVT extension at six months (5.4 mm±2.7 mm *vs* 3.6 mm±1.7 mm, p=0.016). Diagnostic delay of at least one month was associated to higher rate of RVT at six months (OR 6.46, CI 1.17 – 35.74, p=0.029) and to greater RVT extension at three months (7.3±3.6 *vs* 4.8±2.8, p=0.02). Obesity was associated to lower rate of RVT at three months (OR 0.12, CI 0.22 – 0.65, p=0.011) and at six months (OR 0.1, CI 0.01 – 0.87, p=0.03). Main results are shown in Figure 1. **Conclusions**: Male gender is confirmed as independent predictor for persistence of RVT in patients treated with DOACs. In our experience, extension of DVT is associated to a reduced recanalization as previously described during VKA treatment. Diagnostic delay as well seem to be associated to a reduced recanalization. On the contrary obesity does not seem to influence negatively the recanalization. Unlike previous reports, prevalence of RVT in our cohort is similar to published experience in VKA patients.



RVT diameter in millimeters in three categories at 6 weeks (T1), 3 months (T2) and 6 months (T3). Red line is the threshold beyond which risk of recurrence increases.

Figure 1.

PO022

CAN RESIDUAL VEIN THROMBOSIS GUIDE CLINICAL PRACTICE AND THERAPY IN PATIENTS WITH PREVIOUS THROMBOTIC EVENT? COMPARISON BETWEEN CUS AND DOPPLER US DATA FROM "READ" MONOCENTRIC PROSPECTIVE STUDY

A. Pizzuto, M. Simoncini, P. Martinelli, M. Bardetta, C. Sella, C. Dainese, F. Valeri, B. Bruno, A. Borchiellini

Università degli Studi di Torino, Italy

The advent of new therapeutic strategies, *i.e.* the Direct Oral Anticoagulants (DOACs), to treat Deep Vein Thromboses (DVT), rises questions concerning both timing and dose. In patients with a previous DVT, Residual Vein Thrombosis (RVT) is taking more and more ground as a possible risk factor of recurrence, and on the other hand as a prognostic tool to guide clinical practice. In this context, Compression Ultrasonography (CUS) could be applied to assess RVT thanks to its simplicity, accessibility, even in bedside setting, and increasingly widespread use among non-radiology clinicians. We designed a prospective study, named READ ("RVT in DOAC therapy"), to evaluate the predictive factors of pathological RVT and, secondly, we are also verifying reliability between our CUS determination and external center Doppler Ultrasonography (DU) reports. We are here presenting the latter results. The Ethics Committee approved our study in November 2019. All patients signed an informed consent based on the Declaration of Helsinki. Patients have been evaluated at T1 (6 weeks), T2 (3 months) and T3 (6 months) through clinical and demographic variables and CUS. This technique consists in the evaluation, under compression, of two major thrombogenic sites of DVT of lower limbs: the common femoral vein (from great saphenous to subsartorial vein) and popliteal vein (from very proximal tract to tibial vein). We defined CUS test positive when RVT measured at least 4 mm in transverse diameter, as widely accepted. The study is still ongoing and here are the interim results of 50 patients at time T2 of follow up in DOAC therapy. Reliability between internal (CUS) and external (DU) observations has been computed according to Cohen's kappa coefficient. In our cohort, RVT rate at T1 was 77%, at T2 58% and at T3 43%. Comparing results obtained at the same time in the two different settings, CUS *versus* DU, for an amount of 84 total observations, 21 out of 84 (25%) have discordant results. The degree of agreement between CUS and DU was just over "moderate" (κ=0.42, p=<0.0001). Only 27.9% (19 out of 68) of all positive DU were confirmed at CUS. On the other hand, the deviation was only 12.5% (2 out of 16) in case of negative DU (Figure 1). Low level of agreement shown between DU performed by independent specialists without defined specific criteria and our CUS evaluations, highlights the importance of a shared and reproducible elements to define RVT for clinical use. Indeed, the main difference consists of RVT smaller than 4 mm, so insufficient to be considered as CUS positive but well documented in DU. Highest agreement in negative reports may suggest a well negative predictive value of CUS. The role of CUS to evaluate RVT and to guide clinical practice and therapy is still debated but first analyses show possible important future developments in this field, also thanks to the large spreading of CUS among clinicians.

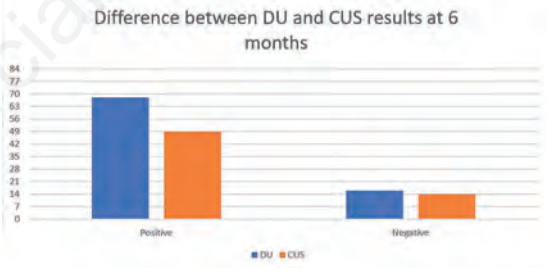


Figure 1.

PO023

UPDATED SURVEY ON TREATMENT AND SECONDARY PROPHYLAXIS OF VENOUS THROMBOEMBOLISM WITH ANTI XA IN PATIENTS WITH SEVERE HEREDITARY THROMBOPHILIA- THE EXPERIENCE OF THREE SOUTHERN ITALY CENTRES EXPERIENCE

P. Madonna[1], A. Guida[1], A. De Sena[1], P. Tirelli,[1] A. Vitale[2], G. Papa[2], S. Mangiacapra[3], M. Amitrano[3], G. Landi[2] C. Bologna[1], M.G. Coppola[1], N. Vitelli[2], A. Tufano[2]

[1]UOC Medicina Interna Ospedale del Mare ASL Napoli 1 Centro; [2]Dipartimento di Medicina Clinica e Chirurgia AOU Federico II; [3]UOC Medicina Interna AORN GMoscati, Avellino, Italy

Background and Aims: Severe Inherited throm-

bophilia (SIT): antithrombin (AT), protein-C (PC), protein-S (PS) deficiency, homozygousity or compound heterozygosity Factor-II (FII)-G20210A and FV-Leiden (FVL) polymorphism although rare, increases the risk of venous thromboembolism (VTE), annual incidence >1%. A paucity of data is available in VTE treatment with direct oral anticoagulants (DOACs) in these setting, aim of our survey was to observe the efficacy and safety of DOACs in SIT. **Methods**: 36-subjects (19M;17F, mean age:40,89) with VTE: confirmed diagnosis of acute deep vein thrombosis (DVT) and/or pulmonary embolism (PE), referred between March 2014 and March 2022 to three Campania thrombosis-centres, were enrolled. All patients were prescribed DOACs for at least 3 months. All subjects were screened for AT, PC, PS deficiencies, FVL, FIIG20210A polymorphisms. Hyperhomocysteinaemia and antiphospholipids were excluded. VTE was treated with anti-Xa anticoagulants. 3-months clinical follow-up was scheduled. **Results**: We identified 4 AT-deficiencies, 2 PC-deficiencies, 7 PS-deficiencies, 13 combined heterozigosity for FVLeiden and FIIG20210A, 4 FVL homozigosity, 3 FII-G20210A homozigosity and 2 combined FVL-heterozigosity and FIIG20210A homozygosity. 22/36 received Rivaroxaban, 5/36 Edoxaban and 9/36 Apixaban; 9/36 switched from VKA to anti-Xa. 28/36 were prescribed lifelong full-dose therapy, two patients withdrawn therapy after 12 and 24 months respectively. During the 8-years follow-up, neither thrombotic recurrences nor major hemorrhagic events were observed. No differences in sex, type of SIT, or drug. (Table 1) **Conclusions**: Our survey suggests efficacy and safety of anti-Xa anticoagulants for treatment and secondary prophylaxis of VTE in patients with SIT. Furthermore extensive multicentric studies are needed.

Table 1.

Unic	Patient	Sex	Age (°event)	VTE	Provoked/ Unprovoked	Drug/Dose reduction	Months of therapy	Type of trombophilia	VTE Bleeding	Center
1	DO	F	59	PE	U	Rivr.	86	AT III.	0/0	ODM
2	DA	F	49	DVT	U	Rivr.	63	AT III.	0/0	ODM
3	BP	F	41	DVT rec	P (Contraceptive)	Rivr.	63	PS	0/0	ODM
4	MB	F	22	PE	P (Estrogens)	Rivr.	18 and stop	PC	0/0	ODM
5	DI	F	28	PE	P (Puerperium)	Apix.	12 and stop	FVL/FII +/-	0/0	ODM
6	MI	F	36	DVT	P (Estrogens)	Rivr. 20/10	24 +25	PS	0/0	ODM
7	ME	M	34	DVT rec	U	Apix.	45	PC	0/0	ODM
8	IA	F	59	DVT+PE	U	Rivr.	46	PS	0/0	ODM
9	IF	M	49	DVT	U	Rivr.	17	FVL/FII +/-	0/0	ODM
10	OC	M	20	DVT	P (Trauma)	Edox	12	FVL +/+	0/0	ODM
11	MM	F	17	PE	U	Rivr.	12	FVL/FII +/-	0/0	ODM
12	DD	F	39	DVT+PE	P(Surgery)	Rivr.	17	PS	0/0	ODM
13	PG	M	39	PE	U	Edox	5	FVL +/+	0/0	ODM
14	GG	M	55	DVT	U	Rivr.	14	F II +/+	0/0	ODM
15	MI	F	50	DVT+PE	U	Rivr. 10.	6+4	FVL/FII +/-	0/0	ODM
16	GE	M	48	DVT	P (Trauma)	Rivr.	4	F II +/+	0/0	ODM
17	VT	F	59	DVT	U	Rivr.	49	FVL/FII +/-	0/0	U
18	ER	M	30	DVT+PE	U	Edox	61	FVL +/+	0/0	U
19	AG	M	29	DVT-PE	P(Trauma)	Edox	37	FVL/FII +/-	0/0	U
20	DL	M	59	DVT	P (Accid)	Apix.	34	ATIII	0/0	U
21	GE	M	42	DVT	U	Rivr.	45	F II +/+	0/0	U
22	SA	M	19	DVT+PE	U	Rivr.	29	FVL +/+	0/0	U
23	EL	M	25	DVT	U	Rivr.	93	FVL/FII +/-	0/0	U
24	MP	M	53	DVT+PE	U	Apix.	36	FVL/FII +/+ PS	0/0	U
25	MA	F	18	DVT	P(Estrogen)	Apix.	71	FVL/FII +/-	0/0	U
26	BL	M	43	DVT+PE	U	Rivr.	51	FVL/FII +/-	0/0	U
27	CA	M	17	DVT+PE	U	Rivr.	77	FVL/FII +/- PS	0/0	U
28	CA	M	33	DVT	U	Rivr.	39	FVL/FII +/-	0/0	U
29	IG	F	25	DVT+PE	U	Apix.	4	AT III	0/0	U
30	FC	F	55	DVT	U	Apix.	12	FVL/FII +/-	0/0	U
31	EG	M	57	DVT	U	Apix. 10/5	61	FVL/FII +/-	0/0	U
32	GI	F	27	DVT	U	Rivr 20/10	20	FVL/FII +/-	0/0	U
33	CA	M	54	DVT+PE	U	Apix 10/5	28	PS	0/0	U
34	SB	F	47	DVT	U	Rivr 20.10	27	FVL/PS	0/0	U
35	MS	F	40	DVT	U	Edox	27	FVL/FII +/-	0/0	AV
36	RL	M	38	DVT	U	Edox	37	PS	0/0	AV

PO024

IDIOPATHIC PORTAL VEIN THROMBOSIS IN HOMOZYGOUS MTHFR C667T CARRIERS DEVELOPS 20 YEARS EARLIER THAN IN WILD TYPE

P.R.J. Ames[1,2], G. D'Andrea[3], A. Arcaro[4], V. Marottoli[5], L. Iannaccone[5], M. Maraglione[3], F. Gentile[4]

[1]Immune Response & Vascular Disease Unit, NOVA Medical School, Universidade NOVA de Lisboa, Lisboa, Portugal; [2]Dumfries & Galloway Royal Infirmary, Dumfries, UK; [3]Medical Genetics, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; [4]Department of Medicine and Health Sciences 'VTiberio', University of Molise, Campobasso, Italy; [5]Multimedica Srl, Naples, Italy

Background and Aims: Studies on idiopathic portal vein thrombosis (PVT) and inherited thrombophilia are mostly case-control; in our cohort we 1) compared age at PVT and plasma homocysteine (HC) in PVT patients devoid of circumstantial factors for PVT, genotyped for methylentetrahydrofolate reductase (MTHFR) C667T; 2) identified predictors of age at PVT. **Methods**: 298 PVT patients were genotyped for MTHFR; we excluded 212 patients with different risk factors for thrombosis, including factor V Leiden, protein C and S deficiency. Of the remaining 86 participants, 24 were MTHFR CC, 45 MTHFR TC and 17 MTHFR TT; age, sex, age at PVT, smoking status, plasma HC and natural anticoagulant concentrations were recorded. **Results**: Age at PVT was lower and plasma HC was higher in MTHFR TT than in other genotypes; natural anticoagulant concentrations were similar (Table 1).

Table 1. Demographics, clinical and laboratory variables by MTHFR genotypes.

MTHFR	CC		CT		TT		P
No	24		45		17		
M/F	13/11		21/24		10/7		
Age years ($\bar{x} \pm \sigma$)	69±15		64±15		48±11		0.001
Age at PVT, years ($\bar{x} \pm \sigma$)	52±14		46±16		31±8		0.001
	No	%	No	%	No	%	
Smokers	7	29.1	17	68.0	6	35.2	
Cigarettes per day ($\bar{x} \pm \sigma$)	8±3		7±3		8±4		
Cavernoma	2	8.3	14	31.3	2	11.7	
PT 20210 No	2	8.2	10	22.2	4	23.5	
AT IU/dl ($\bar{x} \pm \sigma$)	92±15		92±12		91±15		
PC IU/dl ($\bar{x} \pm \sigma$)	90±17		89±21		88±18		
PS IU/dl ($\bar{x} \pm \sigma$)	84±16		84±19		83±14		
HC µmol/L ($\bar{x} \pm \sigma$)	13±6		11±7		18±7		0.001

Abbreviations. MTHFR: methylentetrahydrofolate reductase; No: number; M/F: male/female; PT: prothrombin; AT: antithrombin; PC: protein C; PS: protein S; HC: homocysteine.

MTHFR TT and protein C predicted age at PVT ($p<0.0001$ & $p=0.04$ respectively); MTHFR TT predicted plasma HC ($p=0.05$). Smoking and sex predicted cavernoma ($p=0.002$ and $p=0.02$ respectively). Plasma HC inversely related to protein C in the MTHFR TT group ($r=-58$, $p=0.01$). Age at PVT of the MTHFR TT group was lower than that that of 19 PVT with liver cirrhosis carrying the same genotype (31.3±8.5 *vs* 56±13, $p<0.001$) (cohort presented in separate abstract). **Conclusions**: MTHFR TT anticipates age at PVT by an

average of 20 years compared to other MTHFR genotypes; protein C predicts age at PVT and is adversely affected by plasma HC; smoking is associated with cavernoma.

PO025

CEREBRAL VENOUS SINUS THROMBOSIS IN A PATIENT WITH WISKOTT-ALDRICH SYNDROME DURING THERAPY WITH ELTROMBOPAG

T. Martini[1], P. Pedrazzi[1], L.D. Notarangelo[2], E. Bertoni[3], R. Biguzzi[1], C. Biasoli[1]

[1]Centro MEC Romagna, Medicina Trasfusionale Ospedale Bufalini Cesena; [2]Direzione medica, Presidio Ospedale dei Bambini, ASST-Spedali Civili, Brescia; [3]U.OOncoematologia Pediatrica, ASST-Spedali Civili, Brescia, Italy

Background and Aims: Wiskott-Aldrich Syndrome (WAS) is a rare X-linked immunodeficiency characterized by microthrombocytopenia, eczema, recurrent infections and autoimmunity. In its classical form HSCT or gene Therapy are the treatments of choice. Its milder form (X-linked Thrombocytopenia, XLT) could be managed conservatively. Eltrombopag, a TPO-receptor agonist, has been used in patients with Immune Thrombocytopenia as well as in WAS/XLT but rare cases of atypical venous thrombosis have been reported. Here we describe a case of a young patient with XLT, in therapy with Eltrombopag, who developed a Cerebral Venous Sinus Thrombosis (CSVT) concomitantly with a febrile episode. **Methods**: We evaluated the medical record of the patient, collecting data about clinical history, laboratory results and radiological exams. **Results**: A 19-years old XLT patient started treatment with Eltrombopag in 2018 (base platelet value around 15.000/mmc) with an effective dose of 50 mg OD (platelet count at steady state around 50.000/mmc) in absence of bleeding. One month ago he went to Emergency Room for the onset of headache since 3 days, not sensitive to analgesics, and fever, without any other symptom. Complete blood count showed 184.000 platelet/mmc. A Covid swab was negative. Cerebral AngioCT documented a complete thrombosis of sigmoid sinus and jugular bulb, involving the proximal third of the internal jugular vein, accompanied by a thin subdural hemorrhagic effusion. Suddenly after the exam he had an episode of generalized seizure. Anticoagulation with LMWH (Low Molecular Weight Heparin was promptly started and Eltrombopag suspended for 5 days (platelet count 124.000/mmc). To maintain an adequate platelet count for safe anticoagulation it was gradually reintroduced (25 mg OD on day 5, incremented to 50 mg on day 7) with a platelet count ranging between 35.000 and 45.000/mmc (Figure 1); this strategy allowed us to use a therapeutic dosage of LMWH for the first 3 days, followed by a subtherapeutic dosage (50 U/Kg BID). An AngioCT performed on day 7 resulted in a really good initial response with partial recanalization of left sigmoid and transverse sinus, complete recanalization of right transverse sinus; subdural effusion stable. The patient was discharged after 26 days with a prophylactic dosage of LMWH, still ongoing. Currently the patient is receiving Eltrombopag 50 mg OD. A further MRI control will be performed within 2 months, after 3 months of anticoagulant. **Conclusions**: We report a rare episode of CSVT in a patient with XLT during therapy with Eltrombopag, possibly related to a concomitant febrile episode of unknown origin and a sudden large increase of the platelet. Even if Eltrombopag could have had a causative role as already reported in literature, it was reintroduced to provide a safe and adequate platelet for LMWH treatment. A close radiological control showed a good response, allowing us to reduce LMWH to a prophylactic dosage that should be safe and effective until the complete resolution of the thrombosis.

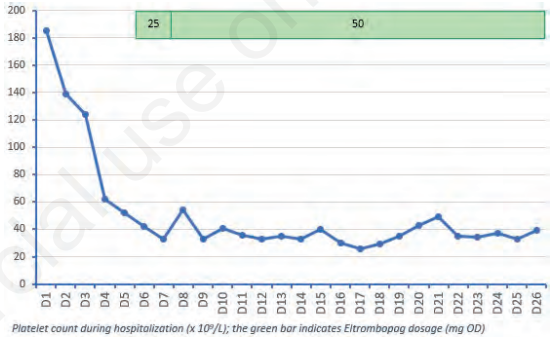


Figure 1.

PO026

IS HYPER-HOMOCYSTEINE AN INDEPENDENT RISK FACTOR FOR THROMBOSIS? RETROSPECTIVE ANALYSIS OF A SINGLE-CENTRE PATIENTS' POPULATION

G.M. Nicolò[1], G. Sottilotta[1], F. Luise[2], A. Piromalli[2]

[1]Hemophilia Centre - Thrombosis and Hemostasis ServiceGreat Metropolitan Hospital of Reggio Calabria, Italy; [2]Analysis LaboratoryGreat Metropolitan Hospital of Reggio Calabria, Italy

Background and Aims: Homocysteine (HCY) is formed from the dietary amino acid methionine and plays a pivotal role in folate metabolism and methyl group transfer. Hyper homocysteine (hHCY) is associated with arterial and venous thrombosis via several mechanisms such as enhanced platelet reactivity, increased tissue factor expression and thrombin generation, and endothelial dysfunction. The aim of our study was to evaluate if hHCY may be considered as an independent thrombosis risk factor and its incidence in different sites of thrombosis. **Methods**: Clinical data of 319 patients were retro-

spectively analysed, 184 with hHCY and 135 with normal HCY (nHCY), followed by our centre during the last 6 years. We excluded any other conditions of thrombophilia: the Factor V Leiden and Prothrombin G20210 polymorphisms, Protein C and S deficiency, presence of lupus anticoagulant and increased anticardiolipin or anti-beta2 glycoprotein-I antibody levels. **Results**: 29/184 with hHCY were found with thrombosis. Of these: 18/29 (62.1%) had venous thrombosis (DVT), 11/29 (37.9%) had arterial thrombosis. We considered three hHCY severity levels: mild 15-29 µmol/L, moderate 30-100 µmol/L and severe>100 µmol/L. 22/135 (16.3%) nHCY patients were diagnosed with thrombosis. Of these: 17/135 (12.6%) had DVT and 5/135 arterial thrombosis (3.7%). Even in this case, we observed more DVT (17/22,77.3%) than arterial thrombosis (5/22,22.7%). The comparison about incidence of thrombosis between hHCY and nHCY groups evidenced similar percentages (15.8% and 16.3% respectively). Results are resumed in Table 1. We found different thrombosis percentages comparing the subjects with normal and mild hHCY to those with moderate hHCY: people with moderate hHCY had a much higher percentage of arterial thrombosis (61.5%) than those with nHCY (22.7%) and patients with mild hHCY (15.8%), even if these data were not sufficient to hypothesize a correlation between levels of HCY and sites of thrombosis. **Conclusions**: Our analysis did not demonstrate that hHCY can be considered an independent risk factor for thrombosis. Previous reports have shown that hHcy-promoting thrombotic events require a first hit, such as dyslipidemia or hypertension. In our opinion, as reported by other authors, hHCY could be only considered as a prothrombotic cofactor. Further prospective studies with dedicated designs are needed to clarify if hHCY may play an independent prothrombotic role in the etiopathogenesis of thrombosis.

Table 1.

Correlation between different sites of thrombosis and homocysteine levels.

	Arterial Thrombosis	PE	Stroke	MI	CVT	DVT	RVT	Patients with thrombosis	Patients without thrombosis	Total Subjects
Mild hHCY	1 (1 m) [57y]	1 (1 m) (35y)	2 (2 m) (53y)	0		7 (5m,2f) (51y)	5 (3m,2f) (63.4y)	16 (12m, 4 f) (52.1y)	100 (42m-58f) (37.8y)	116 (54m,62 f) (40.1y)
Moderate hHCY	1 (1m) (48y)	0	3 (2m,1f) (52.3y)	4 (3m,1f) (57y)	0	5 (5m) (52.3y)	0	13 (11 m. 2 f) (48.6y)	54 (32m, 22 f) (37.1y)	67 (43m, 24 f) (39.4y)
Severe hHCY									1 (1m) (35y)	1 (1m) (35y)
Total hHCY	2 (2m) (48.5y)	1 (1 m) (35y)	5 (4m, 1 f) (55y)	4 (3m,1f) (57y)	0	12 (10 m, 2 f) (50.4y)	5 (3m,2f) (63.4y)	29 (23 m, 6 f) (50.6y)	155 (75 m, 80f) (37.5y)	184 (98m, 86f) (38.8y)
nHCY	0	0	4 (2m, 2f) (44.2y)	1 (1f) (59y)	1 (1f) (47y)	10 (2m, 8 f) (50.5y)	6 (3m, 4 f) (61.8y)	22 (6m, 16 f) (54.5y)	113 (15m, 98 f) (42y)	135 (21m, 114 f) (40.7y)

PE: Pulmonary Embolism; MI: Myocardial Infarction; CVT: Cerebral Venous Thrombosis; DVT: Deep Venous Thrombosis; RVT: Retinal Venous Thrombosis. Hcy: homocysteine; nHcy: normal homocysteine; hHcy: hyperhomocysteinemia

PO027

ANTIXA DIRECT ORAL ANTICOAGULANTS IN ATYPICAL SITE VEIN THROMBOSIS: A SINGLE CENTRE EXPERIENCE

A. Vitale[2], A. Guida[1], A. De Sena[1], P. Tirelli[1], N. Vitelli[2], M. Bottiglieri[2], A. Cardamone[2], O. Iacono[2], G. Landi[2], L. Palmisano[2], M. Lugarà[1], M.G. Coppola[1], P. Madonna[1]

[1]UOC Medicina Interna Ospedale del Mare ASL Napoli 1 Centro; [2]Dipartimento di Medicina Clinica e Chirurgia AOU Federico II Napoli, Italy

Background: Direct oral anticoagulants (DOAC) are currently recommended as initial treatment of Venous Thromboembolism (VTE). Although the evidence on the use of these drugs in patients with unusual-site venous thromboembolism (VTE) is limited to a few, small studies, these drugs are currently used in clinical practice. Aim of our survey was to evaluate the efficacy and safety of anti Xa-direct oral anticoagulants (aXa-DOAC s) in treatment and secondary prevention of VTE in 9 non neoplastic patients. **Methods**: We enrolled 9 patients (2F, 7 Mmean age 53,44) referred to our thrombosis centre since march 2019 to march 2022. All patients were screened for inherited and acquired thrombophilia, Jak-2 polymorphism, autoimmunity and neoplasm. Diagnosis of VTE was obtained with contrast-CT-Scan. 5 patients experienced splanchnic vein thrombosis, in 3 cases secondary to abdominal infection (diverticulitis, cholecystitis) 3 patients had vena cava thrombosis, 2 cases were provocked VTE (1 spondilodiscitis, sepsis and inferior vena atresia) 1 patients presented FII-G20210A heterozigousity, 1 patient Factor V Leiden heterozigousity no other thrombophilic abnormalities were detected. All patients performed a Total body CT-Scan and endoscopy was done in 5/9 patients no solid tumors emerged. 3/9 were prescribed apixaban the others received edoxaban. **Conclusions**: Neither thrombotic events nor haemorrhagic complication were observed in this 3 years followup. All patients were prescribed longterm anticoagulant therapy after informed consent. In a our small case series we observed effectiveness and safety of anti Xa-DOACs in patients with atypical site vein thrombosis

PO028

A CASE OF TRICUSPID VALVE LIBMAN-SACKS ENDOCARDITIS IN A PREGNANT WOMAN WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME

S. Migliorini[1], C. Santoro[2], A. Scatteia[3], S. Dellegrottaglie[4], V. Cuomo[5], E. Pilato[6], G. Comentale[6], M. D'armiento[7], M. Guida[1], L. Sarno[1], A. Tufano[5]

[1]Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy; [2]Department of Advanced Biomedical Sciences, Federico II University Hospital, Naples, Italy; [3]Division of Cardiology "Villa dei Fiori" Hospital, Acerra, Naples, Italy; [4]Zena and Michael AWiener, Cardiovascular Institute/Marie-Josee and Henry RKravis Center for Cardiovascular Health, Icahn School of Medicine at Mount Sinai, New York, USA; [5]Department of Clinical Medicine and Surgery, Federico II University Hospital, Naples, Italy; [6]Departmenttof Advanced Biomedical Science, Division of Adult and Pediatric Cardiac Surgery, University of Naples

Federico II, Naples, Italy; [7]Pathology Unit, Department of Public Health, School of Medicine, University of Naples Federico II, Naples, Italy

Background: Antiphospholipid syndrome (APS) is a systemic autoimmune disorder with acquired hypercoagulability, characterized by venous, arterial and microvascular thrombosis and increased pregnancy morbidity, in the presence of persistently positive antiphospholipid antibodies. Cardiac involvement may be present as heart valve disease, affecting approximately a third of patients, or, less frequently, as intracardial thrombosis, nonbacterial thrombotic endocarditis (*i.e.* Libman-Sacks endocarditis), pulmonary hypertension, right or left ventricular dysfunction, microvascular thrombosis, coronary artery or micro-vascular disease with overt or silent clinical presentation. **Case Report**: We report a case of Libman-Sacks tricuspid valve endocarditis in a 38-year-old pregnant woman at 15 weeks gestation with previously unknown primary APS. During a routine cardiac examination and echocardiography, performed for a previous episode of pleuro-pericarditis, a large mobile mass with irregular edges was found at the level of the tricuspid valve. Three main differential diagnoses for intramyocardial mass were examined: malignancy, infective endocarditis, nonbacterial thrombotic endocarditis. Cardiac magnetic resonance imaging (CMR) with contrast has raised the suspicion of a thrombus. The patient was urgently admitted at the Cardiac Intensive Care Unit of the Federico II University Hospital, and anticoagulant and antiplatelet therapy (Enoxaparin sodium at a dose of 6000 IU every 12 hours and Acetylsalicylic Acid 100 mg daily) was started. Laboratory examination showed prolonged partial thromboplastin time (PTT): 63.6 seconds. Anticardiolipin antibodies IgG and anti-β2 glycoprotein-1 IgG tested positive and they were respectively 63 U/mL (cut off value <20 U/mL) and 200 U/mL (cut off value <20 U/mL) with lupus anticoagulant (LA) positive. The laboratory examination and the medical history induced to a diagnosis of primary APS, pending confirmation of the laboratory results at 12 weeks. Despite adequate anticoagulation for three weeks, guided by the anti–factor Xa assays for dose adjustment, transthoracic three-dimensional echocardiography showed the persistence of the vegetation attached to the tricuspid valve, without changes in its size. A multidisciplinary consultation with obstetricians, cardiologists, and cardiac surgeons was required. The patient decided not to terminate the pregnancy despite the risk to her health and to undergo cardiac surgery during pregnancy. Histological examination confirmed the presence of nonbacterial thrombotic endocarditis. Weekly obstetric controls were performed after surgery to verify foetal well-being. Pregnancy was complicated by foetal growth restriction. At 28 weeks of gestation, foetal weight was below 5th centile, with normal amniotic fluid and normal foetal Doppler velocimetry. At 32 weeks of gestation, a PI of Umbilical Artery (UA) above 95th centile was found, with normal Doppler velocimetry of Ductus Venosus (DV) and reduce PI in Middle Cerebral Artery (MCA) (Brain Sparing). From admission to the hospital, she underwent daily computerized-cardiotocography (c-CTG). Foetal Doppler velocimetry in UA and MCA remained stable until 34 weeks. At 34 weeks, patient developed gestational hypertension that was controlled with nifedipine (20 mg every 8 hours). At 34+3 weeks an emergency caesarean section was performed. A female new born weighed 1290 grams was born. The new born was admitted to Neonatal Intensive Care Unit and she did not require ventilatory support. Already after a week, she reached the weight of 1500 grams and started oral feeding. She has been discharged after two months. Currently enjoys good health. **Conclusions**: Intracardiac thrombosis or nonbacterial thrombotic endocarditis is a rare complication of APS. A correct management of pregnancy in patients with this syndrome is fundamental: a multidisciplinary approach should be required.

PO029

INTRINSIC ALTERATION AND INFLAMMATORY MICROENVIRONMENT AFFECT PROLIFERATION CAPABILITY OF CD8 T CELLS IN A MOUSE MODEL OF HEMOPHILIA A

P.E. Di Simone, V. Kalandadze, A. Follenzi, C. Borsotti

Department of Health Sciences, Histology laboratory, Università del Piemonte Orientale, Novara, Italy

Background and Aims: Hemophilia A (HA) is a recessive X-linked bleeding disorder caused by the lack of coagulation factor VIII (FVIII). One of the main complications of the replacement therapy is the appearance of specific anti-FVIII antibodies (inhibitors) due to the B and T cells activation upon FVIII administration in hemophilic patients. Instead, little is known about any intrinsic T cell alteration especially prior to any FVIII exposure. Moreover, recently it has been reported the presence of pro-inflammatory cytokines in naïve $FVIII^{-/-}$ mice associated to subclinical bleedings and in HA previously untreated patients (PUPs) compare to healthy controls, suggesting that there could be a local environment predisposing them to inhibitor formation. Additionally, our lab observed in an hemophilic murine model a slight decrease in number of HA splenic CD8 T cells compare to wild type (wt) age- and sex-matched mice. In order to study the activation threshold of HA CD8 T cells we sought to investigate *in vitro* and *in vivo* their proliferation capability. **Methods**: Splenic CD8 T cells from HA and wt Balb/c mice were isolated and labelled with a fluorescent proliferation dye. An *in vitro* assay was performed by anti-CD3/CD28±IL2 stimulation while the *in vivo* test was run by adoptively transferring the CD8 T cells into immunodeficient HA NSG or NSG recipient mice. **Results**: HA CD8 T cells showed higher expansion compare to their wt counterparts. This difference was evident by the greater number of HA CD8 T cells recovered after 72 hours of anti-

CD3/CD28 *in vitro* stimulation (18410±3920 HA *versus* [vs] 10298±1968 wt, p=0,001). On the other hand, IL2 addition in the culture medium eliminated the HA CD8 T cells proliferative advantage, suggesting a different intrinsic threshold of activation. Similarly, 72 hours after the adoptive transfer, the splenic HA CD8 T cells recovery from the HA NSG recipient mice was 4-fold higher (425350±95312) than the wt CD8 T cells recovery from NSG animals (91936±31319). Surprisingly, tracking the number of cell divisions we did not only found more HA than wt CD8 T cells in each cell cycle/division but also a different proliferation kinetic with the highest number of proliferating cells in the fourth and fifth cycle for HA cells and third and fourth ones for wt. In addition, the role played by the host microenvironment was investigated by injecting HA CD8 T cells into non-hemophilic NSG and wt CD8 T cells into HA NSG: HA CD8 T cells recovery was higher from HA NSG than from NSG mice (425350±95312 *vs* 229419±46944 respectively, p=0,11) while wt CD8 T cells number were low from both HA NSG and NSG (117324±38776 *vs* 91936±31319 respectively, p=0,68). Therefore, a combination of intrinsic and extrinsic factors might contribute to reduce the threshold of proliferative activation in hemophilic CD8 T cells. **Conclusions**: Our preliminary results showed a higher proliferation capability of HA than wt CD8 T cells, supporting the hypothesis of a possible intrinsic HA T cell alteration and/or the influence of a tissue pro-inflammatory microenvironment. Our preliminary observations, which require further confirmations, could have an impact on the development of new strategies for preventing or eradicating inhibitors in HA patients since it is possible that they already hold a pro-inflammatory signature prior to any FVIII treatment.

PO030

IDEAL STUDY: A REAL-WORLD ASSESSMENT OF PATTERN OF USE AND CLINICAL OUTCOMES WITH RECOMBINANT FACTOR IX ALBUMIN FUSION PROTEIN (RIX-FP) IN PATIENTS WITH HAEMOPHILIA B IN ITALY

G. Castaman[1], A. Tagliaferri[2], A.C. Molinari[3], F. Peyvandi[4], A. Coppola[2], F. Demartis[1], C. Biasoli[5], A. Borchiellini[6], D. Cultrera[7], R. De Cristofaro[8], F. Daniele[9], P. Giordano[10], E. Marchesini[11], M. Margaglione[12], R. Marino[13], B. Pollio[14], P. Radossi[15], C. Santoro[16], R.C. Santoro[17], S. Siragusa[18], G. Sottilotta[19], A. Tosetto[20], L. Valdrè[21], M.R. Villa[22], E. Zanon[23], A. Loraschi[24], I. Schiavetti[25,26], D. Vaccari[26]

[1]Center for Bleeding Disorders and Coagulation, Department of Oncology, Careggi University Hospital, Florence, Italy; [2]Regional Reference Centre for Inherited Bleeding Disorders, University Hospital of Parma, Italy; [3]Regional Reference Center for Hemorrhagic Diseases, GGaslini Children's Hospital, Genova, Italy; [4]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi, Hemophilia and Thrombosis Center, and Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy; [5]Haemophilia and Transfusion Centre, "Bufalini" Hospital, Cesena, Italy; [6]Regional Reference Centre for Bleeding and Thrombotic Disorders, "Città della Salute e della Scienza" University Hospital, Turin, Italy; [7]Haemophilia Regional Reference Centre, Haematology Unit, Policlinico "GRodolico" - SMarco Hospital, Catania, Italy; [8]Haemorrhagic and Thrombotic Disease Service, Area of Haematological and Oncological Sciences, IRCCS Fondazione Policlinico Universitario "AGemelli", Rome, Italy; [9]Haemostasis and Thrombosis Service, Ospedale Civile dell'Annunziata, Cosenza, Italy; [10]"BTrambusti" General and Specialized Pediatrics Unit, "Giovanni XXIII" Hospital, University of Bari, Italy; [11]Haemophilia Centre, Internal and Cardiovascular Medicine, "Santa Maria della Misericordia" University Hospital, Perugia, Italy; [12]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; [13]Haemophilia and Thrombosis Centre, University Hospital, Bari, Italy; [14]Regional Reference Centre for Inherited Bleeding and Thrombotic Disorders, Transfusion Medicine, "Regina Margherita" Children Hospital, Turin, Italy; [15]Hematology Unit, Castelfranco Veneto Regional Hospital, Castelfranco Veneto, Italy; [16]Hematology, University Hospital Policlinico Umberto I, Rome, Italy; [17]Regional Reference Center for Hemophilia and Coagulation Diseases, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy; [18]Haematology Unit, Thrombosis and Haemostasis Reference Regional Center, University of Palermo, Palermo, Italy; [19]Hemophilia Centre, Grande Ospedale Metropolitano, Reggio Calabria, Italy; [20]Haemostasis and Thrombosis Unit, Haematology Department, San Bortolo Hospital, Vicenza, Italy; [21]SSD Congenital haemorrhagic diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [22]Hemophilia and Thrombosis Center, Hematology Unit, "Ospedale del Mare" Hospital, Naples, Italy; [23]Haemophilia Centre, Department of Medicine, University Hospital of Padua, Padua, Italy; [24]CSL Behring, Milan, Italy; [25]Hippocrates Research, Genova, Italy; [26]Department of Health Sciences, University of Genoa, Italy

Background and Aims: Factor IX replacement therapy is used for treatment and prophylaxis of bleeding in hemophilia B. rIX-FP is a long-acting albumin-fusion protein, which, in clinical studies, has demonstrated prolonged dosing intervals up to 21 days for routine prophylaxis, providing therapeutic benefit. The aim of the IDEAL study was to describe dosing frequency and consumption (primary endpoint), efficacy and safety of rIX-FP treatment during routine clinical practice in Italy. **Methods**: Patients with moderate/severe hemophilia B on prophylaxis with rIX-FP for ≥6 months, providing written informed consent, were enrolled in this observational study from October 2017 to February 2019 and

followed-up for two years. Descriptive analysis included prospective and retrospective data (12 months prior to switching to rIX-FP). The study was approved by competent Ethics Committees. **Results**: Data were collected from 59 male patients (median age 30.1 ±16.20 years, 75% ≥18 years) enrolled by 23 hemophilia treating centers. Of them, 50 (84.7%) were on prophylaxis during the entire observation period and completed the study. The infusion frequency changed from every 2-3 times/week in 86.0% of patients with previous treatment to longer than once a week in 84.0% of patients receiving rIX-FP at the second-year follow-up. In particular, 54% of the patients were on prophylaxis regimens every 8 to 10 days, 24.0% every 11 to 14 days and 6% every 15 to 21 days. The prescribed dose remained quite stable during the entire 2-years of follow up (mean value ranging from 43.4 IU/kg to 46.0 IU/kg), as well as the frequency of the infusions. At the end of the study, the annualized total dose decreased from 4098.2±1340.10 IU/kg with previous FIX, to 1562.6±433.23 IU/kg of rIX-FP. With an annual number of infusions decreased of about 70%, mean FIX activity trough level increased from 3.8% to 14.4%. (Table 1). Median Annualized Bleeding Rate of 0.00 was achieved across all prophylaxis regimens. Of note, the max ABR value decreased from 12 with the previous treatment, to 7.7 with rIX-FP. Subjects with zero bleedings increased from 66.0 % to 78.0% with rIX-FP. In the 2-year follow-up, the rate of patients with chronic joint pain decreased from 44.0% with previous treatment to 16.0%, while that of patients with target joints from 30.0% to 8.0%. No safety concerns were identified, no patient developed inhibitors. **Conclusions**: In this real-world setting, approximately 80% of patients receiving rIX-FP are maintained on prophylaxis regimens with infusion frequency less than once a week, showing higher FIX levels, increased number of subjects with zero bleedings, zero median ABR, decreased chronic Joint pain and target joints and notably reduced FIX consumption *vs.* previous treatment.

Table 1.

		Previous treatment	rIX-FP 2y Follow Up
Factor IX activity level (%)	Valid N	28	20
	Mean	3.8	14.4
	SD	2.58	7.23
	Median	3.1	11.7
	Min	0.3	7.0
	Max	11.0	33.8

Factor IX trough level (%): previous treatment vs rIX-FP 2Y Follow Up
SD - Standard Deviation
rIX-FP - recombinant factor IX albumin fusion protein

PO031

IMPACT OF COVID-19 ON PEOPLE WITH HEMOPHILIA: NEEDS AND SHORTINGCOMINGS DUE TO PANDEMIC

G. Sottilotta[1], D. Messina[2], A. Buzzi[3]

[1]Centro Emofilia – Servizio Emostasi e Trombosi, Grande Ospedale Metropolitano di Reggio Calabria, Italy; [2]AIMeF, Italy; [3]Fondazione Paracelso, Milan, Italy

Background and Aims: The large allocation of human and economic resources to face the pandemic and the lockdown mobility restrictions have limited the patients access to in-person care at Hemophilia Treatment Centres (HTC) engaging in adopting new methods to ensure their continuity of care. Although the community of persons with haemophilia (PWH) has shown to be resilient and adaptable, a large proportion of adults have faced financial struggles due to job losses or suspensions. These multiple challenges have likely had a negative impact not only on PWH's physical health but also on their psycho-social well-being7. An online survey was conducted in 2021 with the aim to evaluate the emotions which have affected the adult haemophiliacs daily life, from different countries. **Methods**: An 18-item anonymous online international survey evaluated the feelings of adult hemophiliacs, representing a variety of cultural and economic settings, and different health care systems, during the first year of pandemic and what behaviours have been adopted to face the socio-economic restrictions including lockdown, social distancing and self-isolation, as well as the limited access to HCT. **Results**: The participating patients were 204 from 24 different countries. The demographic and clinical characteristics are summarized in Table 1. Regarding the questions about emotions and feelings in relation to the pandemic, the sample of people interviewed replied as follows: 23.1% "not at all", 45.6% "a little bit", 16.6% "moderately", 10.8% "quite a bit", 3.9% "extremely". To question "Did you find it difficult to manage the relationships with the professionals?" the people interviewed replied: 48.5% that nothing had changed, 16.2% found no difficulty, 19.6% answered that it was difficult, 7.3% that it was very difficult, 8.4% gave other answers. To question about which aspect of the current situation has had the greatest impact on their own life, the sample of people interviewed replied as follows: 10.8% had "difficulties related to the condition", 8.3% "difficulties related to the job, 11.8% "the obligation to stay at home", 25.3% "adapting to new habits and lifestyles", 10.3% "obligation to use face masks, keep the distance and wash hands frequently", 12.7% "fear for my own and other people's health ", for 6.4% it was the economic impact, 5.4% replied "the restrictions when traveling", 7.8% "inability to meet the loved ones". To question about whether and what kind of help the interviewee needed, 21.1% replied that "the support of friends or family, even if virtual", 47.1% replied that they needed more information; 28.4% reported "psychological help". 7.8% said they needed

help in managing daily expenses. 15.7% reported needing a greater presence of the HCT, 6.4% by the GP, and 25.5% said they did not need help. About the check-ups and the planned activities at their HCT had been affected by the pandemic: for 40.2% there was no change; for 24% there was a reduction in all activities; 27.4% were delayed only in programmable activities such as check-ups; 4.9% answered that the pandemic caused a postponement of the switch to a new drug, while 3.5% gave other responses. **Conclusions**: The survey revealed that in a condition of social isolation, it is the experience of a sense of uncertainty that prevails, which, although it is not possible to eliminate completely, can however be managed, if continuity in relationships and support is guaranteed even at a distance.

Table 1.

Demographics and health-related characteristics of the survey participants (n=204)

Age		Education level		Employment	
18-30	63 (28.8%)	Elementary school	9 (4.4%)	Unemployed/ unoccupied	36 (17.6%)
31-50	96 (45.5%)	Junior high school	16 (7.8%)	Free-lance	43 (21.1%)
51-70	48 (22.8%)	High school	90 (44.1%)	Clerk	62 (30.4%)
>70	4 (1.9%)	University Degree	86 (42.2%)	Retired	26 (12.8%)
		PhD	3 (1.5%)	Student	37 (18.1%)

Haemophilia type	Total 204	Africa	Asia	Europe	Central America	North America	South America
A severe	117 (57.4%)	3	19	48	6	8	33
A moderate	29 (14.3%)		7	12	2		8
A mild	20 (9.8%)		5	9	1	4	1
B severe	19 (9.3%)		1	7	2	4	6
B moderate	5 (2.4%)		1		2	1	1
B mild	2 (0.9%)		1	1			
A with inhibitors	12 (5.9%)		1	7	1		3
B with inhibitors	0						
Treatment							
Prophylaxis	121 (59.3%)	1	3	59	4	11	43
On demand	83 (40.7%)	2	33	25	9	6	8

PO032

EMICIZUMAB PROPHYLAXIS: EXPERIENCE OF A SINGLE CENTER

G. Maestrini, E. Baldacci, A. Ferretti, R. Mormile, S. Olivieri, A. Pallotta, F. Viola, A. Chistolini, C. Santoro

Dipartimento di Medicina Traslazionale e di Precisione, Istituto di Ematologia, Università "La Sapienza", Roma, Italy

Background and Aims: The use of emicizumab as prophylaxis for severe HA, with or without inhibitor, is increasing in routine clinical practice. The aim of the study is to describe our center experience with emicizumab prophylaxis in severe hemophilia A (HA) patients without and with inhibitors. **Methods**: All the participants signed an informed consent; data were collected from clinical charts. **Results**: 10 severe HA patients received emicizumab prophylaxis (Table 1). We observed 1 drug-related adverse event, a relapsing orticarioid reaction at the injection site. Non-inhibitor patients: 6. Four patients were on rFVIII prophylaxis, 1 was on demand therapy, 1 was never treated. Reason for starting emicizumab: need to facilitate the therapy administration in young children. Just one traumatic tongue lesion occurred which was treated with one dose of rFVIII. None surgical or invasive procedures were performed. Inhibitor patients: 4. Before switching to emicizumab: 3 on bypassing agents on demand; one patient on rFVIII prophylaxis developed a high titer inhibitor. Two patients received ITI. All patients switched to emicizumab for a better control of the disease. No spontaneous bleeding were reported. A traumatic fracture of the homerus occurred, treated with 4 doses of rFVIIa (90mcg/kg/dose) without any complication. One patient underwent a colonoscopy without additional prophylaxis (no biopsies performed). Two patients underwent dental extraction: just one received prophylaxis with tranexamic acid; no hemorrhagic complications were observed. **Conclusions**: We confirm the efficacy and safety of emicizumab prophylaxis. No spontaneous bleedings occurred. Two traumatic bleedings were easily managed with rFVIIa or FVIII concentrate. Dental extractions in inhibitor patients were performed without rFVIIa therapy. No serious adverse events observed. The different median age and follow-up between inhibitor and non-inhibitor patients depends on timelines of regulatory indications for prescription and previous participation in clinical studies. We observed an improvement in quality of life of patients and their families.

Table 1.

	Non inhibitor patients	Inhibitor patients
Median age at emicizumab start (years) (range)	7.5 (1.1-8.4)	62.8 (27.3-67.7)
Median follow-up (months)	8.4 (6.8-17.7)	36.8 (8.8-46)
Median loading dose (mg) (range)	75 (30-135)	255 (210-300)
Maintenance dose 1.5 mg/kg/week (%)	2/6 (33)	3/4 (75)
Maintenance dose 3 mg/kg every two weeks (%)	4/6 (67)	1/4 (25)
Median ABR one year before emicizumab (range)	1 (0-3)	3 (1-4)
Median ABR two year before emicizumab (range)	0 (0-3)	4 (0-4)
Median AJBR one year before emicizumab (range)	0	1 (0-5)
Median AJBR 2 year before emicizumab (range)	0	2 (0-4)
Median ABR during emicizumab (range)	0 (0-1)	0 (0-1)
Median AJBR during emicizumab	0	0

PO033

MANAGEMENT OF PATIENTS WITH BLEEDING DISORDERS AND EDUCATIONAL NEEDS REGARDING HEMOPHILIA A IN 23 ITALIAN EMERGENCY DEPARTMENTS – RESULTS FROM A SURVEY CONDUCTED IN PIEDMONT

B. Pollio[1], I. Ricca[1], C. Dainese[2], F. Valeri[2], A. Valpreda[3], C. Linari[3], A. Borchiellini[2]

[1]Centro di Riferimento Regionale Malattie Emorragiche e Trombotiche Ereditarie in Età Pediatrica, AOU Città della Salute e della Scienza -Ospedale Infantile Regina Margherita di Torino; [2]Centro di Riferimento Regionale Malattie Trombotiche ed Emorragiche dell'aAdulto, Ematologia Universitaria, AOU Città della Salute e della Scienza di Torino; [3]Struttura Complessa di Biochimica Clinica, AOUCittà della Salute e della Scienza di Torino, Italy

Background: Haemophilia and von Willebrand disease are the bleeding disorders most frequently encountered in the emergency department (ED), that are often the first point of contact for patients. evidence suggests that management in the ED is currently suboptimal, mainly because the physicians have few opportunities to deal with this kind of patients. We carried out a survey to investigate the management of patients with haemophilia A in Emergency Departments (EDs), and to understand the training needs of the involved physicians. **Methods**: Overall, in Piedmont Region there are 32 EDs, and considering that our survey was conducted on 21 physicians working in 23 Emergency Departments (EDs), this number is representative of the Region's reality. The interviews were conducted through face-to-face meetings, including general aspects regarding the clinical characteristics and the management of patients, and self-evaluation of knowledge and interest in receiving information about the disease. **Results**: In 2019, 131 patients with haemophilia A were admitted (108 adults, 23 paediatric). The best-known and most widely available and used treatments were plasma derivatives, followed by first- and second-generation recombinant FVIII. More recent recombinant and bypassing agents were less known. Half of the interviewees considered their knowledge of bleeding disorders in general and haemophilia in particular to be "basic", and only one third defined it as "good"; however, 86% expressed great interest in receiving information about the topic (Figure 1). **Conclusions**: The survey confirms the needs related to the clinical management of rare inherited clotting disorders in EDs. The physicians involved are keen to overcome this lack of knowledge, and proper initiatives should be implemented.

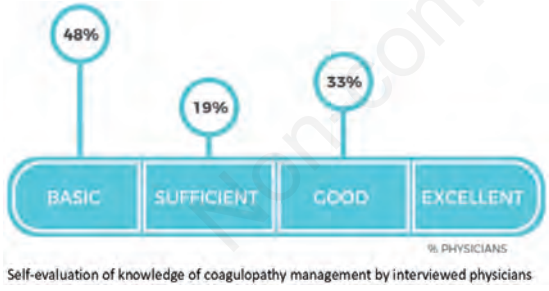


Figure 1.

PO034

MESENTERIC ARTERIAL THROMBOSIS IN A PATIENT WITH HAEMOPHILIA A

A. Casoria[1], M. Amitrano[2], G. Antignani[2], M. Nunziata[2], I. Puca[2], F. Cannavacciuolo[2], M. Mastroianni[2], E. Cimino[1], A. Tufano[1]

[1]Department of Clinical Medicine and Surgery, Federico II University Hospital, Naples, Italy; [2]Internal Medicine Ward, San Giuseppe Moscati Hospital, Avellino, Italy

Background: Haemophilia A is a recessive, X-linked inherited blood disorder caused by a deficiency of coagulation factor VIII (FVIII). The severity of haemophilia is inversely related to the level of functional FVIII and is categorized into mild, moderate, and severe disease, as follows: severe (factor level <1% of normal), moderate (level 1–5% of normal) and mild (level >5%). Although haemophilia patients appear to have lower mortality due to vascular diseases compared to the general population, the prevalence of cardiovascular risk factors (*i.e.* arterial hypertension) in these patients is similar to the prevalence in the general population. Nonetheless, haemophilic patients have the same degree of atherosclerosis as the general population and the incidence of vascular thrombosis in these patients is increasing, as life expectancy of these patients approaches nowadays that of the general population. We report on a mesenteric arterial thrombosis in a mild haemophilia A patient, with concomitant acquired and inherited thrombophilia. **Case Report**: A 51-year-old male patient was admitted because of severe abdominal pain and diarrhea. Computer tomography (CT) revealed thrombotic occlusion of the superior mesenteric artery. The clinical history of the patient included arterial hypertension, dyslipidemia, and smoking habit. Laboratory examination showed a prolonged activated partial thromboplastin time (aPTT): 41,4sec (ratio: 1.4), with normal prothrombin time (PT). Coagulation factor IX (FIX), FXI, and FXII, and fibrinogen were normal. The FVIII level was 33%. The patient reported the presence of heamophilia A in a family member. Molecular analysis showed the presence, in hemizygosis, of the sequence variant c.1649G>A (p.Arg550His), in exon 11 of the F8 gene, present in other members of the same family. Lupus anticoagulant was positive (1.76), anti-β2Glycoprotein I antibodies IgG were positive (13672 GPL), anticardiolipin antibodies IgG were also present at high titres (1487 GPL), suggesting a diagnosis of antiphospholipid syndrome. The search for inherited thrombophilia showed the presence of the FII G20210A variant. A re-evaluation of antiphospholipid antibodies was planned at 3 months. The patient was treated with the association of warfarin (target INR 2-3) and acetylsalicylic acid (100 mg daily), without bleeding complications. **Discussion**: The management of adult haemophilic patients with thrombotic events is often challenging because of coexistence of the high bleeding risk and the multiple co-morbidities and prothrombotic risk factors. The levels of FVIII (mild hemophilia) and the coexistence of a pro-thrombotic state prevented bleeding complications of the antithrombotic treatment in this patient.

PO035

PLASMA CONCENTRATION OF EMICIZUMAB IN CHILDREN WITH HAEMOPHILIA A WITH AND WITHOUT INHIBITORS

M. Leotta, A. Strangio, A. Ierardi, C. Spadafora, R.C. Santoro

Hemostasis and Thrombosis Center, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy

Emicizumab is a bispecific, humanized, monoclonal antibody mimicking the factor (F) VIII cofactor activity in mediating the generation of FXa by FIXa in patients with hemophilia A (HA).. This subcutaneous non-factor agent has been extensively approved for the prophylaxis of patients of HA patients with and without FVIII-inhibitors of all ages. Once weekly (QW) administration of emicizumab increased plasma emicizumab concentrations, and reached steady-state after 4 weeks of weekly loading dose of 3 mg/kg bodyweight followed by QW maintenance dose of 1.5mg/kg bodyweight. Subsequently in patients without inhibitors, maintenance regimens were used which included, as an alternative to the dosage of 1.5 mg / kg / week, dosages of 3 mg / kg every 2 weeks or 6 mg / kg every 4 weeks. Maintenance doses allow,then, to maintain constant plasma levels of emicizumab over time and are not recommended changes in the dosage of the drug. Furthermore, no dosage adjustments are required in relation at the age of the patient or any comorbidities. Therefore, it is not necessary to determine the plasma concentrations of the drug over time, unless there is suspicion of the formation of anti-drug antibodies. **Methods**: We wanted to verify the plasma concentrations of emicizumab and their variations in predetermined times (after the 4 loading doses, after 6 months, after 1 year and after 2 years) in 3 children with hemophilia A. Two of the children, aged 8 and 6, respectively, suffering from haemophilia A with a high-titre inhibitor, were maintained at a dosage of 1.5 mg / kg / body weight. The third child, 3 years old, with severe haemophilia A without inhibitor, practiced maintenance at a dosage of 3 mg / kg / body weight every 2 weeks. **Results**: The median plasma concentration of emicizumab after loading doses was 52.3 µ /ml (50.5- 59.4). Dopo 6 mesi 60,5 µ /ml (48,7 - 84,4). Dopo 12 mesi 59,7 µ /ml (51,2- 94). Dopo 24 mesi 65,6 µ /ml (42,3-89,5). The 24 month data is not available for the patient without inhibitor. In our patients, the median plasma concentration of emicizumab is consistent with the literature data and remains constant over time, also confirming adherence to treatment. In a single patient, with inhibitor, the values are consistently higher (Table 1). **Conclusions**: We confirm that in our young patients, suffering from haemophilia A with and without inhibitor, the plasma levels of emicizumab remain constant over time and always above the minimum levels reported in the literature.

Table 1.

	Age	Dosage	Emicizumab concentration post loading dose	Emicizumab concentration 6 months	Emicizumab concentration 12 months	Emicizumab concentration 24 months
HA With INH	8	1,5 mg/kg/bw / week	59,4	84,4	94	89,5
HA With INH	6	1,5 mg/kg/bw / week	50,5	48,7	51,2	42,3
HA	3	3mg/kg/bw every two weeks	52,3	60,5	59,7	.....

PO036

MILD HAEMOPHILIA A AND B: FROM BLEEDING PHENOTYPE TO ILLNESS PERCEPTION

M. Mattana[1], C. Rotolo[1], R. Tomasello[1], C. Cammarata[1], F. Romano[1], A. Lucchesi[2], G. Giordano[3], S. Siragusa[1], M. Napolitano[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo, Palermo, Italy; [2]IRCCS Istituto Romagnolo per lo studio dei tumori (IRST) "Dino Amadori", Meldola (FC), Italy; [3]Division of Internal Medicine, Hematology Service, Regional Hospital "ACardarelli", Campobasso, Italy

Background and Aims: Haemophilia is an X-linked recessive disease, which mainly affects men. Disease severity depends on the plasma levels of factor VIII (FVIII) in Haemophilia A (HA) or factor IX (FIX) in Haemophilia (HB), mild haemophilia (MH) is defined by FVIII or FIX plasma levels between 5% and 40%. MH is often diagnosed accidentally due to excessive bleeding secondary to trauma or surgery. Patients with MH do not usually require prophylaxis and are treated on-demand, they are less regular in follow-up visits and may underestimate their haemorrhagic risk. There is a paucity of data focused on MH; clinical evidence and guidelines are predominantly derived from severe haemophilia data. The current study aimed to describe clinical, laboratory characteristics and quality of life and illness perception of a cohort of patients affected by MH; we have furthermore evaluated differences, if any, between patients with FVIII:c and FIX:c levels below and above 10%. **Methods**: This retrospective study enrolled all consecutive patients affected by mild haemophilia A or B, evaluated at our reference center from January 1, 2012, to November 30, 2021. Age, sex, FVIII and FIX levels, spontaneous or provoked bleeding episodes were collected. Patients were divided into two groups: A for patients with factor levels less than or equal to 10%, B for patients with factor levels higher than 10%. The Chi-square test was used for normally distributed continuous data analysis. A p-value <0.05 was considered statistically significant. Patients with MH filled also the SF36 and ED-5Q questionnaires, to assess health related quality of life and aspects of illness experience and perception and the results expressed as a percentage. **Results**: Forty-two male patients with MH were analysed. The average age was 42.6 +/- 23.4 years. Of these patients, 14 had FVIII or FIX levels less than or equal to 10%; the remaining 28 had levels greater than 10%. Haemorrhagic manifestations and related therapies are shown in Figure 1. Seventeen patients received short-term prophylaxis for dental extraction and 12 for surgery. All the enrolled patients were treated on demand except for two, under continuous prophylaxis for recurrent bleeding. The analysis revealed in-group A a statistically significant ($p <0.05$) higher incidence of hemarthrosis and spontaneous bleeding than in-group B. There were no statistically sig-

nificant differences regarding bleeding secondary to trauma or surgery. SF36 and EQ5D were available for 20 patients: 8 patients from group A and 12 from group B. SF36 questionnaire scored above average (50 SD +/-10) in the area of physical function, and below average in the area of emotional aspects, with a 5/6 point difference. The impact of the disease on daily life is thus important, and unrelated to Factor levels. The ED-5Q data are congruent with the SF36 data. Perceived health status, which mirror the state of disease awareness (Insight), was also assessed, with results consistently above 70%. **Conclusions**: The results of our analysis confirm that among patients with mild haemophilia, the most frequent bleeding events are secondary to trauma or surgery. Our analysis found that spontaneous bleeding and hemarthrosis are more common in patients with factor levels below 10%, MH negatively impacts patients' quality of life, especially for their emotional sphere.The haemophilia community is called to act on patient education and improvement of disease perception.

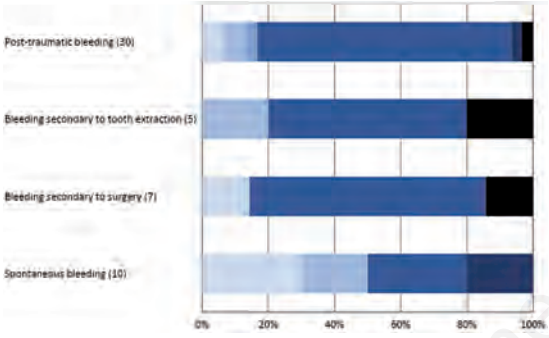


Figure 1.

PO037

A PARTICULAR CASE OF EPILEPSY IN A CHILD WITH HAEMOPHILIA AFTER SARS-COV2 INFECTION

M.L. Tramontano[1], G. Tesone[1], M. Pandolfi[2], M. Della Corte[2], E. Savastano[2], M. Schiavulli[1]

[1]Bleeding and Thrombosis Care Regional Reference Center Santobono-Pausilipon Children's Hospital, AORN, Naples, Italy; [2]Pediatric Neurology Unit Santobono-Pausilipon Children's Hospital, AORN, Naples, Italy

Background: Hemophilia A (HA) is an inherited bleeding disorder that, if not properly treated, is associated with debilitating joint damage due to recurrent hemarthroses as well as life-threatening bleeds including intracranial hemorrhage. The report describes an unusual case of epileptic seizure in a child affected by severe hemophilia after sars Cov2 infection. **Case Report**: A 7 years old male affected by Severe Hemophilia A. Heterozygous Prothrombin mutation (G20210A). Autistic spectrum disorder. The patient started prophylaxis with recombinant FVIII -Fc from 2016 to 2020, afterwards he was switched with Emicizumab prophylaxis. In January 2022 the patient contracted a paucisymptomatic Sars-CoV2 infection. In March 2022 the patient presented acute onset of right hemiplegia and loss of consciousness; trasported to the hospital, he presented low O2 saturation and Glasgow Coma Scale (GCS) 9 and was immediately intubated and moved into Intensive Care Unit (ICU). The EEG performed in ICU showed few slow and spiky/sharp potentials in the context of hypnic patterns with left prevalence. The brain MRI with angiographic sequences showed an uncertain thrombosis of the right transverse sigmoid jugular venous axis: for this reason he started antithrombotic prophylaxis with enoxaparine (100 UI/kg). After few hours the patient recovered, thus he was extubated and when awaked he appeared in good general and neurological condition (no focal signs on neurological examination). He performed another EEG where no epileptic anomalies were detected and another brain MRI showing an improvement of the previously described findings and, moreover, absence of recent onset areas of tissue suffering. Coagulation lab test were normal, including d-dimer. According to these findings, he discontinued antithrombotic therapy and started valproic acid to prevent seizures. After 12 days patients experienced a new and similar critical event with right hemiplegia and loss of consciousness with a duration of 10 minutes without compromising respiratory activity, so he didn't need intubation. After the first critical episode, emicizumab prophylaxis was stopped. Therefore, the patient has undergone to a strict follow up (once a week) by monitoring coagulation assays, including thrombotic parameters (activated partial thromboplastin time, FVIII one-stage clotting assays, FVIII chromogenic assays, d-dimer, anti-phospholipid antibodies). The patient has resumed rFVIII-Fc prophylaxis (30 UI/kg) only after 25 days from last emicizumab infusion and continued with a weekly infusion for 4 weeks. After this period he resumed regular frequency of prophylaxis three times/week according to FVIII chromogenic assays with a trough level of 1.9% according to pk assessment. **Conclusions**: The patient, despite having mild genetic thrombophilia, did not experience thrombotic events during prophylaxis with FVIII and also with emicizumab. We do not yet have elements to correlate with the previous SarsCoV2-infection but we suppose that this condition, even if after about two months, may have played a role in determining the onset of such serious episodes.

PO038

CLINICAL AND MOLECULAR CHARACTERIZATION OF PATIENTS WITH INHERITED DYSFIBRINOGENEMIA FOLLOWED IN A SINGLE CENTER

A. Strangio, A. Ierardi, M. Leotta, C. Spadafora, R.C. Santoro

Hemostasis and Thrombosis Center, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy

Background and Aims: Fibrinogen is a 340 KDa glycoprotein that is encoded by three genes namely Fibrinogen Aα (FGA), Bβ (FGB), and γ (FGG). Inherited Dysfibrinogenemia is a rare functional fibrinogen disorder in which the fibrinogen protein is present but with a reduced function. The disorder is characterized by a wide spectrum of clinical phenotypes, ranging from asymptomatic to mild to severe bleeding or thrombotic manifestations and recurrent miscarriages. The aim of our study is to describe the clinical phenotype in relation to genotype in our cohort. **Methods**: We conducted a retrospective analysis of 22 patients from 14 families with inherited dysfibrinogenemia followed by our center. All patients were asked for consent to make a molecular diagnosis, but consent was obtained from only 12 patients from 7 families. To assess the clinical phenotype, data was collected that included coagulation and molecular screening, gender, bleeding and thrombotic events. **Results**: 8 patients are male and 14 female. 4 of the male and 8 female patients were asymptomatic. The remaining male patients had experienced thrombotic episodes, particularly ischemic hearth disease in 2 patient at the age of 42 and 48, respectively. Another patient had an ischemic stroke at age 50 and the last patient had idiopathic cerebral haemorrhage at age 40 and cerebral ischemia at age 65. 8 female were asymptomatic. 2 women had post-partum haemorrhages and two other women had hematomas after surgery, all of them before diagnosis. One woman had cerebral venous thrombosis and another had post-partum deep vein thrombosis. Whole exone sequencing analysis which included testing of the fibrinogen genes FGA, FGB and FGG, was performed in 12 patients. Five pathogenic mutations were found in the FGG gene: c.901 C>T in exon 1 in three cases; homozygosity for the mutation c.673 G>T in exon 7 in another case and finally c.902 G>A in exon 8; in the latter case, a further associated mutation, of uncertain significance, was found c.401 G>A in exon 4. Seven pathogenic mutations were found in the FGA gene: c.394_405 in exon 4 in three cases; c.103 C>T in exon 2 in two cases and c.160 T>G in exon 2 in two cases (Table 1).

Tabella 1.

Patient/Sex	Genetic mutation	Thrombosis	Bleeding	Asyntomatic
1) F	c.902 G>A FGG c.401 G>A FGG			x
2) F	c.901 C>T FGG			x
3) M	c.901 C>T FGG	myocardial infarction		
4) F	c.901 C>T FGG		post surgery hematoma	
5) F	c.673 G>T FGG			x
6) F	c.160 T>G FGA		Post-partum hemorrhage	
7) M	c.160 T>G FGA			x
8) F	c.103 C>T FGA			x
9) F	c.103 C>T FGA			x
10) F	c.394_405 FGA	cerebral venous thrombosis		
11) F	c.394_405 FGA	deep vein thrombosis (TVP) post partum		
12) M	c.394_405 FGA	cerebral ischemia	cerebral hemorrhage	

Conclusions: Patients carrying the c.901 C>T mutation in exon 8 in FGG gene had both thrombotic and hemorrhagic manifestations; patients with c.394_405 in exon 4 in FGA gene only thrombotic episodes; patients with c.160 T>G in the exon 2 in FGA gene only hemorrhages, all others were asymptomatic. Further studies to assess phenotype-genotype correlation of Dysfibrinogenemia are needed.

PO039

OBSTETRICAL MANAGEMENT OF PATIENTS WITH FACTOR XI DEFICIENCY: RETROSPECTIVE ANALYSIS OF A SINGLE CENTER

A. Ierardi, A. Strangio, M. Leotta, C. Spadafora, R.C. Santoro

Hemostasis and Thrombosis Center, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy

Background and Aims: Factor XI (FXI) deficiency was first described in the 1950s. Managing patients with FXI deficiency presents several challenges, including a lack of correlation of bleeding symptom with FXI activity levelsThis study aimed to evaluated pregnancy management of FXI deficiency patients. **Methods**: We performed a retrospective chart review of patients with FXI deficiency who underwent childbirth or had miscarriages. Data on age, ethnicity, family or personal history of bleeding, type of anesthesia and wheter used or not, any compications, and type and timing of blood product or hemostatic agent administered in the peri-operative period were collected. **Results**: We identify 13 women with FXI deficiency who had 31 pregnancy ending in childbirth or miscarriage (21 vaginal deliveries, 7 caesarean sections and 3 miscarriages). At the moment of the event the mean age was 27 years (20-37). 7/13 women, for a total of 16 pregnancies, did not know they had a FXI defect at the time of their first pregnancy and one of them became aware of the defect after both of her deliveries. Their median level of FXI was 34% (25-37). One pregnancy resulted in miscarriage, 2 caesarean sections under neuro-axial anesthesia, 13 spontaneous vaginal deliveries. None of them had a history of bleeding. One woman, with anamnesis of heavy menstrual bleeding, had a first pregnancy with partial placental abruption and concomitant haemorrhage at 20 weeks of gestation. On that occasion, a Factor XI defect (1,4%) was diagnosed. The patient carried the pregnancy to term with spontaneous delivery. Subsequently the patient had 3 other pregnancies, two completed with spontaneous birth, 1 abortion. In all cases, FFP was administered, with no adverse events. Other three women, without a history of bleeding and with mild FXI defect (27%, 40% and 40% respectively) had 6 pregnancies, 4 with vaginal delivery and two with caesarean delivery (with general anesthesia). In none of these cases were blood products or hemostatic agents used. Finally, two women with severe FXI defect and

no history of bleeding (FXI 0.5 and 0.7 respectively) had 5 pregnancies. The first had 3 caesarean deliveries and 1 miscarriage (Table 1). The second just one pregnancy carried out with spontaneous birth. In all cases both patients were prepared with FFP. No haemorrhagic or thrombotic events were reported. **Conclusions**: In our patients higher FXI levels correlate with a lower odds of bleeding. All of our patients with mild defects have gone through childbirth without the need for replacement therapy. All the other patients were prepared with the administration of FFP.

Tabella 1.

Patient	Factor XI	Spontaneous Delivery	Caesarean section	Miscarriage	Previous bleeding	Bleeding	Hemostatic agents	Type of anesthesia
1	1.4	3	/	1	Heavy Menstrual bleeding	Partial placental abruption and bleeding at 20 week of pregnancy	FFC	/
2	26	2	/	/	/	/	/	/
3	36	2	/	/	/	/	/	/
4	34	2	/	/	/	/	/	/
5	25	3	/	/	/	/	/	/
6	34	2	/	1	/	/	/	/
7	37	/	2	/	/	/	/	Neuro-axial anesthesia
8	31	2	/	/	/	/	/	/
9	40	2	/	/	/	/	/	/
10	34	2	/	/	/	/	/	/
11	40	/	2	/	/	/	/	General
12	0.5	/	1	1	/	/	FFC	General
13	0.7	1	/	/	/	/	FFC	/

PO040

IMPACT ON VON WILLEBRAND FACTOR SURVIVAL OF DIFFERENT AMINO ACID CHANGES AT P.1205 IN D3 DOMAIN OF VWF MOLECULE

L. Pieri[1], S. Linari[1], F. Demartis[1], R. Fjerza[1], F. Salvianti[1], M. Coppo[1], M. Attanasio[1], M.C. Di Stefano[1], E. Dalla Porta[2], G. Castaman[1]

[1]Centre for Bleeding Disorders and Coagulation, Department of Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [2]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy

Background and Aims: Von Willebrand factor (VWF) variant p.Arg1205His in D3 domain of VWF is associated with von Willebrand disease (VWD) Vicenza. This variant is by far that associated with the highest clearance of VWF, as demonstrated by very high ratio between VWF propeptide and VWF antigen (VWFpp/VWF:Ag) in circulation in several studies. Little is known about the possible influence, if any, of different amino acid substitution at the same location. The aim of this study is to report the phenotypic FVIII/VWF changes associated with amino acid variants at this location in patients with VWD. **Methods**: We identified 3 patients with p.Arg1205His, 2 with p.Arg1205Cys and 2 with p.Arg1205Leu. Laboratory tests included FVIII, VWF:Ag and VWF:RCo at baseline and after a desmopressin test trial. The level of VWF propeptide was also assessed. Desmopressin was administered subcutaneously at 0,3 µg/kg and FVIII/VWF measurements assayed 1 and 4 hours post-administration. **Results**: At baseline, patients with p.Arg1205His and p.Arg1205Leu showed very low FVIII and VWF measurements (FVIII:C 10-15 U/dL, VWF:Ag 7-11 U/dL and VWF:RCo 6-12 U/dL), while those with p.Arg1205Cys showed clearly higher levels (FVIII:C 34 – 45 U/dL, VWF:Ag 17 – 29 U/dL and VWF:RCo 21 – 28 U/dL). VWFpp/VWF:Ag ratio was >10 in all the patients with p.Arg1205His/Leu variants while those with p.Arg1205Cys had only a slight increase (VWFpp/VWF:Ag 2.9 – 4.4). After desmopressin, a similar pattern was observed in patients with p.Arg1205His/Leu variants, with rapid return to baseline after 4 hours and an average VWF half-life of 2.1 hours, while those with p.Arg1205Cys variant had levels 2-3 fold greater than baseline still after 4 hours, with a VWF half-life of 6-8 hours. **Conclusions**: Amino acid substitutions at p.Arg1205 location in VWF may induce variable decrease of VWF at baseline and different clearance after desmopressin. In particular, the substitution with a cysteine residue seems to improve significantly the laboratory phenotype, mitigating the increased clearance of mutant VWF, again highlighting the important role of cysteine residues in this multimeric molecule.

PO041

ORAL AND GYNAECOLOGICAL MANIFESTATIONS IN TWO WOMEN WITH PLASMINOGEN DEFICIENCY

L. Pieri[1], S. Linari[1], F. Demartis[1], R. Fjerza[1], M.C. Di Stefano[1], R. Marcucci[2], G. Castaman[1]

[1]Centre for Bleeding Disorders and Coagulation, Department of Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [2]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy

Background: Plasminogen (PLG) deficiency is a rare disorder associated with development of fibrin-rich pseudomembranes on mucosal surfaces, mainly affecting the conjunctiva (ligneous conjunctivitis), gums (ligneous gingivitis), cervix and endometrium, ears, nasopharynx, respiratory, gastrointestinal and genitourinary tracts. Abnormal wound healing and infertility have also been reported. The estimated prevalence of homozygous or compound heterozygotes is 1.6 affected individuals per million population. Currently, there is no specific replacement treatment approved for congenital PLG deficiency. We collected the medical history

and describe therapeutic approach in two patients with PLG deficiency referred to our center. The laboratory data included PLG activity measured with chromogenic assay and genetic test. **Case Report 1**: A 35 year-old woman with history of ligneous conjunctivitis and recurrent pseudomembranes of the gingiva treated by surgical excisions with limited success. The gums involvement led to multiple dental loss. The patient also presented vaginal and cervical pseudomembranes. The PLG level was 19% (normal value 80-110%). The genetic analysis showed a compound heterozygosity for aTrp616Cys and an Arg386* mutations. The Trp616Cys is located at the catalytic domain of PLG gene in exon 15, is reported with an allele frequency 0.001% without data on the clinical significance, while Arg368*, located in exon 10, results in a stop codon and it has not been reported previously. The patient performed a tooth extraction and implantology using prophylaxis with fresh frozen plasma 25 mL/kg before the procedure and after 24 hours (h), without complications. The PLG level rised to 38% after 24 h and to 30% after 48 h. The patient is currently following an *in vitro* fertilization program for primary infertility. **Case Report 2**: A 20 year-old woman presented with post coital bleeding and severe dysmenorrhea due to ligneous cervicitis and vaginal pseudomembranes. PLG level was 16%. The genetic evaluation identified compound heterozygosity for two novel unreported mutations: a Lys38Glu and a Gly30Val substitutions, both in critical domains for the protein function and estimated as likely pathogenetic by the bioinformatic programs. **Conclusions**: PLG deficiency diagnosis is often delayed due to its rarity. Both patients underwent multiple bioptic procedures before appropriate diagnosis. The lack of specific therapy makes difficult the management of the clinical manifestations, that can be invalidating with consequent poor quality of life. The diagnosis can be easily performed by the PLG plasma level determination, once a clinical suspicion is made. Genetic test could be useful for the diagnosis confirmation and for a better understanding of the disorder, with an assessment of structure/function relationships.

PO042

EFFECTIVENESS OF PROPHYLAXIS USING WILFACTIN® IN PATIENTS WITH TYPE 2A VON WILLEBRAND DISEASE AND ISCHEMIC HEART DISEASE IN TREATMENT WITH ANTIPLATELET THERAPY: A CASE REPORT

E. Zanon, C. Simion, E. Campello, L. Spiezia, P. Simioni

Haemophilia Center and General Internal Medicine, Department of Medicine – DIMED, University-Hospital of Padua Medical School, Padua, Italy

Backgound: Management of von Willebrand disease (VWD) in patients with normal FVIII levels and cardiovascular comorbidities treated with antiplatelets remains a matter of debate. **Case Report**: We report the case of a 79-year-old patient with type 2A VWD. VWD was diagnosed at the age of 32 years because of prolonged bleedings after minor wounds. The laboratory evaluation showed FVIII:C 64.5% (normal range 60-160%), VWF:Ag 37.3% (normal range 60-160%,) VWF:RCo 21.1% (normal range 60-160%) and VWF:CB 19.1% (normal range 65-150%). In 2010, the patient underwent a cholecystectomy and an excision of a benign lesion of the neck complicated by bleeding, which required hospitalization and infusion of plasma-derived VWF/FVIII concentrate (Haemate P®) for a few days. Moreover, the patient had a history of arterial hypertension, type 2 diabetes mellitus and hypercholesterolemia. In 2015, following the onset of exertion-induced chest pains, the patient was admitted to Padua University Hospital and resulted positive to inducible myocardial ischemia. A coronary angiography revealed a critical multi-vessels disease involving the common arterial trunk, which required a multiple coronary artery bypass. Cardiac surgery was successful, and no hemorrhagic complications occurred. After surgery, the patient started antiplatelet therapy with Cardioaspirin 100 mg. In 2016, under antiplatelet treatment, the patient underwent multiple tooth extractions complicated by slight post-procedure bleeding, despite the administration of a high VWF levels FVIII concentrate (Fanhdi®). A pharmacokinetics test with Wilfactin®, a highly purified plasma-derived VWF almost devoid of FVIII, was performed (Figure 1), and the patient started an on-demand regimen because he refused prophylactic treatment. From 2016 to 2021, the patient suffered from recurrent epistaxis (up to 3-4 episodes a year); moreover, because of the development of benign prostatic hypertrophy, and urinary incontinence associated with many diverticula, a bladder catheter was placed. However, the patient continued to refuse a prophylaxis regimen. In the last year, we recorded 4 episodes of epistaxis and 6 episodes of hematuria, of which at least 3 required hospitalization. In October 2021, the patient agreed to start prophylaxis with Wilfactin® at a dose of 2000 IU twice a week (less than 30 IU/kg). In the last six months, only a single episode of bleeding (hematuria), requiring an additional infusion of Wilfactin®, has been reported. **Conclusions**: Prophylaxis with Wilfactin® appear to be effective in preventing bleeding in patients undergoing antiplatelet treatment.

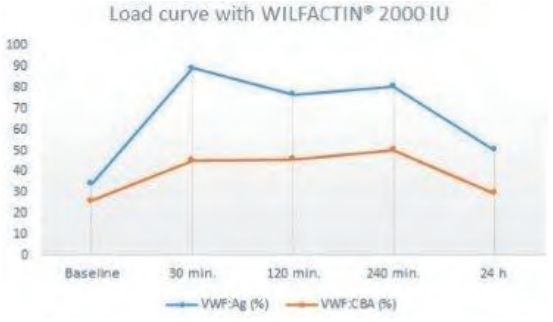


Figure 1.

PO043

TWO CASES OF HERMANSKY-PUDLAK SYNDROME ASSOCIATED WITH NOVEL MUTATIONS IN HPS3 AND HPS6 GENES

L. Pieri, M. Attanasio, M. Coppo, F. Salvianti, S. Linari, F. Demartis, R. Fjerza, M.C. Di Stefano, G. Castaman

Centre for Bleeding Disorders and Coagulation, Department of Oncology, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

Hermansky-Pudlak syndrome (HPS) is characterized by oculocutaneous albinism, a bleeding diathesis, and, in some individuals, pulmonary fibrosis, granulomatous colitis, or immunodeficiency. HPS is a rare disorder with an estimated worldwide prevalence of 1-9 per 1,000,000 individuals. The prevalence per subtype can differ due to founder variants. The prevalence of HPS1-related HPS in northwestern Puerto Rico is 1:1,800. A variable bleeding diathesis associated with platelet function abnormalities has been reported in some patients with the disorder. We report two novel cases of HPS in two children. The first patient was born in 2019. At 3 months of age he developed nystagmus with photophoby, no bleeding symptoms were reported. Two novel heterozygous variants were identified in HPS3 gene (c.1457G>A and c.1813G>T). This gene codes for a protein of the complex BLOC-2 responsible for lysosome-associated organelle byosynthesis. As expected, ADP and ATP release from platelets was almost absent. The platelet count was normal and light transmission aggregometry revealed only reduced aggregation after stimulation with epinephrine. The second patient was born in 2017 and soon after birth had nystagmus with foveal hypoplasia resulting in oculo-cutaneous albinism. No bleeding symptoms were reported. Compound heterozygosity for two novel mutations has been identified, both in the HPS6 gene which also codes for a protein of the complex BLOC-2 responsible for lysosome-associated organelle byosynthesis. The first variant (c.1A>G) was identified in the asymptomatic father and the second (c.210_211insGGGCC) in the asymptomatic mother. No release from platelet delta granules was detected in the patient after stimulation with ADP 2 and 10 microM and epinephrine, as a reduced ATP release after stimulation with arachidonic acid and collagen. Platelet count was normal, and light transmission aggregometry showed slight impaired aggregation after stimulation with ADP 2 microM and epinephrine. HPS is a very heterogeneous disorder, although clinical oculocutaenous albinism is present in almost all patients. It is also confirmed that although low or absent delta granules are detected, HPS3 and HPS6 variants are usually associated with no or mild bleeding tendency.

PO044

BLOOD PLATELETS AT THE CROSSROAD BETWEEN ALZHEIMER'S DISEASE AND AGEING-RELATED FRAILTY

M. Vismara[1], S.M.G. Trivigno[1,2], L. Galgano[1,2], G.F. Guidetti[1], I. Canobbio[1], M. Brambilla[3], M. Camera[3], S. Momi[4], P. Gresele[4], M. Torti[1]

[1]Department of Biology and Biotechnology, University of Pavia, Italy; [2]University School for Advanced Studies IUSS, Pavia, Italy; [3]Centro Cardiologico Monzino IRCCS, Milano, Italy; [4]Department of Medicine and Surgery, Division of Internal and Cardiovascular Medicine, University of Perugia, Italy

Background and Aim: Frailty represents a general state of excessive vulnerability frequently associated to aging in humans, and significantly affects the quality of life. Age-related frailty is more commonly associated to comorbidities such as obesity, diabetes, and Alzheimer's Disease (AD), but the underlying physiopathological mechanisms remain poorly understood (Chen *et al.*, Clin Interv Aging 2014; 9:433-441). It is generally recognized that a low grade chronic systemic inflammation plays a major role in the development of frailty (Soysal *et al.*, Ageing Res Rev 2016:31:1-8). Beside their role in haemostasis and thrombosis, platelets act as true inflammatory cells, and are implicated in some age-related pathologies, including AD (Catricalà *et al.*, Immun Ageing 2012; 9:20). Therefore, platelets appear to be strategically located to link aging, frailty, and AD. We have undertaken a longitudinal study to investigate the correlation between platelet reactivity and the onset of frailty in a mouse model of AD. **Materials and Methods**: Transgenic APP23 mice, a consolidated murine model for AD, were analysed during their lifespan and experimental observations and analysis were collected at 3, 9 and 18 months of age in comparison with control wild type (WT) of the same genetic background. Development of frailty was assessed according to the methodology proposed by Whitehead *et al.* (J Gereontol 2014; 69:621-632) based on the non-invasive evaluation of clinical signs of deterioration of phenotypic and physiological functions (*i.e.* tegument, ocular, nasal respiratory systems, etc.). Platelet activation and reactivity as well as platelet-leukocytes aggregates were evaluated by flow cytometry and lumiaggregometry. **Results**: Clinical assessment of 31 specific clinical signs of deterioration in mice allowed the calculation of a cumulative frailty index that progressively increased with age in both WT and APP23 mice demonstrating that this approach is a valuable method to follow the progression of frailty in longitudinal studies on aged mice. In 18 months old mice, the frailty score for some, but not all the specific evaluated parameters was higher in APP23 mice compared to WT control animals of the same genotype, indicating that the development of AD is associated to a stronger propensity to develop frailty. Circulating platelets from aged APP23 mice displayed an overall hyperactivation state compared to age-matched controls, as demonstrated by a slightly higher degree of integrin alpha(IIb)beta(3) activation and P-selectin exposure. Aged APP23 platelets also revealed an evident hyperreactivity in terms of aggregation and secretion upon stimulation with soluble agonists including thrombin and convulxin. Importantly, the formation of platelet-leukocyte aggregates was significantly

enhanced in blood from aged APP23 mice compared to WT controls. **Conclusion**: These results show that age-related frailty is more pronounced in a mouse model of AD and is associated to hyperactivation and hyperreactivy of circulating platelets. These observations point toward a possible novel role of platelets in linking AD with ageing-related frailty.

PO045

INSIGHT INTO PROTHROMBOTIC PHENOTYPE AND PLATELET ACTIVATION IN PATENT FORAMEN OVALE (PFO) PATIENTS WITH AND WITHOUT MIGRAINE

P. Canzano[1], D. Trabattoni[1], M. Brambilla[1], A. Becchetti[1], M. Conti[1], M. Camera[1,2]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy; [2]Department of Pharmaceutical Sciences, Università degli Studi di Milano, Italy

Background and Aims: Patent foramen ovale (PFO) is a interatrial communication with a prevalence of 25-35%. Patients with cryptogenic stroke (CS) have a 2.3-fold increased relative risk of having PFO, compared with age-matched subjects with a clear-cause-stroke, suggesting the PFO causative role of CS. Furthermore, a strong relationship links PFO and migraine, a chronic neurovascular disorder with a multifactorial aetiology. We recently showed that PFO patients with migraine with aura (MHA) are characterized by a unique hyperactive platelet state, featured by increased platelet expression of Tissue Factor (TF) and phosphatidylserine (PS). This procoagulant phenotype is completely reverted by PFO closure with a significative regression of migraine symptoms, suggesting a biologic causative link between PFO and MHA. To date, there are no studies focused on platelet activation and procoagulant phenotype of CS PFO patients without migraine. Thus, aim of this study was to perform a comparative analysis of platelet activation and prothrombotic potential between these patients and PFO patients with migraine. **Methods**: 33 PFO with migraine and 27 ischemic PFO without migraine patients on aspirin treatment were enrolled before PFO closure. Pselectinpos-, activated-glycoproteinIIbIIIa (aGPIIbIIIa)pos-, TFpos-, Tissue Factor Pathway inhibitor (TFPI) pos-, reactive oxygen species (ROS)pos-platelets, platelet-leukocyte aggregates (PLA) and microvesicles (MVs) were evaluated by whole blood flow cytometry. Flow cytometry data were reported as % of positive cells and levels of Mean Fluorescence Intensity (MFI). TF-dependent Thrombin generation (TG) was assessed by Calibrated Automated Thrombogram assay in the presence of TF and TFPI neutralizing antibodies. **Results**: Expression of Pselectin and aGpIIb/IIIa, and the number of PLA, was similar in PFO patients with and without migraine. In migraineurs, the number of TFpos platelets was comparable to ischemic PFO patients without migraine, but the latters showed a significantly higher MFI (1.11±0.44 in migraineurs *vs* 1.64±0.5 in ischemic PFO; p<0.0001). Conversely TFPI expression was comparable in the 2 groups of patients thus resulting in a comparable platelet-associated TG. The number and the MFI of ROSpos platelets was slightly increased in ischemic PFO patients without migraine, suggesting a higher oxidative stress status compared to migraineurs. The total number of M*Vs* was comparable between the 2 groups of patients and the most abundant of them were from platelets. TF+-M*Vs* were slightly higher in ischemic patients (22.231±6671 in migraineurs *vs* 23.182±5140 MVs/µl in ischemic PFO); PS+TF+-M*Vs* behaved similarly (4236±1556 in migraineurs *vs* 4777±125 MVs/µl in ischemic PFO). This resulted in a higher MV-associated TF-dependent TG in ischemic compared to PFO patients with migraine (p=0.0495). **Conclusions**: This study shows that ischemic and migraineur PFO patients share a similar platelet prothrombotic phenotype. By contrast they differ in terms of MV-associated TG, being that of ischemic patients significantly higher than that of migraineurs.

PO046

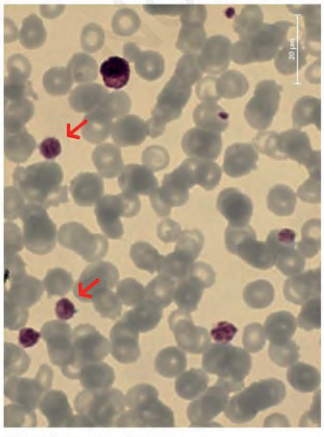
ADVANCES IN DIAGNOSIS OF HEREDITARY MACROTHROMBOCYTOPENIA: THE ROLE OF NEXT-GENERATION SEQUENCING

P. De Bonis, A. De Laurenzo, E. Chinni, F. Cappucci, L. Fischetti, D. Colaizzo, E. Grandone, G.L. Tiscia

Unità di Emostasi e Trombosi, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG), Italy

Background: About half of all cases of hereditary macrothrombocytopenia are due to MYH9 (Myosin Heavy Chain 9) variations. However, as this condition involves different stages of platelet development/differentiation and respective controlling genes can be involved in the pathogenesis of the disease, the diagnosis may be challenging. On the other hand, molecular diagnosis is needed, due to different management strategies according the gene involved. **Methods**: We describe two cases of macrothrombocytopenia observed at Thrombosis and Hemostasis Centre of our research Institute. Personal and family histories were collected, with a focus on bleeding history. Laboratory investigations included complete blood count (BC) and blood smear. Next-Generation Sequencing (NGS) was performed using an Illumina platform targeting a custom hereditary macrothrombocytopenia panel gene. **Results**: The first case here described is a 50-years-old male with a previous diagnosis of immune thrombocytopenia (ITP). He had no personal or family history of spontaneous or post-traumatic bleedings (tooth extractions, minor surgery, etc.). He was diagnosed with ITP eight years before and treated with thrombopoietin (TPO) mimetics with partial response. Therefore, he underwent a comprehensive thrombocytopenia check

for ITP differential diagnosis. Blood smear examination showed a platelets diameter of 6-7 µm (n.r. 2.0-3.0 µm) (Figure 1); no leukocyte inclusions were observed. BC revealed following data: platelet count $41x10^9$/L [normal range (n.r.): 150-450], mean-platelet-volume (MPV) 13.6 fL (n.r.: 6.0-13.0). Hereditary macrothrombocytopenia was, then, hypothesized. NGS demonstrated the presence of heterozygosis in the MYH9 gene of allele variant c.5629C>T, responsible for the novel p.Arg1877Trp amino acid exchange, supporting the diagnosis of May-Hegglin disorder. The mature protein shows a replacement of a very basic residue (arginine) with a nonpolar residue (tryptophan), possibly impacting myosin-9 structural features. Indeed, *in silico* annotations indicated a p.Arg1877Trp deleterious effect on protein structure (Polyphen-2 score: 1.00; SIFT score: 0). Of note, according to the American College of Medical genetics (ACMG) recommendations, this variant is likely to be pathogenic, as it involves a mutational residue. The second case was a 57-years-old asymptomatic woman with two previous at term pregnancies in absence of any bleeding complications. She was referred because of a failed response to TPO mimetics after an ITP diagnosis. The patient underwent a series of diagnostic tests showing a platelet count of $20x10^9$/L and MPV value of 22.7 fL. Morphological examination confirmed a larger platelet size [6-7 µm (n.r. 2-3 µm)]. However, clinical judgment guided toward the suspicion of a congenital macrothrombocytopenia, mostly because of the poor response to classical ITP therapies. NGS investigation showed a heterozygous allele variant within the ACTN1 gene, coding for actinin alpha 1. The woman showed the allele variation c.1349G>A, causing p.Arg450His. The variation has been previously described in subjects with a patient's phenotype and to impair actinin alpha-1 dimerization and, in turn, platelet shape and internal organization. **Conclusions**: Massively sequencing technologies, such as the NGS, is improving diagnostic approaches and enabling for important clinical applications in the context of hereditary thrombocytopenia, whose genetic determinants are rapidly expanding.



Blood smear image.
Legend: Arrows point giant platelets

Figure 1.

PO047

LOW C-MPL EXPRESSION AND IMPAIRED TPO SIGNALING PATHWAYS IN ANKRD26-RELATED THROMBOCYTOPENIA: THERAPEUTIC IMPLICATIONS.

S. Barozzi[1], F. Melazzini[1,2], V. Bozzi[1], F. Fumoso[1], C. Zaninetti[3], A. Pecci[1,2]

[1]Medicina Generale, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [2]Department of Internal Medicine, University of Pavia, Italy; [3]Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany

Background and Aims: *ANKRD26*-related thrombocytopenia (*ANKRD26*-RT) is an autosomal-dominant thrombocytopenia associated with predisposition to myeloid malignancies. Patients present mild to severe thrombocytopenia with normal platelet size and function, mild bleeding tendency, and increased serum thrombopoietin (TPO) concentrations. The disorder is caused by single nucleotide substitutions in the 5'UTR of the *ANKRD26* gene. A previous study showed that the variants interfere with the physiologic repression of ANKRD26 during megakaryocyte differentiation, resulting in its persistent expression in late megakaryocytes: this, in turn, associates with hyperactivation of the MAPK/ERK pathway and reduced proplatelet formation. We recently conducted a clinical trial on the efficacy of the TPO-mimetic Eltrombopag in inherited thrombocytopenias (ITs). The study showed a significant variability in the response to the drug among *ANKRD26*-RT patients; moreover, overall, *ANKRD26*-RT showed a lower extent of response compared to other forms of IT. We therefore conducted a systematic analysis of the TPO signaling pathways in the largest series of patients with *ANKRD26*-RT analyzed for this aspect, including subjects who had received Eltrombopag within the clinical trial. **Methods**: We studied platelets from 11 *ANKRD26*-RT patients, 7 of whom had participated in the trial. We analyzed the phosphorylation state of the main kinases of TPO signaling, STAT5, ERK and AKT, in basal conditions and after stimulation with TPO or TPO plus Eltrombopag. Platelet expression of c-MPL, the receptor for TPO, was measured by immunoblotting. **Results**: In resting conditions, *ANKRD26*-RT platelets showed a significantly higher activation of STAT5 and ERK compared to control platelets ($p=0.01$). The analysis of the same pathways following stimulation with TPO and Eltrombopag showed a markedly reduced response to the stimuli of patients' platelets, expressed as the ratio between induced and baseline phosphorylation. The activation of STAT5 in *ANKRD26*-RT platelets was reduced by 80% following stimulation with TPO ($p<0.005$) and 84% after stimulation with TPO and Eltrombopag ($p<0.005$) compared to control platelets. Similarly, ERK activation was reduced by 51% ($p<0.05$) after TPO stimulation and by 67% ($p<0.005$) when Eltrombopag was added to TPO. We then explored whether these alterations were associated with altered c-MPL expression.

Indeed, c-MPL was found to be significantly reduced in *ANKRD26*-RT platelets, with a mean decrease of 63% ($p<0.01$) compared to controls. When we analyzed the patients previously treated with Eltrombopag, we found a direct correlation between c-MPL expression and the extent of response to the drug *in vivo* ($R^2=0.73$, $p<0.01$). **Conclusions**: In *ANKRD26*-RT, the constitutive activation of signaling downstream c-MPL is associated with a reduced expression of the receptor itself in platelets; this results in a defective response to stimulation with c-MPL ligands, such as TPO and Eltrombopag. Of note, we found a direct relationship between the amount of c-MPL expressed in platelets and the response to Eltrombopag *in vivo*, providing a plausible explanation for the variability of the response to the drug among *ANKRD26*-RT individuals. Finally, the reduced expression of c-MPL on platelet surface could result in a lower internalization of TPO, contributing to the markedly increased serum TPO levels observed in *ANKRD26*-RT patients.

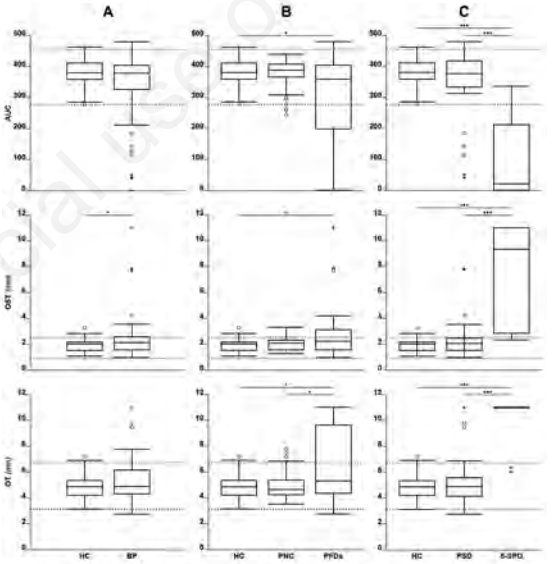
PO048

FLOW-CHAMBER DEVICE, (T-TAS) TO DIAGNOSE PATIENTS SUSPECTED OF HAVING PLATELET FUNCTION DEFECTS

S. La Marca[1], A. Lecchi[1], L. Padovan[1], M. Boscarino[1], F. Peyvandi[1,2], A. Tripodi[1]

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milano, Italy; [2]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milano, Italy

Background and Aims: Patients suspected of having platelet defects represent a diagnostic challenge for the clinical laboratory, mainly due to the complexity and poor standardization of methods used for investigation. We provide herewith a comparative face-to-face results evaluation for a new flow-based chip-equipped point-of-care device, Total-Thrombus-Analysis-System (T-TAS), meant to measure the overall primary hemostatic function *vs* the more complicated and time consuming lumi-aggregometry and platelet delta granule measurement. **Patient population**: Object of the study was a group of patients (n=96) suspected of having platelet defects. They displayed a mild-to-moderate bleeding diathesis of variable severity, characterized by mucocutaneous bleeding, menorrhagia, and excessive post-surgical and post-traumatic blood loss. This cohort was characterized as having the following parameters within normal limits: platelet count and size; prothrombin and activated partial thromboplastin time; VWF (antigen and ristocetin cofactor activity) and factor VIII activity. We also investigated a second group of patients (n=26) referred for the evaluation of residual platelet hyperactivity whilst on antiplatelet therapy. **Results**: Forty-eight (PFDs) of 96 patients (BP) displayed abnormal platelet function by lumi-aggregometry and 10 of the 48 displayed defective delta granule content and were classified as δ-storage pool disease (δ-SPD) (Figure 1). T-TAS compared favourably with lumi-aggregometry in diagnosing the most severe forms of platelet function defects (*i.e.*, δ-SPD) [test agreement (Lumi-LTA-vs-T-TAS) for the δ-SPD subgroup amounted to 80% and K CHOEN to 0.695. T-TAS was less sensitive to the milder defects [*i.e.*, primary secretion defects (PSD)]. Concerning patients on antiplatelet drugs, test agreement (Lumi-aggregometry-vs-T-TAS) in detecting patients, who were responders to the therapy amounted to 54% and K CHOEN to 0.150. **Conclusions**: Results indicate that T-TAS can diagnose the more severe forms of platelet function defects (*i.e.*, δ-SPD). There is limited agreement T-TAS *vs* Lumi-aggregometry to identify responders to antiplatelet drugs, but this poor agreement is commonly shared by Lumi-aggregometry and other devices owing to the lack of test specificity and prospective data from clinical trials linking platelet function and therapeutic efficacy.



Distribution of T-TAS results for patients with normal coagulation and previous history of bleeding, presumably due to platelet dysfunction.
Panel A: *(HC)* 50 healthy controls; *(BP)* all 96 bleeding patients (irrespective of the final diagnosis based on Lumi-LTA).
Panel B: *(HC)* 50 healthy controls; *(PNC)* 48 bleeding patients without confirmed platelet defect based on Lumi-LTA; *(PFDs)* 48 bleeding patients with confirmed platelet function defect based on Lumi-LTA.
Panel C: *(HC)* 50 healthy controls; *(PSD)* 38 bleeding patients diagnosed as having Platelet Secretion Defect, *(δ-SPD)* 10 bleeding patients diagnosed as having δ-Storage Pool Disease.
*p < 0.05, ** p < 0.01, *** p < 0.001. Dashed lines: reference range (2.5th- 97.5th centile of the distribution of HC).

Figure 1.

PO049

PLATELET DISTRIBUTION WIDTH AS A MORTALITY RISK PREDICTOR: PROSPECTIVE FINDINGS IN AN ITALIAN ADULT GENERAL POPULATION

S. Costanzo[1], B. Izzi[1], A. Gialluisi[1,2], A. De Curtis[1], S. Magnacca[3], T. Panzera[1], A. Di Castelnuovo[3],

F. Gianfagna[2,3], M.B. Donati[1], C. Cerletti[1], G. de Gaetano[1], L. Iacoviello[1,2], for the Moli-sani Study Investigators

[1]*Department of Epidemiology and Prevention, IRCCS NEUROMED, Pozzilli, IS, Italy;* [2]*EPIMED Research Center, Department of Medicine and Surgery, University of Insubria, Varese, Italy;* [3]*Mediterranea Cardiocentro, Napoli, Italy*

Background and Aims: Platelet Distribution Width (PDW) is a marker of platelet size heterogeneity recognized as a diagnostic marker for diseases with impaired megakaryocyte differentiation and thrombopoiesis, as it represents a readout from the combination of processes leading to platelet production and destruction. Recently, PDW was also found to tag platelet activation variability, both *ex vivo* and *in vitro* and to be a prognosis predictor of cardiovascular disease (CVD), cancer, diabetes and neurological disorders. We therefore evaluated the possible relationship, at population level, between PDW values and all-cause and cause-specific mortality. **Methods**: Longitudinal analysis was performed on 17,334 participants (52% women, mean age 55.6 ±12 years) from the Moli-sani study (enrollment 2005-2010), without a history of haematological diseases and for whom baseline PDW measurements were available. All-cause and cause-specific mortality was validated by using the Italian Death Registry. PDW measurements were categorized in tertiles, the first acting as the reference. Multivariable Cox-proportional hazard model was used to estimate the association of PDW and mortality, using multiple imputation technique for missing data. **Results**: In the whole population sample, the mean value of PDW was 16.4 ±0.58 fL. Over a median follow-up of 11.6 years (interquartile range 10.7-12.5), 1,535 deaths (37.7% CVD and 36.5% cancer) were identified. As compared to those in the first tertile of PDW (14.6-16.0 fL), individuals within the highest tertile (16.6-20.4 fL) had an increased risk of all-cause (HR: 1.20; 95% CI: 1.04-1.37) and CVD mortality (HR: 1.29; 95% CI: 1.03-1.62). (Figure 1). In contrast, no association between PDW and cancer mortality was found. Additionally, all-cause and CVD mortality increased by 5% and 7%, respectively, for each increase in 1 standard deviation (0.58 fL) of PDW (HR: 1.05; 95% CI:1.00-1.10 and HR: 1.07; 95% CI: 0.99-1.16, respectively). No difference in the association between PDW and all-cause and CVD mortality was observed by subgroup analyses by gender, history of CVD/cancer and major risk factors of non-communicable diseases. However, subgroup analyses by age classes (35-65 years, ≥65 years) showed that the association of PDW with all-cause mortality was more evident in the elderly (HR: 1.34; 95% CI: 1.14-1.58, p for interaction=0.028, Figure 1). Similar results were found when cancer mortality was investigated (age ≥65 years: HR: 1.37; 95% CI: 1.01-1.85, p for interaction=0.020). **Conclusions**: A PDW-associated increase of all-cause and CVD mortality risk, could be related to accelerated/altered platelet activation, production or destruction, all age-dependent processes leading to several clinical conditions and death.

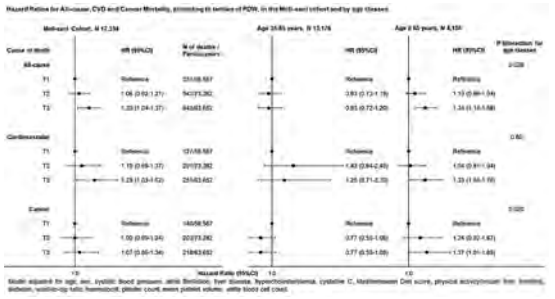


Figure 1.

PO050

IMPORTANCE OF THE SCREENING FOR VARIANTS CAUSING INHERITED THROMBOCYTOPENIAS PREDISPOSING TO HEMATOLOGICAL MALIGNANCIES IN POTENTIAL BONE MARROW DONORS, A CASE REPORT

L. Bury[1], E. Falcinelli[1], A.M. Mezzasoma[1], A. Carotti[2], P. Gresele[1]

[1]*Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Perugia, Italy;* [2]*Department of Medicine and Surgery, Division of Hematology and Clinical Immunology, University of Perugia, Perugia, Italy*

Background and Aims: Inherited platelet disorders predisposing to hematological malignancies are autosomal dominant forms characterized by thrombocytopenia, most often mild, with normal or slightly increased platelet volume, a bleeding diathesis of widely variable severity and an enhanced risk of developing hematological malignancies. Three disorders have been identified so far: familial platelet disorder with propensity to acute myelogenous leukemia (FPD-AML), *ETV6*-related thrombocytopenia (*ETV6*-RT) and *ANKRD26*-RT.
FPD-AML is caused by mutations of *RUNX1*, a transcription factor crucial for megakaryocyte maturation. About 45% of FPD-AML patients evolve to a hematological malignancy during their life, mostly to myeloid leukemia or myelodysplastic syndromes (MDS), but also to T-cell or B-cell lymphoproliferative disorders. Next-generation sequencing (NGS) has revolutionized the diagnostic approach to these disorders providing a rapid molecular diagnosis but it has also raised discussion about the ethical implications of discovering unexpected variants associated with leukemic risk. We report a case showing the importance of the prompt identification of these variants. **Methods**: Platelet function was studied by light transmission aggregometry (LTA), flow cytometry and lumiaggregometry. Variants affecting genes involved in bleeding and platelet disorders were assessed by NGS, performing targeted sequencing of 91 TIER-1 genes. Germinal variants were confirmed by Sanger sequencing of DNA extracted from finger nails. **Results**: A patient came to our

attention for mild thrombocytopenia found on the occasion of a blood donation. He did not refer hemorrhagic manifestations. We confirmed very mild thrombocytopenia ($121x10^9$/L) associated with defective platelet aggregation in response to adrenaline, U46619 and collagen and defective content and release of α and δ granules. Expression of platelet glycoproteins was normal. NGS identified a rare variant in *RUNX1*, p.Gln262Ter, classified as pathogenic in ClinVar and present in the literature and was then diagnosed FPD-AML. Our patient reported that his brother had been affected by AML in the '90s and was treated by allogeneic hematopoietic stem cells transplantation for which he was the donor. We therefore wondered if the brother with AML who received his bone marrow was also a carrier of the same genetic variant. Given that the brother circulating white blood cells would necessarily present the mutation, we analyzed DNA from his finger nails and found the same *RUNX1* variant. Platelet function tests revealed the same type of platelet function defect observed in the bone marrow donor brother. **Conclusions**: The study of this family shows that the brother of our propositus developed AML because carrier of FPD-AML and he received hematopoietic stem cell transplantation from our propositus carrier of FPD-AML too. This poses the patient at risk of further leukemic transformation. At the time of transplantation (1990s) FPD-AML was still unknown but nowadays we know that platelet disorders predisposing to hematological malignancy are more common than expected, therefore: 1) after obtaining explicit informed consent, patients with suspected platelet disorders predisposing to hematological malignancies should be genetically screened for these disorders; 2)mutational screening of siblings should be promptly performed to avoid to transplant patients with cells carrying the same genetic variant.

PO051

PLATELET THROMBOXANE INHIBITION BY LOW-DOSE ASPIRIN IN POLYCYTHEMIA VERA: *EX VIVO* AND *IN VIVO* MEASUREMENTS AND *IN SILICO* SIMULATION

G. Petrucci[1], A. Giaretta[2], P. Ranalli[3], V. Cavalca[4], A. Dragani[3], B. Porro[4], D. Hatem[1], A. Habib[5], E. Tremoli[6], C. Patrono[1], B. Rocca[1]

[1]Department of Safety and Bioethics, Section of Pharmacology, Catholic University School of Medicine, Rome, Italy; [2]Department of Pathology, University of Cambridge, Cambridge, UK; [3]Department of Hematology, SSpirito Hospital, Pescara, Italy; [4]Centro Cardiologico Monzino, IRCCS, Milano, Italy; [5]Department of Basic Medical Sciences, QU Health, Qatar University, Doha, Qatar; [6]Maria Cecilia Hospital, Cotignola, Italy

Background and Aims: Low-dose aspirin is currently recommended for patients with polycythemia vera (PV), a myeloproliferative neoplasm (MPN) with increased risk of arterial and venous thromboses. Based on aspirin pharmacodynamics in essential thrombocythemia, a twice-daily regimen is recommended for PV patients deemed at particularly high thrombotic risk. **Methods**: We investigated the effects of low-dose aspirin on platelet cyclooxygenase activity and *in vivo* platelet activation in 49 PV patients, as assessed by serum thromboxane (TX)B_2 and urinary TXA_2/TXB_2 metabolite (TXM) measurements, respectively. A previously described pharmacokinetic-pharmacodynamic *in silico* model was used to simulate the degree of platelet TXA_2 inhibition by once-daily (qd) and twice-daily (bid) aspirin, and to predict the effect of missing an aspirin dose during qd and bid regimens. **Results**: Serum TXB_2 averaged 8.2 [1.6-54.7] ng/ml and significantly correlated with the platelet count (rho=0.39) and urinary TXM (rho=0.52) in multivariable analysis. One-third of aspirin-treated PV patients displayed less-than-maximal platelet TXB_2 inhibition, and were characterized by significantly higher platelet counts and platelet-count corrected serum TXB_2 than those with adequate inhibition. Eight PV patients were sampled again after 12±4 months, and had reproducible serum TXB_2 and urinary TXM values. The *in silico* model predicted complete inhibition of platelet-derived TXB_2 by bid aspirin, a prediction verified in a PV patient with the highest TXB_2 value while on aspirin qd and treated short-term with a bid regimen. Conclusions: In conclusion, one in three PV patients on low-dose aspirin display less-than-maximal inhibition of platelet TXA_2 production. A personalized approach to antiplatelet therapy can be guided by serum TXB_2 measurements.

PO052

EFFICACY OF THROMBOPOIETIN RECEPTOR AGONISTS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA AND MEGAKARYOCYTES WITH MYELODYSPLASTIC FEATURES

R. Tomasello[1], F. Romano[1], M. Mattana[1], C. Rotolo[1], C. Cammarata[1], A. Lucchesi[2], G. Giordano[3], S. Siragusa[1], M. Napolitano[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo, Palermo, Italy; [2]IRCCS Istituto Romagnolo per lo studio dei tumori (IRST) "Dino Amadori", Meldola (FC), Italy; [3]Division of Internal Medicine, Hematology Service, Regional Hospital "ACardarelli", Campobasso, Italy

Background and Aims: Immune Thrombocytopenia (ITP) is a bleeding disorder characterized by persistently low platelet counts (PLT). ITP is a diagnosis of exclusion that may require bone marrow (BM) examination to rule out a unilinear myelodysplastic syndrome (MDS). BM histopathology of patients with ITP

usually shows megakaryocyte (MK) hyperplasia with aggregation of cells to form diffuse clusters, sometimes associated to mature lymphoid cells infiltration. In a few cases, BM examination shows signs of MK dysplasia with no other criteria compatible with MDS. There is currently no difference between the treatment of patients with BM compatible with ITP and those with isolated myelodysplastic features of MKs. **Methods**: We performed a retrospective analysis that included all patients diagnosed with ITP from 2011 to 2021, treated with thrombopoietin receptor agonists (TPO-RAs) as 2nd (or further) line treatment, whose BM examination (performed before TPO-RA administration) showed signs of MK dysplasia. Treatment with TPO-RAs started in this group of patients in a period ranging from 2016 to 2021. Patients with a prior diagnosis of MDS, HCV-related liver disease and autoimmune diseases were excluded from the study. All patients included that were not treated with TPO-RAs on first relapse ultimately developed resistance to steroids (PLT persistently <50000/mmc under treatment with maximum tolerable dose of steroid) or intolerance (to the minimum effective dose of steroid able to maintain a PLT >50000/mmc), some of them were also unresponsive to Rituximab and splenectomy. We evaluated response to TPO-RAs at 2 weeks, 3 months, 6 months and 1 year from start of treatment. **Results**: We evaluated 16 patients of age at diagnosis ranging from 40 to 83yo (median age 58.5y), M:F ratio of 1.3:1, diagnosed with ITP who became unresponsive to steroids and were ultimately treated with either Eltrombopag (ETP, n=8) or Romiplostim (RPS, n=8). In all patients, a BM examination showed myelodysplastic features of MKs. All patients were treated with corticosteroids with or without high dose intravenous immunoglobulins (IVIGs) on first line. Three patients started TPO-RAs in 2nd line. Twelve patients started TPO-RAs in 3rd or further lines due to a loss of response to previous immunosuppressive treatments. Two patients treated with ETP experienced sustained response (PLT >100000/mmc for more than 3 months off therapy). Response at 2 weeks and 1 year were better in the ETP group. BM aspiration and biopsy were performed again on 2 patients out of 15 due to development of other cytopenias, after 2 years and 1 year of treatment with TPO-RAs respectively, and showed overt MDS (Table 1). **Conclusions**: Limited to the small number of patients enrolled, this study demonstrates the efficacy of long-term treatment with TPO-RAs in patients with underlying MK myelodysplastic features in the BM observed at diagnosis. The overall response to immunosuppressive treatments such as Rituximab or steroids appears to be short lived and hard to maintain in the analyzed cohort, thus suggesting a role of MK dysplasia in the mechanism of thrombocytopenia in addition to autoimmunity. Due to their mechanism of action, TPO-RAs actually represent the optimal treatment for these patients, as they can stimulate thrombopoiesis while also inducing a degree of immunotolerance. Furthermore, this study shows the possibility of a sustained response in patients affected by ITP with signs of MK dysplasia.

Table 1.

GENDER (N)	AGE AT DIAGNOSIS	COMORBIDITIES (N)	2nd LINE THERAPIES (N) (excluding TPO-RAs)	3rd LINE THERAPIES (N) (excluding TPO-RAs)	START OF TPO-RA (N)
Male (9) Female (7)	40-83 y.o Median 58.5	High blood pressure (6) History of cancer (2) Ischemic cardiopathy (2) Diabetes mellitus type 2 (2) Hypomyxedema (1) Epatic cirrhosis (1) Glaucoma (1)	Steroids +/- IVIG (7), Rituximab (6)	Steroids +/- IVIG (3) Rituximab (3)	2nd line (5) 3rd line (10) 4th line (3)
PATIENT GROUP (N)	MEDIAN PLATELET COUNT AT 2W (PLT/MMC)	MEDIAN PLATELET COUNT AT 3M (PLT/MMC)	MEDIAN PLATELET COUNT AT 6M (PLT/MMC)	MEDIAN PLATELET COUNT AT 1Y (PLT/MMC)	SUSTAINED RESPONSE
Eltrombopag + Romiplostim (16)	60/500	70000	100000	200000	2/16
Eltrombopag (8)	140500	97000	106000	202000	2/16
Romiplostim (8)	45500	77500	120000	110000	0/16

PO053

PLATELET FUNCTION IN PATIENTS WITH PTEN-MUTATIONS

S. Sorrentino[1], A. Pasquarella[2], R. Onesimo[2], C. Leone[2], A. Curatola[2], G. Zampino[2,3], I. Lazzareschi[2,3], E. De Candia[1,4]

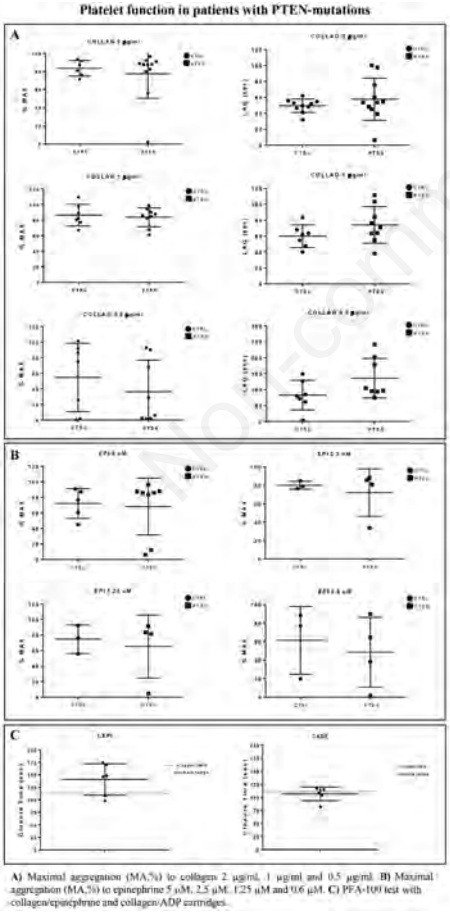
[1]Unità Malattie Emorragiche e Trombotiche, Fondazione Policlinico Universitario Agostino Gemelli IRCSS, Roma, Italy; [2]UOC Pediatria, Fondazione Policlinico Universitario Agostino Gemelli IRCSS, Roma, Italy; [3]Dipartimento di scienze della Vita e della Sanità Pubblica, Università Cattolica del Sacro Cuore, Roma, Italy; [4]Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Roma, Italy

Background and Aims: Phosphatase and tensin homolog (PTEN) Hemartoma Tumor Syndrome (PHTS) is a group of very rare diseases due to mutations in PTEN gene and characterized by amartomatosis tumors. A previous study in mouse models showed that PTEN is involved in the negative regulation of collagen-induced platelet activation As a consequence, PTEN-deficient mice had increased platelet aggregation to collagen compared to control mice (Weng, 2010). Due to a collaboration with the Rare Diseases Center in our institution, we could perform a pilot study on PHTS patients with the aim of evaluating platelet function in these patients, with a special focus on platelet aggregation by low dose collagen. **Methods**: The study population included 12 patients (with proven genetic diagnosis of PHTS and not in treatment with antiaggregant/anticoagulant drugs) and 11 controls (volunteer patients with no PHTS diagnosis). Platelet function was investigated using: 1) PFA 100 System collagen/epinephrine (CEPI) and collagen/ADP (CADP) test cartridges, 2) light transmission aggregometry (LTA) and platelet rich plsma (PRP) stimulated by ADP (2 µM), collagen (2, 1, 0.5 µg/ml), epinephrine (5, 2.5, 1.25, 0.6 µM). Maximal aggregation (MA, %) for all agonists and lag phase (LP, seconds) for collagen were measured (Figure 1). **Results**: Platelet aggregation by ADP 2 µM was not significantly different in PTEN patients compared to the control group (MA >75%, data not shown). Platelet aggregation by collagen 2 µg/ml, 1 µg/ml and 0.5 µg/ml was >70% in both populations with a longer LP in PTEN patients (LP >70 sec), however no statistically significant differences were found. Platelet

aggregation by low dose epinephrine, *i.e.* 5, 2.5, 1.25 and 0.6 μM also was not different in PTEN patients compared to controls. PFA-100 CEPI and CADP closure times were prolonged in 67% and 50% of PTEN patients, respectively (normal values CEPI <140 seconds; CADP <120 seconds). **Conclusions**: Although previous studies in animal models suggested that PTEN regulation of PI3K/Akt pathway could negatively affect collagen-induced platelet aggregation, we did not confirm increased aggregation by low collagen concentrations in patients with PTEN-related diseases. Despite the small number of patients analyzed, this pilot study is the first study in human subjects affectd by these very rare diseases. Our results suggest that PTEN could regulate collagen-induced aggregation to a lesser extent in humans rather than in mice. Studies on a larger number of patients and using a wider range of agonists concentrations are needed to elucidate the role of PTEN in the regulation of platelet function.

Reference:

Weng Z, Li D, Zhang L, Chen J, Ruan C, Chen G, Gartner TK, Liu J. PTEN regulates collagen-induced platelet activation. Blood. 2010 Oct 7;116(14):2579-81. doi: 10.1182/blood-2010-03-277236.



A) Maximal aggregation (MA,%) to collagen 2 μg/ml, 1 μg/ml and 0.5 μg/ml. B) Maximal aggregation (MA,%) to epinephrine 5 μM, 2.5 μM, 1.25 μM and 0.6 μM. C) PFA-100 test with collagen/epinephrine and collagen/ADP cartridges.

Figure 1.

PO054

IDENTIFICATION OF A TUBB1 MISSENSE MUTATION IN A CONGENITAL MACROTHROMBOCYTOPENIA FAMILY BY CLINICAL EXOME SEQUENCING (CES)

G. D'Andrea, A. Ariano, M. D'Apolito, G. Cordisco, I. Adipietro, G.P. Palladino, M.F. D'Ambrosio, M.A. Di Stefano, M. Curcetti, R. Santacroce, M. Margaglione

Genetica Medica, Dipartimento di Scienze Mediche e Sperimentali, Università di Foggia, Foggia, Italy

Background: Congenital macrothrombocytopenia is a heterogeneous group of rare disorders with an autosomal-dominant inheritance, characterized by increased platelet size and a decreased number of circulating platelets.Several genes are associated with macrothrombocytopenia, in particulary, the genes implicated, encode proteins that vary widely in function and include transcription factors, proteins involved in cytoskeleton rearrangement and organization.One of less frequent forms of macrothombocytopenia is determined by component defects of the cytoskeleton that regulate proplatelet formation and morphology through microtubules compounds of α/β-tubulin. Abnormal assembly and incorporation of β1-Tubulin is caused by molecular defects of TUBB1 gene.The aims of this study were to characterize molecularly a family affected by macrothrombocytemia using a new sequencing method (CES), then, to validate its importance in the molecular diagnostics of genetically heterogeneous pathology. **Materials and Methods**: This report describes a 15 year-old girl with macrothrombocytopenia, diagnosed from the first years of the life and inherited from mother family branch. Indeed,The mother and a maternal aunt are platelets deficiency too,The young men, after obtainig the parental consent informed, was subjected to a molecular investigation. DNA was extracted from peripheral blood leukocytes and subsequently submitted to an Clinical Exome Sequencing (CES) analysis by a kit DNAPrep with enrichment Illumina on platform NextSeq. 550.The results, at first, were analyzed by an Illumina Variant Interpret platform,then,to verify candidate mutations and examine their segregation among family members, Sanger sequencing was performed using standard methods on an ABI 3130 automated sequence. **Results**: Molecular analysis revealed a heterozygous variant p.Gln191Pro in the exon 4 of TUBB1 gene with unknown clinical significance (VUS). Direct Sanger sequencing confirmed the mutation both in the patient and in the mother, but none in the maternal aunt due to the DNA unavailability. *In silico* analysis as Polyphen-2 and SIFT Web Server assign to the mutation an pathogenic effect on β1-tubulin protein.The replacement of a polar amino acid with a non-polar one constitutes a destabilizing element of the tertiary structure of protein and determines, probably, deficient functional microtubules that might lead to defective proplatelet for-

mation and abnormal protrusion-like platelet release, resulting in macrothrombocytopenia. **Conclusions**: Our results underline the usefulness of the Clinical Exome Sequencing in clinical practice as well as that it is an efficient method for a quick and a cheaper molecular analysis of pathogenic variants in inherited macrothombocytopenia or for most heterogeneous genetically pathologies. In any case, family segregation and *in vitro* molecular studies are indispensable to demonstrate the effective role of the mutation in the development of macrothrombocytopenia in the family we have analyzed.

PO055

LUSUTROMBOPAG TREATMENT IN A PATIENT WITH THROMBOCYTOPENIA CIRRHOSIS RELATED PRIOR TO RADIOTHERAPY FOR OROPHARYNGEAL SQUAMOUS CELL CARCINOMA: A CASE REPORT

F. Valeri[1], C. Dainese[1], M. Bardetta[1], C. Sella[1], B. Bruno[2], A. Borchiellini[1]

[1]CRR Malattie Emorragiche e Trombotiche dell'Adulto, Ematologia Universitaria, AOU Città della Salute e della Scienza di Torino; [2]Ematologia Universitaria, Dipartimento di Oncologia, AOU Città della Salute e della Scienza di Torino, Italy

Background: Lusutrombopag (Mulpleo®) is an orally bioavailable, small molecule thrombopoietin receptor agonist approved for the treatment of thrombocytopenia in adult patients with chronic liver disease who are scheduled to undergo a procedure. In placebo-controlled phase 3 clinical trials, lusutrombopag (at the dose of 3-mg tablet once daily for up to 7 days) significantly increased the proportion of patients who did not require a platelet transfusion prior to the procedure or rescue therapy for bleeding up to 7 days after the scheduled procedure. In the present report, we discuss a case in which radiotherapy (who we considered comparable to procedure) was performed reaching target platelet levels following oral administration of lusutrombopag. **Case Report**: We report the case of a 69-year-old woman affected by chronic liver disease HBV related who developed an oropharyngeal squamous cell carcinoma. The patient underwent first line chemotherapy (cisplatin (CDDP) and 5-fluorouracil (5-FU) with a 30% reduction dose due to a thrombocytopenia cirrosis related (basal value 76 x 10^9/L) but this treatement was early stopped because of worsening of thrombocytopenia (39 x 10^9/L). Oncologist decided then to shift treatement to radiotherapy (RT), but platelet values were inadequate. 5 days prior to radiotherapy, oral lusutrombopag was initiated at a dosage of 3 mg/day (day 1) and was continued for 7 days. Platelet count on day 5 (first day of RT) was 62 × 10^9/L and on day 21 (the last day of RT) was 70 × 10^9/L, allowing the entire treatment. **Conclusions**: Use of lusutrombopag in this setting allowed our patient to safely perform radiotherapy despite initial thrombocytopenia. We believe that this drug can serve as a potential treatment option when a short period of platelet increase is needed for clinical reasons other than invasive procedures in patients with chronic liver disease complicated by thrombocytopenia.

PO056

CORRELATION BETWEEN BLEEDING SEVERITY AND LABORATORY TEST RESULTS IN PATIENTS WITH INHERITED PLATELET FUNCTION DISORDERS (THE BAT-LAB SUBSTUDY)

E. Falcinelli[1], M.C. Alessi[2], L. Bury[1], G. Podda[3], G. Guglielmini[1], M. Fiore[4], T. Sevivas[5], N. Bermejo[6], E. De Candia[7,8], M. Chitlur[9], M. Lambert[10], L. Barcella[11], P.G. Heller[12,13], C. Falaise[2], P. Gresele[1]

[1]Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Italy; [2]Centre for CardioVascular and Nutrition research (C2VN), INSERM 1263, INRA 1260, Marseille, France; [3]Medicina III, ASST Santi Paolo e Carlo, Dipartimento di Scienze della Salute, Università degli Studi di Milano; [4]Laboratory of Hematology, University Hospital of Bordeaux, Pessac, France; [5]Servicio de Hematologia Clinica- Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal; [6]Hospital San Pedro de Alcantara, Caceres, Spain; [7]Hemostasis and Thrombosis Unit, Fondazione Policlinico Agostino Gemelli IRCCS, Roma; [8]Institute of Internal Medicine and Geriatrics, Università Cattolica del Sacro Cuore, Roma; [9]Children's Hospital of Michigan, Detroit, USA; [10]Children's Hospital of Philadelphia, Philadelphia, USA; [11]Immunohematology and Transfusion Medicine (SIMT) & Hemostasis and Thrombosis Center ASST Papa Giovanni XXIII, Bergamo, Italy; [12]Instituto de Investigaciones Médicas ALanari, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina; [13]Departamento Hematología Investigación, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad de Buenos Aires, Instituto de Investigaciones Médicas (IDIM), Buenos Aires, Argentina

Background and Aims: The diagnostic approach to inherited platelet function disorders (IPFD) is based on the bleeding history (ISTH-BAT) and on a streamlined panel of laboratory tests. Few studies have assessed the predictive value of platelet laboratory tests for bleeding manifestations in patients with IPFD, with inconclusive results. This is an ad hoc designed substudy of the multicentric international ISTH BAT-validation study promoted by the Platelet Physiology SSC of the ISTH (BAT-VAL) including patients with a well-defined diagnosis of IPFD. Aim of the present study was to assess whether there is an association between the bleeding score (BS) and results of platelet function tests in patients with IPFD. **Methods**: Participating centers were asked to fill in a standardized form on laboratory results of the platelet function studies performed to diagnose

the enrolled IPFD patients. Laboratory data included bleeding time (BT), PFA-100® closure time, light transmission aggregometry (LTA), alpha granule release and platelet glycoprotein expression by flow cytometry. Results were classified as abnormal when out of the internal laboratory reference range. **Results**: 72 patients (37% of the initially enrolled IPFD population), with 16 different IPFD forms, were included. Median baseline BS was 7.5 (IQR 2-12) (BS of healthy controls: 0, IQR 0-1). A LTA defect in response to at least one platelet agonist was found in 71% individuals. The % of patients with high BS and defective LTA in response to ADP was significantly higher compared to that of patients with normal BS and defective LTA (59.4±4.7% *vs* 35.5±4.8%, p<0.05). BT and PFA-100® closure time were altered, respectively, in 64% and 88% of patients. BT was significantly more prolonged in patients with a high BS compared to those with a normal BS (27.1±6.2min *vs* 15.1±10.6min, p<0.01), while PFA-100® closure times was not different between patients with normal or high BS. A ROC curve analysis revealed moderate discriminative ability of the BT for pathologic BS (AUC 0.795, p<0.001). Flow cytometry showed that 27% of patients had abnormal αIIbβ3 expression and 39% defective αIIbβ3 activation (measured as PAC-1 binding). The % of patients with high BS and defective αIIbβ3 expression was significantly higher compared to that of patients with normal BS and defective αIIbβ3 (90% *vs* 10%, p<0.05). Moreover, the % of patients with defective PAC-1 binding and pathologic BS was significantly higher than that of patients with defective PAC-1 binding but normal BS (66% *vs* 33%, p<0.05). A ROC curve analysis revealed a moderate discriminative ability of abnormal PAC-1 binding for pathologic BS (AUC 0.778, p<0.001). α-granules content was reduced in 29% of patients and was significantly more common among patients with a pathologic BS compared to those with a normal BS (80% *vs* 20%, p<0.05). **Conclusions**: This study shows that an abnormal ISTH-BAT BS is associated with altered laboratory platelet function test results in IPFD. In particular, impairment of LTA in response to ADP, prolongation of the BT, reduced α-granules content, reduced αIIbβ3 expression and decreased ADP-induced PAC-1 binding were associated with increased BS. Despite the relatively small patient number of this study suggests a predictive value of some platelet assays for bleeding in patients with IPFD. An ad hoc designed prospective study on the predictive value of platelet function tests for subsequent bleeding in patients with IPFD is highly warranted.

PO057

RISK STRATIFICATION IN PATIENTS WITH ATRIAL FIBRILLATION IN TREATMENT WITH ORAL ANTICOAGULANTS: AN NMR-BASED METABOLOMICS AND LIPOPROTEOMICS STUDY

A. Vignoli[1,2], F. Cesari[3], L. Tenori[1,2], E. Salvadori[4], B. Formelli[5], A. Kura[3], A.M. Gori[3], B. Giusti[3], R. Marcucci[3], A. Poggesi[5]

[1]Magnetic Resonance Center (CERM) and Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino, Italy; [2]Consorzio Interuniversitario Risonanze Magnetiche MetalloProteine (CIRMMP), Sesto Fiorentino, Italy; [3]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [4]IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy; [5]NEUROFARBA Department, University of Florence, Florence, Italy

Background and Aims: Atrial fibrillation (AF) is the most common cardiac arrhythmia with a clinical relevance observed in medical practice[1]. Long-term oral anticoagulation is the mainstay therapy for ischemic stroke prevention in AF patients[2]. However, benefits of anticoagulation need to be carefully balanced against the risk of bleeding, with intracranial hemorrhage being the most dreaded scenario. Stroke risk in AF is not a homogeneous phenomenon, currently decision making regarding antithrombotic drug administration relays on available stroke and bleeding risk stratification schemes (*e.g.*, CHADS2VASC2, HAS-BLED scores) which are based on clinical information[3]. However, their validity is still controversial, thus the identification of novel stratification approaches able to recognize patients at high risk of bleeding is pivotal for AF patient management. **Methods**: The Strat-AF study is a single center, longitudinal observation study evaluating elderly AF patients on oral anticoagulation for thromboembolism prevention. The goal of this project is to evaluate if biological markers, either clinical, circulating and neuroimaging-based, and their possible combinations, can improve the prediction of bleeding risk in AF patients under treatment with any type of oral anticoagulants[4]. Serum samples of 177 AF patients (mean age 78.2 years, 55.3% males, 70% on DOAC treatment) were analyzed via high resolution 1H-NMR spectroscopy[5]. Data on 27 metabolites and 112 lipoprotein-related fractions were obtained and analyzed with multivariate and univariate statistical approaches. **Results**: We characterized, for the first time to best of our knowledge, how different kinds of oral anticoagulation, Vitamin K and coumarin anticoagulants, affect the serum metabolomic profiles of patients, obtaining a good discrimination between the two treatments (71% accuracy). Moreover, several statistically significant associations (FDR adjusted p-value <0.05) between metabolomic/lipoproteomic features and clinical outcomes (*i.e.* memory disturbances, stroke events, CHADS2VASC2 score) emerged from the regression analyses. **Conclusions**: Our results support the usefulness of NMR-based metabolomic approach in the AF setting both for the description of the pharmacological effects on patients' metabolism and for the improvement of the risk stratification of AF patients.

References:

1. Kornej, J., Börschel, C. S., Benjamin, E. J. & Schnabel, R. B. Epidemiology of Atrial Fibrillation in the 21st Century. Circulation Research 127, 4-20 (2020).
2. Lau, Y. C. & Lip, G. Y. H. Which drug should we use for stroke prevention in atrial fibrillation? Curr Opin Cardiol 29, 293-300 (2014).

3. Lip, G. Y. H. & Lane, D. A. Stroke prevention in atrial fibrillation: a systematic review. JAMA 313, 1950-1962 (2015).
4. Poggesi, A. *et al.* Role of Biological Markers for Cerebral Bleeding Risk STRATification in Patients with Atrial Fibrillation on Oral Anticoagulants for Primary or Secondary Prevention of Ischemic Stroke (Strat-AF Study): Study Design and Methodology. Medicina (Kaunas) 55, E626 (2019).
5. Vignoli, A. *et al.* High-Throughput Metabolomics by 1D NMR. Angew. Chem. Int. Ed. Engl. 58, 968-994 (2019).

PO058

ANALYTICAL PERFORMANCE OF DIFFERENT LABORATORY METHODS FOR MEASURING SUSOCTOG-ALPHA

C. Novembrino[1], I. Quaglia[2], M. Boscolo-Anzoletti[1], E. Galbiati[1], A. Valpreda[3]

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; [2]Istituto Clinico Humanitas, Milan, Italy; [3]Azienda Ospedaliera Città della Salute e della Scienza, Turin, Italy

Background and Aims: Acquired haemophilia A (AHA) is an often severe bleeding disorder caused by the development of antibodies (inhibitors) to factor VIII (FVIII). Recombinant porcine factor VIII (rpFVIII, susoctocog alfa, Obizur®, Baxalta US Inc.-Takeda company- Bannockburn, IL, USA) is indicated for the treatment of acute bleeding episodes in AHA, but there are few data regarding the laboratory methods for properly monitoring the treatment. This study involving 3 different laboratories aimed to evaluate the analytical performance of different assays for measuring rpFVIII. **Methods**: Five spiked samples at different rpFVIII concentrations (from 0.05 U/mL to 1.5 U/mL) were analysed on three distinct days, in triplicate every day, with nine different combinations of reagents (SynthasIL and SynthaFax -Werfen- for one-stage assay, Chromogenix Coamatic FVIII for chromogenic assay), FVIII depleted plasmas (with or without von Willebrand factor –vWF-) and calibrators (HemosIL human calibrator plasma, porcine calibrator diluted in FVIII deficient plasma with or without vWF). All the assays were performed on ACL Top analysers (Werfen, Bedford, MA). Intra- and inter assay and inter-laboratory Coefficient of Variation (CV%) were calculate together with percentage of recovery (% recovery) on the expected value. **Results**: The results of the three laboratories are reported in Table 1 as total inter-laboratory CV% (mean of CV% obtained for all the measures of the 5 samples in the three laboratories) and % recovery (mean of % recovery obtained for all the measures of the 5 samples in the three laboratories). **Conclusions**: The present study highlights the impact of the calibrator and the deficient plasma used in the assay on the accuracy of the results. The use of porcine standard (when available) and FVIII deficient plasma with vWF is recommended.

Table 1.

METHOD/REAGENT	CV %	% RECOVERY
OSA/SynthasIL (human standard) FVIII def plasma+vW	**4.04**	**99.43**
OSA/SynthasIL (porcine standard) FVIII def plasma	46.47	52.14
OSA/SynthasIL (porcine standard) FVIII def plasma+vW	**2.75**	**111.04**
OSA/SynthaFax (human standard) FVIII def plasma+vW	2.84	77.89
OSA/SynthaFax (porcine standard) FVIII def plasma	13.94	66.32
OSA/SynthaFax (porcine standard) FVIII def plasma+vW	21.45	146.63
Chromogenic (Coamatic FVIII) (human standard)	5.12	61.50
Chromogenic (Coamatic FVIII) (porcine standard in FVIII def plasma)	4.67	129.79
Chromogenic (Coamatic FVIII) (porcine standard FVIII def plasma+vW)	**8.32**	**96.57**

PO059

A COMPARISON BETWEEN NON-ACTIVATED THROMBOELASTOMETRY AND THROMBIN GENERATION ASSAY FOR EMICIZUMAB MONITORING

S. Arcudi[1], E. Scalambrino[2], M. Clerici[2], C. Novembrino[2], A. Tripodi[2], F. Peyvandi[1]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano and Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy; [2]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy

Background and Aims: The ability of coagulation assays to monitor severe hemophilia A (HA) patients on emicizumab prophylaxis has been poorly investigated. Given this background, our study aims to report the results of non-activated rotational thromboelastometry (NATEM) and thrombin generation assay (TGA) performed in patients on emicizumab prophylaxis and to correlate findings with plasma emicizumab concentration (EmiC). **Methods**: Consecutive HA patients on emicizumab prophylaxis at steady state were enrolled in an observational prospective study. TGA was assessed with a homemade method upon addition to plasma of tissue-factor (1pM) and synthetic phospholipids (1μM). NATEM was measured for whole blood after addition of CaCl2 (100mM). Emicizumab plasma concentrations (EmiC) were measured by the modified calibrated one stage FVIII assay. We considered for TGA lag-time (LT), endogenous thrombin potential (ETP) and peak-thrombin (PH); for NATEM, clotting-time (CT), clotting-firmness-time (CFT), time-to-maximum-clotting-firmness

(MCF-t). Spearman's Rho coefficient was used to assess the correlation between variables. **Results**: Sixteen patients on emicizumab prophylaxis (aged 17-64 years) were included in the study. Three were positive for FVIII inhibitors. Results for NATEM and TGA are reported in Table 1. When assessing the correlation with EmiC, NATEM-CT, NATEM-CT+CFT and NATEM-MCF-t were inversely correlated with EmiC (CT rho=-0.536, p<0.05; CT+CFT rho=-0.515, p<0.05; MCF-t rho=-0.561, p<0.05). TGA-ETP and TGA-PH were positively correlated with EmiC (ETP rho=0.507, p<0.05; TGA-PH rho=0.643, p<0.01). Moreover, TGA and NATEM assays resulted to be inversely correlated (CT *vs* ETP, rho=-0.771, p<0.001; CFT *vs* ETP, rho=-0.765, p<0.001; CT+CFT *vs* ETP rho=-0.805, p<0.001). **Conclusions**: Our analysis of 16 patients on emicizumab prophylaxis shows that both assays, NATEM and TGA, correlate with the plasma concentration of emicizumab. Dose-adjustment of emicizumab based on laboratory testing is currently not required routinely. However, our preliminary data suggest that NATEM and TGA might be useful to help managing special situations requiring monitoring with a global test, such as surgeries or concomitant treatment with bypassing agents.

Table 1.

Test		number	Median (IQR)
	Emic (µg/ml)	16	61 (38-74)
NATEM	CT (sec)	16	848 (713-1068)
	CFT (sec)	16	194 (151-233)
	MCF (mm)	16	60 (56-63)
	MCF-t (sec)	16	1681 (1403-1745)
	CT +CFT (sec)	16	1070 (864-1291)
TGA	Lagtime (min)	16	8 (6-9)
	ETP (nM*min)	16	1125 (710-1337)
	PEAK (nM)	16	74 (44-100)

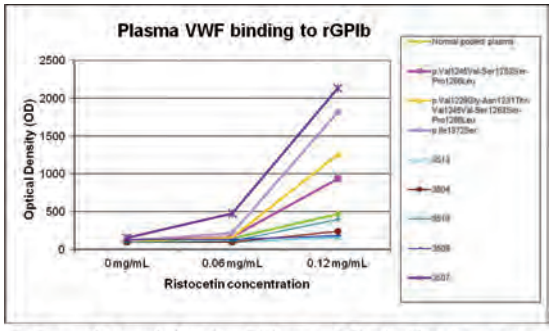
PO060

A SCREENING ASSAY FOR TYPE 2B VON WILLEBRAND FACTOR VARIANTS USING AN ELISA ASSAY WITH RISTOCETIN AND A RECOMBINANT WILD-TYPE GLYCOPROTEIN IB

P. Colpani[1], L. Baronciani[1], G. Cozzi[1], M.T. Pagliari[2], A. Cairo[1], E. Biguzzi[1], F. Peyvandi[1,2]

[1]Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; [2]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy

Background and Aim: Type 2B patients are usually detected using the first level assays for von Willebrand disease (VWD), because of their loss of high molecular weight multimers (HMWM). Ristocetin-induced platelet agglutination assay (RIPA) or genetic analysis of von Willebrand factor (VWF) exon 28 is then performed to confirm diagnosis. Type 2B with HMWM (*e.g.*, p.Pro1266Leu) might be missed at the first screening or misdiagnosed as type 1/low-VWF and RIPA or genetic analysis are rarely performed in these cases. Due to the relative common presence of type 2B with HMWM variants in our type 2B population (25%), we developed a screening assay to identify the type 2B variants using patients' frozen plasma. **Methods**: A wild-type recombinant glycoprotein Ib (rGPIb) was immobilized on a 96-wells ELISA plate using the 2D4 monoclonal antibody. A normal pooled plasma (NP), type 2B controls and patients' plasma were normalized to the lowest VWF antigen level. Each sample was seeded in presence of ristocetin at 2 different concentrations (0.06 and 0.12 mg/mL; Figure 1) and incubated for 2 hours. VWF was detected with an anti-VWF HRP–labeled polyclonal antibody. Seventy patients with VWF levels borderline or reduced, at their first clinical evaluation due to bleeding diathesis, were assessed *versus* a group of 14 type 2B patients. Sanger sequencing of exon 28 was performed in patients with VWF increased binding to rGPIb. **Results**: We calculated the mean values of the optical density (OD) ratios between patient 's samples and NP, in comparison with those obtained with the type 2B patients. Considering ristocetin 0.06 mg/mL concentration, patients with VWF increased affinity to GPIb shown a mean OD ratio higher or equal to 1.87±0.70 *versus* mean OD ratio higher or equal to 0.69±0.14 in patients with VWF normal affinity to GPIb. **Conclusions**: This GPIb ELISA assay, that has the advantage to use frozen plasma, could help to identify type 2B variants and can be used to select patients to be investigated at molecular level.



The Figure reports an example of the in-house ELISA assay results. Three type 2B samples were used as controls, their OD were above the OD of the normal pooled plasma (green line). Patient 3507 was identified to carry the type 2B variant p.Arg1341Gln. VWF, von Willebrand factor; rGPIb, recombinant wild-type glycoprotein Ib.

Figure 1.

PO061

POPULATION PHARMACOKINETICS OF DIFFERENT CLOTTING FACTOR CONCENTRATES IN FIFTY-SIX PATIENTS WITH SEVERE HEMOPHILIA A: A SINGLE CENTER EXPERIENCE

S. Pasca[1], E. Zanon[2]

[1]*Biomedical Sciences Department (DSB), Padua University Hospital;* [2]*Hemophilia Center, General Medicine, Padua University Hospital, Italy*

Background and Aims: There are currently many factor VIII (FVIII) concentrates, either standard or extended half-life, available to clinicians that can certainly improve the quality of life of patients with hemophilia A. However, given the great inter and intra-individual variability, a correct pharmacokinetic evaluation is needed to better find the most suitable drug for each patient. Population pharmacokinetics (PopPK) make it possible to easily obtain these data using a few samples and in a short time. The aim of our study was to compare the PK results obtained with different FVIII concentrates in different patients with hemophilia A. **Methods**: We have analyzed, described, and compared all PopPK profiles of different FVIII concentrates, standard (including recombinant FVIII single chain – rFVIII-sc) and extended half-life, obtained in a group of severe hemophilia A patients using the Web-Accessible Population Pharmacokinetics Service (WAPPS). For all products FVIII activity was assessed using a one-stage assay (OSA) with ellagic acid (Actin®FS). The rFVIII-sc values were multiplied by the conversion factor 2.0. **Results**: Overall, we have collected 121 PopPK in 56 different patients with severe hemophilia A. 88 of which were obtained with standard half-life (SHL) concentrates in 47 patients, while the remaining 33 with extended half-life (EHL) products in 26 subjects. 17 patients had both evaluations and switched from SHLs to EHLs after PopPK assessment. Mean age at the time of PopPK was similar between SHL and EHL groups, 23 years *vs* 26 years; and the mean dose of concentrate used in the two groups was 34.3 IU/kg and 35.0 IU/kg, respectively. Mean half-life was 1.38-fold higher (Figure 1-c) with EHL products (16.8 hours) than SHL (12.2 hours), with many differences between the different FVIII concentrates analyzed. Figures 1-a and 1-b show the various half-lives obtained with the different concentrates, respectively with SHLs and EHLs. The 17 patients who underwent the switch had an improvement in the mean half-life of the drugs used which went from 12.5 to 17.1 hours, allowing them to reduce their infusions by a mean of one third. No significant differences in blood group or in mean vWF:Ag/vWF:RCo were observed between the different two groups, SHLs *vs* EHLs, or among patients treated with the different drugs. **Conclusions**: Our study comprising over a hundred PK analyzes, performed in 56 different patients, showed a great PK variability existing between drugs. The OSA used to evaluate PK profiles is usually considered acceptable for all the tested drugs, therefore it did not significantly influence the data we obtained, although in any case, great attention must always be paid to the assays and reagents used when deciding to perform a PK assessment. rFVIII-sc showed a mean half-life very close to damoctocog-alfa, despite not being considered an EHL. Among the other SHLs, the best result in terms of half-life was obtained from the only tested plasma-derived FVIII, while among the EHLs turoctocog-alfa-pegol emerges with a half-life 1.25 times higher than those of the other drugs with extended half-life. These results are sometimes different from those obtained in the pivotal studies, therefore before starting a prophylaxis with any clotting factor concentrates, it is very useful to carry out a PK evaluation, which together with an appropriate medical history, can guarantee the patient an adequate treatment for his needs and lifestyle.

Figure 1-a

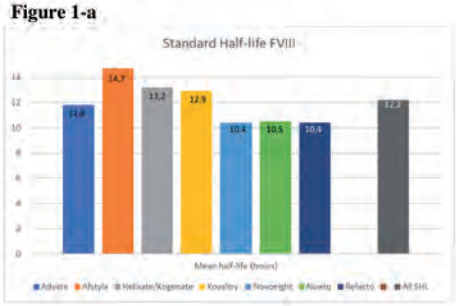


Figure 1-b

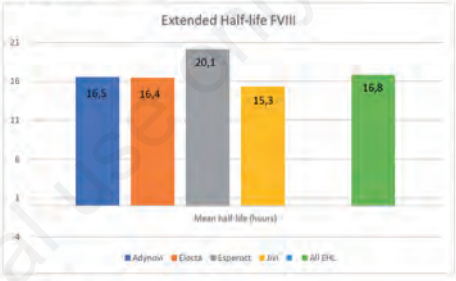
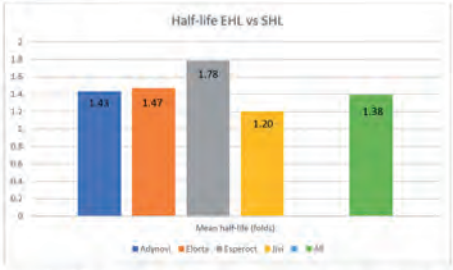


Figure 1-c



1-a: mean half-lives with SHL concentrates; 1-b: mean half-lives with SHL concentrates; 1-c Average half-life of each EHLs and of their totality, compared with the men half-life of all the SHLs tested (expressed in folds).

Figure 1.

PO062

ANALITYCAL EVALUATION OF PRO II AND PLATFORM POCT

C. Dellanoce, A. Zerbi, C. Zecca, N. Portesi, M. Di Gioia, O. Paoletti, R. Morandini, M. Tala, S. Testa

Haemostasis and Thrombosis Center; Department of Laboratory Medicine, ASST Cremona, Italy

Background and Aims: AVK therapy is monitored through PT-INR to ensure the therapeutic range stability.

Portable coagulation systems are an easy and immediate methods to perform PT-INR test on capillary blood. Many differences due to reagents, coagulometers and calibrations can allow different INR results. Agreement between portable monitors and reference systems should be always evaluated before their routine use for patient management. Aim of the study was evaluate two commercial portable monitors, Pro II and Platform, in comparison with PT-INR provided by central laboratory reference system. **Methods**: PT-INR was performed on two portable monitors: Coagucheck Pro II (Roche Diagnostics, Basel, Switzerland) Platform (LumiraDx, UK, Limited) which both use recombinant human thromboplastin with ISI=1.00. The local reference system was STAR Max2 (Stago, France) a magneto-mechanical analyzer with rabbit thromboplastin (NeoPTimal, Stago France, ISI=1.04). 126 AVK patients, with a stable PT-INR ranging from 2.0 to 4.0, were evaluated. In the same daily session venous blood samples were tested with the reference system and capillary PT-INR test was performed on the two portable monitors. Linear regression and Bland&Altman method were used for the statistical analysis. **Results**: The correlation among different systems was good. Coefficient of regression (R^2) of NeoPTimal *vs* Pro II=0.914 and *vs* Platform=0.875; Pro II *vs* Platform=0.844 (see Table 1). Differences in PT INR≥±0.5 were observed in about 12% of the total population, specifically 10.3% between NeoPTimal and Platform, 3.96 % between NeoPTimal and Pro II, 3.96 % between the two POCT. Bland&Altman method showed a bias as follow: NeoPTimal *vs* Coagucheck Pro II=-0.3 while the same thromboplastin *vs* Platform=2.6; between the two POCT=2.6. **Conclusions**: Results showed good correlation between the different methods. Pro II showed a better correlation and agreement compared to the laboratory reference system. These differences could be due to different thromboplastins, calibrations and reference systems. It is advisable that each center performs analytical evaluations before introducing POCT in clinical practice.

Table 1.

	R^2	Linear Regression Equation	Bland Altman Bias	INR≥0.5 (%)
NeoPTimal vs Pro II	0.914	Y=0.3758+0.8400x	0.3	3.96
NeoPTimal vs Platform	0.875	Y=0.5132+0.8051x	2.4	11.9
Pro II vs Platform	0.844	Y=0.07033+0.9480 x	2.6	3.96

Linear regression, Bland&Altman analysis and percentage of discrepancies between the POCT.

Background and Aims: Prothrombin Time (PT INR) is the test used for monitoring Anti-Vitamin K Antagonists (VKA). The detection of the Clot Waveform Analysis (CWA) was introduced for studying the dynamic process of clot formation. The aim of this study was to measure the PT 1st and 2nd derivatives (D) in anticoagulated patients for achieve information on the clotting process. **Methods**: We studied a total of 780 patients (426 men and 354 women, median age 75 years, range 13-100). PT ratio (RecombiPlasTin 2G, (Werfen), was performed using an ACL TOP 500 (Werfen) which uses a turbidimetric method for clot detection. CWA detected the time at which maximum velocity of clot formation was reached (1st D, as mAbs/s), and the time at which maximum acceleration of clot formation was reached (2nd D, as mAbs/s2). **Results**: PT INR was not different between men and women (2.63, 0.95-9.46 and 2.99, 1.01-11.05 respectively, p=0.356) while a difference was found between both derivatives of men and women: 1st D 203.38, 45.24-693.57 and 223.57, 28.51-622.17 mAbs/s respectively (p<0.0001) and 2nd D (752.18, 161.17-5359.55 and 818.68, 77.53-5049.58 mAbs/s2 respectively (p<0.006). A nonlinear regression showed a significant correlation between PT INR and both 1st DER (R2=0.339) and 2nd D (R2=0. 501). A trend to a plateau is present from INR from 3.0 to above 4.00 for the 2nd D (Figure 1). **Conclusions**: 1st and 2nd D highlights a more reactive clot formation in women. CWA also shows that the deep of anticoagulation did not change much for INR above 3.0.

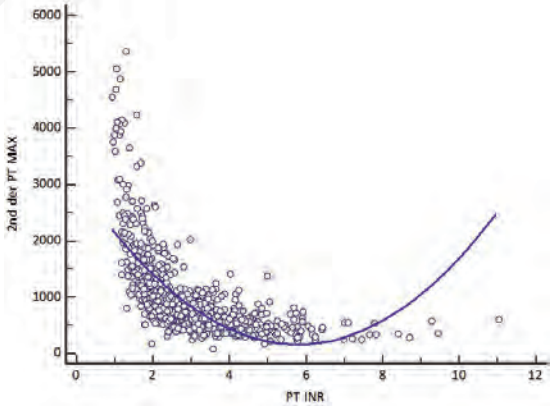


Figure 1.

PO063

CLOT WAVE ANALYSIS OF PROTHROMBIN TIME DURING ANTI-VITAMIN K ANTAGONISTS

M.F. Ruberto[1], F. Marongiu[1], A. Mameli[2], P. Schirru[2], S. Cornacchini[2], D. Barcellona[1]

[1]Department of Medical Sciences and Public Health, University of Cagliari; [2]Azienda Ospedaliero-Universitaria di Cagliari, Italy

PO064

EVALUATION OF A POCT SYSTEM FOR TESTING D-DIMER IN THE EXCLUSION OF THROMBOEMBOLIC EVENTS

A. Fassina, L. De Valentin, S. Luzi, R. Turrini, K. Proko, L. Zardo

Laboratorio Analisi, ULSS 2, Marca Trevigiana, Treviso, Italy

Background and Aims: D-Dimer is a protein fragment detectable within 4-6 hours in blood, from the beginning of the fibrinolysis process. D-Dimer test is an integral part of validated algorithms for the diagnosis of deep-vein thrombosis (DVT) and pulmonary embolism (PE) avoiding invasive and expensive imaging tests. The importance of D-Dimer test during COVID-19 pandemic is testified by the doubling of D-Dimer requests. The aim of this study is to evaluate the performance of a Point of Care Testing (POCT) for D-Dimer determination compared to the laboratory assay. **Methods**: The D-Dimer plasma concentration, obtained from POCT (microfluidic immunofluorescent, LumiraDx), was compared to the one resulted from the laboratory method (turbidimeter, ACL-TOP, Werfen). The results were represented in Bland-Altman Plot. 105 samples from Emergency Room (E.R.), hospital units and external patients were analyzed for D-Dimer assay on the reference instrument ranging from 184 to 4965 ng/mL FEU. The evaluation was performed using sodium citrate plasma samples collected for D-Dimer assay, firstly on the laboratory instrument and then on POCT, within approximately 4 hours. **Results**: 37 of the 105 samples tested were negative with the reference method and 68 were positive. Age-modified cut-off (500 ng/mL FEU up to 50 years and 10 ng/mL more for each year of age) was used, as recommended by qualified publications (*Lippi et al., D-Dimer Testing for Suspected Venous Thromboembolism in the Emergency Department, Clin Chem Lab Med 2014*), to define positive or negative results. The agreement between reference method and POCT was 88.6%, considering positive-positive and negative-negative. POCT results evidenced 4 out of 105 false negatives and 8 out of 105 false positives. In all the 4 false negative cases D-Dimer determinations were just below the age-adapted cut-off and with small quantitative differences compared to the reference method. Sensitivity and specificity were respectively 94.1% and 78.4%. Bland Altman Plot is used to evaluate methods' agreement. The difference mean line is very close to zero (44 ng/mL), so there is no clear tendency to underestimate or overestimate. In the Bland-Altman Plot points near the age-modified cut-off are very close to the mean line showing minimum differences between methods; whereas, for concentrations >500 ng/mL, points are far from the mean line. Disagreement results only emerged near the age-modified cut-off where the patient classification is difficult using only D-Dimer results, so any important clinical impact can be excluded. **Conclusions**: Our evaluation shows a good agreement of POCT system and reference method. POCT systems are particularly useful in E.R. and situations in which using laboratory test is difficult. In these contexts, the use of POCT is important in order to reduce Turn Around Time and help early decision making for patient management. In the determination of D-Dimer, high sensitivity is essential to avoid false negative results, so that DVT or PE potentially patients can be correctly screened. Considering POCT as an exclusion test, its performance should be evaluated in selected patients already diagnosed for DVT or PE, just to verify whether false negatives occur in these cases too. In conclusion, POCT system is a valid alternative to the laboratory method, in particularly for clear positive or negative results. For borderline results, confirmation by laboratory testing may be suggested.

PO065

PROTHROMBIN TIME (PT) AND ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) TESTING: RAPID CENTRIFUGATION *VERSUS* CLASSICAL CENTRIFUGATION

M. Gagliardi[1], L. Capone[2], P. Migliaccio[2], M.R. Cianciulli[2], V. Marottoli[3], A. Ciampa[1]

[1]Haemostasis Centre, S.GMoscati Hospital, Avellino; [2]Laboratory of Clinical Pathology, S.GMoscati Hospital, Avellino; [3]Multimedica SrL, Napoli, and Mediterranea Diagnostica, Castellammare di Stabia (NA), Italy

Background and Aims: Prothrombin time (PT), and activated partial thromboplastin time (APTT) are basic tests performed for coagulation screening disorders, and for monitoring patients on anticoagulant therapy. In coagulation tests, sample processing and preparation is one of the most critical steps affecting reporting times. Centrifugation is the pre-analytical phase that most limits speed in sample processing. The aim of this work is to demonstrate that it is possible to reduce the reporting time for coagulation tests by using a centrifugation with a higher centrifugal force and a lower rotation time that one recommended by Laboratory Guidelines, obtaining a qualitatively suitable plasma. **Methods and Patients**: Three hundred samples obtained from outpatients and inpatients for PT and APTT estimation were included. Overfilling samples, insufficient and lipemic filling samples were excluded. Tube 1 was centrifuged in a benchtop centrifuge (JOUAN B4i) at 1500 g for 20 minutes. Tube 2 was centrifuged with the same centrifuge at 3026 g for 4 minutes. No brakes were used. The plasma obtained with the two methods was tested for PT and APTT using the automated method on the ACL TOP 500 coagulometer (Werfen). To confirm the adequacy of platelet-poor plasma, platelet counts were performed on plasma obtained from 40 random samples out of 300 samples included. Residual platelets were evaluated in accordance with CLSI recommendations, and the sample test results were less than 10 000/µl. Platelet count was performed on the automated hematological analyzer COULTER LH 780. A routine internal quality control for the analyzer including platelet count was performed daily, using BIORAD three-level controls. **Results**: PT and APTT results obtained by conventional centrifugation and rapid centrifugation were compared. A comparison was also made among INR values that were subtherapeutic (<2.0), INR values within the therapeutic range (between 2.0 and 3.0), and that ones above the therapeutic range (INR >3.0). The data were analyzed using descriptive statistics, Student

t-tests, correlation coefficient (r) and Bland-Altman graphs for comparison of methods (Figure 1). The mean values for PT and APTT for both conventional and rapid centrifugation were comparable, without statistically significant differences. **Conclusions**: The present study has shown that by rapid centrifugation of samples, 3026 g for 4 minutes, it is possible to obtain PT and APTT values, almost identical to those values obtained by conventional centrifugation (1500 g for 20 minutes). No statistically or clinically significant difference between PT and APTT values was observed for either type of centrifugation. This means that reporting time for routine PT/APTT tests can be reduced of 16 minutes: this is a significant time reduction in performing clotting tests, and it can have a positive impact on diagnostic and therapeutic decisions in patients care, particularly in those patients hospitalized in intensive care settings such as trauma units and operating theatres. This will also reduce the waiting time of outpatients for monitoring oral anticoagulant therapy.

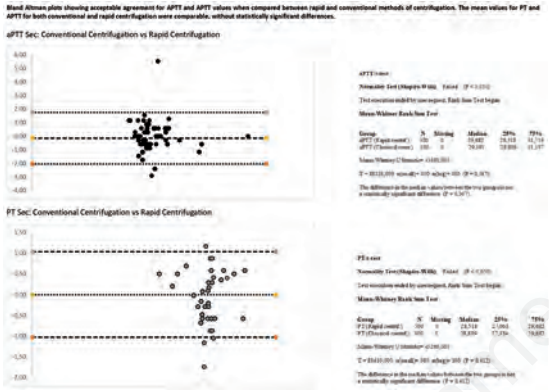


Figure 1.

PO066

DEFECTIVE BINDING OF ETS1 AND STAT4 TO THPO PROMOTER IN CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA

V. Capaci[1], E. Adam[2], I. Bar-Joseph[3], M. Faleschini[1], A. Pecci[4,5], A. Savoia[1,6]

[1]Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy; [2]Department of Pediatric Hematology, Oncology and Bone Marrow Transplant, Sheba Medical Center, Tel Hashomer, Israel; [3]The Center for Cancer Research, Sheba Medical Center, Tel Hashomer, Israel; [4]Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation, Pavia, Italy; [5]University of Pavia, Pavia, Italy; [6]Department of Medical Sciences, University of Trieste, Trieste, Italy

Congenital amegakaryocytic thrombocytopenia (CAMT) is a recessive disorder characterized by severe reduction of megakaryocytes and platelets at birth, which evolves toward bone marrow aplasia in childhood. CAMT is mostly caused by mutations in MPL, the gene encoding the receptor of thrombopoietin (THPO), a crucial cytokine regulating hematopoiesis. CAMT can be also due to mutations affecting the THPO coding region. In a child with CAMT clinical picture, we identified the homozygous c.-323C>T substitution, affecting a potential regulatory region of THPO. Though mechanisms controlling the THPO transcription are not characterized, bioinformatics and *in vitro* analysis showed that c.-323C>T prevents the binding of transcription factors ETS1 and STAT4 to the putative THPO promoter, impairing THPO expression. Accordingly, in the proband the serum THPO concentration indicates defective THPO production. Based on these findings, the patient was treated with the THPO-mimetic agent eltrombopag, inducing a significant increase in platelet count and stable remission of bleeding symptoms. Herein, we report a novel pathogenic variant responsible for CAMT and provide new insights into the mechanisms regulating the transcription of the THPO gene.

PO067

MRP-4 (MULTIDRUG RESISTANCE PROTEIN-4) PROTEIN EXPRESSION IN PATIENTS WITH ACUTE CORONARY SYNDROME UNDER CHRONIC ASPIRIN TREATMENT

E. Schiera[1], M. Curreli[1], V. Cesario[2], F. De Felice[2], G. Tocci[3], F.M. Pulcinelli[1]

[1]Dipartimento di Medicina Sperimentale Sapienza Università di Roma; [2]UOC Cardiologia Interventistica, AOSCamillo Forlanini; [3]Dipartimento di Medicina Clinica e Molecolare Sapienza Università di Roma, Italy

Background and Aims: Aspirin reduces cardiovascular events. However, some patients under chronic aspirin treatment present «High-on Aspirin Residual Platelet Reactivity» (HARPR) phenomenon. Platelet MRP-4 overexpression correlate with HARPR, suggesting that MRP-4 overxpression is an additional CV risk factor. To evaluate platelets over-expression as an additional CV risk factor, we performed a study in which we investigated amounts of platelets MRP4 between the ACS population and patients under chronic aspirin treatment without ACS population. **Methods**: We analyzed platelet protein expression in patients diagnosed with ACS on chronic aspirin therapy (100 mg/die) for at least two months (SCA) and patients on chronic aspirin therapy (100 mg/die) for at least two months, in the absence of ACS (ASA). Between 1st December 2021 and 1st march 2022 we enroled patients with ACS on chronic aspirin therapy (100 mg/die) for at least two months (N=51)) and patients on chronic aspirin therapy (100 mg/die) for at least two months, in the absence of ACS (ASA; N=30) from San Camillo Interventional Cardiology Unit and Platelet physiopatology Lab, Policlinico Umberto I respectively. Levels of platelets

MRP4 expression were evaluated with Western Blot analysis comparing the densitometric values of protein expression of each individual belonging ACS and ASA populations. Data were expressed as percentage of the densitometric mean (ImageJ, NIH) of each sample compared to the average of the densitometric values obtained from three healthy volunteers (HV). Each gel data were normalized using the same HV samples. **Results**: In platelets obtained from ACS patients we found a higher amount of MRP4 platelets expression (50% higher) compared to those obtained in ASA patients. In Figure 1 is reported a representative case that show a higher platelet MRP4 level *vs* ASA patients in a patient with acute ST segment elevation myocardial infarction secondary to a thrombus that stopped one week before double antiplatelet treatment. **Conclusions**: Reduced sensitivity to aspirin by MRP-4 could be one of the causes of higher risk for SCA events. In the context of precision medicine platelet MRP4 levels may be a useful tool to identify patients less sensible to aspirin treatment and at high CV risk factor. In addition it could be important to identify patients that required antiplatelet treatment variation adding MRP4 inhibitor to aspirin treatment, so as to create a personalized treatment in order to reduced CV complications.

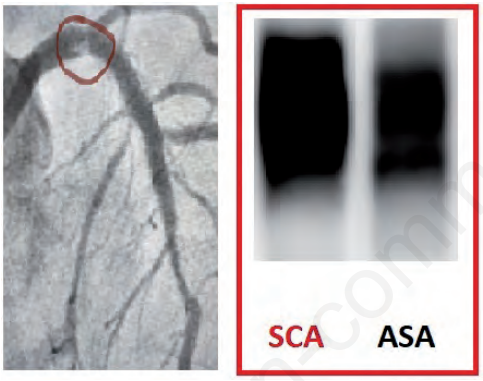


Figure 1.

PO068

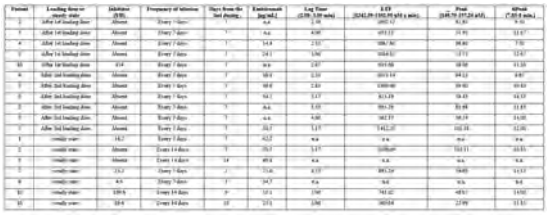
EVALUATION OF EMICIZUMAB CONCENTRATION AND THROMBIN GENERATION IN A COHORT OF SEVERE HEMOPHILIA A PATIENTS WITH AND WITHOUT INHIBITOR DURING EMICIZUMAB PROPHYLAXIS

M. Mormile, E. Baldacci, A. Ferretti, G. Maestrini, F. Viola, A. Chistolini, C. Santoro

Dipartimento di Medicina Traslazionale e di Precisione, Istituto di Ematologia, Università "La Sapienza", Roma, Italy

Background and Aims: Although emicizumab is administered at fixed dose, its measurement could be occasionally required. Thrombin generation assay (TGA) can be used to monitor the global hemostatic response in emicizumab treated patients. Aims. To measure emicizumab plasma levels and TGA in severe hemophilia A (HA) patients without and with inhibitors. **Methods**: A modified one-stage FVIII clotting assay has been used to measure emicizumab concentration. The assay is calibrated against emicizumab using a dedicated plasma emicizumab calibrator and two levels of control. Thrombin generation was determined by Calibrated Automated Thrombography (CAT) using PPP-Reagent (phospholipids [4 uM], TF [5 pM]). **Results**: 10 patients (non-inhibitor 6; inhibitor 4) received emicizumab. At start, 6 were without and 4 with inhibitor. No spontaneous bleedings occurred. Two patients experienced traumatic bleedings. One was treated with rFVIII concentrate (14th dose of life) and subsequently developed a high titer inhibitor. At the time of blood drawn he was considered an inhibitor patient. Emicizumab plasma levels increase during loading phase; median concentration at steady state is 42.2µg/mL (25.3-75). Lag time is in normal range after the first dose; ETP is variable among patients, almost always below normal range, and does not significantly differ between loading and steady state phase (Table 1). Comparing non inhibitor and inhibitor patients, at steady state median emicizumab concentration is 58.4 µg/mL (46.1-70.7) and 34.7 µg/mL (25.3-75), respectively. In non-inhibitor patients, median ETP and peak are higher than in inhibitor patients: ETP 1073.14 nMxmin (562.17-1412.23), peak 92.67 nM (38.43-107.11) *versus* ETP 688.64 nMxmin (369.64-895.24), peak 43.065 nM (22.99-56.66). **Conclusions**: We observed variability among patients as regards emicizumab concentration and TGA, but the drug was efficacious in all. It has to be explored if variables such as inhibitors, BMI, patients age, adherence to treatment, time period from the last infusion, influences emicizumab concentration and/or TGA.

Table 1.



PO069

EVALUATION OF THE ROLE OF NEW LABORATORY PARAMETERS IN THE MANAGEMENT AND THE PREVENTION OF RELAPSES IN PATIENTS WITH ACQUIRED TTP TREATED WITH CAPLACIZUMAB AND ANTI-CD20

R. Tomasino[1], M. Napolitano[3], F. Bonura[1,2], F. Schiralli[1], E. Zora[1], M. Ciaccio[1,2]

[1]Department of Laboratory Medicine, University Hospital "Paolo Giaccone", Palermo, Italy; [2]Department of Biomedicine, Neurosciences and Advanced Diagnostics, Institute of Clinical Biochemistry, Clinical Molecular Medicine and Clinical Laboratory Medicine, University of Palermo, Palermo, Italy; [3]Department of Haematology, University Hospital "Paolo Giaccone", Palermo, Italy

Background: Acquired Thrombotic Thrombocytopenic Purpura (aTTP) is an acute, severe, rare syndrome of difficult diagnosis related to antibodies anti-ADAMTS13. In about one-third of patients, aTTP relapses most often during the first two years. The aim of the current study was to evaluate, by clinical and laboratory parameters, the response to the treatment of relapsing forms of aTTP treated with Caplacizumab and subsequent administration of anti-CD20 (Rituximab). **Case Report**: A cohort of 5 patients affected by relapsed aTTP was followed over a period of 18 months. The response to treatment with Caplacizumab and anti-CD20 was assessed by laboratory parameters, including platelets count, ADAMTS13 Activity levels, and titers of anti-ADAMTS13 antibodies, measured by both chemiluminescence (CLIA) and immunoenzymatic techniques. The HemosIL AcuStar ADAMTS13 Activity Kit by IL Werfen was used for the evaluation of the ADAMTS13 Activity in CLIA and titration of neutralizing ADAMTS13 antibodies was performed by applying the modified Bethesda Nijmegen protocol; for the titration of total antibodies the ELISA technique was used. The cohort consists of one patient at the clinical onset and four patients with aTTP diagnosed in previous years, relapsing, undergoing traditional therapies (PEX, Rituximab and corticosteroids) plus Caplacizumab. All five patients received Caplacizumab during PEX and after its suspension, according to recommended indication. Three of them were also treated with anti-CD20 during hospitalization at the end of PEX, while the other two received anti-CD20 in the following months during follow-up for ADAMTS13 Activity below 20%. **Conclusions**: ADAMTS13 Activity is the cornerstone of the correct diagnostic framing of aTTP, but it does not seem to be a valid predictive indicator of relapse. For this purpose, the antibody titer, inversely proportional to the ADAMTS13 Activity, may be a more useful tool. Bethesda method by CLIA to show the inhibitory function of anti-ADAMTS13 autoantibodies is important to confirm the diagnosis of immune-mediated TTP, even in cases where the antibodies are not neutralising, since their presence does not necessarily exert an inhibitory effect on ADAMTS13 Activity. The determination of ADAMTS13 Activity, however, is recommended during follow-up to apply preventive therapeutic protocols. Data have been useful to the clinician to monitor patients and to define the effectiveness of the treatments administered, especially in cases of multiple relapses. Specifically, for the two patients who have benefited from treatment with Rituximab only in the months following the treatment with PEX+Caplacizumab for various reasons, one might venture the hypothesis that they have been subject to several relapses close in time. This could be due to the delay in the introduction of the Rituximab and the laboratory parameters may be able to give an objective explanation for the fact that the only therapy with PEX+Caplacizumab is not effective for prevent the relapse. The course of the disease in the other three patients would confirm the hypothesis that the closest administration overtime of anti-CD20 to Caplacizumab favors a complete remission and does not predispose to further relapses, at least in the short term. The preliminary data described show that the new laboratory parameters are configured as excellent candidates to improve the clinical-diagnostic scores, making them more effective in preventing relapses.

PO070

DECOMPENSATED CIRRHOSIS WITH BACTERIAL INFECTIONS SHOWS A PECULIAR DERANGEMENT OF THE HEMOSTATIC BALANCE

E. Campello[1], A. Zanetto[2], L. Spiezia[1], C. Bulato[1], S. Gavasso[1], G. Saggiorato[1], S. Shalaby[2], P. Burra[2], M. Senzolo[2], P. Simioni[1]

[1]Department of Medicine, University of Padova, Italy; [2]Department of Surgery, Oncology, and Gastroenterology, University of Padova, Italy

Background and Aims: Bacterial infections (BIs) in cirrhosis are associated with increased bleeding risk. To assess the factors responsible for bleeding tendency in BIs, we conducted a prospective study comparing all components of hemostasis (platelets, coagulation, and fibrinolysis) in hospitalized patients with decompensated cirrhosis with *vs.* without BIs. **Methods**: Primary hemostasis assessment included whole blood platelet aggregation and von Willebrand factor (VWF). Coagulation assessment included procoagulant factors (fibrinogen, factor II, V, VII, VIII, IX, X, XI, XII, XIII), natural anticoagulants (protein C, protein S, antithrombin) and thrombomodulin-modified thrombin generation test. Fibrinolysis assessment included fibrinolytic factors (plasminogen, t-PA, PAI-1, α2-AP, TAFIa/ai) and plasmin-antiplasmin complex (PAP). **Results**: Eighty patients with decompensated cirrhosis were included (40 with and 40 without BIs). Severity of cirrhosis and platelet count were comparable between groups. At baseline, patients with cirrhosis and BIs had a significantly lower whole blood platelet aggregation, consistent with impaired platelet function, without significant differences in VWF. Regarding coagulation, BIs were associated with reduced procoagulant factors VII and XII, and a marked reduction of all natural anticoagulants. Thrombomodulin-modified thrombin generation, however, was comparable between groups. Finally, although mixed potentially hypo-fibrinolytic (low plasminogen) and hyper-fibrinolytic (high t-PA) changes were present in BIs, comparable levels of PAP were detected in both groups. Upon resolution of infec-

tion (n=29/40), platelet aggregation further deteriorated whereas coagulation and fibrinolysis factors returned to levels observed in patients without BIs (Table 1). **Conclusions**: In hospitalized patients with decompensated cirrhosis, BIs are associated with reduced whole blood platelet aggregation and a marked decrease of all natural anticoagulants, which may unbalance hemostasis and potentially increase the risk of bleeding and thrombosis.

Table 1. Hemostatic alterations in patients with cirrhosis with vs. bacterial infections at baseline.

	Infection (n=40)	No infection (n=40)	P value
Platelets			
Platelet aggregation, AUC			
ADP	35 (24-50)	47 (35-64)	0.003
ASPI	33 (22-48)	38 (27-47)	0.2
TRAP	60 (36-94)	100 (72-122)	<0.001
VWF:Ag, %	303 (244-383)	276 (223-346)	0.06
VWF:RCo, %	369 (264-526)	325 (243-417)	0.07
Coagulation			
Fibrinogen, mg/dL	194 (111-339)	167 (121-242)	0.3
Factor II, %	44 (24-52)	40 (26-59)	0.7
Factor V, %	56 (40-81)	65 (51-91)	0.3
Factor VII, %	29 (17-42)	40 (25-61)	0.01
Factor VIII, %	245 (176-283)	231 (186-265)	0.4
Factor IX, %	60 (44-92)	60 (42-87)	0.8
Factor X, %	53 (44-67)	55 (43-74)	0.7
Factor XI, %	48 (30-68)	53 (39-72)	0.2
Factor XII, %	47 (38-65)	60 (48-85)	0.02
Factor XIII, %	47 (38-71)	50 (46-73)	0.1
Protein C coagulometric, %	21 (12-38)	31 (17-46)	0.03
Protein C chromogenic, %	26 (21-51)	40 (27-59)	0.05
Protein S, %	56 (42-72)	68 (56-85)	0.001
Anti-thrombin, %	32 (21-47)	38 (31-55)	0.001
ETP, nmol/L*min	903 (774-1117)	965 (789-1156)	0.8
ETP + TM, nmol/L*min	853 (709-1054)	865 (698-958)	0.7
ETP ratio	0.95 (0.91-0.99)	0.90 (0.87-0.92)	0.001
TAT, ng/mL	2.6 (2.3-3.7)	3.1 (2.2-3.8)	0.4
Fibrinolysis			
Plasminogen, %	39 (29-53)	47 (37-64)	0.004
t-PA, ng/mL	22 (19-32)	17 (11-22)	0.001
PAI-1, ng/mL	33 (20-54)	29 (19-42)	0.5
α2-AP, %	50 (43-70)	62 (47-80)	0.2
TAFIa/ai, ng/mL	26 (23-33)	24 (20-33)	0.1
PAP, ng/mL	41 (38-48)	42 (39-44)	0.8

Median values reported with 25th and 75th percentile values in parenthesis. Abbreviations: ADP: adenosine diphosphate; ASPI: arachidonic acid; TRAP: thrombin receptor agonist peptide; VWF:Ag: Von Willebrand factor antigen; VWF:RCo: ristocetin cofactor activity; ETP: endogenous thrombin potential; TM: thrombomodulin; TAT: thrombin-antithrombin complex; t-PA: tissue-type plasminogen activator; PAI-1: plasminogen activator inhibitor; α2-AP: α2-antiplasmin; TAFIa/ai: activated inactivated thrombin-activatable fibrinolytic inhibitor; PAP: plasmin-antiplasmin complex.

PO071

THE USE OF HUMAN PLASMINOGEN EYE DROPS IN SUBJECTS AFFECTED BY LIGNEOUS CONJUNCTIVITIS IN ITALY

M.T. Sartori[1], A. Bartuli[2], R. Caputo[3], F. Cortellessa[2], A. Leonardi[4], S.M. Siboni[5], P. Valente[6], L. Pino[7], A. Lotti Suffredini[8], P. Mathew[7], R. Crea[7]

[1]Clinical Medicine I, Department of Medicine, Padua University Hospital, Padua, Italy; [2]UOC Rare Diseases and Medical Genetics, University Pediatric Dpt., Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy; [3]Pediatric Ophthalmology Unit, AMeyer Children's Hospital, Florence, Italy; [4]Ophthalmology Unit, Department of Neuroscience, University of Padua, Padua, Italy; [5]Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; [6]UOC Ophthalmology, Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy; [7]Medical Affairs Group, Kedrion SpA, Lucca, Italy; [8]Clinical Research Group, Kedrion SpA, Lucca, Italy

Background and Aims: Congenital plasminogen (PLG) deficiency is an ultrarare autosomal-recessive systemic disorder caused by mutations in PLG gene that affects ~1.6 million people worldwide. It is characterized by severely impaired extravascular fibrinolysis leading to woody (ligneous) fibrin-rich pseudomembranous formation on mucous membranes, such as eyelids, respiratory tract, oropharynx, gums, middle ear, female reproductive tract, renal collecting system, central nervous system and skin. Ligneous conjunctivitis (LC) appears to be the most common clinical manifestation (80%) and presents with redness of the conjunctivae and progress to pseudomembranes' formation on the palpebral surfaces that replace the normal mucosa and if untreated, can result in corneal involvement and related damages with visual impairment or blindness. Topical treatment with corticosteroids, cyclosporine, azathioprine, and heparin have been used with limited success; systemic Fresh Frozen Plasma was found to be effective, but it is hampered by the need for repeated infusions; topical plasminogen concentrate has demonstrated efficacy in several case studies. Six subjects are currently on home treatment in Italy with human plasma-derived plasminogen eye drops (Ked-hPLG; Kedrion Biopharma, Italy) to prevent recurrence of LC. This study describes these 6 subjects' clinical course before and after use of Ked-hPLG eye drops. **Methods**: Review of medical records of the 6 subjects with confirmed diagnosis of LC at four centers, as per the 648/96 Law in force in Italy. These subjects are on home treatment with Ked-hPLG eye drops based on the phase II/III clinical trial (KB046) results. Two subjects came from the KB046 study. **Results**: All subjects are female and their age range at initiation of treatment were 4-71 years (median 7.5 years). All subjects underwent between 1-50 surgical interventions (median 5) for removal, peeling, or debridement of pseudomembranes before initiating Ked-hPLG eye drops treatment. These surgical procedures were followed by recurrences of pseudomembranes in all 6 subjects despite several topical treatments, including steroids, antibiotics, heparin, cyclosporine and FFP. The treatment with Ked-hPLG eye drops started with two drops for each affected eye 4-8 times/day as loading dose and then continued with a maintenance regimen of 2 drops per eye 4 times/day. Subjects' follow up range is 22-141 months (median 102 months). The results, as shown in Table 1, provide evidence of the effectiveness of long-term treatment with no post-operative recurrence in all subjects on treatment with Ked-hPLG eye drops. No major safety concerns, including decrease in efficacy or

hypersensitivity reactions, have been reported so far (Apr/2022) confirming the favorable safety and tolerability profile of Ked-hPLG eye drops. The 2 subjects from the KB046 study developed transient anti-aprotinin antibodies and 1 of them also developed transient anti-PLG antibodies. However, none of them required modification, interruption or discontinuation of treatment. Data on the other 4 subjects are not available. **Conclusions**: Congenital PLG deficiency is an ultrarare disease probably underdiagnosed requiring more attention to identify symptomatic subjects that might benefit from newer therapeutic options. Our study confirms long term effectiveness from the use of Ked-hPLG eye drops, and is well tolerated and effective in preventing pseudomembrane recurrence in subjects with LC.

Table 1.

Ked-hPLG eye drops regimens and response to treatment

ID	Center	Age at treatment start (years)	No. of surgeries followed by recurrences before treatment with Ked-hPLG	Follow-up (months)	Loading dose (2 drops per eye)	Maintenace dose (2 drops per eye)	Recurrence (Y/N)
1*	Padua University Hospital	7	5	102	8 times/day	4 times/day	N(1)
2	Padua University Hospital	32	50	22	4 times/day	4 times/day	N
3	Bambino Gesù Pediatric Hospital, Rome	4	1	141	8 times/day	5 times/day	N(1)
4*	A. Meyer Children's Hospital, Florence	8	2	102	8 times/day	4 times/day	N(1)
5	A. Meyer Children's Hospital, Florence	5	3	131	8 times/day	4 times/day	N(1)
6	Policlinico Hospital, Milan	71	8	82	8 times/day	4 times/day	N(1)

Abbreviations: N: no; Y: Yes
(1) Dosed with betamethasone, at the beginning of treatment with Ked-hPLG. After the membranes showed no recurrence had been reported
*Subject from KB046 study

PO072

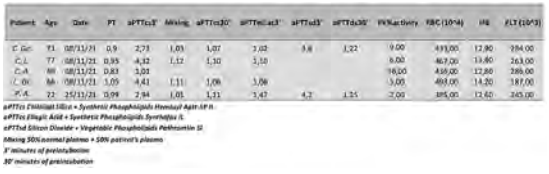
PREKALLIKREIN DEFICIENCY: DESCRIPTION OF TWO CASES

M. Gagliardi[1], L. Capone[2], A. Uva[2], M.R. Cianciulli[2], V. Marottoli[3], A. Ciampa[1]

[1]Haemostasis Centre, S.GMoscati Hospital, Avellino
[2]Clinical Pathology Laboratory, S.GMoscati Hospital, Avellino; [3]Multimedica SrL, Napoli, and Mediterranea Diagnostica, Castellammare di Stabia (NA), Italy

Background: Prekallikrein (PK) is a zymogen that is converted to kallikrein (PKa) by FXIIa. Prekallikrein deficiency is a rare autosomal recessive defect caused by KLKB1 mutations, whose main characteristic is an isolated prolongation of the aPTT, which becomes normal after prolonged preincubation, due to autoactivation of FXII. This deficiency is not associated with bleeding tendency. Even though PK and FXII reciprocally activate each other, and FXIIa initiates the intrinsic pathway of the coagulation system via the cleavage of FXI to FXIa, which then activates FIX, Kallikrein (PKa), in a dose and time dependent manner, can directly activate FIX to generate FIXa, resulting in thrombin generation and clot formation independently of FXIa. **Methods**: A 71-year-old male patient (1) was admitted to our hospital to undergo a surgical procedure. The preoperative screening showed a prolonged aPTT ratio of 2,94 with a normal reference range of 0,85 to 1,20, while the PT ratio (0,99) was normal. Some years ago, he received a radical prostatectomy and some dental avulsions, without any bleeding. Another 71-year-old male patient (2) presented to our haemostasis laboratory for a preoperative screening before undergoing a prostatectomy. The aPTT was prolonged with a ratio of 2,73 while, also in this case, the PT ratio (0,90) was normal. His past medical history was negative for minor and major bleeding events. Two brothers of this patient,(aPTT of 4,32 and 4,41)had a prekallikrein clotting activity of 6% and 5%, respectively. **Results**: By mixing an aliquot of normal plasma with plasma of each patient in equal proportions, aPTTs normalized from 2,94 and 2,73 to 1,01 and 1,03, respectively. Clotting activities of FXII, FXI, FIX, FVIII and an immunoassay of vWFAg were normal. After a preincubation of 30 m' aPTTs normalized from 2,94 and 2,73 to 1,11 and 1,07, respectively. Patient (1) showed a prekallikrein clotting activity of 2% and patient (2) of 9%. The sensitive reagents were colloidal silica and synthetic phospholipids (Hemosil APTT-SP), with an aPTT ratio of 2,94 patient (1) and of 2,73 patient (2), and silicon dioxide and vegetables phospholipids (Pathromtin SL) with an aPTT ratio of 4,2 (patient 1) and 3,8 (patient 2), which normalized to 1,25 and to 1,22, respectively, after 30 m' of preincubation, while aPTTs performed with ellagic acid and synthetic phospholipids (Synthafax) were normal (Table 1).

Table 1.



Conclusions: About all patients with prekallikrein deficiency have a personal and family history negative for bleeding events. Some arterial thrombotic events observed in some patients seem related to the increased incidence of arterial hypertension and endothelial dysfunction, due to lack of bradykinin formation, normally released from High Molecular Weight (HMW) Kininogen by kallikrein (PKa). Bradykinin causes vasodilation inducing release of powerful vasodilators such as prostacyclin, endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide (NO), which regulates endothelial homeostasis too. Bradykinin causes also release of tissue plasminogen activator, that regulates fibrinolysis. Notably, because PK and FXII reciprocally activate, and because PKa contributes to thrombin generation also through activation of FIX independently of FXII and FXI, the development of new anticoagulants,

which act as inhibitors of FXIIa may contribute to reduce PKa generation, and consequently to reduced FIXa with subsequent reduced thrombin formation.

PO073

ACQUIRED HEMOPHILIA A IN A 74-YEAR-OLD WOMAN AFTER THE THIRD DOSE OF M-RNA SARS-COV-2 VACCINATION

I. Costanzo, M. Mazzetti, M.M. Poggi, G. Emmi, E. Silvestri, C. Tamburini, D. Prisco

Medicina Interna Interdisciplinare, AOU Careggi, Italy

Background: Acquired hemophilia A (AHA) is a rare and potentially life-threatening bleeding disorder characterized by circulant autoantibodies directed against factor VIII, resulting in an isolated prolongation of activated partial thromboplastin time (aPTT). Different conditions have been associated to AHA such as neoplastic and autoimmune diseases, pregnancy, infections, and drugs. Between 2021 and 2022 13 reports of AHA following Sars-CoV-2 mRNA vaccine have been reported. We report a case of AHA arose with skin hematomas and macroscopic hematuria in a 74-year-old woman about a month after the third dose of mRNA Sars-CoV-2 vaccination. **Case Report**: A 74-year-old woman presented to our hospital for persistent hematuria onset on 10th February 2022. She had received three mRNA Pfizer SarS-CoV-2 vaccines, the last one on 12th December 2021, followed by spontaneous hematomas and macroscopic hematuria respectively arose one and two months after the vaccination. Laboratory tests showed normocytic anemia with Hb 10.5 g/L and isolated aPTT prolongation (53.6 s) with normal PT (11,5 s) and normal platelets count (215k). The coagulation factor assay showed an isolated factor VIII deficiency with activity of 6.3% and inhibitor titer of 2.5 BU, which was not corrected by the dilution test. AHA with low anti-factor VIII antibody titer was diagnosed. Initially, she was treated with factor aVII Eptacog alfa and prednisone 75 mg/die. After three days, due to persistent hematuria, she was treated with the new recombinant VIII porcine factor Susoctacog alfa. Bleeding interruption was obtained with the restoration of hemoglobin levels. We excluded an anti-phospholipid antibodies' syndrome, other autoimmune diseases and a parainfectious origin. A paraneoplastic form was ruled out: neoplastic markers assay and occult stool's blood research, abdominal ultrasound, total body CT and PET, mammography and breast ultrasound exams were negative. The abdominal CT showed clots in the right renal pelvis; a cysto-uretero-endoscopy was performed with negative result for neoplastic lesions. The immunophenotype showed a slight increase in CD8+ and CD4+ activated lymphocytes, in absence of significant quantitative changes in the lymphocyte and leukocyte subpopulations. We measured the anti S IgG too, positive with high titer (12,800), as expected in a recent SarS-CoV-2 vaccination, with negative IgM and RNA. Factor VIII levels increased up to 38.3% after 14 days of combined therapy with Susoctacog alfa and steroids. After 4 weeks, rfVIII administration was interrupted and the patient was discharged after 32 days of corticosteroid therapy with no other spontaneous bleeding events, a factor VIII dosage of 20% and an inhibitor's titer reduction. **Conclusions**: AHA has been associated with several clinical conditions. We found 13 Sars-CoV-2 mRNA vaccination cases associated with the onset of AHA, occurring after a minimum of 7 to a maximum of 52 days after the vaccination. In our case, the first spontaneous bleeding occurred 30 days after the third dose with negative investigations for infectious, autoimmune, or neoplastic diseases. Even though the association still remains purely temporal and pathogenetic mechanisms have only been hypothesized, the temporal association, the exclusion of other possible trigger causes and the similar cases reported in the recent literature suggest a possible connection between this case and Sars-CoV-2 mRNA-based vaccine.

PO074

ACQUIRED FACTOR XI INHIBITOR IN A CHILD WITH MEMBRANOUS GLOMERULONEPHRITIS

V. Nosratian, L. Banov, A. Naim, J. Svahn, A.C. Molinari

Centro Emostasi e Trombosi, IRCCS G.Gaslini Genova, Italy

Background: Acquired deficiency of coagulation factors is rare, and is mainly associated with autoimmune disorders or malignancies. We describe a case of acquired factor XI deficiency in a child affected by autoimmune disorder. **Case Report**: A 21 months old male with autoimmune thyroiditis showed prolonged aPTT (3,92 ratio). Not incubated mixing tests showed partial aPTT correction. During subsequent follow up he always showed persistent prolongation of aPTT no more corrected by mixing test; second level tests at local institution revealed severe deficiency of factor XI (<1%) and positive Lupus Anticoagulant test (dRVVT). No Factor XI gene mutation was found. His parents had normal Factor XI activity. Family history was unremarkable and personal history did not reveal any bleed. At the age of 53-months due to generalized edema, increased body weight and nephrotic proteinuria he received oral prednisone 60 mg/m^2/die. Proteinuria decreased, but its persistence the child received intravenous (IV) metil-prednisolone boluses 10 mg/kg/die for three days and prednisone was tapered. He was referred to our Institution to undergo kidney biopsy (KB). Two weeks from the steroid IV boluses a milder prolongation of aPTT was observed (1,33 ratio) and the factor XI activity resulted increased (33.24%). Acquired FXI deficiency secondary to autoantibodies against FXI was supposed. Given the hemorrhagic risk associated with the KB we anyway decided to give oral tranexamic acid and low dose rFVIIa before and three hours after KB that was safely performed. Membranous

glomerulonephritis was diagnosed. Serial aPTT, FXI assays and FXI inhibitor were performed. While severe Factor XI deficiency was persistently documented low titer FXI inhibitor (1.4 Nijmegen Bethesda Units) was detected only once. Thromboelastometric global coagulation test (Rotem) showed normal clot activation time and strength. Thrombin generation assay will be performed to better document the bleeding risk. Proteinuria recurred two months after IV steroid boluses and combined therapy was started with tacrolimus and, till now, two doses of rituximab. Coagulation parameters remained unchanged. The patient was therefore sent to outpatient follow up initially every 2 weeks and then every 3 months (Table 1). **Conclusions**: The bleeding phenotype in FXI deficiency does not correlate with the FXI activity level, with evidence of bleeding reported in heterozygotes with mild deficiency (FXI levels 20%–60%). Most of patients with severe deficiency do not bleed spontaneously but can bleed seriously during or after surgery. This lack of correlation between bleeding risk and FXI activity poses a significant therapeutic challenge. Acquired coagulation inhibitors are autoantibodies that bind to coagulation factors and neutralize their activity or accelerate their clearance. They include inhibitors against coagulation factors I, II, V, VII, VIII (acquired hemophilia A), IX (acquired hemophilia B), X, XI, and XIII. Acquired deficiency of FXI having been reported with autoimmune disorders (systemic lupus erythematosus, rheumatoid arthritis, Crohn disease), or malignancies (chronic myelomonocytic or lymphocytic leukemias, and gastrointestinal adenocarcinoma) but only one case of FXI inhibitor is reported in a child affected by membranoproliferative glomerulonephritis. Inhibitors should be suspected when coagulation factor(s) deficiency is found in autoimmune diseases.

Tabella 1.

Coagulation tests over time

Age (months)	aPTT (ratio)	aPTT mix (ratio)	FXI:C (%)	Inhibitors (NB IU)	LAC
21	3,68 (0,82 - 1,2)	1,09 (0,82 - 1,2)	4		POSITIVE
25	3,92 (0,82 - 1,2)	1,73 (0,82 - 1,2)	<1		
38	3,80 (0,82 - 1,2)		1		
44	3,35 (0,82 - 1,2)	2,68 (0,82 - 1,2)	<1		
49	3,65 (0,82 - 1,2)	2,90 (0,82 - 1,2)	<1		
53	3,75 (0,82 - 1,2)	2,02 (0,82 - 1,2)	0		
54	1,33(0,82 - 1,2)	2,02 (0,85 - 1,23)	33,24		Negative
55	3,08(0,82 - 1,2)	1,39 (0,85 - 1,23)	6,21	1,4	Negative
55	3,15(0,82 - 1,2)	1,16 (0,85 - 1,23)	6	negative	Negative
56	4,26(0,82 - 1,2)	1,18 (0,85 - 1,23)	5,51		Negative
58	2,27(0,82 - 1,2)	1,22 (0,85 - 1,23)	8,35	negative	Negative

PO075

DO INFECTIONS POSE A SIGNIFICANT RISK OF RELAPSE OF IDIOPATHIC THROMBOCYTOPENIC PURPURA? A CASE SERIES

C. Rotolo[1], M. Mattana[1], C. Cammarata[1], R. Tomasello[1], G. Giordano[2], A. Lucchesi[3], S. Siragusa[1], M. Napolitano[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo, Palermo, Italy; [2]Division of Internal Medicine, Hematology Service, Regional Hospital "ACardarelli", Campobasso, Italy; [3]IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST)"Dino Amadori", Meldola (FC), Italy

Background: Idiopathic thrombotic thrombocytopenic purpura (iTTP) is an acquired thrombotic microangiopathy caused by inhibition of the ADAMTS13 protein (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) by an inhibitory antibody. iTTP is a rare disease and recurrences are even rarer. The etiopathogenesis of iTTP relapses is still not completely clear and available evidences are scarce. Infections, often viral, seem frequently triggering factors of iTTP relapses. Both laboratory (ADAMTS13<10%) and clinical recurrences of iTTP have been associated with paucisymptomatic or complicated episodes of CMV and HSV mononucleosis, tuberculosis, urinary tract infections and SARSCoV2 infection. **Case Report**: We here report a series of 4 patients with a history of iTTP, followed up at our Institute, with a history of clinically significant infections during clinical remission of iTTP. Case 1: 33-yo man diagnosed with iTTP in June 2016, treated with steroid and PEX with the achievement of clinical and laboratory remission. On April 201,7 the patient had laryngitis successfully treated with antibiotic therapy, ADAMTS13 activity in July 2017 was 102%. In February 2018 the patient had pharyngitis successfully treated with antibiotic therapy, ADAMTS13 dosage on March 2018 was 100%. Case 2: 55-yo man diagnosed with iTTP in May 2020, treated with Caplacizumab and PEX with the achievement of partial clinical remission. In October 2020, the patient had a subcutaneous abscess resulting from an infection of hernia mesh, for which he was treated with antibiotic therapy and vacuum-assisted closure therapy (VAC) with success. During hospitalization, the patient had a clinical (Hb 9.2g/dL and PLT 34000/mmc) and laboratory (ADAMTS13 1.3%) relapse of iTTP disease, treated with Caplacizumab + PEX and Rituximab, with the achievement of hematological remission. Case 3: 43-yo woman diagnosed with iTTP in March 1998, treated with steroid, immunosuppressants and FFP with complete hematological remission. At follow-up in November 2021 and January 2022 ADAMTS13 was 5.1% and 7.7% respectively, with normal haematochemical parameters. In February 2022 the patient had pneumonia successfully treated with antibiotic therapy. During the hospital stay, the range of ADAMTS 13 values already detected before the infection remained within the last control visit (ADAMTS13 was found to be 7.7%, in March 2022 it was 5.7%, and in April 2022 it was 6.8%), without any other detectable abnormality. Case 4: 52-yo woman diagnosed with iTTP in September 2019, treated with steroids, Rituximab, FFP and PEX with the achievement of hematological remission. In September 2021 the patient had paucisymptomatic SARSCoV2 infection, resolved without specific therapy, in the course of which the blood count and ADAMTS13 levels remained normal.

Conclusions: Limited to the extreme rarity of the disease, the real-life data collected in our case series do not show a marked tendency for iTTP patients to relapse within the course of localized or systemic infections. This is in contrast to the available literature, according to which activation of the immune system could trigger the production of autoantibodies. The pathogenetic mechanisms of ADAMTS13 inhibitor and iTTP relapse need further investigation.

Table 1.

	CASE 1	CASE 2	CASE 3	CASE 4
TREATMENT OF iTTP AT DIAGNOSIS	Steroid + PEX	Caplacizumab + PEX	Steroid + immunosuppressant + FFP	Rituximab + PEX + FFP
ADAMTS13 LEVELS DURING FOLLOW UP	> 50%	< 50%	< 50%	> 50%
INFECTIONS FROM FIRST REMISSION OF DISEASE	2	1	1	1
RELAPSE (CLINICAL OR LABORATORY)	0	1	0	0

PO076

EMICIZUMAB IN ACQUIRED HAEMOPHILIA A

F. Demartis, L. Pieri, R. Fjerza, S. Linari, G. Castaman

Sodc Malattie Emorragiche, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy

Background: Acquired haemophilia A (AHA) is a rare bleeding disorder caused by autoantibodies to FVIII resulting in low FVIII. Bleeding is common, ranging from 70 to 90% of cases at diagnosis. Activated prothrombin complex concentrates(APCC) and recombinant activated FVII (rFVIIa) are usually required to control bleeding, while plasma-derived or recombinant FVIII concentrates, are effective only with low inhibitor titers (<5 Bethesda Units). In recent years the use of recombinant porcine FVIII concentrates is an option for patients without cross-reactivity. Immunosuppressive therapy with corticosteroids (1 mg/Kg), cyclophosphamide (2 mg/Kg) and /or rituximab (375 mg/m^2), is used for inhibitor eradication, however the time required to eradicate could be significantly long in patients with very high titer at presentation, thus putting the patient at risk of bleeding becuse of lack of effective prophylaxis meanwhile. Emicizumab is a bispecific antibody recognizing activated FIX and Factor X, with FVIIIa-cofactor activity which allows effective prophylaxis in patients with congeniotal hemophilia A with and without inhibitors. A few cases of AHA treated with this agent have been recently reported. **Case Report**: A 66 year old male with a history of treatment-refractory AHA (four rituximab weekly infusions [375 mg/m^2]; cortison [1 mg/kg/day]) and multiple bleedings, treated with rhFVIIa (90ug/Kg/4-6-h) came to our attention. The patient was diagnosed two months earlier with high inhibitor titre 2480 B.U.; aPTT 96 m“ and no detectable FVIII. The cross-reactivity for recombinant porcine FVIII (233 B.U.) excluded the susoctocog alfa option. The patient had several recurrent hematomas on the shoulders and back, requiring packed red cells and treatment with rFVIIa 90 ug/kg/ 6-8 hours. After 6 hours of wash out, Emicizumab 3mg/kg subcutaneously weekly for 3 doses and then 1,5 mg/kg/weekly was started. The day after the first Emicizumab dose the aPTT was reduced by 60% (from aPTT 91 m“ to 26 m“). The patient did not require additional rFVIIa infusions, the hematomas reduced and on Day 6 since the Emicizumab had been started, the patient was discharged (Table 1). The patient is actually coming in for weekly follow up. **Conclusions**: Acquired haemophilia A can be very challenging, even for experienced clinicians. Common bypassing agents as activated prothrombin complex concentrates (APCC) and rFVIIa or recombinant porcine FVIII concentrates in AHA bleeding patients require very close monitoring and hospitalization. We describe a treatment for a refractory patient that had been hospitalized for a long time and that had been discharged after just two weekly Emicizumab 3 mg/kg s.c. injections, with reduced hematomas, aPTT in range and no bleeding.

Table 1.

21/4/2022	30/4/2022	2/5/2022	3/5/2022	5/5/2022
aPTT Ratio 2,94	aPTT Ratio 0,86	aPTT Ratio 0,75	aPTT Ratio 0,73	aPTT Ratio 0,74
EMICIZUMAB	-	-	16,5 ug/ml	-

PO077

UNCONVENTIONAL USE OF RECOMBINANT PORCINE FVIII IN ACQUIRED HAEMOPHILIA: THREE CASE REPORTS

C. Sella[1], M. Bardetta[1], C. Dainese[1], F. Valeri[1], A. Valpreda[1,4], D. Grimaldi[3], P. Martinelli[1], D. Stella[2], B. Bruno[2], A. Borchiellini[1]

[1]Regional Centre for Haemorrhagic and Thrombotic Diseases, Haematology Unit, City of Health and Science University Hospital of Molinette, Turin, Italy; [2]Haematology Unit, City of Health and Science University Hospital of Molinette, Turin, Italy; [3]Haematology Unit, Santa Croce e Carle Hospital, Cuneo, Italy; [4]Central Laboratory Baldi and Riberi, City of Health and Science University Hospital of Molinette, Turin, Italy

Background: Acquired haemophilia A (AHA) is due to autoantibodies which interfere with the activity of clotting factor VIII (FVIII). AHA is frequently caused by underlying conditions like systemic rheumatic disease, malignancy, drugs and postpartum. Treatment of AHA consists of tempestive control of bleeding and inhibitor eradication with immunosuppressive therapy (IST). Hemostatic treatments include recombinant activated factor VII (rFVIIa), activated prothrombin complex concentrate (aPCC), both known as bypassing agents, and susoctocog-alfa, a recombinant porcine FVIII (rpFVIII).

The last one has been recently licensed for management of acute bleeding in AHA. Unlike treatment with bypassing agents, rpFVIII can be monitored to provide a successful haemostatic effect. This advantage is important in patient with high thrombotic risk or those who need to undergo surgery. Nevertheless, as shown by the literature, only two case reports illustrate the use of rpFVIII in perioperative setting (ocular and abdominal surgery). Instead, in patients with high haemorrhagic risk, after resolution of acute bleeding, is reasonable the usage of rpFVIII in prophylaxis schedule; only one case has been described using prophylaxis dosage in outpatients. **Case Report**: We report three cases of AHA in which was used susoctocog-alfa in non-conventional schedule (Table 1).

Table 1.

	Case 1	Case 2	Case 3
Sex	Female	Male	Female
Age	70	79	31
Comorbidities	Myasthenia gravis, Hashimoto Thyroiditis and Glaucoma	Stomach Carcinoma, Urothelial neoplasia, Polymyalgia rheumatica	Episode of major depression after son death
Bleeding type/site	Right upper limb hematoma	Ileo-psoas hematoma, upper and lower limbs hematomas, hematuria	1: Right lower limb hematoma; 2: ankle and ileo psoas hematomas
Surgery during AHA	Left hemicolectomy with terminal stomia	Tissue biopsy and laser ablation in the nearby of neoplastic lesion	None
Hb at diagnosis (g/dL)	9.1	7.4	1: 6; 2: 9.2
aPTT ratio	3,07	2,57	1: 3.26; 2: 1.8
FVIII at diagnosis	0.9%	1,7%	1: 1%; 2: 19%
FVIII inhibitors titer	76 UI/mL	23 UB/mL	1: 64 UB/mL; 2: 11,2 UB/mL
Lines of IST	2	4	1
First hemostatic therapy	rFVIIa 90 mcg/Kg	rFVIIa 90 mcg/Kg	rFVIIa 90 mcg/Kg
Days on rFVIIa treatment	7 days	5 days	1: 2 days 2: 3 days
Second hemostatic therapy	rpFVIII	rpFVIII	rpFVIII
rpFVIII loading dose	200 U/Kg (11.500 U)	200 U/Kg (10.000 U)	200 U/Kg (10.000 U)
rpFVIII inhibitors pre-therapy	44 UB/mL	12 UB/mL	11 UB/mL
Peak FVIII	255%	177%	142%
rpFVIII prophylaxis dose	200 U/Kg (11.500 U)	100-200 U/Kg (5.000-10.000 U)	100 U/Kg (5.000 U)
Prophylaxis schedule	Prolongation of administration interval (every 12-36-48 h) until ending	Succession of full dose and half dose and then half dose until ending	Half dose starting from 16h after first dose, then half dose every other day
Days on rpFVIII treatment	11 days	44 days	22 days
Relapse	No	No	No
Adverse events	None	None	None

AHA: Acquired hemophilia A; Hb: hemoglobin; aPTT: activated partial thromboplastin time; FVIII: factor VIII of coagulation; IST: immunosuppressive therapy; rFVIIa: recombinant factor VII activated; rpFVIII: recombinant porcine factor VIII; UK: unknown.

Case 1: A 70-year-old woman with myasthenia gravis, Hashimoto thyroiditis and glaucoma was hospitalized for right upper limb hematoma in AHA. Initially treated with steroid than cyclophosphamide. During hospitalization she underwent surgical intervention for diverticular bowel perforation performed under rFVIIa coverage. In post-operative, for elevated rFVIIa dependency, was administered rpFVIII without haemorrhagic complications. **Case 2**: A 79-year-old man with history of stomach carcinoma, suspected urothelial neoplasia and polymyalgia rheumatica arrived at our attention for haematuria and diffuse hematomas. He was diagnosed with AHA and treated with rFVIIa, subsequently changed with rpFVIII for inadequate response. He underwent ureteroscopy with biopsy and laser ablation in the nearby of neoplastic lesion under rpFVIII coverage without complication. In the meanwhile, he received four lines of IST for persistent higher titre of inhibitors. **Case 3**: A 31-year-old woman, 60 days later partum, was admitted to emergency room for anemia and right lower limb hematoma. She started therapy with rFVIIa and steroids and she obtained immediate response. Therefore, she was dismissed eight days later. She was hospitalized again 5 days later for new bleeding, and she was treated with rFVIIa, than replaced with rpFVIII. The last one was continued in ambulatory setting at reduced dosage. She had no relapse of AHA. **Conclusions**: We report a case of successful peri-procedural haemostatic coverage with rpFVIII in prevention of bleeding and another one after abdominal surgery (in immediate post-operative), both despite the presence of porcine FVIII inhibitors. Indeed the presence of rpFVIII inhibitors titre and the effect of rpFVIII do not have correlation, as seen in previous studies. Furthermore we describe use of rpFVIII in ambulatory setting with reduced dosage (100 U/kg) every other day to prevent relapse. This schedule represents a favourable cost-efficacy alternative in management for patients with high risk of re-bleeding (risk depends on site and extension of previous bleeding or concomitant conditions).

PO078

THALIDOMIDE AS EFFECTIVE DRUG FOR THE CONTROL OF GATROINTESTINAL BLEEDINGS IN CONGENITAL AND ACQUIRED BLEEDING DISORDERS

A. Ferretti[1], M. Basso[1], C. Santoro[3], D. Feliciani[4], M.E. Riccioni[4], S. Sorrentino[1], E. De Candia[1,2]

[1]Malattie Emorragiche e Trombotiche, Fondazione Policlinico Universitario Agostino Gemelli-IRCSS, Roma, Italy; [2]Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Roma, Italy; [3]Ematologia, Azienda Ospedaliera Universitaria Policlinico Umberto I, Roma, Italy; [4]Gastroenterologia, Fondazione Policlinico Universitario Agostino Gemelli-IRCSS, Roma, Italy

Background and Aims: Gastrointestinal bleeding (GIB), both upper and lower, can be a well-recognised complications of congenital and acquired forms of von Willebrand disease (vWD) and Glanzmann Thrombasthenia (GT), generally arising in older patients and being a challenge for clinicians. The aim of this study is to describe the experience of GIB management in patients with congenital and acquired coagulation defects and platelet alterations. **Methods**: Between 2016-2020, 5 patients affected by congenital and acquired form of vWD and GT and severe GIB [3F/2M; median age 66 years (66-77)] have been diagnosed and treated successfully with thalidomide 50 or 100 mg/die. Diagnosis was made with clinical/laboratory features. Data were collected from clinical charts. **Results**: Gastrointestinal bleeding is, potentially, a fatal condition due to loss of large volumes of blood. Angiodysplasia is thought to be one of the most common gastrointestinal vascular malformations associated to GIB. Generally, the therapy is represented: 1) in congenital vWD by prophylaxis with VWF/factor VIII concentrates, 2) in congenital GT by platelet tranfusions or rFVIIa infusions in patients with platelet alloantibodies, 3) in acquired form variability of response to standard therapy is reported. In some cases, these therapies were not always successful. Possible rescue therapies such as octreotide, thalidomide, lenalidomide, and tamoxifen were described in a few case reports. It is well known the thalidomide effect on angiogenesis counteraction by its effects on vascular endothelial growth factor

(VEGF) and basic fibroblastic growth factor (bFGF) and, in few cases has been reported a positive effect in treatment of patients affected by vWDs and GT with GIB. Notably, we described 5 cases with vWD type 2 (1), GT (1) and acquired vWD associated to MGUS (3) with recurrent severe GIB successfully treated, with different posology of thalidomide. In particular, two patients with congenital disease exhibited GIB at older age, caused by angiodysplasia, in absence of severe bleeding history. After few months by starting thalidomide 50 mg/die, acute and severe GIB were stopped. Three patients with acquired VWD and concomitant IgG K MGUS had recurrent hospitalizations for acute and severe melena caused by angiodysplasia. Two of them were primarily treated with intravenously immunoglobulins with good response even for a small period of time. After that, thalidomide administration was started resulting in increased hemoglobin levels. Thalidomide treatment is still ongoing in all the patients with an improvement in the quality of life, obtaining a striking and stable reduction of GIB and of the requirement of platelet and blood transfusions. **Conclusions**: Although the exact mechanism by which thalidomide exerts antiangiogenic effects is not fully understood, we can describe its successfully use in this particular contest of patients with severe GIB. This preliminary evaluation needs to be carried out on a larger population to better understand both good and side effects of thalidomide in patients with vWD and GT with GIB considering the possibility to standardize this treatment.

PO079

INCREASED INCIDENCE OF ACQUIRED HAEMOPHILIA A DURING THE SARSCOV2 VACCINE CAMPAIGN: PATHOPHYSIOLOGICAL RELATIONSHIP OR INCREASED DIAGNOSIS VIGILANCE?

J. Agnelli Giacchello, P. Sciancalepore, L. Contino, R. Santi

SSD Emostasi e Trombosi, Ospedale S.SAntonio e Biagio e CArrigo, Alessandria, Italy

Background and Aims: Acquired Hemophilia A (AHA) is a rare autoimmune disorder caused by autoantibodies affecting clotting factor VIII activity leading to a bleeding disorder. Population-based unselected cohorts have calculated an incidence of acquired factor VIII inhibitor of 1.3 to 1.5 cases per million population per year. An increased incidence of AHA from October 2021 to March 2022 was observed in Alessandria area: 5 new diagnoses in a population of about 400.000 residents (approximately 20 times more than expected). Previously, several reports of possible correlation between vaccines and immune disorders onset have been reported. In recent few months at least 10 cases of AHA post SarCcov2 vaccine were reported. Considering the wide vaccine campaign, a possible correlation between SarsCov2 vaccines and AHA was supposed. **Methods**: Data about vaccines (type, dose and timing) and AHA diagnosis and management were retrospectively collected. Patients' features were summarized in Table 1. **Results**: Our small study cohort consists of 5 females with median age at diagnosis of 77 years (range 34-80). Median FVIII activity and median inhibitor titer at diagnosis was 0.019 IU/ml (range 0.01-0.08) and 30 Bethesda Units/ml (range 2.6-100), respectively. In 2 patients, relevant autoimmune comorbidities were found (Polymyalgia rheumatica and Scleroderma currently off-therapy). SarsCov2 vaccine was performed in all patients before AHA diagnosis (2/5 after Vaxzevira® II dose, 2/5 after Cominarty® II dose and 1/5 after booster). No patients experienced SarsCov2 infection before AHA diagnosis; SarsCov2 nasal swab turned positive after 2 weeks of hospitalization in one patient. Median time from vaccine to AHA diagnosis was 121 days (range 74-222); 2/6 and 1/6 patients had cutaneous and muscular-cutaneous bleeds at diagnosis respectively. Median time from vaccine to diagnosis and from vaccine to clinical onset was 97 days and 56 days, respectively. Two Patients had no bleeds and AHA has been diagnosed after routine blood exams. In these cases, AHA onset remains unknown and time from vaccine to diagnosis was longer (134 and 121 days). All patients failed first line immunosuppressor therapy (steroid, prednisone 1mg/kg/day) so a second line immunosuppressor therapy (Rituximab® 375mg/sq weekly, 4 weeks) was required with 5 complete responses; in particular, 3/5 patients didn't achieve remission during steroids and 2 patients relapsed after steroid discontinuation. Recombinant porcine FVIII concentrate (Obizur®) was used in 3/5 patients with very good bleed control. Median follow-up is 5.8 months (range 3.8-6.4). **Conclusions**: Correlation between SarsCov2 vaccine and AHA onset can not be demonstrated by our small sample size. However, without other triggers it is reasonable to assume a possible role of sarscov2 immunization in AHA pathophysiological process. Furthermore, a delayed diagnosis of AHA it is common due to low incidence, inadequate clinician awareness and to large variety of the bleeding symptoms (asymptomatic case *vs* life-threating bleeds). This trend could explain the wide range in time from vaccine to diagnosis in our population. Efficacy and safety of Obizur® and Rituximab® was confirmed.

Table 1.

	Case 1	Case 2	Case 3	Case 4	Case 5
Age at diagnosis	34 y	77 y	75 y	80 y	80 y
Sex	F	F	F	F	F
FVIII activity Inhibitor titer	0.08 IU/ml 13 BU/ml	0.019 IU/ml 30 BU/ml	0.019 IU/ml 2.6 BU/ml	0.01 IU/ml 40 BU/ml	0.02 IU/ml 100BU/ml
SarsCov2 vaccine	Cominarty®	Vaxzevria®	Vaxzevria®	Cominarty®	Cominarty®
Dose	II	II	II	Booster	II
Comorbidities	-	Polymyalgia rheumatica	-	Scleroderma	-
Bleeds	Cutaneous e muscolar hematoma	-	-	Cutaneous	Cutaneous
Time from vaccine to diagnosis	97 days	121 days	134 days	74 days	222 days
Time from vaccine to clinical onset	56 days	-	-	32 days	179 days

P0080

ACQUIRED TYPE 2 WILLEBRAND'S DISEASE IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

D. Dragonetti[1], M. Becce[1], S.P. Pascale[2], S. Coluzzi[2], R. Nuccorini[2], M. Giuseffi[1], A.M. Calabrese[1], A. Picerno[1], A. Matturro[2], A. Amendola[2], I. Chitarrelli[2], R. Iula[2], K. Ferrara[2], D. Caputo[2], M. Cimminiello[2], N. Filardi[2], D. Vertone[2], M. Pizzuti[2]

[1]Department of Clinical Laboratory; [2]Department of Hematology; Regional Hospital "San Carlo", Potenza, Italy

Background and Aims: Patients with Essential Thrombocythemia (TE) can have both thrombotic and hemorrhagic events. In a study of 809 patients, 65% experienced thrombosis, 15% both thrombosis and bleeding, and 1.4% only bleeding, that is often gastrointestinal and muco-cutaneous, as occurs in middle-severe Willebrand's disease, and is promoted by surgery. It is more common with marked thrombocytosis, but can also occur with platelets slightly above 400,000. One of the causes of bleeding is a reduction in vWF-RCo due to a decrease in high molecular weight multimers, with normal antigenic concentration and reduced vWF-RCo/vWF-Ag ratio. In these cases Desmopressin (DDAVP) can induce a dose-dependent increase in high molecular weight multimers of vWF with improvement in vWF: Ag/vWF:RCo. After few hours, there is clearance of multimers, whose rapidity is correlated with platelet count. so it may be necessary to readminister DDAVP.We report the case of two patients, with TE, both with Calreticulin type ins 5 mutation, not on cytoreductive therapy. The first patient needed laparoscopic multiple myomectomy, the second had bleeding after dental extraction. In both of them on screening examinations there was a reduction of vWF:RCo, with normal value of vWF:Ag, so we performed a test with DDAVP 0.3 μg/kg with improvement of vWF:RCo. **Methods**: For the quantitative determination of vWF: Ag and of vWF-RCo, we used two automated latex enhanced immunoassay in human citrated plasma on IL Coagulation Systems. **Results**: The results obtained from the laboratory tests (Table 1), for the two patients showed a remarkable increase in vWF-Ag concentrations, and in its activity, with an increase in the ratio vWF-RCo / vWF: Ag and improvement in PFA. The first patient underwent surgery after administration of DDAVP every 12 hours for 3 days, without hemorrhagic complications. The second patient, is waiting for a new dental surgery. **Conclusions**: We confirm the evidence in published literature on the possibility of hemostatic alterations in patients with TE due to the reduction of vWF-RCo (and that this phenomenon is possible even when platelet values are less than one million) and underline that the administration of DDAVP can re-establish a proper, although transient, balance between vWF-RCo/vWF-Ag and optimize hemostatic mechanisms. This will allow the patient to safely undergo surgery, avoiding or reducing hemorrhagic manifestations.
We consider useful to evaluate the vWF-RCo/vWF-Ag ratio in all patients with TE, to limit the risks of antithrombotic prophylaxis with aspirin as much as possible. Finally, literature reports a higher incidence of Willebrand type 2 in patients with the JAK2 mutation, but both our patients have the Calreticulin type 2 mutation, so we will further investigate all our TE patients to see if there may be a correlation with this type of mutation as well.

Table 1.

Test	Patient 1			Patient 2		
	Time 0	90 min	180 min	Time 0	90 min	180 min
vWF-Ag (%)	80.9	195.1	193	80	179	135.5
vWF-RCo (%)	26	135	132	25	92	68
vWF-RCo/vWF-Ag (ratio)	0.32	0.69	0.68	0.31	0.5	0.5
PFA (Epinephrine) (sec)	198	82	111	>200	94	128
PFA (ADP) (sec)	>201	61	68	>200	>200	>200
Factor VIII (%)	84	426	293	72	210	140
Platelet count at diagnosis (*10³/µL)	1.971					

P0081

A CASE OF CONGENITAL GLANZMANN THROMBASTHENIA WITH A NEW MUTATION IN ITGA2B GENE AND EFFECTIVE CONTROL OF GASTROINTESTINAL BLEEDING BY THALIDOMIDE

S. Sorrentino[1], D. Feliciani[2], A. Ferretti[1], A. Minucci[3], E. De Paolis[3], P. Concolino[3], E. De Candia[1,4]

[1]Unità Operativa Malattie Emorragiche e Trombotiche, Area di Ematologia, Fondazione Policlinico Universitario "Agostino Gemelli", IRCCS, Rome, Italy; [2]UOC di Medicina Interna e Gastroenterologia, Dipartimento di Scienze Mediche e Chirurgiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [3]Molecular and Genomic Diagnostics Unit, Fondazione Policlinico Universitario AGemelli IRCCS, Rome, Italy; [4]Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica Del Sacro Cuore, Rome, Italy

Background: Glanzmann Thrombasthenia (GT) is an autosomal recessive disorder of platelet function caused by quantitative or qualitative defects in integrins αIIb and β3. The most common symptoms of GT are spontaneous mucocutanous bleedings, unremitting bleeding after injury or surgery, menorrhagia in women. Spontaneous upper gastrointestinal bleeding (GIB), causing recurrent episodes of severe anemia and requiring hospitalization for invasive procedures for bleeding control and red blood cells transfusions, may also occurr. Angiodysplasias sustaining GIB have been described in GT patients. There is no specific therapy to control GIBs, except for local treatments, and systemic hemostatic therapy such as platelet transfusions, antifibrinolytitcs, rFVIIa. We describe a case of GT with a new mutation in the ITGA2B gene who showed a good GIB control under therapy with the antiangionic drug thalidomide. **Case Report**: A 62-year-old woman was hospitalized in our hospital for

rectal bleeding and melena sustained by multiple gastrointestinal angiodysplasia (GIA) and active bleeding at caecum. She reported history of spontaneous mucocutaneous hemorrhagic manifestations, nosebleeds, gingivitis, frequent cutaneous bleeding, anemia since childhood. The patient reported that an undefined platelet dysfunction was diagnosed and platelet transfusions were required during some bleeding episodes in other hospitals. She also reported history of systemic lupus erythematosus and fluctuating thrombocytopenia responsive to corticosteroids in the last years. Platelet function studies during hospitalization in our institution showed severe reduction of platelet aggregation to all stimuli except to ristocetin together with strong reduction of glicoproteins αIIb and IIIb expression (Figure 1) and TG was diagnosed. The genetic test demonstrated a new undescribed homozygotic mutation in the ITGA2B gene [c.1096C>T and p.(Arg366*)]. Actively bleeding angiodysplasia was demonstrated and treated by argon plasma coagulation on two different episodes at a distance of one month. Due to progressive increase of GIB episodes requiring hospitalization in the last two years, the patient was treated off-label with thalidomide 50 mg/day. The patient was stable for 6 months, after which a new hospitalization for GIA of cecal fundus was necessary. The thalidomide posology was increased to 100 mg/day, but it was reduced to 50 mg/day after two months because of side effects such as drowsiness, mental confusion and constipation. After 12 months of treatment, she is in good general clinical conditions, hemoglobin is stable and no new GIBs occurred. **Conclusions**: GIB is a recurrent symptom in some GT patients, requiring frequent hospitalizations and urgent medical therapy, thus severely affecting their quality of life. Thalidomide is an antiangiogenic drug that has been used for the control of GIB in patients with congenital and acquired vonWillebrand diseases and in patients with hereditary hemorrhagic teleangectasia. One case of GT patient treated with thalidomide has previously been described, resulting in a significant decrease of blood transfusion, bleeding episodes and hospitalizations (ref). We found a new homozygotic mutation in the ITGA2B gene. In addition, we confirm that gastrointestinal angiodysplasias may sustain recurrent GIB in GT patients and that thalidomide can be effective for the control of GIB in these patients, although possible adverse effects may limit its use.

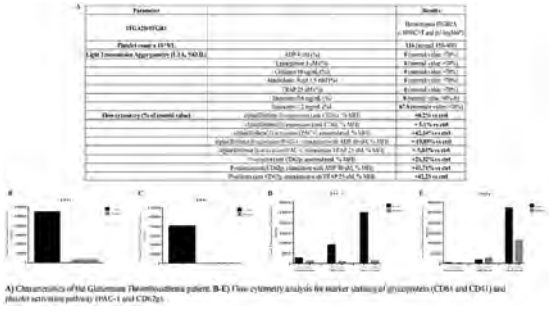


Figure 1.

PO082

PROPHYLAXIS WITH PDVWF IN A OBESE WOMAN AFFECTED BY VWD AND MUTIFOCAL ANGIODYLASIA: A CHALLENGING SCENARIO

R. Tomasello[1], M. Mattana[1], C. Rotolo[1], C. Cammarata[1], A. Lucchesi[2], G. Giordano[3], S. Siragusa[1], M. Napolitano[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo, Palermo, Italy; [2]IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola (FC), Italy; [3]Division of Internal Medicine, Hematology Service, Regional Hospital "ACardarelli", Campobasso, Italy

Background: von Willebrand disease (VWD) is the most common inherited coagulopathy worldiwide. VWD has a strong association with congenital angiodysplasias of the ileum, vascular malformations with a tendency to cause intestinal haemorrhages resulting in melena. More than 90% of patients with ileal angiodysplasia are eventually diagnosed with VWD. The management of a haemorrhagic coagulopathy associated with vascular malformations poses several challenges to clinicians. **Case Report**: A 60-yo obese patient with a known diagnosis of VWD type 1, in the past medical history treated on demand with plasma-derived coagulation factor VIII-rich Von Willebrand factor (pdVWF/FVIII) for mucosal bleedings in September 2021 was admitted to the E.R. for diarrhoea with melena and severe anemia. She was transfused with 2 pRBC and discharged. Due to persistence of melena, she was admitted to the Department of Internal Medicine and started replacement therapy with pdVWF/FVIII. During her hospitalization, following the onset of bilateral pain and oedema of the upper limbs, superficial vein thrombosis (SVT) was diagnosed by Ultrasound, FVIII:c levels resulted 195%. The patient thus needed anticoagulant treatment with Low Molecular Weight Heparin (LMWH). During the hospital stay endoscopic evaluation of the gastrointestinal tract were performed, videocapsular endoscopy revealed multiple angiodysplasias at medial and distal ileal level. She was discharged after two weeks with resolution of melena. Concurrently, for the indication to LMWH treatment of SVT, prophylaxis with pdVWF/FVIII was administered. In November 2021, due to the recurrence of melena, the patient was admitted to the Gastroenterology unit where, due to severe sideropenic anemia, she was treated with pRBC and intravenous iron carboxymaltose, subcutaneous octeotride and pdVWF/FVIII were administered to control bleeding. Surgical treatment of angiodypslasia was deemed too risky due to severe obesity. At discharge, a purified plasma derived vWF(pdvWF) concentrate prophylaxis was chosen to counterbalance the high thrombotic risk. Prophylaxis was effective and safe. In February 2022, the patient was however again admitted

to the E.R. for melena with severe anemia. Endoscopic treatment of angiodysplasia was than planned. On April 2022, the endoscopic procedure was perfomed under treatment with pdvWF with a partial removal of the tumors without complications. Prophylaxis was then tapered on FVIII:c, vWF:Ag and vWF:Ricof levels, in absence of bleeding. Currently then patient receives pdvWF twice weekly, mantaining stable haemoglobin levels, in absence of melena (Figure 1). **Conclusions**: The association between VWD and ileal angiodysplasia is not infrequent and difficult to manage, it carries a high risk of severe anemia due to martial deficiency caused by chronic haemorrhages. In the treatment of gastrointestinal bleeding in this patient with high thrombosis risk, exposure to high doses of pdVWF/FVIII was associated with SVT and required a difficult combination with LMWH, while prolonged prophylaxis with pdVWF resulted safe and equally effective in controlling bleeding. This finding may be related to the difference in FVIII:c levels reached during therapy with pdWVF/FVIII. Prophylaxis in these patients is not independent from the specific therapy of the vascular lesions, which is crucial to control bleeding. In the above reported case, a partial removal of the tumors led to the resolution of bleeding.

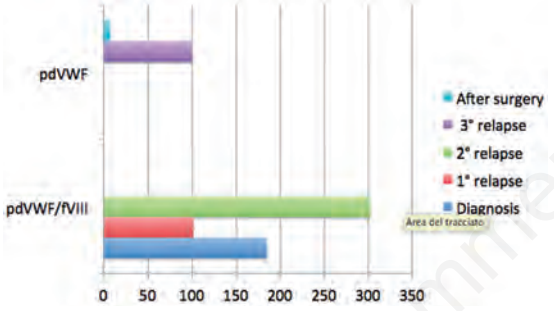


Figure 1. FVIII:c according to therapy.

PO083

THE UTILITY OF BLEEDING ASSESSMENT TOOLS IN FIBRINOGEN DEFICIENCIES

S. Mohsenian[1], O. Seidizadeh[2,3], R. Palla[3], M. Jazebi[4], A. Azarkeivan[1], S. Moazezi[4], M.R. Baghaeipour[4], M. Menegatti[2], F. Peyvandi[2,3]

[1]Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran; [2]Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; [3]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy; [4]Iranian Comprehensive Hemophilia Care Center, Tehran, Iran

Background: Bleeding-assessment-tools (BAT) or bleeding-score-systems (BSS) are developed to address the challenges associated with the evaluation of patients. A small number of patients with congenital fibrinogen deficiencies (CFD) are evaluated with these tools without definitive results. **Aims**: To compare the results of the ISTH-BAT with the EN-RBD-BSS in patients with CFD. We further evaluated the correlation between bleeding scores (BS) and fibrinogen levels. **Methods**: In total, 100 (55% females) Iranian patients with CFD were included. Routine coagulation and fibrinogen-specific tests including antigen (Fg:Ag) and its functional activity (Fg:C) were performed. Patients were classified using Fg:C/Fg:Ag ratio and they were divided into four categories of bleeding severity (asymptomatic, grade I, grade II, grade III). The ISTH-BAT and the EN-RBD-BSS were used to assess the BS of all patients. Pearson's correlation was performed using IBM-SPSS Statistics 26. **Results**: The mean age of patients were 18.5 (SD±15.9) years. The median (range) of the ISTH-BAT and EN-RBD-BSS were 4 (0-16) and 2.21 (-1.49-6.71), respectively. In general, a moderate positive correlation (r=0.597, P<0.001) was found between the ISTH-BAT score and EN-RBD-BSS. The correlation between Fg:Ag and the ISTH-BAT scores was weak negative (r=-0.407, P<0.001), comparable to the correlation between Fg:C and ISTH-BAT scores (r=-0.423, P<0.001). There was a weak negative correlation between Fg:Ag and EN-RBD-BSS (r=-0.466, P<0.001), and between Fg:C and EN-RBD-BSS scores (r=-0.446, P<0.001). Hypodysfibrinogenemic patients had the strongest correlation between the EN-RBD-BSS and ISTH-BAT (r=-0.916, P=0.001). Excluding dysfibrinogenemic patients, there was a significant association between bleeding severity and Fg:C (r=-0.402, P<0.001), and Fg:Ag (r=-0.316, P=0.005). **Conclusions**: Based on the good correlation between the two systems, it appears that in addition to the ISTH-BAT score, the EN-RBD-BSS could also be useful in CFD patients. The BS showed a significant negative correlation with fibrinogen antigen/activity levels.

PO084

GENDER DIFFERENCES IN THROMBOTIC AND HEMORRHAGIC ISSUES AFTER COVID-19 VACCINATION

E. Grandone[1,2], S. Chiocca, S. Castelvecchio, R. Nappi on behalf of representatives for Gender Medicine of Scientific Hospitalization and Treatment Institutes–Italian Ministry of Health

[1]Thrombosis and Haemostasis Unit, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG); [2]Ob/Gyn Department, University of Foggia, Foggia; [3]IEO, Istituto Europeo di Oncologia IRCCS, Milano; [4]IRCCS Policlinico San Donato, San Donato Milanese (MI); [5]IRCCS Istituto Ortopedico Rizzoli, Bologna; [6]IRCCS Fondazione Policlinico S. Matteo, Pavia, Italy

Gender medicine deals with differences in approaching

diagnostic work-up and management according to gender. Although the issue is relevant in every field of medicine, it is often neglected. The recent pandemic due to SARS-CoV-2 virus raises this matter even more urgently. In fact, available literature has suggested a higher number of deaths among infected males than in females and more side effects in women than in men recipients of certain vaccines against COVID-19. In this narrative review, we critically revised sex-disaggregated data on thrombotic and bleeding events associated with vaccination against COVID-19. Thrombotic complications are by far more frequently reported than bleeding events after vaccination and are mostly observed in young women who are administered with viral-vectored vaccines. Bleeding complications are mainly reported as aggregated data, whereas thrombocytopenia is reported to occur in the presence or absence of thrombotic complications. This information is relevant, as it underlines the need to differentiate thrombocytopenia occurring with- or without -thrombosis; indeed, management and prognosis are different according to the presence of thrombotic events. Conflicting data are available on the prevalence of bleeding events according to sex in recipients of viral-vectored vaccines. Lastly, clinical data on vaccination against SARS-CoV-2 during pregnancy show that its safety-efficacy profile is high. However, further data are needed and will be obtained from ongoing trials. In conclusion, available literature on sex -disaggregated data and on the possible role of hormones in thrombotic and bleeding complications after vaccination against COVID-19 is still largely lacking. In view of the role played by platelets in the pathogenesis mechanism of both thrombotic and bleeding complications, future studies should be focused on platelets and on global tests as thrombin generation assays.

PO085

TRENDS IN THE INCIDENCE OF LOWER LIMB DEEP VEIN THROMBOSIS IN PATIENTS WITH COVID-19 PNEUMONIA DURING THE EARLIER AND LATER WAVES OF SARS-COV-2 PANDEMIC: A MULTICENTER PROSPECTIVE STUDY

L. Corbo[1], F. Mani[1], R. Marcucci[2], G. Guazzini[1], L. Lastraioli[1], F. Luise[1], L. Maddaluni[1], A. Milia[1], L. Sammicheli[1], A. Fortini[3], F. Pieralli[1]

[1]COVID-19 Intermediate Care Unit, Careggi University Hospital, Florence, Italy; [2]Department of Clinical and Experimental Medicine, University of Florence and Careggi Hospital, Florence, Italy; [3]Internal Medicine COVID-19 Unit, San Giovanni di Dio Hospital, Florence, Italy

Background and Aims: The aim of this study was to evaluate the trend of the incidence of deep vein thrombosis (DVT) of the lower limbs, using serial compression ultrasound (CUS) surveillance, in patients hospitalized with COVID-19 pneumonia in a non-ICU setting in different waves of the pandemic. **Methods**: Multicenter, prospective study of patients with COVID-19 pneumonia admitted to Internal Medicine units. All patients were screened for DVT of the lower limbs with serial CUS. The primary end-point of the study was to assess the incidence of DVT diagnosed by CUS in the different waves of SARS-CoV-2 pandemic. **Results**: Two periods of time, named first wave and subsequent waves were considered, and a total of 363 consecutive patients with moderate-severe COVID-19 pneumonia were enrolled. The pooled incidence of DVT in the two cohorts was 8%; incidence of DVT was 13.5% (6.8% proximal, 6.7% distal) in the first wave, and 4.2% (2.3% proximal, 1.9% distal) in the subsequent waves (p=0.002). Patients enrolled in the first and the second wave had similar clinical characteristics, and thrombotic risk profile as assessed by PADUA score (mean score 6.14 *vs* 6.31; p=0.155); a significant difference in anticoagulant regimen and initiation of thromboprophylaxis at home (8.1% *vs* 25.1%; p<0.001) was observed. **Conclusions**: The incidence of DVT, diagnosed through a surveillance protocol by serial CUS of the lower limbs, in acutely ill patients with COVID-19 pneumonia hospitalized outside the ICU showed a significant reduction over time during the different waves of the pandemic.

PO086

LABORATORY EVALUATION OF POST-COVID19 PATIENTS WITH ACUTE VENOUS THROMBOEMBOLISM DURING HOSPITALIZATION: THE BERGAMO EXPERIENCE

C. Ambaglio[1], S.V. Benatti[2], C.J. Tartari[1], C. Giaccherini[1], L. Russo[1], M. Marchetti[1], A.M. Palladino[3], F. Schieppati[1], S. Venturelli[2], L. Barcella[1], M. Rizzi[2], A. Falanga[1,3]

[1]Division of Immunohematology and Transfusion Medicine, Papa Giovanni XXIII Hospital, Bergamo; [2]Division of Infectious Diseases, Papa Giovanni XXIII Hospital, Bergamo; [3]Medicine and Surgery, University of Milan-Bicocca, Milan, Italy

Background and Aims: In a cohort of COVID-19 survivors who experienced venous thromboembolism (VTE) during the acute phase and followed up at out outpatient clinic, we evaluated whether alterations in coagulation and inflammatory parameters were persisting after hospital discharge. **Methods**: An outpatient service specifically dedicated to follow up COVID-19 survivors was set up at our institution. A retrospective analysis of a cohort of 1,563 patients enrolled from May 2020 to November 2020 was performed. Data were obtained on the occurrence or not of acute COVID-19 associated VTE, the site of thrombosis, ongoing anticoagulant treatment and the results of laboratory tests performed at the first post-discharge assessment visit, including blood cell count, D-dimer, fibrinogen and CRP. Statistical analysis

was performed using SPSS v21 software. Results of laboratory tests were expressed as median values (5th-95th percentile). **Results**: Fifty-six out of 1,563 COVID-19 survivors [43M/13F, median age 62 years (40-80.5) 3.58%] experienced VTE during the acute phase as follows: 9 isolated proximal DVT (4 in upper extremity), 45 isolated PE (7 subsegmental PE, 27 segmental PE, 11 central pulmonary artery thrombosis), 2 DVT + PE, with subsegmental and segmental PE representing 60.7% of all VTE. The first outpatient evaluation was performed between 41 and 222 days post-admission (average 181). Treatment of VTE consisted in LMWH (n=11), vitamin K antagonist (n=2) and DOAC (n=43). Only two patients had already stopped anticoagulant therapy at the time of laboratory evaluation and presented normal values of D-Dimer. Laboratory data analyses revealed that 13/56 patients had persistent D-dimer >500 ng/ml, 11/56 had D-dimer >age-adjusted cut-off. 23,6% of VTE subjects had increased levels of both D-Dimer (>500 ng/ml) and fibrinogen (>450 mg/dl), and 11% had elevated levels of CRP (>1 mg/dl), while no abnormalities were observed in the platelet and leucocyte counts. CRP significantly correlated with fibrinogen and D-Dimer levels (r=0.791, p<0.0001). No patients had VTE recurrence or bleeding complications at the time of first follow up visit. **Conclusions**: Anticoagulant treatment of COVID-19 VTE seems to be clinically safe and effective, nonetheless laboratory analyses suggest a persistence of procoagulant and inflammatory alterations in spite of ongoing anticoagulants. Further observations are needed to establish on long-term persistence of these laboratory features and long-term risk of VTE recurrence even after the end of therapy.

PO087

NATURAL ANTICOAGULANTS AND ENDOTHELIAL MARKERS IN COVID-19 PATIENTS: A LONGITUDINAL EVALUATION

P. Della Valle, C. Tresoldi, N. Maugeri, L. Apruzzi, S. Calvisi, M. Ripa, G.A. Ramirez, P. Rovere-Querini, G. Landoni, A. Castagna, M. Tresoldi, G. Martino, F. Ciceri, A. Manfredi, A. D'Angelo, on behalf of COVID-BioB Study Group

Scientific Institute Ospedale San Raffaele and Vita e Salute University, Milano, Italy

Background and Aims: A combination of cytokines, activated complement, platelets, endothelial cells and microvescicles appear to sustain the coagulopathy in severe covid-19 infection. We evaluated changes in natural anticoagulants and selected endothelial markers in patients with different severity of covid-19 infection during the first wave of the pandemic. **Methods**: We retrospectively analyzed antigen levels of d-dimer (DD), protein C (PC), total protein S (PS), total tissue factor pathway inhibitor (TFPI), antithrombin (Diagnostica Stago, France) and of the endothelial markers sCD146 (Biocytex, France), s-thrombomodulin (sTM) and se-selectin (R&D Systems, MN, US) in 190 samples from 101 patients with laboratory proven coronavirus 2 (SARS-CoV-2) infection accessing the ER of the San Raffaele Hospital in March 2020. Control samples were from 45 apparently healthy volunteers. Eighteen patients were not hospitalized (NH); 46 patients were admitted to medical wards (MW), and 37 to intensive care units (ICU). Samples from 25 ICU patients (9 survivors and 16 non-survivors) were additionally tested after a median interval of 7 days (T2). Samples from 40 MW patients and 19 ICU patients (all survivors) were tested at a median interval of 3 months (T3). The hospital's EC approved the study, registered on ClinicalTrials.gov (NCT04318366). **Results**: At baseline DD, TFPI, and sTM were higher and sCD146 lower in patients than in controls (p<0.0001). Trends according to the severity of infection were only observed for DD and sCD146 (Table 1). T2 samples showed increased levels of sTM, se-selectin, and decreased levels of PC and PS (p≤0.01), with no significant difference between survivors and non-survivors. T3 samples showed increased PC and PS and reduced DD and TFPI (p<0.0001) compared to T1 samples. **Conclusions**: Compared to changes in DD levels, changes in endothelial markers and natural anticoagulants are relatively moderate in covid-19 patients. The later increase in PS and PC antigen levels suggests a rebound phenomenon.

Table 1.

	Controls	NH	MW	p	ICU	ANOVA
sTM (ng/ml)	3.35 (2.55-3.90)	4.5 (3.4-5.4)	3.8 (2.6-4.6) †	.017	4.0 (3.0-5.3) ‡	.00003
sCD146 (ng/ml)	424 (295-556)	308 (244-364)*	277 (220-322) ‡	0.42	230 (177-341) ‡	.00003
TFPI (ng/ml)	89 (79-99)	134 (122-201) ‡	104 (84-120)* <0.0001 *vs* NH	.00014	116 (99-135) ‡	.00003
D-dimer (mg/L)	0.22 (0.12-0.38)	0.59 (0.43-0.88)	2.75 (1.12-5.03) ‡ 0.019 *vs* NH	.00001	3.38 (1.83-9.1) ‡ <0.0001 *vs* NH	.00003

*<0.01; †<0.001; ‡<0.0001 *vs* controls

PO088

THROMBOTIC THROMBOCYTOPENIA FOLLOWING SYSTEMIC INJECTION OF THE RECOMBINANT ADENOVIRAL VECTOR SIMIAN ADENOVIRUS Y25 SEROTYPE (CHADY25): REPORT OF TWO CASES AND A PERSPECTIVE ON PATHOGENESIS

C. Cammarata[1], M. Mattana[1], C. Rotolo[1], R. Tomasello[1], A. Lucchesi[2], G. Giordano[3], S. Siragusa[1], M. Napolitano[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE), University of Palermo, Palermo, Italy; [2]IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola (FC), Italy; [3]Division of Internal Medicine, Hematology Service, Regional Hospital "A. Cardarelli", Campobasso, Italy

Background: Several cases of unusual deep vein thrombotic (DVT) events and thrombocytopenia have been observed after vaccination with Vaxzevria® (ChAdOx-1

nCov19) from the end of 2020 to early 2021. Vaccine-induced thrombotic thrombocytopenia (VITT) is more common in young females without any relation to hormonal contraceptives or pre-existing coagulopathy. Symptoms begin from 1 to 2 weeks after the administration of the first dose and are initially mild, simulating a flu-like syndrome, until their worsening leads to hospitalization. This adverse effect has been shown to occur only after the first dose of Vaxzevria®. **Case Report**: We here report atypical site DVT associated with severe thrombocytopenia after exposure to the first dose of Vaxzevria®. **Case 1**: In March 2021, a 45-year-old woman, obese, was admitted to the emergency area ten days after the first dose of Vaxzevria® for abdominal pain unresponsive to analgesics. Blood tests showed severe thrombocytopenia and increased D-dimer. A CT scan of the abdomen showed extensive thrombosis of the spleno-mesenteric-portal axis. Low molecular weight heparin (LMWH) was initiated. After one week, treatment switched to high molecular weight heparin (HMWH). PLT transfusions were given to maintain PLT above 30x109/L. Severe hematemesis with acute anemia occurred about 36 hours after the beginning of HMWH; endoscopy detected hemorrhagic foci in the mucous membranes of the pharynx without detectable lesions. The latest blood tests suggested a pattern of disseminated intravascular coagulation (DIC). A new CT scan confirmed DVT plus abundant abdominal hemorrhages. Therefore, therapy switched to Fondaparinux with high-dose intravenous immunoglobulin (IVIGs), and off-label use of the anti-IL-6 antibody Tocilizumab. Despite treatment, the patient died (Table 1). **Case 2**: Late in March 2021, a 61-year-old woman was admitted to our center after two weeks after the first dose of Vaxzevria®. The echo-color-doppler showed popliteal and tibial DVT. A CT scan showed left internal jugular vein and bilateral pulmonary embolisms. Blood tests found moderate/severe thrombocytopenia and increased D-dimer. The patient, therefore, started therapy with corticosteroids, IVIGs, and Fondaparinux. Five days after treatment, she experienced a clinical-laboratory improvement. The patient was discharged asymptomatic under treatment with direct oral anticoagulants (DOACs). In both cases ADAMTS13 activity, evaluated immediately after patient admission was normal, thrombophilia screening was negative, the anti-PF4-heparin complex immunoassay was negative, autoimmune assay was unremarkable (Table 1). **Conclusions**: According to the available literature on immune response to Adenovirus and Ad-induced thrombocytopenia, and the correlation between estrogens and thrombotic risk, it can be assumed that the Adenoviral vector based on Y25 serotype may cause a systemic inflammatory response characterized by a massive platelet activation leading to thrombotic thrombocytopenia, especially in women as estrogens alone favor the instauration of a prothrombotic state. The largest studies conducted on VITT demonstrated the presence of anti-PF4 antibodies in nearly all the patients enrolled. In our 2-cases-experience the assay for the anti-PF4-heparin complex was negative. Early recognition of the symptoms related to disease onset has allowed prompt and successful management of the disease.

Table 1.

	CASE 1	CASE 2
EARLY SYMPTHOMS	Abdominal pain, unresponsive to analgaesics	Headache, right lower limb pain
DAYS FROM VACCINE INOCULATION TO SYMPTHOMS ONSET	10	14
DAYS FROM SYMPTHOMS ONSET TO ADMITTANCE INTO OUR DEPARTMENT	7	Immediately
SITES OF DVT CONFIRMED	Spleno-mesentheric-portal	Popliteal, tibial, left internal jugular
PULMONARY EMBOLISM AT DIAGNOSIS	No	Yes
SECONDARY PULMONARY EMBOLISM	No	No
PLATELET COUNT AT DIAGNOSIS	28000/mmc	43000/mmc
D-DIMER LEVELS AT DIAGNOSIS	100000ng/ml	52842ng/ml
HIT immunoassay (anti-PF4-heparin complex)	Negative	Negative
ADAMTS13 activity	>150%	>150%
cPLT TRANSFUSIONS NEEDED	5	0
FIRST LINE THERAPY	Unfractioned heparin (continuous infusion)	HD Corticosteroids + HD IVIG + Fondaparinux
RESPONSE TO FIRST LINE THERAPY	Upper pharynx mucous bleeding	Improvement
SECOND LINE THERAPY	Tocilizumab	N.A.
RESPONSE TO SECOND LINE THERAPY	N.A.	N.A.
MAINTENANCE THERAPY	N.A.	Dabigatran

PO089

HEPARIN-INDUCED THROMBOCYTOPENIA (HIT) AFTER SARS COV 2 INFECTION: A CASE REPORT

F. Pelucchini[1], C. Pestelli[2], A. Bertelli[1], A.A. Rogolino[1], F. Cesari[1], E. Sticchi[1], B. Giusti[1], F. Pieralli[2], A.M. Gori[1], R. Marcucci[1]

[1]Dept of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [2]COVID-19 Intermediate Care Unit, Careggi University Hospital, Florence, Italy

Introduction: Heparin-induced thrombocytopenia (HIT) is a severe, life-threatening drug reaction associated with decrease in platelet count and a high risk of thrombosis caused by platelet-activating antibodies against PF4/heparin complexes. The atypical clinical and therapeutic context of the COVID-19 pandemic, with a broader indication for heparin treatment, could lead to a higher prevalence of HIT. Here we report a 72 years old woman operated on for severe aortic valve stenosis, treated with heparin, who, after contracting COVID-19, developed a severe HIT. **Methods and Results**: A 72-year-old woman with a history of hypertension, hiatus hernia, rheumatic polymyalgia and valvulopathy, was admitted for aortic valve replacement surgery. She started heparin treatment the same day of hospitalization with a platelet count of $202x10^9$/L. After 5 following surgery, platelet count dropped to 83 $x10^9$/L. 4T score was 4. Therefore, heparin was discontinued and test for anti PF4/heparin antibodies was carried out by a chemiluminescent assay (Acustar Werfen), obtaining a negative result (0.04 U/mL). As the immunological assay failed to demonstrate the presence of antibodies anti PF4/heparin complex negative, the patient was again treated with heparin. On the next day, warfarin was added to the therapy and the patient tested positive for SARS-COV-2 on nasopharyngeal swab, therefore she was transferred to the COVID department. The patient was asymptomatic for

Covid-19 infection. The day after platelets dropped again to 47 x10^9/L, so it was decided to stop the heparin treatment and perform again the immunological assay for anti PF4/heparin antibodies, which resulted positive (>128 U/mL). Subsequently the HIPA (Heparin Induced Platelet Activation) functional test demonstrated that the anti PF4/heparin antibodies detected by the immunological assay were able to activate platelets. In the following days after heparin suspension the platelet count returned to normal values. One week later, a thrombosis from the basilica vein to the left axillary vein was found, probably originating from the peripheral venous catheter. **Discussion**: We report the case of a 72-year-old woman who developed thrombocytopenia after cardiac surgery with a negative test for PF4/heparin antibodies. Subsequently, concomitant with a positive swab for SARS-COV-2, patient developed anti PF4/heparin antibodies, able to functionally activate platelets, thus suggesting that SARS-COV-2 infection triggered the formation of anti PF4/heparin antibodies leading to a clinical presentation of HIT.

PO090

VENOUS THROMBOEMBOLISM-RELATED MORTALITY IN COVID-19 PATIENTS ADMITTED IN INTENSIVE CARE UNIT: A RETROSPECTIVE SINGLE-CENTER ANALYSIS

G. Turatti, D. Ceccato, F. Boscaro, C. Tirrito, S. Ragazzo, G. Avruscio

U.O.CAngiologia, Azienda Ospedale Università di Padova, Italy

Background and Aims: The strong association between COVID-19 and venous thromboembolism (VTE) has been widely described, and anticoagulant treatment seems to improve overall survival. Otherwise, the effect on severe forms is scarce, and the impact of COVID-related VTE in intensive care unit (ICU)-patients in terms of mortality remains unclear. Our aim was to investigate the presence and entity of this association in Padua ICU patients. **Methods**: We retrospectively revised ICU patients admitted in our university hospital for COVID-19-related respiratory failure, from January 2020 to April 2022. All patients underwent a complete Doppler ultrasound (jugular veins, upper and lower extremities). Descriptive data are reported with means and standard deviations (SD). Association between VTE and death was explored via odds ratio (OR) calculation with 95% confidence interval (CI), and a survival analysis was performed. **Results**: We report the exploratory analysis of 185 patients. Mean age was 65 years and women were slightly underrepresented (65, 35.1%). VTE occurred in 87 (50.3%) patients. Of these, 56 (30.3%) patients had deep vein thrombosis (DVT), respectively 44 distal and 15 proximal, 49 (26.5%) patients had catheter-related thrombosis (CRT), and 4 (4.6%) patients had pulmonary embolism (PE). Among the 44 patients with distal DVT, 14 patients also had involvement of other districts (3 had concomitant proximal DVT, 3 had PE, and 10 had CRT, of which 2 with concomitant PE). Death occurred in 35 (18.9%) patients. There was no relationship between overall VTE and death (OR 1.2, 95% CI 0.6 - 2.6). **Conclusions**: In our cohort we observed a high incidence of VTE events. This confirmed the strong relationship between COVID-19 and thrombosis, especially in the critically ill patients. However, we observed no differences in the 30-day mortality rate between VTE and non-VTE patients. This observation may in part be due to the localization of the index event (mostly distal DVTs or CRTs). Although VTE is a common complication of COVID-19 infection, its impact on COVID- related mortality is still unclear.

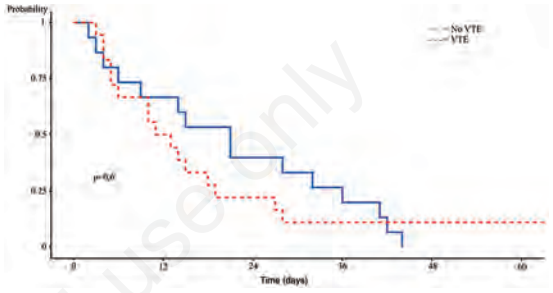


Figure 1.

PO091

ACUTE PORTAL VEIN THROMBOSIS TRIGGERED BY SARS-COV2 INFECTION

A. Dalla Salda[1], A. Soriano[2], R. Giacosa[2], M. Iotti[1], A. Ghirarduzzi[1]

[1]Cardiovascular Medicine, Department of Internal Medicine, USL-IRCCS Reggio Emilia; [2]Gastroenterology and Digestive Endoscopy Unit, USL-IRCCS Reggio Emilia, Italy

Background: Portal vein thrombosis (PVT) is a rare condition occurring in patients with hepatic cirrhosis, solid or hematological malignancies or abdominal inflammation. Infection by SARS-COV-2 is associated with respiratory symptoms but also with various thrombotic complications ranging from deep vein thrombosis /pulmonary embolism to cases of PVT, secondary to a virus induced hypercoagulable state. Notably, patients affected by hereditary thrombophilia can experience major thromboembolic events during SARS-COV2 infection, which can require prompt diagnosis and management. **Case Report**: A 48-year-old male presented at Emergency Department with persistent epigastric pain. He had recently experienced fever, dry cough and diarrhea in relation to a mild symptomatic SARS-COV-2 infection and his naso-pharynx SARS-COV-2 swab had resulted negative a week before the onset of abdominal symptoms. His medical history was characterized by previous splenectomy secondary to an abdominal trauma

and mild gastroesophageal reflux. He had been previously vaccinated against COVID-19 with one dose of vaccine. During Emergency Department visit an urgent abdomen X-ray resulted normal and the liver ultrasound examination revealed PVT. An urgent abdomen contrast-enhanced computed tomography (CT) confirmed the PVT showing intra- and extrahepatic portal vein thrombosis (24 mm caliber), superior mesenteric vein and splenic vein thrombosis. On laboratory workup, a mild increase in blood cell count (WBC) and reactive C protein (RCP) were present, aspartate aminotransferase were slightly elevated. Medical therapy with low-weight molecular heparin and pump protonic inhibitors was promptly started. During hospital stay, an upper gastrointestinal endoscopy ruled out portal hypertension-related esophageal varices. A thrombophilia workup finally revealed heterozygous prothrombin G20210A mutation. After warfarin bridging, he was discharged home and, a month later, he underwent an abdomen ultrasound examination, which confirmed extensive portal vein thrombosis with low portal outflow and the presence of collateral circles. He is actually on Warfarin treatment, and he will go on anticoagulant therapy for a minimum of six months. To date, acute thrombotic complications have been frequently reported in association with SARS-COV-2 infection but few data about PVT have been reported in this setting. Nevertheless, the real incidence of this event is perhaps higher than expected, especially in male patients. Inherited thrombophilia may play a role in increasing thrombotic risk in healthy and non-cirrhotic patients and a comprehensive thrombophilia screening should be performed. With regard to therapy, the goal of treatment is avoiding early and late complications of portal hypertension, but evidences about the optimal anticoagulant regimen are lacking. Even if limited data are still available in non-cirrhotic patients, either direct oral anticoagulants (DOAC) or warfarin can be used. **Conclusions**: Portal vein thrombosis represents a rare complication of SARS-COV-2 infection. A prompt diagnostic workup is necessary in patients with acute or recent SARS-COV-2 infection complaining acute abdominal pain or abdominal symptoms in order to exclude/confirm splanchnic thrombosis. Anticoagulant treatment is the corner stone of treatment, but the duration of therapy must be individualized on the clot resolution and recurrent thrombotic risk.

PO092

VACCINE-ASSOCIATED CEREBRAL VENOUS SINUS THROMBOSIS (CVST) AS A MANIFESTATION OF VACCINE-INDUCED IMMUNE THROMBOTIC THROMBOCYTOPENIA (VITT) IN TWO YOUNG WOMEN. ROLE OF ORAL CONTRACEPTIVES

C. Franzetti[1], L. Tajana[1], F. Piovella[2]

[1]Unità di Medicina Legale e Scienze Forensi "A. Fornari", Dipartimento di Sanità Pubblica, Medicina Sperimentale e Forense, Università di Pavia, Pavia; [2]Angioematologia, Casa di Cura Lecco "Beato Luigi Talamoni", Lecco, Italy

Background: The etiology of cerebral venous sinus thrombosis (CVST) involves hereditary and acquired factors. Acquired factors include brain tumors, brain trauma, infections, immune system diseases, pregnancy, postpartum complications, trauma, and drugs. Oral contraceptives are the most common drugs responsible for causing CVST. Rapid anticoagulant therapy with low molecular weight heparin (LMWH) is the preferred anticoagulant treatment for CVST, and the disease generally has a favourable outcome, with a complete functional recovery in about 75% of patients. In some cases, CVST develops in patients under high dosage progestative drugs taken for reasons different from contraception. Recently, new syndrome of vaccine-induced immune thrombotic thrombocytopenia (VITT) has emerged as a rare side-effect of vaccination against COVID-19. Cerebral venous thrombosis associated with severe hemorrhage is the most common manifestation of this syndrome. The VITT syndrome seemed more frequent to develop in young woman after the first dose of vaccines utilizing a non-replicating recombinant chimpanzee adenovirus to deliver the genetic material of the spike protein to the recipient (Vaxzevria, Oxford–AstraZeneca and Jcovden, Johnson & Johnson/Janssen). Differently from "classic" CVST, early reports in which patients were treated with heparins described clinical worsening, including death, and early recommendations from health autorities were to avoid heparin because of the resemblance of VITT to HIT. **Case Reports**: We studied the autopsies of two young women under estroprogestative treatment cases of VITT on assignment from the Public Prosecutor Office at the Court. Patient T. F. autopsy diagnosis: temporo-parieto-occipital cerebral hemorrhage following thrombosis of the dural venous sinuses (thrombosis of the right transverse venous sinus, sigmoid sinus, jugular gulf and upper sagittal sinus), occurring 10 days after administration of the first dose of Vaxzevria vaccine in subject being treated with estroprogestins. Laboratory tests: D-dimer 120,800 µg/L, platelets 35x109/L, presence of anti-PF4 antibodies. These data allow to classify the patient as belonging to the category of certain VITT syndrome. Patient C.C. autopsy diagnosis: cerebral death following massive thrombosis of the venous sinuses and cortical veins, complicated by widespread subarachnoid hemorrhage and extensive cerebral intraparenchymal infarction occurring a week after administration of the first dose of Vaxzevria vaccine. The patient started combined estroprogestin with high doses didrogesterone and estradiol due to ovarian polycystosis and menstrual irregularities. Laboratory tests: D-dimer 34,575 µg/L; platelets 22x109/L, presence of antibodies against PF4. These data allow to classify the patient also as belonging to the category of VITT syndrome certain. In conclusion, the cases of thrombosis observed to date during the world vaccination campaign are very rare. It is possible to hypothesize that an inflammatory reaction triggered by the viral vector vac-

cine at the level of the endothelium, which seems to be linked to the generation of antibodies against platelet factor 4 (PF4) in response to the vaccine itself, overlaps with an activation of coagulation factors with consequent local thrombosis determined by the presence of estroprogestin drugs.

PO093

ROLE OF THE PROLINE-RICH TYROSINE KINASE PYK2 IN PLATELET ACTIVATION IN A MURINE MODEL OF ENDOTOXEMIA

L. Galgano[1,2], M. Vismara[2], S.M.G. Trivigno[1,2], S. Rustichelli[2], G.F. Guidetti[2], M. Torti[2], I. Canobbio[2]

[1]Istituto Universitario di Studi Superiori IUSS, Pavia, Italy; [2]Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Università di Pavia, Pavia, Italy

Background and Aims: Endotoxemia induced by lipopolysaccharide (LPS) in mice leads to thromboinflammation. It is accompanied by hypercoagulability, increased platelet and leukocyte activation, and disseminated intravascular coagulation [1]. Pyk2 is a non-receptor tyrosine kinase highly expressed in platelets where it controls adhesion, activation, aggregation, thrombus formation, and platelet-leukocyte/neutrophil aggregate (PLA/PNA) formation [2,3]. In this work, the role of Pyk2 in platelet and neutrophil activation was investigated in a murine model of endotoxemia. **Materials and Methods**: LPS (*Escherichia coli*, O111:B4) was injected to induce endotoxemia in WT and Pyk2-KO mice. After four hours, vital parameters were checked, and blood was collected. PLA and PNA formation, platelet integrin $\alpha_{IIb}\beta_3$ activation, P-selectin expression and neutrophil $\alpha_M\beta_2$ activation were analysed by flow cytometry. Phosphorylation of signalling proteins was analysed *ex vivo* on isolated platelets in immunoblotting and aggregation was monitored in a Born lumiaggregometer. **Results**: LPS-injected Pyk2-KO mice show a reduced disease score, a more severe thrombocytopenia, and a lower number of circulating PLA/PNA because of a reduced P-selectin exposure on platelets compared to WT mice. LPS injection increases phosphorylation of MAPK and Akt induced by Thrombin Receptor Activating Peptide 4 (TRAP4) in WT platelets, which is abolished in Pyk2-KO platelets. No differences in term of aggregation, platelet $\alpha_{IIb}\beta_3$ and neutrophil $\alpha_M\beta_2$ activation were observed in the absence of Pyk2. **Conclusions**: Our results demonstrate that Pyk2 regulates MAPK and Akt phosphorylation, platelet P-selectin expression and PLA/PNA formation in a mouse model of endotoxemia, thus modulating the first trigger of thromboinflammation.

References:

1. Shannon, Platelets. 2015;26:302-8.
2. Canobbio *et al.*, Blood. 2013;121:648-57.
3. Momi *et al.*, Haematologica. 2022; in press.

PO094

YOU HAVE TO THINK ABOUT IT: HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

N. Costantini

Ospedale Giovanni Paolo II, Olbia, Italy

Background: Fever of unknown origin is a challenge for internist, because it is particularly confounding and the underlying disease of the patient not always can explain it. There is a strong link between coagulation and immune system. **Case Report**: A 78-year-old woman with rheumatoid arthritis was hospitalised to the Department of Internal Medicine, in Olbia, in October 2021 for the evaluation of fever. It began one months earlier, of 39-40°C, which occurred almost daily: she had not joints manifestations and no other symptoms. Abatacept, her agent for rheumatoid arthritis, was permanently discontinued at admission to the hospital. The initial laboratory findings revealed: blood cultures were always negative; the levels of C-reactive protein and the erythrocyte sedimentation rate, serologic and immunological analyses were within reference values; elevation of tumour markers (CA125 150 U/ml; CA15.3 49,1 U/ml), a progressive anemia (hemoglobin 8,3 g/dL), reduction in platelet level up to 101×10^3/microL, leukopenia (2.9×10^3/microl), hypertriglyceridemia (triglycerides level up to 563 mg/dL) and marked elevation in ferritin (>2000 ng/mL), d-dimer (17129 ng/ml) and lactate dehydrogenase (up to 1357 U/L) without evidence of thrombosis. There was an hypogammaglobulinemia (IgG 442 mg/dl, IgM 25 mg/dl), hypofibrinogenemia (up to 89 mg/dl), slightly elevated activity of aminotransferases, increased total bilirubin level and beta 2 microglobulin. Echocardiography, gastroscopi and colonoscopy were all unremarkable. By using computed tomography of the chest and abdomen no lymphadenopathy was recorded, the size of spleen was normal but there was a mural thickness in the sigmoid colon with increased density in adjacent adipose tissue: however the intraoperative aspect was normal. Two lines of empirical antibiotic terapie were used, but they were then discontinued as infectious workup was always negative. Bone marrow biopsy revealed also reactive alterations. Patient was treated with oral methylprednisolone, 40 mg per day, with rapid resolution of fever; it came back on reducing the dose of the steroid. On December the patient was transferred to another department of Internal Medicine, in Cagliari, where a new bone marrow biopsy was done, almost two months after the first: it exhibits typical Chronic lymphocytic leukemia phenotype.The patient met 5 of 8 HLH-2004 criteria for diagnosis of acquired hemophagocytic syndrome, including 1 clinical (fever) and 4 laboratory (cytopenia, hyperferritinemia, hypertriglyceridemia and hypofibrinogenemia,high levels of soluble IL-2 receptor) criteria. HLH patient had a clinical response to systemic dexamethasone and she was admitted to hematology. **Conclusions**: HLH is likely

under-recognized and may not be visible until late in disease progression; bone marrow biopsies performed early in the course of secondary disease may be normal, so we have to think about it. We must not be satisfied with the initial diagnosis. Secondary hemophagocytic lymphohistiocytosis is a potentially life-threatening complication of rheumatic disease, therefore in our patient we should not have looked for alternative diagnoses: instead HLH etiology was ascribed to haematological malignancy. D-dimers levels were highly increate probably for the demonstrated fibrinolytic activity, a coagulation disorders described in HLH.

PO095

ENDOTHELIAL CELLS FROM UMBILICAL CORD OF WOMEN AFFECTED BY GESTATIONAL DIABETES: AN *IN VITRO* VASCULAR MODEL TO STUDY THE ANTI-INFLAMMATORY AND PRO-ANGIOGENIC ROLE OF AMNIOTIC MEMBRANE IN DIABETIC FOOT ULCER

C. Pipino[1], I. Cappellacci[1], P. Di Tomo[1], Á. Bernabé-García[2], J. Stelling-Férez[2,3], N. Di Pietrantonio[1], D. Mandatori[1], M.P.A. Baldassarre[4], F.J. Nicolás[2], A. Pandolfi[1]

[1]Center for Advanced Studies and Technology-CAST (ex CeSI-MeT), Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, StemTeCh group, Chieti, Italy; [2]Regeneration, Molecular Oncology and TGFß, IMIB-Arrixaca, Murcia, Spain; [3]Department of Nutrition and Food Technology, UCAM, Murcia, Spain; [4]Center for Advanced Studies and Technology-CAST (ex CeSI-MeT), Department of Medicine and Aging Sciences, University Gd'Annunzio Chieti-Pescara, Chieti, Italy

Background and Aims: Diabetic foot ulcer (DFU) is a severe complication of diabetes which affects 15% of patients with advanced stages of the disease. Despite improvements in the treatment, DFU remains the main cause of lower limb amputation. One of the most relevant diabetic wounds healing issues is the reduced peripheral blood flow, the diminished neovascularization and chronic inflammation. Amniotic membrane (AM) has shown promising results in the treatment of DFU. Here, the potential role of AM on endothelial cells isolated from umbilical cords of gestational diabetes affected women (GD-HUVECs) has been investigated. Indeed, GD-HUVECs, considering their epigenetic memory and pro-inflammatory phenotype induced by *in vivo* hyperglycemia during pregnancy, could be a proper model to investigate the AM promoting mechanisms in repairing diabetic chronic non-healing wounds. **Methods**: For Control (C)- and GD-HUVECs, stimulated with low doses of the pro-inflammatory Tumour Necrosis Factor-α (TNF-α, 1ng/ml), in basal condition and after 24h incubation with AM, monocytes adhesion assay was performed to assess the adhesion of monocytes to endothelial cells. Gene expression of Vascular and Intercellular adhesion molecules 1 (VCAM-1, ICAM-1), E-selectin (SELE) and chemokine CC ligand-2 (CCL2) were evaluated by real time PCR. VCAM-1 and ICAM-1 membrane exposure was analysed by flow cytometry. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) phosphorylation and nuclear translocation were evaluated by western blot and immunofluorescence staining, respectively. Nitric oxide bioavailability through cyclic guanosine monophosphate (NO/cGMP) levels was measured by ELISA. Cell migration was performed by scratch assay and by measuring proteins involved in the focal adhesion turn-over such as Paxillin and Vinculin. Vessel formation was evaluated by Matrigel tube formation assay. **Results**: AM significantly reduced TNF-α stimulated monocyte-endothelium interaction and the expression of VCAM-1, ICAM-1, SELE and CCL2 in C- and GD-HUVECs. That was also reflected on a reduction on membrane exposure of VCAM-1 and ICAM-1 in both cell types upon AM stimulation. This was associated with a significant decrease of phosphorylation of NF-kB and its cytoplasm-nucleus translocation. Finally, AM-increased NO bioavailability was affected by eNOS inhibitor L-NAME, suggesting that AM activation of eNOS could be a possible way of regulating this phenomenon. Of note, C- and GD-HUVECs migration capability was ameliorated after incubation with AM in wound healing scratch assay. Moreover, the increased number of focal adhesions involving both Paxillin and Vinculin after AM treatment suggests a rapid turnover of these structures supporting cell migration improvement. Furthermore, several angiogenic parameters, analyzed following Matrigel tube formation assay, were improved in AM stimulated GD-HUVECs, thus promoting better interconnected network areas. **Conclusions**: Overall, these results indicate that in our cell model AM is effective in reducing hyperglycemia and improving vasculogenesis, possibly through the modulation of NO bioavailability, which plays a key role in the vascular homeostasis balance. This study suggests that AM improves chronic wound healing by both enhancing angiogenesis and decreasing inflammation, thus supporting its clinical application for diabetic foot ulcers.

PO096

INTRACARDIAC THROMBOSIS IN A PATIENT WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME[/B]

E. Marrone[1], A.M. Cerbone[2], D. D'Auria[1], F. Gallucci[1], C. Romano[1], A. Tufano[2], P. Morella[1], G. Di Minno[2]

[1]Internal Medicine Unit 3, Cardarelli Hospital, Naples, Italy; [2]Department of Clinical Medicine and Surgery, Federico II University Hospital, Naples, Italy

Background: Antiphospholipid syndrome (APS) is a

systemic autoimmune disorder characterized by venous and arterial thrombosis, recurrent abortion, and thrombocytopenia. Cardiac involvement in APS may be presented as right or left ventricular dysfunction, pulmonary hypertension, accelerated atherosclerosis, occlusive arterial disease (angina, myocardial infarction), valve abnormalities or less frequently as intracardial thrombosis and myxoma. Few cases of intracardiac thrombosis associated with primary APS are described and the management of these patients is challenging. We describe a case of intracardiac thrombosis in a young patient with primary APS. **Case Report**: A 28-year-old female patient was admitted to our hospital because of swelling and pain of the right leg. Venous Doppler ultrasound showed femoral, popliteal and great saphenous vein thrombosis. CT scan showed pulmonary embolism (right pulmonary artery). It was negative for active malignancy. Anticoagulant treatment with Fondaparinux (7.5 mg daily) was started. She had never smoked, did not take any medication, in particular oral contraceptives. She was not pregnant. Anticoagulant protein S, protein C, and Antithrombin were normal. Factor V Leiden and prothrombin G20210A mutation were negative. A prolonged activated partial thromboplastin time (aPTT) (Ratio 4.11, with normal values 0.7-1.3), along with a positive lupus anticoagulant and high titer of anticardiolipin (aCL) IgG antibodies at 44 GPL-U/mL (normal range <10 GPL-U/mL) and aβ2 glycoprotein I (aβ2GPI) IgG antibodies at 2038 U/mL (normal range ≤20 U/mL) suggested for APS. During hospitalization, the patient underwent a transthoracic echocardiogram that revealed the presence of a cardiac mass in the right atrium, mobile and attached to the interatrial septum. Differential diagnosis was between thrombi and myxoma. A comprehensive transesophageal echocardiographic examination and cardiac magnetic resonance imaging confirmed a thrombosis in right atrium. Consultation with cardiac surgeons ruled out surgical treatment. She was discharged with oral anticoagulant therapy with warfarin maintaining an international normalized ratio (INR) between 3 and 3,5. Repeated blood tests after 12 weeks confirmed the diagnosis of APS. At 3-6-12 months of follow-up echocardiograms showed a progressive reduction of thrombosis, without cardiac complication. Long-term treatment with warfarin and acetilsalicilic acid was recommended. **Conclusions**: Cardiac manifestations of APS may be associated with increased cardiovascular mortality. A prompt and correct identification of APS is needed.

PO097

D-DIMER LEVELS AFTER SARS-COV-2 VACCINATION CAN BE FALSELY ELEVATED WITHOUT MIRRORING TRUE HYPERCOAGULABILITY

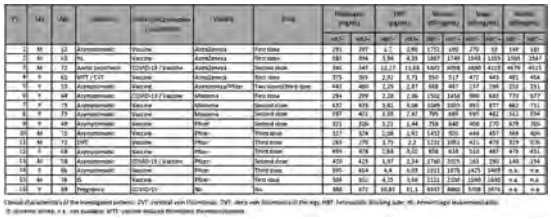
V. De Stefano[1], S. Lancellotti[2]

[1]*Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica, Fondazione Policlinico Universitario AGemelli IRCCS, Roma;* [2]*Servizio Malattie Emorragiche e Trombotiche, Fondazione Policlinico Universitario AGemelli IRCCS, Roma, Italy*

Background and Aims: Measurement of D-dimer (D-D) can vary according to different instruments and testing methods; moreover, results can be falsely increased by heterophilic antibodies, which exhibit multispecific reactivity. D-D is persistently increased in 15-20% of pts after COVID-19; conversely, D-D after SARS-CoV-2 vaccination was reported normal. We report a series of pts with D-D increased after SARS-CoV-2 vaccination. **Methods**: We investigated 16 pts (M/F 7/9, median age 64, range 32-76) referred for D-D >500 ng/ml (median 1502, range 550-5802) after SARS-CoV-2 vaccination and/or COVID-19 infection; in 11 D-D was >1000 ng/ml. Vaccination was adenoviral (Ad) vector-based in 4 pts, mRNA-based in 10, and heterologous Ad vector-/mRNA-based in 1. Three pts had COVID-19 before vaccination; one was pregnant and had COVID-19 without being vaccinated. Eleven pts were asymptomatic. Five have had vascular complications after vaccination; in 3 the association with the Pfizer vaccine was doubtful (deep vein thrombosis=2, ischemic stroke=1), whereas in 2 the association with the AstraZeneca vaccine was likely (hemorrhagic leukoencephalitis=1, vaccine-induced thrombotic thrombocytopenia with cerebral vein thrombosis=1) (Table 1). Three different assays measured D-D levels: Innovance (Siemens Healthineers, Erlangen, Germany), Liatest D-Di Plus (Stago, Asnières, France), and AcuStar D-D (Werfen, Milan, Italy). Fibrin and fibrinogen degradation products (FDPs) were measured by an immunoturbidimetric assay (Stago). All tests were performed in plasma samples not treated and treated with a heterophilic blocking tube reagent (HBT, Scantibodies Laboratory Inc, USA); moreover, the Liatest D-Di Plus declares a blocking agent in the buffer. **Results**: D-D using the Siemens assay with HBT diminished from a median of 1467 ng/mL (range 550-5802) to 1043 ng/mL(range 190-6098) (p=0.002). Only 2 pts had D-D <500 ng/mL after HBT (No. 1 and 5). The Stago assay induced a decrease of D-D in comparison with Siemens either without HBT (median 602 ng/mL, range 165-3880, p<0.0001 *vs.* Siemens HBT-) and with HBT (median 484 ng/mL, range 33-4219, p<0.0001 *vs.* Siemens HBT+). The Werfen assay showed in 13 pts a median D-D of 481 ng/mL (range 140-4679) without HBT (p=0.0009 *vs.* Siemens HBT-) and 454 ng/mL (range 154-4515) with HBT (p=0.01 *vs.* Siemens HBT+). The Stago and Werfen assays with no HBT showed D-D <500 ng/mL in 7/16 and 7/13 pts, respectively. HBT induced no difference between the values obtained by Stago (p=0.66) or Werfen (p=0.19). FDPs were normal (<5 µg/mL) in 14/16 pts; 9 had D-D <500 ng/mL using an HBT or a comparative system, and 5 had elevated D-D by all systems but without increased FDP. The remaining 2 pts had elevated D-D accompanied by FDPs double the normal (No. 3 and 16): both cases

had a clinical condition possibly associated with true hypercoagulability (aortic prosthesis in pt. No. 3 and pregnancy at term in pt. No. 16). Notably, the patient with VITT after adjustment for age had normal D-D and FDPs. **Conclusions**: D-D measurement can be not reliable after SARS-CoV-2 vaccination, and falsely elevated levels can be demonstrated in half of pts by blocking heterophilic antibodies. However, in all pts true hypercoagulability is not demonstrable. Such results should prompt to use of confirmatory laboratory systems to avoid undue anxiety and unuseful and costly examinations in asymptomatic pts with falsely elevated D-D after SARS-CoV-2 vaccination.

Table 1.



PO098

CAPLACIZUMAB FOR THE MANAGEMENT OF ACUTE IMMUNE-MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA: REAL WORLD DATA FROM THE MILAN TTP REGISTRY

P. Agosti[1], P. De Leo[2], M. Capecchi[2,3], A. Artoni[2], I. Mancini[1], M. Biganzoli[2], B. Ferrari[2], S. Gattillo[4], S.M. Trisolini[5], E. Rinaldi[6], G. Podda[7], L. Prezioso[8], S. Prassede[9], L. Facchini[10], D. Caramazza[11], G. Tolomelli[12], F. Peyvandi[1,2]

[1]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, and Fondazione Luigi Villa, Milan, Italy; [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; [3]Università degli Studi di Milano, Department of Biomedical Sciences for Health, Milan, Italy; [4]Immuno-Hematology and Transfusion Medicine Unit, San Raffaele Hospital, Milan, Italy; [5]Hematology, Department of Translational and Precision Medicine, "Sapienza" University of Rome, Rome, Italy; [6]Hematology Unit, APerrino Hospital, Brindisi, Italy; [7]U.OMedicina III, ASST Santi Paolo e Carlo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy; [8]Haematology and BMT Unit, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy; [9]Ospedale Civile "Spirito Santo," Pescara, Italy; [10]Hematology Unit, Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy; [11]Hematology, University Hospital "Ospedale di Circolo e Fondazione Macchi" - ASST Sette Laghi, Varese, Italy; [12]Unit of Hematology, Rimini Hospital "Infermi", Rimini, Italy

Background and Aims: Acute immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare but life-threatening thrombotic microangiopathy. In 2018 caplacizumab, an anti-von Willebrand factor nanobody, was approved by the European Medicines Agency for the treatment of acute iTTP. To date, data stemming from real-world experience are limited. This study aims to describe the efficacy and safety endpoints of caplacizumab treatment in Italian iTTP patients from a real-world clinical setting. **Methods**: In this observational retrospective study, 26 patients from the Milan TTP Registry receiving caplacizumab treatment for an acute iTTP episode between 2018 and August 2021 were enrolled. Clinical signs/symptoms at the time of onset were categorized as follows: systemic (fatigue, fever, abdominal pain, headache, vomiting, jaundice); neurological (ischemic stroke, transient ischemic attack, epileptic seizures, coma, personality disorders, focal neurological signs); hemorrhagic (skin bleeds, mucosal bleeds, hematuria, meno-metrorrhagia, gastrointestinal hemorrhages); cardiovascular (acute coronary syndrome, myocardial infarction, electrocardiographic ischemic changes); renal (acute renal failure, anuria, need for dialysis, emoglobinuria, proteinuria, oliguria). Clinical response was defined as the sustained normalization of platelet count (≥150,000/μL) for at least two consecutive days. Exacerbation was defined as a platelet count decrease (to less than 150,000/μL) occurring after a clinical response and before a clinical remission within 30 days from stopping PEX or caplacizumab. Partial ADAMTS13 remission was defined when ADAMTS13 activity was ≥20%. Total ADAMTS13 remission was defined as ADAMTS13 activity ≥45%. **Results**: The main patient characteristics and acute event features are shown in Table 1. All patients were treated with plasma-exchange (PEX), corticosteroids and caplacizumab, except for one patient with mild thrombocytopenia (113000/μL) treated with steroids and caplacizumab. Rituximab was used in 16/24 patients (66.7%). Caplacizumab was started a median of 1 day after PEX initiation (Table 1). Thirty percent of patients started caplacizumab 7 days or more after PEX start to treat refractory TTP or exacerbation. All patients achieved clinical remission. In patients treated with caplacizumab within 24 hours after PEX start, the median time to platelet count normalization was 3.5 days (3.0-4.3), with a median of 6 days of PEX (4.3-7.8) to achieve clinical response. One patient experienced acute TTP exacerbation 11 days after stopping caplacizumab. Two caplacizumab-related adverse events leading to drug discontinuation were observed, an episode of hemoptysis treated with a plasma-derived von Willebrand factor/factor VIII-containing concentrate and artery embolization, and a gastrointestinal bleeding requiring blood transfusion in a patient with several conditions predisposing to bleeding. **Conclusions**: With a median of 4 days to achieve platelet count normalization, caplacizumab proved effective and safe for the treatment of acute iTTP in the real-world clinical setting of the Milan TTP Registry, consistently with the results of randomized clinical trials.

Table 1.

patient characteristics, episode clinical manifestations and laboratory parameters	N=26
First TTP episode, n (%)	15 (57.7)
Age, median (IQR)	53.0 (47.3-61.5)
Female, n (%)	20 (76.9)
White ethnicity, n (%)	24 (92.3)
Platelet count/μL, median (IQR)	14,000 (9,500-23,500)
Hemoglobin, g/dl, median (IQR)	9.0 (7.6-10.9)
Creatinine, mg/dl, median (IQR)	1.05 (0.86-1.25)
LDH, IU/L, median (IQR)	1285 (456-2157)
Total bilirubin, mg/dl, median (IQR)	2.2 (1.5-3.5)
Systemic signs/symptoms, n (%)	21 (80.8)
Neurological signs/symptoms, n (%)	15 (57.7)
Hemorrhagic signs/symptoms, n (%)	12 (46.2)
Cardiovascular signs/symptoms, n (%)	5 (19.2)
Renal signs/symptoms, n (%)	4 (15.4)
caplacizumab-related features and clinical endpoints	
Time to receive the 1st dose of caplacizumab after PEX initiation, median (IQR), days	1 (0-8)
Lenght of caplacizumab treatment, median (IQR), days	30 (21-36)
Time to normalization of platelet count after caplacizumab initiation, median (IQR), days	4 (3-4)
Total days of PEX to achieve clinical response, median (IQR)	8 (5-15)
Number of days of hospitalization, median (IQR), days	16 (8-30)
Exacerbation, n (%)	1 (4.3)
Partial ADAMTS13 remission, n (%)	23 (92.0)
Total ADAMTS13 remission, n (%)	19 (79.2)

PO099

THE INCIDENCE AND RISK FACTORS OF THROMBOSIS IN AGGRESSIVE LYMPHOMA AND MYELOMA PATIENTS: A 6-YEAR SINGLE-CENTER EXPERIENCE

E. Morsia, E. Torre, F. Marrara, S. Morè, S. Cacciagiù, V.M. Manieri, G. Guidi, M. Offidani, A. Olivieri, S. Rupoli

Clinica di Ematologia, Ospedali Riuniti di Ancona,Italy

Background and Aims: The risk of venous thromboembolism (VTE) in patients with cancer is higher compared to healthy people. Recent studies demonstrated a high incidence of VTE in patients with hematologic malignancies as well as in patients with solid cancers. Although some, very well validated scores delineate the risk of VTE by cancer subtype and other risk factors, hematologic malignancies are less represented in these models. Moreover, this setting of patients represents a peculiar entity that undergoes therapy that can be thrombogenic. The aim of our study was to analyze VTE incidence and factors related to thrombosis risk, in a retrospective cohort of patients with aggressive lymphoma and multiple myeloma. **Methods**: We reviewed the medical records of 227 patients diagnosed with aggressive lymphoma and 231 patients with multiple myeloma who referred to our institute from January 2015-December 2020. Among patients with lymphoma, the most common type was diffuse large B-cell lymphoma (n=191), followed by mantle cell lymphoma (n=19), peripheral T cell lymphoma (n=10), Burkitt lymphoma (n=4), and primary mediastinal large B-cell lymphoma (n=3). **Results**: Among 227 patients with diagnosis of aggressive lymphoma, the incidence of VTE was 19.8% (n=45) in a median follow up of months 28.87 (range, 0.23-177.18). Only about half of patients (55.5%) were treated with thromboprophylaxis. VTE events occurred in the older patients (pV=0.029), with concomitant HIV infection (pV=0.031), and with spleen involvement (pV=0.022). At diagnosis, higher level of LDH (pV=0.042), leukocytosis (pV=0.004) and higher neutrophil-to-lymphocyte ratio (NLR) (pV=0.022) are risk factor of development of VTE. While the VTE did not significantly affect the overall survival, it caused increased morbidity with an incidence of recurrent VTE of 9%. During a follow-up period of 23.09 month (range,1.01-135.95), 46 of 231 patients (19.9%) with diagnosis of multiple myeloma experienced a VTE. Twenty-three (50%) events were TVP, followed by EP in 28% and T*VS* in 22% of patients. There was no effect of clinical and laboratory features at diagnosis on the occurrence of thrombosis, with the exception of older age (pV=0.027), bone disease (pV=0.002), and the presence of central venous catheter (pV<0.001). Interesting, treatment of myeloma patients with Lenalidomide-based therapy, Carfizomib-based therapy and monoclonal antibody-based therapy increase the risk of VTE (HR 2.55 (1.31-4.95), pV=0.005; HR 6.1 (2.8-13.6), pV<0.001; HR 2.36 (1.07-5.19), pV=0.037; respectively). **Conclusions**: VTE is a frequent complication in patients with aggressive lymphoma and multiple myeloma. In our cohort of aggressive lymphoma patients, the new inflammatory status markers NLR is an independent risk factor for VTE. However, in patients affected by multiple myeloma the therapeutic approach seems play a crucial role in development of thrombosis. Emerging clinical and therapeutic risk factors were found, but further studies are needed to develop a predictive model for identifying patients with lymphoma and myeloma who are at high risk for developing thrombosis and for planning a tailored prophylactic strategy.

PO100

COMPARISON OF STANDARD VS. LOW DOSE DIRECT ORAL ANTICOAGULANTS FOR THE SECONDARY PROPHYLAXIS OF VENOUS THROMBOEMBOLISM IN PATIENTS AFFECTED BY HEMATOLOGIC MALIGNANCIES

F. Malfona, A. Serrao, G.M. Assanto, S. Olivieri, S. Ligia, C. Santoro, A. Chistolini

Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy

Background: Venous thromboembolism (VTE) is one serious complication in patients with hematologic malignancies due to the release of procoagulant factors by neoplastic cells, central venous catheter and chemotherapy. Considering that hematologic malignancies represent a non-transient VTE risk factor, the treatment and secondary prophylaxis of VTE are an important step in the therapeutic approach of hematologic patients. Although direct oral anticoagulants (DOACs) demonstrated their efficacy and safety in the treatment of acute phase of VTE cancer related, there are few data in the setting of the secondary prophylaxis. **Aims**: The aim of our study was to evaluate the efficacy and safety of DOACs in the VTE secondary prophylaxis in hematologic patients, comparing full and low dose of DOACs. **Methods**: This retrospective monocentric study collected data of patients affected by hematologic malignancies with VTE (pulmonary embolism and deep vein thrombosis of typical sites) treated with DOACs. We divided the patients in 2 groups according to DOACs dose performed after the first 6 months of VTE treatment: one group continue with standard dose and the other group shifted to apixaban 2.5 BID or rivaroxaban 10 QD. **Results**: We studied 55 patients (Table 1). After a median time of 6 months from a first VTE, 29 patients continued with DOAC at full dose, 26 patients shifted to DOACs at reduced dose. After a median time of 24 months (range 19-32) no thrombotic recurrences were observed in the two groups. We reported 4 minor bleeding events: 3 in the group treated with full dose and 1 in the other group. We did not report any statistically significant differences in terms of DOACs efficacy and safety between the two groups. **Conclusions**: Our experience supports the use of DOACs also at reduced dosage in the VTE secondary prophylaxis in patients affected by hematologic malignancies.

Table 1. Patients' characteristics.

N of Patients	**55**	
M/F	33/22	
Median age (years)	68 (range 26-93)	
Event		
DVT	40	
PE	6	
DVT+PE	9	
Diagnosis	**Standard dose**	**low dose**
NHL	8	12
MPN	8	7
MM	6	4
HL	2	3
MDS	2	0
CLL	2	0
AML	1	0
DOAC		
Apixaban	12	14
Edoxaban	6	NA
Dabigratan	2	NA
Rivaroxaban	9	12

DVT: deep vein thrombosis, PE: pulmonary embolism, NHL: non Hodgkin lymphoma, HL: Hodgkin lymphoma, MPN: myeloproliferative neoplasm, MM: multiple myeloma, MDS: myelodysplastic syndrome, CLL: chronic lymphoid leukemia, AML: acute myeloid leukemia, DOAC: direct oral anticoagulants, NA: not applicable.

PO101

THE SAFETY AND EFFICACY OF DIRECT ORAL ANTICOAGULANTS FOR ATRIAL FIBRILLATION IN PATIENTS AFFECTED BY HEMATOLOGIC MALIGNANCIES: A SINGLE-CENTER EXPERIENCE

F. Malfona, A. Serrao, G.M. Assanto, S. Ligia, E. Baldacci, C. Santoro, G.O. Chavez, A. Chistolini

Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy

Background and Aims: Pre-existing atrial fibrillation (AF) is not uncommon in cancer patients receiving active therapy, and the risk of AF increases during the period adjacent to diagnosis and during tratment. Managing anticoagulation in onco-hematological patients with atrial fibrillation is a clinical challenge considering the risk of bleeding and thrombosis with limited data, mainly concerning low molecular weight heparin. The aim of our study was to evaluate the efficacy and safety of direct oral anticoagulants (DOACs) in patients affected by hematologic malignancies with AF. **Methods**: A single-center retrospective cohort study was performed by reviewing medical records of patients with a confirmed diagnosis of hematologic malignancy who presented a pre-existing AF or developed AF during the clinical course and treated with DOACs. Oral anticoagulation was continued until a platelet count ≥50x109/l. Results: We included 97 patients (Table 1). DOACs therapy was performed for a median time of 25 months (range 10-108 months). We did not observe any thrombotic adverse event during the follow-up. We observed 14 (14.4%) bleeding complications: 1 major, 12 clinically relevant non major and 1 minor hemorrhagic event. The major complication was a severe gastrointestinal bleeding in a patient affected by non-Hodgkin lymphoma during local disease progression: in this case, DOAC was definitively discontinued. The CRNM bleedings were 5 gastrointestinal haemorrhages and 7 hematomas: in three of the above cases DOAC was temporarily replaced with LMWH (median time 8 days) because of thrombocytopenia with a platelet count between 100X109/L and 50X109/L; in one case DOAC was definitively discontinued in one patient with a platelet count <20X109/L. The minor event was a mild epistaxis, for which no DOAC withdrawal was needed. In 9 cases DOAC was temporary discontinued for a median time of 21 days (range 5-28): 7 for severe transient thrombocytopenia and 2 for chemotherapy-related coagulopathy. Four/14 (28.6%) bleeding complications (1 major and 3 CRNM) were reported during the concomitant administration of BTK inhibitor ibrutinib. No statistically correlation was found between bleeding complications and sex (p=0.129), age (p=0.264), type of DOAC (p=0.527), hematologic disease (p=0.729) stage of the hematologic disease (p=0.405). Conclusions: Our study evaluated the efficacy and safety of DOACs in patients with AF and

hematologic neoplasms. In this setting, in light of the recognized increase of bleeding risk for concomitant thrombocytopenia, coagulopathy and cancer treatment, clinical surveillance is required even in patients at high thromboembolic risk. In our study, we confirm the higher incidence of hemorrhagic adverse events *versus* thrombotic complications. At univariate analysis, none of the variables evaluated were statistically significant, even considering the different type of anticancer therapies: these results could be related to our small sample size. In patients with hematologic malignancies and AF, specific monitoring modalities should be considered. In addition to careful clinical examination for bleeding signs and regular monitoring of liver and renal function, repetitive full blood counts should be evaluated. Our data support the efficacy of DOACs in the treatment of AF in patients with hematologic malignancies but suggest caution in case of thrombocytopenia and disease progression.

Table 1. Patients' characteristics.

NO PATIENTS	97
FOLLOW-UP (MEDIAN, MONTHS)	25 (range 10-108)
M/F	59/38
MEDIAN AGE (RANGE)	77 (34-92)
<=65Y	28
>65Y	69
DIAGNOSIS	
MPN/CLL	41
LYMPHOMA (NHL, HL)	39
MDS	9
ACUTE LEUKEMIA (AML, ALL)	8
LABORATORY PARAMETERS AT DOAC START (RANGE)	
HB (MG/DL)	12.6 (8-18)
PLT (10^9)	269 (38-730)
CREATININE	1 (0.5-1.6)
DOAC	
APIXABAN	30
EDOXABAN	30
RIVAROXABAN	29
DABIGATRAN	8
THERAPY	
CHEMO-IMMUNOTHERAPY (R-CHOP; R-BENDA)	12
IMMUNOTHERAPY (R MAINTANCE)	28
AZACITIDINE	4
VENETOCLAX	2
OTHERS (LENA; HU; CLO)	26
TKI	8
IBRUTINIB	13
RUXOLITINIB	3
DISEASE PHASE	
CHRONIC/STABLE	71
COMPLETE REMISSION	3
ACTIVE	22
THROMBOTIC EVENTS (PTS)	0 (0)
HEMORRHAGIC EVENTS (PTS)	14 (14)
CHADS2-VASC2 DIAGNOSIS <2	2
HAS-BLED DIAGNOSIS ≥3	1
FULL DOSE DOAC AT THE TIME OF EVENT	6
REDUCED DOSE DOAC AT THE TIME OF EVENT	8
DOSE REDUCTION AFTER THE EVENT	2

MPN: mveolooroliferative neoplasms; CLL: chronic lymphocytic leukemia; NHL: non Hodgkin lymphoma; HL: Hodgkin lymphoma; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; HB: hemoglnhin; PLT: Platelet count; DOAC: direct oral anticoagulants; TKI: tyrosine kinase inhibitors; HU: hydroxyurea; CLO: chlorambucile

PO102

THE CLINICAL PATTERNS AND ETIOLOGICAL FEATURES OF CEREBRAL VEIN THROMBOSIS IN SINGLE-CENTER STUDY

E. Torre[1], E. Morsia[1], F. Marrara[1], A. Fiorentini[1], A.R. Scortechini[1], M. Silvestrini[2], L. Da Lio[3], A. Olivieri[1], S. Rupoli[1]

[1]Clinica di Ematologia, Ospedali Riuniti di Ancona; [2]Clinica Neurologica, Ospedali Riuniti di Ancona; [3]Medicina di Laboratorio, Ospedali Riuniti Ancona, Italy

Background and Aims: Cerebral vein thrombosis (CVT), including thrombosis of cerebral veins and major dural sinuses, is a rare disorder accounting for <1% of all strokes. It is three times more common in younger women as compared to men. CVT can appear with a variety of symptoms including neurological deficits and can progress to hemorrhage. Patients affected by myeloproliferative neoplasms (MPNs) including polycythemia vera, essential thrombocythemia, and primary myelofibrosis are prone to develop thrombosis, especially in unusual sites, either at diagnosis or during follow-up. In these patients the prevalence of CVT is 1% and can be the first clinical manifestation. The aim of this study is to analyze, in a retrospective cohort of CVT patients, the prevalence, clinical features and risk factors for thrombosis. Additionally, this study shows the clinical outcome of CVT, with a specific focus on CVT in MPN patients. **Methods**: The patient cohort included 38 patients of our center who experienced CVT during a 20-year period (January 2002-December 2021). Diagnosis of CVT was established with CT or MRI. MPN are defined according to WHO 2016 criteria. Data regarding demographic, clinical and radiological features were retrospectively collected from medical records. **Results**: Our cohort includes 38 patients with CVT (median age 42 years, range 20-79; 65% females) in median follow up of 21.35 months (range, 9,13-255,38). Comprehensive thrombophilia testing was performed in most cases (94%), showing presence of prothrombin G20210A (10.5%), factor V Leiden (7.9%), and hyperhomocysteinemia (26.3%) as the three most common abnormalities. Other risk factors observed were oral contraceptive and mastoiditis in 31.6% and 15.8% of patients, respectively. Notably, one patient received the ChAdOx1-S vaccine 10 days prior to CVT. VTE events occurred most frequently in multiple sites, involving transverse, sigmoid and sagittal sinuses in 65% of cases. The most common presenting symptom of CVT was headache (81.6%) followed by visual loss (36.8%). Post diagnosis, 34 patients received antithrombotic treatment with vitamin K antagonists (VKA). Twenty-five patients (66%) achieved CVT recanalization within a median time of 5.6 months from the appearance of the thrombosis and 5 patients (13%) had recurrent venous thrombosis. However, CNS hemorrhage occurred in three patients (7.9%) and menorrhagia in one. In total, 6 patients with CVT (median age 34 years, range 21-50) were also diagnosed with MPN JAK2 mutated, which in half cases occurred simultaneously with the thrombosis. All these patients were treated with hydroxyurea plus VKA. Two patients had prior VTE in median 11.5 years before MPN while in two patients a recurrent event occurred. Except for smoke (pV=0.048) and provoked thrombosis due to mastoiditis (pV=0.046), no significant difference was found in term of thrombophilia abnormalities and other risk factors for thrombosis comparing CVT with and without MPN. Moreover, patients with MPN and CVT presented at time of thrombosis with higher

platelet (pV=0.001) and hematocrit (pV=0.022) levels, compared to CVT patients without MPN. Finally, within this setting of patients factor V Leiden and prothrombin G20210A were absent. **Conclusions**: Our data suggest the clinical importance of ensuring, using JAK2 mutation screening, the absence of underlying MPN especially in young patients with unprovoked CVT (without smoke attitude) and blood tests abnormalities.

PO103

THROMBOSIS DURING ANTICOAGULANT THERAPY. THINK OUTSIDE THE BOX

D. Tonello, L. Filippi, L. Cecchetto, M. Marchetti

UOC Medicina Generale, Ospedale Alto Vicentino, Azienda Ulss 7-Pedemontana (VI), Regione Veneto, Italy

Male, 76-year-old, in November 2016 develops unprovoked common femoral DVT during therapy with apixaban for atrial fibrillation. Blood tests, neoplastic markers and thrombophilia are negative. CT scans and endoscopic tests are negative for occult neoplasms. Subsequent checks show no recanalization of DVT. Edema in the lower limb does not regress. In November 2018, thrombosis shows expansive growth and internal vascularity. MR angiography and PET-scan confirm a neoplastic intravascular lesion. The histology concludes for a venous leiomyosarcoma. Male, 64 years old, in August 2020, develops unprovoked iliac-femoral DVT. Blood tests, neoplastic markers and thrombophilia are negative. CT scan excludes occult neoplasms or pulmonary embolism. Discharged on rivaroxaban therapy. In May 2021 there is no recanalization of DVT, but sharp increase in the edema of the thigh which poses clinical suspicion of recurrence of thrombosis. Blood tests, neoplastic markers, thrombophilia again negative. New CT scans are unchanged. Endoscopic tests for cancer are negative. In June 2021 thrombosis shows expansive growth and arterial vascularization. MRI and PET scan confirm the suspicion of intravascular neoplasia. Histological examination diagnoses a synovial sarcoma. **Conclusions**: Intravascular malignant tumors can mimic a DVT. Expansive growth, arterial vascularization and resistance to anticoagulant therapy can guide us in the diagnostic suspicion.

PO104

ORAL ANTICOAGULANT THERAPY IN ATRIAL FIBRILLATION. ANALYSIS OF THE DOSING APPROPRIATENESS OF DIRECT ORAL ANTICOAGULANTS FROM THE START2 REGISTRY

L. Ciucciarelli[1], E. Antonucci[2], F. Crudele[3], M. Berteotti[3], S. Testa[4], A. Toma[5], L. Masciocco[6], D. Pastori[7], P. Pignatelli[7], G. Palareti[2], R. Marcucci[3], D. Poli[8]

[1]Medicina Interna, Ospedale di Borgo San Lorenzo, Firenze; [2]Fondazione Arianna Anticoagulazione, Bologna; [3]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze; [4]Dipartimento di Medicina di Laboratorio, Centro Emostasi e trombosi, ASST Cremona, Cremona; [5]UO Laboratorio Analisi Distretto Ovest, ULSS 8Berica, Arzignano (VI); [6]UO Medicina Interna, Presidio Ospedaliero Francesco Lastaria di Lucera - ASL Foggia, Lucera (FG); [7]Department of Clinical, Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Roma; [8]SOD Malattie Aterotrombotiche, Azienda Ospedaliero Universitaria-Careggi, Firenze, Italy

Background and Aims: Direct oral anticoagulants (DOACs) have been marketed in 2 doses. When used for stroke prevention in atrial fibrillation (AF) the dosage of each drug should be chosen following the European Medicines Agency indications. However, inappropriate dose prescrition have been reported with a frequency ranging from 10% to 57% of cases. The purpose of the study is to evaluate the appropriateness of the DOACs dosage and the occurrence of ischemic and haemorrhagic events and death in relation to dose prescription in AF patients enrolled in the observational, prospective START2-Register – Survey on anTicoagulated pAtients RegisTer – an independent,, ongoing database aimed at recording the history of patients starting anticoagulant treatment whatever the indication and the drug. **Methods:** We investigated the clinical characteristics of patients receiving therapy with full, appropriate and inappropriate dosing, and with reduced, appropriate and inappropriate dosing for all the available drugs. Thrombotic events, major and clinically relevant not major bleeding events and death occurred in the follow-up were recorded. **Results**: The population cohort included 2106 patients treated with DOACs, 53.5% of them were on full dose. Compared to patients on full dose, patients on low dose have a higher frailty profile, with increased number of associated diseases and more were frequently treated with associated antiplatelet drugs. The full dose is appropriate in 98.1% of cases while the low dose is appropriate in 63.1% of cases. Inappropriated reduced dose prescrition was found in 19.7% of patients on edoxaban, 20.1% of patients on dabigatran, 39.7% of patients on rivaroxaban, and 63.1% of patients on apixaban. Instead, the appropriateness of full dose prescription was similar between the different drugs. In the follow-up there is no statistically significant difference between thrombotic and haemorrhagic events in patients treated with approprited or not approprited full and low dose. Instead, patients treated with inappropriate dosage showed higher mortality rate [Relative Risk 1.6 (1.1-2.3), p=0.01]. **Conclusions**: In clinical practice, the use of off-label dosing DOACs is quite common, especially for low doses. We found that AF patients treated with off-label low doses of DOACs have an increase in mortality rate. Instead, on the contrary of what expected, the wide use of low dose DOACs is not associated with an increase in thrombotic events, nor in a reduction of bleeding events.

PO105

LOW DOSE RIVAROXABAN IN PATIENTS WITH CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

T. Bucci[1], F. Del Sole[2], G. Galardo[1], F.G. Biccirè[1], A. Farcomeni[3], P. Pignatelli[2], D. Pastori[2]

[1]Department of General Surgery, Surgical Specialties and Organ Transplantation "Paride Stefanini," Sapienza University of Rome, Rome, Italy; [2]Department of Clinical, Internal Medicine, Anaesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy; [3]Department of Economics and Finance, University of Rome "Tor Vergata", Rome, Italy

Background: Low-dose rivaroxaban (LDR) has been recently approved as secondary prevention strategy in patients with coronary artery disease (CAD) and peripheral artery disease (PAD). Pooled data on the risk-benefit profile of using LDR in these patients are not available. **Methods**: We performed a systematic review and meta-analysis of randomized controlled trials (RCT) including CAD or PAD patients treated with LDR. Studies were divided into three groups:1)LDR 2,5 mg bid+aspirin (ASA) or P2Y12 inhibitor *vs* ASA and/or P2Y12 inhibitor,2)LDR 2,5 mg bid+ASA *vs* ASA,3)LDR 5 mg bid+ASA *vs* ASA. Cardiovascular events (CVEs) and major bleeding were the endpoints. Number needed to treat (NNT) and number needed to harm (NNH) were also calculated. **Results**: We included a total of 59,745 patients. Overall, 5,060 CVEs were registered. 7 RCT on LDR were identified. LDR 2,5 mg bid was associated with a reduction of CVEs (HR:0.84,95%CI:0.77-0.92) with a higher major bleeding risk (HR:1.27,95%CI:1.09-1.47). When we analysed only the RCT with LDR + ASA, the LDR 2,5 mg twice was associated with a greater reduction of CVEs (HR:0.79,95%CI:0.71-0.88) while the 5 mg twice was not (HR:0.88,95%CI:0.76-1.02). Major bleedings were associated with both 2,5 mg twice (HR:1.58,95%CI:1.32-1.89) and 5 mg twice (HR:1.4,95%CI:1.20-1.86). The NNT to prevent one CVE for LDR 2,5 mg bid was 66(49-122) and the NNH to prevent major bleeding was 114(-375-751). **Conclusions**: LDR 2,5 mg bid has a favourable risk-benefit profile in patients with CAD/PAD. A careful selection of patients to treat with LDR is needed to reduce the risk of major bleeding.

PO106

CLINICAL HISTORY OF PATIENTS WITH LEFT VENTRICULAR THROMBOSIS RECEIVING VITAMIN K ANTAGONISTS

E. Valeriani[1], T. Bucci[2], F. Baratta[3], D. Menichelli[3], F. Del Sole[3], D. Pastori[4], P. Pignatelli[3]

[1]Azienda Ospedaliero-Universitaria Policlinico Umberto 1, Roma; [2]Department of General Surgery, Surgical Specialties and Organ Transplantation "Paride Stefanini," Sapienza University, Rome; [3]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University, Rome; [4]Department of Clinical, Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University, Rome, ITALY

Background: Left ventricular thrombosis (LVT) is a life-threatening condition responsible for a high risk of stroke and systemic embolism. The aim of our study is to evaluate the effectiveness and safety of vitamin k antagonists (VKAs) in patients with LVT. **Methods**: All consecutive patients were retrospectively included from January 2013 through January 2021 if they received VKAs for at least three months. Exclusion criteria comprehended different treatment regimens and unavailability of relevant data. Primary outcomes included on-treatment thrombus resolution and major bleedings while secondary outcomes on-treatment acute ischemic stroke, acute peripheral embolism, acute myocardial infarction, and clinically relevant non-major bleedings. Follow-up time was calculated from LVT diagnosis up to outcomes development or 12-months follow-up, whichever came first. The frequency of thrombus resolution was expressed as cumulative incidence with 95% confidence intervals (CIs) while the frequency of other outcomes was descriptively reported. **Results**: A total of 21 patients were included in the analysis. Baseline patients' characteristics were reported in Table 1. Median age was 73 year and 90.5% of patients were male. Risk factor for LVT was acute myocardial infarction in 95.2% and primitive dilated myocardiopathy in 4.8% of patients. All patients received warfarin with a median time in therapeutic range (TTR) of 59% and 52.4% of patients received concomitant dual antiplatelet therapy. Cumulative incidences of complete thrombus resolution at 3-months and 12-months were 42.9% (95% CI, 21.3% to 62.9%) and 57.1% (95% CI, 32.8% to 75.5%), respectively (Figure 1). Between patients with thrombus resolution and thrombus persistence, no significative difference was noted about underlying cardiomyopathy (*i.e.*, acute myocardial infarction, primitive dilated cardiomyopathy), comorbidities (*i.e.* atrial fibrillation, diabetes mellitus, smoking history, prior acute ischemic stroke or peripheral arterial disease), and treatment characteristics (*i.e.*, median TTR and concomitant dual antiplatelet therapy). While no patients developed on-treatment acute ischemic stroke, acute peripheral embolism, and acute myocardial infarction, two patients (9.5%) developed an on-treatment major gastrointestinal bleeding (one in the upper and one in the lower gastrointestinal tract) and one patient (4.8%) developed an on-treatment genitourinary clinically relevant non-major bleeding. All bleeding events occurred within the first three months of therapy. **Conclusions**: Despite suboptimal TTR values, the cumulative incidence of thrombus resolution during the three recommended months of VKA therapy was roughly 40% and rose to roughly 60% if anticoagulant therapy lasted for 12-months. Furthermore, AVK thera-

py protected patients against cardioembolic events and is associated with an acceptably low rate of major and clinically relevant non-major bleeding.

Tabella 1.

Baseline patients' characteristics

Variables	Overall population N = 21	Thrombus persistence N = 10	Thrombus resolution N = 11	p-value
Male sex, n (%)	19 (90.5)	8 (80.0)	11 (100.0)	0.415
Median age (IQR)	73.50 [63.75, 79.75]	72.00 [61.00, 75.00]	75.00 [68.00, 80.50]	0.470
Risk factors				
Acute myocardial infarction, n (%)	20 (95.2)	10 (100.0)	10 (90.9)	1.000
Primitive dilated cardiomyopathy, n (%)	1 (4.8)	0	1 (9.1)	1.000
Primitive hypertrophic cardiomyopathy, n (%)	0	0	0	NA
Reduced ejection fraction, n (%)	11 (52.4)	5 (50.0)	6 (54.5)	1.000
Comorbidities				
Smoking history, n (%)				0.278
No	7 (33.3)	5 (50.0)	2 (18.2)	
Actual	2 (9.5)	1 (10.0)	1 (9.1)	
Former	12 (57.1)	4 (40.0)	8 (72.7)	
Diabetes mellitus, n (%)	5 (23.8)	2 (20.0)	3 (27.3)	1.000
Atrial fibrillation, n (%)	4 (19.0)	2 (20.0)	2 (18.2)	1.000
Prior Acute ischemic stroke, n (%)	4 (19.0)	2 (20.0)	2 (18.2)	1.000
Peripheral arterial disease, n (%)	0	0	0	NA
Treatment characteristics				
Median TTR [IQR]	59.00 [51.00, 70.00]	56.00 [47.25, 63.75]	59.00 [57.00, 75.50]	0.245
Concomitant dual antiplatelet therapy, n (%)	11 (52.4)	5 (50.0)	6 (54.5)	1.000
Outcomes of interest, n (%)				
Acute ischemic stroke	0	0	0	NA
Acute peripheral embolism	0	0	0	NA
Acute myocardial infarction	0	0	0	NA
Major bleeding	2 (9.5)	0	2 (18.2)	0.501
Clinically relevant non-major bleeding	1 (4.8)	1 (10.0)	0	1.000

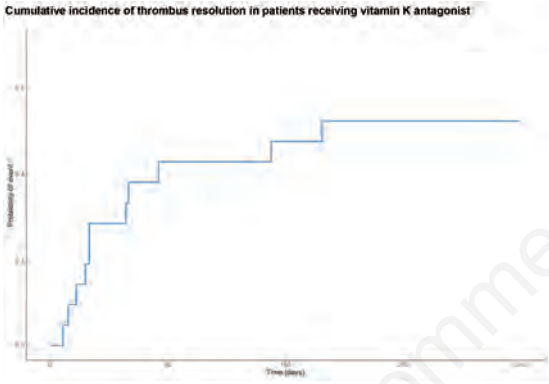


Figura 1.

PO107

ENDOSCOPIC FINDINGS OF PATIENTS WITH DOACS-ASSOCIATED GASTROINTESTINAL BLEEDING: RESULTS FROM PROSPECTIVE COHORT STUDY

M. Verso[1], O. Morelli[2], D. Castellani[2], G. Bassotti[2], A. Vinci[1], C. Becattini[1]

[1]Emergency and Vascular Medicine-Stroke Unit, University of Perugia; [2]Gastroenterology Division, University of Perugia, Italy

Background and Aims: Gastrointestinal bleedings (GIBs) are clinically relevant complications in patients receiving long-term therapy with DOACs. The aim of the study was to describe the endoscopic characteristics of DOACs-related GIB. **Methods**: A prospective cohort study of patients with DOACs-related GIBs was performed. All endoscopic lesions were adjudicated for the probability to be the responsible lesion of GIB, using a modified Saurin classification, by two independent gastroenterologists. **Results**: From November 2013 to March 2022, 208 patients (121 male), consecutively admitted to Santa Maria della Misericordia Hospital in Perugia for spontaneous DOACs-related GIBs were included. At entry, the mean patient age was 83±4 years (range 51 –95 years). 63 (30.1%%) patients presented GIB on dabigatran, 73 (35.1%) on rivaroxaban, 48 (23.1%) on apixaban and 24 (11.5%) on edoxaban. In AF patients, median CHA2DS2VASC was 4.5 and median HAS-BLED was 2.6. According with ISTH criteria, GIB was classified as major in 73.1% of patients. At entry, mean Hb value was 8.8 gr per deciliter, mean creatinine value was 1.47 mg per deciliter. The site of bleeding was in upper GI tract in 79 patients (42.5%), in lower GI tract in 65 patients (34.9%), in both in 42 patients (22.6%). In 10.6%, the endoscopic procedures were not performed or were not able to detect a source of bleeding. 58.7% of endoscopic lesions identified in upper gastrointestinal tract and 68.2% of endoscopic lesions identified in lower gastrointestinal tract were adjudicated as with high probability for being potentially bleeding lesions (of which 27.6% of cases with evidence of active bleeding or clot). A systemic reversal of anticoagulation was performed in 13.5% patients. The endoscopic procedures were performed urgently in 52.0% of cases (of which 73.8% were in upper GI). Fifty-four patients (85% of patients with evidence of active bleeding or clot) received an urgent local haemostasis. **Conclusions**: In this prospective cohort study, the upper GIBs were more frequent. In about 65% of cases endoscopic procedures were able to identify lesions with high probability to be responsible of GIB and 25.9% of patients receiving an urgent local haemostasis.

PO108

FCSAPP: A STRATEGIC NETWORK FOR A CLOSE CONNECTION BETWEEN ANTICOAGULATION CLINICS

E. Antonucci[1], S. Testa[2], D. Barcellona[3], P. Bucciarelli[4], A. Ciampa[5], E. Grandone[6,7], G. Malcangi[8], G. Rescigno[9], A. Squizzato[10], V. Toschi[11], D. Poli[12]

[1]Fondazione Arianna Anticoagulazione, Bologna; [2]Dipartimento di Medicina di Laboratorio, Centro Emostasi e trombosi, ASST Cremona, Cremona; [3]Dipartimento di Scienze Mediche e Sanità Pubblica, Unità Semplice Dipartimentale di Emostasi e Trombosi Azienda Ospedaliero-Universitaria di Cagliari, Cagliari; [4]Centro Emofilia e Trombosi "Angelo Bianchi Bonomi" UOC Medicina Generale Emostasi e Trombosi Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano; [5]UOSD Centro Emostasi AORN SGMoscati, Avellino; [6]IRCCS Casa Sollievo della Sofferenza, Poliambulatorio Giovanni Paolo II, Viale Padre Pio, San Giovanni Rotondo (FG); [7]Dipartimento

di Scienze Mediche e chirurgiche, Università degli Studi di Foggia, Foggia; [8]Unità Operativa Semplice a Valenza Dipartimentale Centro Emofilia e Trombosi Azienda Ospedaliero-Universitaria Policlinico di Bari, Bari; [9]Centro di Emostasi e Trombosi per la Diagnosi e la Cura delle Malattie Tromboemboliche ed Emorragiche DEA PP.OO "Umberto I-A. Tortora-M. Scarlato", ASL Salerno, Nocera Inferiore (SA); [10]Centro di Ricerca Malattie Tromboemboliche e Terapie Antitrombotiche Università dell'Insubria, ASST Lariana, Ospedale Sant'Anna, San Fermo della Battaglia (Como); [11]SIMT e Centro Trombosi ASST Santi Paolo e Carlo, Milano; [12]SOD Malattie Aterotrombotiche, Azienda Ospedaliero Universitaria-Careggi, Firenze, Italy

Background and Aims: In the recent decades, the widespread adoption of smartphones and digital innovations, has greatly changed everyday life and consumer behavior in many ways. In addition, the widespread internet connectivity led to a significant change in human-technology interaction. Mobile health apps and medical apps are becoming increasingly popular in a wide range of health-related applications in almost all sectors of healthcare. The purpose of FCSApp is to provide to physicians who referred to Italian Federation of Centers for the Diagnosis of Thrombotic Disorders and the Surveillance of the Antithrombotic Therapies (FCSA) a tool to actively share clinical suggestions, comments, scientific information helpful for everyday practice. **Methods**: FCSApp is developed by Prospero Multilab and is active from October 2021. The app is available for iOS and Android, it is free, and it works as an interactive chat. To access each physician member of FCSA must be register and create an account; after login each participant can follow and actively participate to the "discussion channels" that meet his/her field of interest. In FCSApp there is a section dedicated to news and communications from the secretary office. Three Institutional channel (FCSA Board, Regional FCSA Representative and FCSApp board) were created to allow a fast communication among members of each group. **Results**: From October 2021 about 200 physicians have signed up; 10 channels have been created. More than 30 clinical cases have been discussed among users. All cases presented in the chat reported specific clinical situation on patients generally excluded from clinical trial; 20 articles on hemostasis and thrombosis have been shared. **Conclusions**: FCSApp is a social networking app aimed to facilitate interaction, research and collaboration between FCSA members. Users demonstrate to be interested mainly in the discussion of the clinical problems from their own practice.

PO109

EMPLOYMENT OF DIRECT ORAL ANTICOAGULANTS IN Β-THALASSEMIA PATIENTS

S. Raso, L. Pitrolo, G. Calvaruso, A. Giangreco, R. Di Maggio, D. Renda, A. Vitrano, G. Carmina, A. Maggio

Department of Hematology and Rare Diseases, Azienda Ospedaliera Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy

Patients (PTs) with β-thalassemia (β-T) show a chronic hypercoagulability state due to numerous factors including iron overload, splenectomy and hemolysis. 1 The increased thrombotic risk requires often the use of antiplatelet and/or anticoagulant agents as primary and/or secondary prophylaxis. Despite the extensive use of direct oral anticoagulants (DOACs) during the past years, there is limited experience regarding the use of DOAC in PTs with β-T. The studies on DOACs efficacy and safety have not been conducted in PTs with β-T, however, they may represent a valuable alternative, given the reduced half-life, decreased bleeding risk and incidence of venous thromboembolism (VTE) recurrences and/or stroke respect to Vitamin K antagonist (VKA). Only one study tested the effectiveness of rivaroxaban in a very small group of PTs with β-T and non-valvular atrial fibrillation (NVAF).2 We herein present our experience with the use of DOACs in 17 PTs with β-T: 9 with NVAF and 8 with history of deep vein thrombosis and pulmonary embolism. The characteristics of PTs are reported in Table 1.

Table 1.

Characteristics of patients who received DOACs

Patients' characteristics	n=17
Age (median, IQR) years	49 (42-55)
Sex M/F	8/9
βTM / βTI	9/8
Time of follow-up (median,IQR) months	36 (16-51)
Laboratory parameters (median, IQR)	
- Hb (g/dl)	9.3 (9.0-9.6)
- Wbc (x 10^3/L)	10 (5.5-14)
- Plt (x 10^3/L)	496(293-573)
- Creatinine (mg/dl)	0.77 (0.59-0.83)
- GOT/GPT (UI/L)	23 (14-33)/22(19-35)
- Bilirubin total (mg/dl)	2.4 (1.8-3.1)
Comorbidity (n°/%)	
- Hypertension	5 (29)
- Cardiopathy	2 (11)
- Diabetes	4 (23)
- Renal impairment	1 (5)
- Respiratory disease	1 (5)
- History of neoplasm	2 (11)
- Pulmonary hypertension	6 (35)
- Hemocromatosis	15 (88)
- Liver disease	8 (47)
βT therapy (n°/%)	
- Transfusion	12 (70)
- HU	5 (29)
- ICT	12 (70)
Splenectomy	8 (47)
Anti-platelet therapy (n°/%)	5 (29)
NVAF (n°/%)	9 (52)
VTE (n°/%)	8 (47)
- DVT	3 (37)
- PE	5 (62)
Type of DOACS (n°/%)	
- Rivaroxaban	8 (47)
- Edoxaban	4 (23)
- Dabigatran	0
- Apixaban	5 (29)

Abbreviation: βTM: β-thalassemia major; β-thalassemia intermedia; Hb: hemoglobin; Wbc white blood cell; Plt: platelets; ICT: iron chlation therapy; HU:hydroxyurea; NVAF:non-valvular atrial fibrillation; VTE; venous thromboembolism. DVT: deep vein thrombosis; PE: pulmonary embolism.

Data was obtained from review of electronic health records. The median time of treatment was 36 months (IQR, 16-51) and during the follow-up 3 (18%) PTs developed superficial vein thrombosis and 1 (5%) intestinal ischemia while none experienced major bleeding, including PTs treated also with antiplatelet drug. Thrombosis and thromboembolic events were treated with low-molecular-weight-heparin (LMWH). Three of 4 PTs showed increased platelet count at the moment of thrombotic episode. None of PTs with thrombotic complications was switched to another DOAC. Seven out of 17 PTs started DOACs as frontline anticoagulant treatment and 10/17 PTs were shifted from a previous VKA treatment. Rivaroxaban was the drug most often used while dabigatran was unemployed. Frequently PTs with β-T present liver and kidney damage, due both to course of the disease and to hemosiderosis. Therefore, using DOACs could be challenging, as they have been reported to cause hepatotoxicity. However, in our experience there were no adverse events reported and no need of dose modification in any PT. In conclusions, in our experience anticoagulant therapy with DOACs in PTs with βT is well tolerated, apparently safe, without recurrences of thrombotic events and significant bleeding complications. Further studies are therefore needed in order to assess the role of DOACs in PTs with β-T.

PO110

STABILITY OVER TIME OF DIRECT ORAL ANTICOAGULANTS

D. Barcellona[1], M.F. Ruberto[1], A. Mameli[2], P. Schirru[2], S. Cornacchini[2], F. Marongiu[1]

[1]Department of Medical Sciences and Public Health, University of Cagliari; [2]Azienda Ospedaliero-Universitaria di Cagliari, Italy

Background and Aims: Direct Oral Anticoagulants (DOAC) are the preferred drugs for treatment and prophylaxis of venous thromboembolism and atrial fibrillation. It is known that a high variability does exist. The aim was to investigate whether the DOAC plasma levels were stable during a long follow up (six months). D-Dimer (DD) was also detected as an indicator of hypercoagulability. **Methods**: A total of 126 patients treated as follows: 52 with Rivaroxaban 20 mg/qd, 53 with Apixaban 5 mg/bid and 21 with Dabigatran 150 mg/bid. Dosages of DOAC were carried out at trough and after 2h (peak). Anti Xa and direct thrombin inhibitor (Hemosil Werfen) assays were used for measuring drugs plasma levels. DD was detected by HS HemosIL (Werfen). All dosages were carried out at the first, third and sixth month of treatment. Healthy controls were 55. **Results**: ANOVA for repeated measures showed no statistical differences among the three different DOAC plasma levels both at trough and peak (Table 1). ANOVA (Kruskal-Wallis) sowed that DD levels (trough and peak) were not different among the dosages and controls during the follow-up of Rivaroxaban (p>0.05) while those of Apixaban were not different at trough (p>0.05) but all lower than controls at peak (p=0,019). Finally, DD levels during dabigatran treatment were lower than controls both at trough and peak (p=0.004 and 0.0016 respectively). **Conclusions**: Plasma levels of DOAC are stable over time. No signs of hypercoagulability were found during the entire follow-up. These findings are reassuring in the long-term treatment with these drugs.

Table 1.

Drugs	Parameters	Mean	95% CI	p
Dabigatran (Pradaxa®) n=21	dTT trough 1st M	75.0	54.5 - 95.4	ns
	dTT trough 3rd M	82.7	63.4 - 102.1	
	dTT trough 6th M	93.4	71.2 - 115.6	
	dTT peak 1st M	218.4	166.2 - 270.6	ns
	dTT peak 3rd M	199.4	156.9 - 241.8	
	dTT peak 6th M	208.7	162.0 - 255.5	
Apixaban (Eliquis®) n=53	anti-Xa trough 1st M	115.7	100.3 - 131.2	ns
	anti-Xa trough 3rd M	115.3	98.5 - 132.1	
	anti-Xa trough 6th M	128.2	112.1 - 144.4	
	anti-Xa peak 1st M	240.2	215.4 - 265.0	ns
	anti-Xa peak 3rd M	233.3	205.8 - 260.8	
	anti-Xa peak 6th M	241.7	218.4 - 265.0	
Rivaroxaban (Xarelto®) n=52	anti-Xa trough 1st M	17.5	10.8 - 24.2	ns
	anti-Xa trough 3rd M	13.4	10.2 - 16.5	
	anti-Xa trough 6th M	16.8	12.9 - 20.8	
	anti-Xa peak 1st M	194.2	169.6 - 218.8	ns
	anti-Xa peak 3rd M	186.4	166.6 - 206.2	
	Anti-Xa peak 6th M	195.5	173.7 - 217.3	

PO111

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) IN THROMBOTIC PRIMARY ANTIPHOSPHOLIPID SYNDROME. THE MULTICENTER ATHERO-APS STUDY

T. Bucci[1], P.R.J. Ames[2,3], V. Cammisotto[4], S. Bartimoccia[4], M. Triggiani[5], R. Parente[5], A. Ciampa[6], P. Pignatelli[4], R. Carnevale[7], D. Pastori[4]

[1]Department of General Surgery and Surgical Specialties "Paride Stefanini", Sapienza University of Rome, Rome, Italy; [2]Immune Response and Vascular Disease Unit, CEDOC, Nova University Lisbon, Rua

Camara Pestana, Lisbon, Portugal; [3]Department of Haematology, Dumfries Royal Infirmary, Cargenbridge, Dumfries, UK; [4]Department of Clinical, Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy; [5]Department of Internal Medicine, Division of Allergy and Clinical Immunology, University of Salerno, Italy; [6]Centro Emostasi A.O.R.N"SG Moscati", Avellino, Italy; [7]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy

Background: The proprotein convertase subtilisin/kexin type 9 (PCSK9) is a novel cardiovascular risk factor. Levels of PCSK9 in thrombotic primary antiphospholipid syndrome (PAPS) have never been investigated. **Methods**: Cross sectional comparison of baseline characteristics of 91 PAPS patients enrolled in the multicenter ATHERO-APS cohort study. PCSK9 levels were categorized into tertiles and the association with arterial and recurrent thrombosis were assessed by univariable and multivariable regression analysis. **Results**: Median age was 51 years and 71.4% (n=65) were women. Overall, 33% (n=30) experienced an arterial event while 31% (n=28) had recurrent thrombotic events. Median PCSK9 levels were 1243 (1100-1650) pg/ml. Patients in the third PCSK9 tertile (>1458 pg/ml) showed a higher prevalence of dyslipidemia, lupus anticoagulant positivity and a history of previous arterial and recurrent thrombosis than patients in the first and second tertile. PCSK9 levels were higher in arterial than venous thrombosis (1502 *vs.* 1180 pg/ml, p=0.002), and in patients with recurrent *vs* isolated thrombosis (1680 *vs.* 1150 pg/m, p<0.001). High plasma PCSK9 levels were associated with a 4-fold increase risk for arterial events and with a 10-fold increase risk for recurrent thrombosis after adjustment for confounding factors. **Conclusions**: These preliminary data suggest that in PAPS, PCSK9 levels are associated with arterial and recurrent thrombosis. Its role as a possible therapeutic target in PAPS needs further studies.

PO112

CHARACTERIZATION OF EDOXABAN EFFECTS ON PLATELET FUNCTION

G. Barbieri[1], A.M. Gori[2], F. Cesari[3], E. Sticchi[2], A. Rogolino[3], R. Orsi[1], A. Bertelli[1], R. De Caterina[4], B. Giusti[2], R. Marcucci[2]

[1]University of Florence, Department of Experimental and Clinical Medicine, Florence; [2]University of Florence, Department of Experimental and Clinical Medicine, Atherothrombotic Diseases Center, Careggi Hospital, Florence; [3]Atherothrombotic Diseases Center, Careggi Hospital, Florence; [4]Cardiology division, University of Pisa, Italy

Background: All anticoagulants are expected to have an indirect effect on platelet function since they interfere with the generation or activity of thrombin, but the impact of Direct Oral Anticoagulants (DOACs) is largely unknown. Previous studies conducted with Dabigatran, Apixaban and Rivaroxaban showed a reduction in endogenous thrombin potential (ETP) and in platelet aggregation induced by thrombin and tissue-factor (TF) in a dose dependent manner. Aim of this study was to evaluate the effects of Edoxaban on platelet function by *in vitro* and *ex vivo* studies. **Methods**: We evaluated platelet aggregation (PA), thrombin generation (TG) and thromboxane B_2 (TXB_2) levels in 20 healthy donors: samples were incubated *in vitro* with increasing concentrations of Edoxaban [E50, E150, E250 (ng/mL)] or vehicle as control. We also investigated the same parameters in 20 patients with Atrial fibrillation (AF) treated with Edoxaban (*ex vivo* study). A PAP-8 aggregometer was used to assess PA on PRP samples, induced by ADP (5 μM), TRAP-6 (10 μM), Human Thrombin (THR, 0.182 mU/μL) and TF. TG was measured (both in the presence and absence of thrombomodulin-TM) using the Calibrated Automated Thrombogram System (CAT). Serum TXB_2 was measured by using the TXB_2 EIA kit, according to the manufacturer's instructions. **Results**: The incubation of PRP with different Edoxaban concentrations significantly reduced TF-induced PA with respect to vehicle [E50 by 21% (p=0.033), E150 by 33% (p=0.004), and E250 by 52% (p<0.001)]. TF-induced PA was significantly lower in patients treated with Edoxaban than in controls (p<0.001). ADP and TRAP-6-induced PA was not inhibited by any Edoxaban concentrations in the *in vitro* study, and also *ex vivo* experiments failed to demonstrate any difference between ADP and TRAP-6-induced PA from AF patients treated with Edoxaban and controls. THR-induced aggregation in E150 group showed a trend towards a reduction, though did not reach the statistical significance. Among the parameters related to TG, Lag Time (-TM) was significantly (p<0.001) and positively related to Edoxaban concentrations. Patients showed more prolonged Lag Time values (p=0.031) with respect to those observed in controls. ETP and Peak (-TM) were significantly reduced *in vitro* (p<0.001) by the incubation of Edoxaban in a dose dependent manner. In *in vitro* study, ETP ratio values were significantly reduced according to increasing Edoxaban concentrations. AF patients showed reduced levels of ETP ratio with respect to controls (p<0.001). We found a 24% decrease in serum TXB_2 concentration in the E250 group *vs* control (p<0.01), while the reduction is not significant in the other Edoxaban concentrations (Figure 1). **Conclusions**: Our data show that Edoxaban is able to interfere with platelet function. In particular, it significantly reduces TF-induced platelet aggregation in a dose-dependent manner and also TG, although thrombin-induced aggregation is not affected by the drug, supporting Edoxaban's indirect effect on thrombin by inhibiting FXa. In addition, the reduction of TXB_2 levels by Edoxaban suggests that the drug is endowed with an antiplatelet effect, which may, in turn, lead to the delayed/reduced formation of coagulation complexes reinforcing its antithrombotic potential.

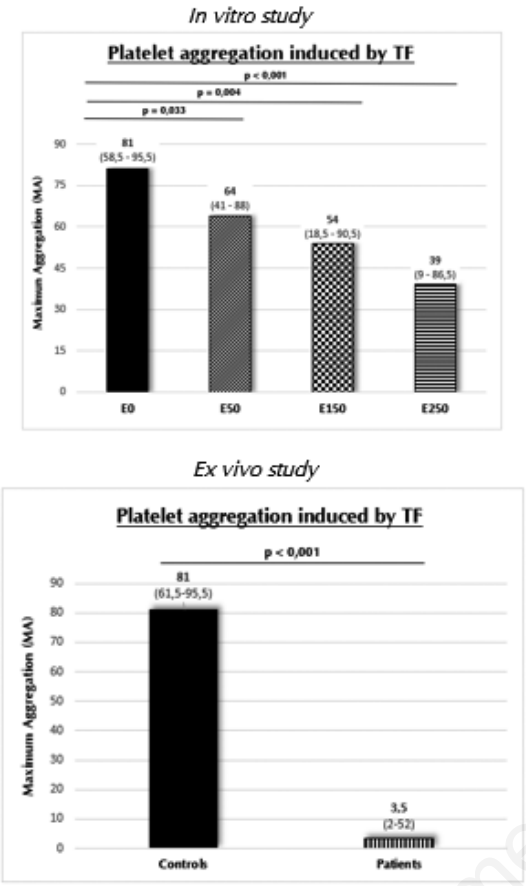


Figure 1.

PO113

HYPERLIPOPROTEIN(A) AND PREMATURE CORONARY ARTERY DISEASE: A CALL FOR ACTION

T. Capobianco, W. Iannotti, A. Sammartino, M. Casini, S. Tedesco, F. Brogni, E. Lotti, M. Berteotti, R. Valenti, A. Migliorini, N. Marchionni, C. Di Mario, R. Marcucci

Dipartimento Cardiotoracovascolare, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italy

Background and Aims: Elevated Lipoprotein (a) levels are well recognized as a genetic risk factor for atherothrombotic events. Recently, new therapeutic approaches – such as small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) – have been developed and are under evaluation in phase 3 trials. Evidences from literature suggest the need for screening Lp(a) in premature coronary artery disease. Aim of our study was to evaluate, in a real world scenario: 1) the percentage of premature CAD in whom clinicians choose to screen Lp(a); 2) the prevalence of hyperLp(a) in premature CAD. **Methods**: We retrospectively evaluated data form patients admitted for acute coronary syndrome at Department of Cardiology of Careggi University Hospital, Florence, between 1st January 2021 and 28th February 2022. Inclusion criteria were: age <50 years for men and <55 years for women; confirmed diagnosis of acute coronary syndrome at hospital discharge. **Results**: We selected 78 patients (12 F and 66 M; median age: 46 years). LDL median values at admission were 121.87 mg/dl). Four out of 78 patients (5.1%) had LDL levels higher than 190 mg/dl, suggesting the diagnosis of Heterozygous Familial Hypercholesterolemia (with respect to the expected prevalence in the general population: 1/250 (0.004%). No patient had hepatic disease or thyroid disease. Twenty out of 78 patients (25.6%) had triglycerides levels higher than 150 mg/dl; 17/78 (21.7%) with levels higher than 200 mg/dl. As regards Lp(a) levels, a dosage was performed in only 34/78 patients (43.5%). Among these, elevated Lp(a) levels were documented in 11/34 (32.4%). In particular, 4/11 (11.8%) had extreme elevated Lp(a) levels (*i.e.* higher than 800 mg/L) (Figure 1). In a patient we documented both extreme Lp(a) levels and LDL levels higher than 190 mg/dl; in the other patients elevated Lp(a) levels were the only lipid parameter out of range, and the only risk factor for premature CAD. At a multivariate analysis adjusted for classical risk factors, elevated Lp(a) levels were significantly associated with the risk of premature CAD (OR: 2.4 95%CI 1.9-3.6); p<0.001). **Conclusions**: these results demonstrated that: 1) it is urgent, from a clinical point of view, to apply screening protocols in premature CAD including Lp(a) dosage; 2) elevated Lp(a) levels, and in particular extreme elevated Lp(a) levels, have an elevated prevalence in a population of premature CAD and are significantly and independently associated with the disease.

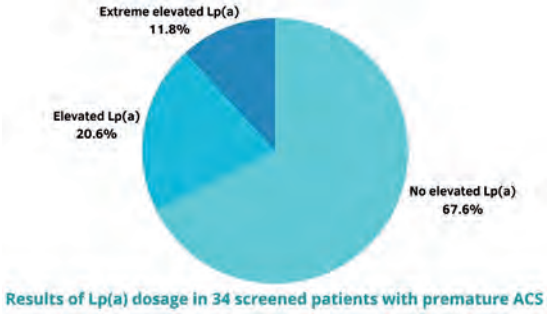


Figure 1.

PO114

PREDICTORS OF ISCHEMIC AND HEMORRHAGIC EVENTS IN PATIENTS WITH ATRIAL FIBRILLATION UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

M. Berteotti[1], A.M. Gori[1], A. Bertelli[1], B. Bandinelli[1],

G. Grossi[2], N. Ciardetti[2], B. Giusti[1], G. Barbieri[1], E. Lotti[1], R. Valenti[2], C. Di Mario[1], N. Marchionni[1], R. Marcucci[1]

[1]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy; [2]Division of Interventional Cardiology, Cardiothoracovascular Department, Careggi University Hospital, Florence, Italy

Background and Aims: Antithrombotic management of patients with atrial fibrillation (AF) undergoing percutaneous coronary intervention (PCI) can be challenging. Recent direct oral anticoagulant (DOAC) trials demonstrated the safety of double antithrombotic therapy (DAT) in comparison with triple antithrombotic therapy (TAT) in this setting. However, this benefit is counterbalanced by a higher risk of ischemic, mainly stent-related, events. We therefore sought to identify possible laboratory predictors of bleeding and ischemic risk in a real-world population with concomitant AF and PCI. **Methods**: All consecutive patients with history of AF discharged from our cardiology ward with DAT or TAT after a PCI from April 2018 to March 2021 were enrolled in an observational registry. For all subjects, blood serum samples were collected and tested for D-Dimer, thrombin generation time, clot lysis time, platelet reactivity by arachidonic acid and ADP, erythrocyte aggregation and deformability, oxidative stress and DOAC concentration if appropriate. Major adverse cardiac and cerebrovascular events (MACCE) and major hemorrhagic or clinically relevant non major bleeding events together with therapeutic changes were recorded for all patients. **Results**: A total of 147 patients were included (70.1% after acute coronary syndromes); the mean age was 78±8 years and 48 (33%) were women. Ninety-one patients (62%) were discharged with TAT. Both in TAT and in DAT group DOACs were preferred (58% and 77%, respectively). In 93.4% of patients, clopidogrel was chosen as P2Y12 inhibitor. The median follow-up was 401 days (IQR 241-588). MACCE occurred in 26 cases (17.7%), while hemorrhagic events in 25 (17%), 14 of which were major (9.5%), with no significant differences between TAT and DAT group. The incidence of all-cause death and cardiovascular death was 12.9% and 7.4%, respectively. The independent predictors of MACCE at COX regression analysis were acute coronary syndrome presentation (HR 8.70, 95% C.I. 1.16 to 65.42, p=0.036), P2Y12 dependent platelet reactivity-PRU (HR 1.65, 95% C.I. 1.15 to 2.38, P=0.007) and D-Dimer (HR 1.34, 95% CI 1.03 to 1.75, p=0.030). Platelet function estimated by PRU, was significantly affected by the *2 genotype: 155.5 (102-218.7) in wild-type *vs* 204 (96-246) in carriers of *2 allele (p<0.05). The independent predictors of bleeding were active smoke (HR 3.31, 95% C.I. 1.04-10.34, p=0.043), previous myocardial infarction (HR 0.27, 95%C.I. 0.08-0.96, p=0.043), left main disease (HR 2.76, 95% C.I. 1.07-7.09, p=0.035) and D-dimer (HR 1.63, 95% C.I. 1.12-2.37, p=0.011). Independent predictors of all-cause mortality were the following: male gender (HR 0.13, 95% CI 0.04-0.41, p=0.001), peripheral artery disease (HR 3.17, 95% C.I. 1.06-9.43, p=0.038), white blood cells count (HR 1.89, 95% C.I. 1.14-3.12, p=0.014), eGRF (HR 0.41, 95% C.I. 0.22-0-76, p=0.005) and D-dimer (HR 1.64, 95% C.I. 1.05-2.58, p=0.031). **Conclusions**: In a real-world unselected population, a significant incidence of ischemic as well as hemorrhagic events was observed both in patients on TAT and DAT early after discharge. Our data documented the relevance of the entity of platelet inhibition in patients with ACS suggesting that a laboratory evaluation of drug response might be useful in personalizing antithrombotic therapy in this setting. Besides D-Dimer resulted an independent predictor of both ischemic and bleeding events, as well as death.

PO115

EXTRACELLULAR VESICLES NUMBER AND CELL SUBTYPE MAY BE INFLUENCED BY DIABETES MELLITUS AND METFORMIN IN PATIENTS AT HIGH CARDIOVASCULAR RISK

P.G. Simeone[1], R. Liani[1], G. Bologna[1], R. Tripaldi[1], A. Di Castelnuovo[2], P. Simeone[1], D. D'Ardes[1], S. Miscia[1], F. Cipollone[1], M. Marchisio[1], A. Consoli[1], P. Lanuti[1], F. Santilli[1]

[1]Department of Medicine and Aging, Center for Advanced Studies and Technology (CAST), Chieti, Italy; [2]Mediterranea Cardiocentro, Napoli, Italy

Background and Aims: Extracellular vesicles (EV) represent a population of small vesicles deriving from all types of cells, generated by the extroflection of the plasma membrane, and released into the circulation. EV can have both pro- and anti-atherothrombotic effects depending on the clinical setting, origin cell, stimuli, and different treatments might affect their levels. The primary endpoint of our study was to compare the amount of circulating EV and specific EV subtypes derived from platelets, endothelial cells, and leucocytes in subject at high CV risk, with and without T2DM, and if any ongoing anti-diabetic drugs could affect the levels of EV. **Methods**: Fifty-nine T2DM patients and forty patients without T2DM with or without prior vascular disease, were enrolled, this was a cross-sectional study, extracellular vesicles were evaluated by flow cytometry analysis. **Results**: After propensity score matching the levels of total EV (Cohen d=0.55, p=0.064), total Annexin+ EV (d=0.49, p=0.043), EV CD41a+ platelet derived EV (d=0.51, p=0.014), CD41a+/AnnexinV+ platelet derived EV (d=0.50, p=0.019) and CD45 leukocyte derived EV (d=0.19, p=0.012) were lower in diabetic patients *vs.* those without T2DM. Interestingly, platelet-derived EV positive for AnnexinV were lower in patients on treatment with metformin (d=0.72, p=0.012) (Figure 1). **Conclusions**: Our study showed that, among high CV risk patients, treated with the state-of-the-art preventive strategies,

T2DM is associated with lower levels of total, platelet- and leucocyte-derived EV, and that this difference may be accounted for, at least in part, by ongoing treatment with metformin, the most widely prescribed oral antidiabetic agent.

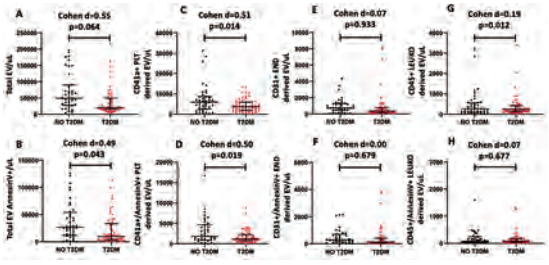


Figure 1.

PO116

LDL TARGET LEVELS IN MACCE PATIENTS: AN (UN)EXPECTED GENDER BIAS. A SNAPSHOT FROM TUSCANY

E. Lotti[1], P. Francesconi[2], E. Mannucci[1], A. Zuppiroli[1], G. Casolo[1], D. Grisillo[1], N. Ferrante[3], F. Profili[2], R. Marcucci[1]

[1]Department of Experimental and Clinical Medicine, University of Florence; Atherothrombotic center, AOU Careggi, Itay; [2]ARS-Agenzia regionale di sanità della Toscana, Firenze, Italy; [3]Novartis FarmaVarese, Italy

Background: Collection of epidemiological data and information on related healthcare is crucial for monitoring healthcare appropriateness and the fulfillment of clinical needs. Limited data are available on the prevalence of LDL cholesterol in Italian population, in particular in the very high risk population of MACCE patients. **Aims**: This project aimed to estimate how many patients had LDL cholesterol at the target levels suggested by the international guidelines by analyzing regional administrative data. **Methods**: By linking health administrative and laboratory data, we collected data from seven Local Health Districts in Tuscany on residents aged ≥45 years. Inclusion criteria were: 1) at least one LDL measurement in the year; 2) a history of major adverse cardiac and cerebrovascular events (MACCE, defined as myocardial infarction and/or stroke and/or revascularization of the coronary arteries, the carotids, or peripheral arteries) and/or type 2 diabetes (T2D). Cohorts were defined at 1st january 2019 and 1st january 2020. Lipid lowering therapy use (defined as ≥2 prescription fillings or ≥75% coverage of treatment days) over the past 6 months if LDL cholesterol (LDL-C) data were available or over the past year if no LDL-C data were available was monitored. The outcome was the number of patients with on-treatment LDL optimal levels, as defined by levels <55 mg/dl for pts with MACCE and <70 mg/dl for pts with diabetes without MACCE. **Results**: A cohort of 174,200 individuals was analyzed (55% M and 45% females). As regards subjects on lipid-lowering therapies, we found that female gender was associated with a significantly lower probability to have LDL levels at target (OR: 0.58-0.01; p<0.000) in patients with MACCE with or without diabetes, in a model adjusted for age, district area, and the presence of cardiovascular risk factors and comorbilities (renal failure, heart failure, atrial fibrillation). This result was confirmed also in the analysis conducted in subjects without lipid-lowering therapies (OR: 0.56-0.01; p<0.000). No significant differences were documented by stratifying for the presence of clinical pathways aimed to the active call of the patients by clinicians ("Initiative Health"). **Discussion**: These results demonstrated that females have a significantly lower probability to have LDL cholesterol levels at targets suggested by guidelines. This datum is confirmed even in the absence of lipid-lowering therapies. These results call for action aimed to: 1) education for general population and for patients with MACCE with a specific target on females; 2) information for clinicians for the need of a lipid-lowering therapy gender-tailored; 3) organization of clinical pathways - from admission to recovery and follow-up - with a specific target on lipid lowering therapies ad gender.

PO117

THE ASSOCIATION BETWEEN PAD AND DIABETES: PRELIMINARY FINDINGS FROM A PROSPECTIVE STUDY

A. Abenante[1], M. Dalle Fratte[1], R. Corso[1], C. Dedionigi[1], G. Fabro[1], C. Fumagalli[1], I. Giarretta[2], R. Landi[3], S. Marino[1], D. Mastroiacovo[2], M.C. Naim[1], E. Nicolini[2], P. Pagani[1], A. Bonaventura[2], F. Dentali[1]

[1]Università degli Studi dell'Insubria di Varese; [2]UO di Medicina Interna, Ospedale di Circolo di Varese; [3]Università degli Studi del Piemonte Orientale, Novara, Italy

Background and Aims: Peripheral artery disease (PAD) refers to a partial or complete occlusion of the arterial vessels of the lower limbs. Patients with diabetes mellitus (DM) have more than two-fold increased prevalence of PAD compared with the general population. Despite its epidemiological and clinical importance, PAD remains largely underdiagnosed and undertreated. Therefore, this study investigated PAD in DM patients by measuring ankle-brachial-index (ABI). **Methods**: Consecutive patients referring to the Diabetes Outpatient Clinics of the ASST Settelaghi (Varese) were screened for PAD since June 2018 and included in the database. Patients with gestational DM or those with a history of type I DM shorter than 10 years were excluded. All patients signed an informed consent. Every subject underwent a full clinical evaluation, ABI measurement and filled the San Diego Claudicatio questionnaire. A further diagnostic evaluation with Doppler ultrasound was planned for all

patients with ABI <0.9 or for those with non-compressible arteries. For the current analysis, patients with ABI <0.9 or those with a known diagnosis of PAD were considered as having PAD. Comparisons between patients were performed using Mann-Whitney U test or Fisher's exact test, as appropriate. All variables with p <0.10 were included in a backward stepwise logistic regression model to evaluate independent risk factors for PAD occurrence. Values were expressed as odds ratio (OR) with 95% confidence interval (CI). A two-sided p<0.05 value was considered as statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Mac version 27.0 (IBM Co., Armonk, NY, USA). **Results**: A total of 797 consecutive DM patients were screened. Among them, 316 were women (39.6%). One hundred and seven (13.4%) patients were diagnosed with PAD, while 690 (86.6%) were not affected. The multivariate logistic regression analysis showed that age >65 years (OR 2.1; 95% CI, 1.25 to 3.62; p=0.005), chronic kidney disease (OR 2.8; 95% CI, 1.30 to 3.31; p=0.002), retinopathy (OR 2.39; 95% CI, 1.40 to 4.07; p=0.001) and heart failure (OR 1.80; 95% CI, 1.01 to 3.19; p=0.044) were significantly associated with PAD diagnosis. Furthermore, carotid atherosclerosis showed a positive association with PAD (OR 1.55; 95% CI, 0.98 to 2.45; p=0.057). **Conclusions**: This preliminary analysis of our ongoing study showed that several common comorbidities are significantly associated with PAD. Moreover, the rate of undiagnosed PAD among DM patients is not trivial. An early screening in this high-risk subgroup of patients should allow easier diagnosis and more effective treatment. Hence, a larger number of patients and further investigation is eagerly needed in the next future.

PO118

VERTEBROBASILAR DOLICHOECTASIA IN THE CHOICE OF ANTICOAGULANT THERAPY: A CASE REPORT

F. Crudele[1,2], G. Guidotti[1,2], E. Lotti[1,2], D. Poli[1,2], L. Mannini[1,2], C. Sarti[3], F. Meucci[1,4], R. Marcucci[1,2]

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [2]Atherothrombotic Center, AOU Careggi, Florence, Italy; [3]Department of NEUROFARBA, University of Florence, Florence, Italy; [4]Structural Interventional Cardiology, AOU Careggi, Florence, Italy

Background: Dolichoectasia of the vertebro-basilar artery (VBD) is defined by Smoker's criteria as dilation greater than 4.5 mm, elongation and tortuosity of the basilar artery. It is found in 9% of people over 60 years old. Etiology is unknown and it is associated with female sex, arterial hypertension and diabetes mellitus. It is a possible cause of cerebrovascular events, both ischemic and hemorrhagic, and its mortality at 5 years after the first clinical manifestation is about 30%. **Case Report**: A 69 y/o woman is admitted into the emergency room complaining of impaired movement of left arm and leg upon awakening. Upon arrival, she is alert, normotensive with a rhythmic pulse and a left hemiparesis and hemianesthesia is noted. She has a history of arterial hypertension, diabetes mellitus, hypercholesterolemia and hypothyroidism. Imaging shows subcortical ischemic outcomes in the left frontal lobe and right cerebellum and two bilateral subacute thalamic lesions, the most recent on the right. The patient is not a candidate for acute phase therapy and therefore acetylsalicylic acid 300 mg/die and hight dose statin is started. During hospitalization, she experiences chest pain radiating to the back. A contrast-enhanced CT scan shows multiple filling defects of the pulmonary circulation, multiple splanchnic aneurysms and dissection of the hepatic artery. In agreement with the neurologist, anticoagulant therapy with LMWH is set up. The study of cerebral circulation reveals a VBD with a maximum diameter of 6.2 mm. A coronary angiography shows multiple aneurysms at this level as well. PET with 18F-FDG is also considered to rule out vasculitis. Transesophageal echocardiogram is performed, detecting patency of the foramen ovale. No signs of deep venous thrombosis are found with doppler of the lower limbs. Vascular surgeons recommended follow-up of splanchnic aneurysms without any present surgical indication. A diagnosis suspect Loeys-Dietz syndrome was made after a full evaluation with an expert in collagen disease. **Conclusions**: The pulmonary embolism associated with cerebral ischemia in a patient with patent foramen ovale and coronary aneurysms, would lead to a long-term anticoagulant therapy in secondary prevention, but VBD increases complexity in this case. In this patients, the few data available show no evidence of superiority between DOACs and VKAs in the prevention of ischemic events. On the other hand it emerges that the greatest risk of bleeding complications is related to dilations greater than 6.5 mm or in case of a growth greater than 2 mm per year, regardless the antithrombotic therapy. Therefore, the therapeutic choice with the highest safety profile seemed to be Dabigatran 150 mg/db and a radiological follow-up to monitor the evolution of VBD. VBD is a variable to consider when choosing antithrombotic therapy in patients with cerebrovascular events. In our opinion a subgroup of the complications related to anticoagulant therapy may be related to the presence of undiagnosed VBD. Further studies are needed to provide more valuable data on this specific topic which may affect a discrete number of patient.

PO119

THROMBO-EMBOLIC RISK IN CARDIAC TRANSTHYRETIN AMYLOIDOSIS: A CASE REPORT

F. Crudele[1], G. Guidotti[1], A. Argirò[2], E. Lotti[1], D. Poli[1], L. Mannini[1], F. Perfetto[2], F. Cappelli[2], R. Marcucci[1]

[1]Department of Experimental and Clinical medicine,

University of Florence; Atherothrombotic Center, AOU Careggi, Florence, Italy; [2]Tuscan Regional Amyloidosis Centre, AOU Careggi, Florence, Italy

Introduction: Systemic amyloidosis is an extracellular storage disease of insoluble protein fibrils. Its prevalent cardiac involvement subtypes are due to the deposition of amyloid from immunoglobulin light chains or from transthyretin (ATTR). In recent years, ATTR based amyloidosis incidence has had a marked increase thanks to the greater diagnostic capacity: it is estimated that up to 13% of subjects with heart failure with preserved ejection fraction may be affected by aTTR. Other possible complications include arrhythmic events, mostly atrial fibrillation (AF), and an increased risk of cardiogenic thrombo-embolic events. In these patients intracavitary thrombi are more frequently detected at the time of an attempt at AF cardioversion, despite adequate anticoagulation therapy. The increased thrombotic risk appears to be related to the degree of amyloid infiltration and the severity of cardiac dysfunction. **Case Report**: In March 2022, we evaluated Mr. F.S., aged 81, affected by transthyretin cardiac amyloidosis from January 2020 and heart failure with preserved ejection fraction and AF as complications. Patient's comorbidities are stage III renal failure, as well as arterial hypertension, diabetes mellitus and dyslipidemia. He is also suffering from non-critical carotidopathy in follow up. At the time of diagnosis, warfarin therapy was initiated in the presence of a CHA_2DS_2-VASc score of 5. Subsequently, in February of the same year, he had an ischemic stroke during well-conducted therapy (INR 3.01 at the time of the event). For this reason, warfarin was replaced with Dabigatran 110 mg / bd, according to renal function. He came to our attention for an episode of sudden blanching of the forearm and left hand, associated with intense pain and burning paresthesia, which occurred about 15 days earlier. At the time of the event, all the therapy was assumed regularly, including Dabigatran, and any other associated symptoms or significant events was not reported. He did not turn to the general practitioner for spontaneous resolution of the event in a few days. Subsequently, the reference cardiologist highlighted stability of the echocardiographic findings and good haemodynamic balance, interpreting the episode as a spontaneously resolved acute ischemia. At the time of our evaluation, the patient complained of residual numbness in the hand, in the absence of clear signs of ischemia. An anti-IIa activity dosage was fully in the therapeutic range (with normal trough values of 140 ng/ml). Since it was fully in the therapeutic range, a therapy based on acetylsalicylic acid 75 mg/day was prescribed in addition to anticoagulation. **Conclusions**: Few data are available on patients with aTTR cardiac amyloidosis with recurrent ischemic events on anticoagulation therapy. Data from literature and from randomized trials are not able to define the better antithrombotic strategy in this clinical setting. Therefore we decided to increase the intensity of the therapy adding an antiplatelet agent, because of multiple atherothrombotic risk factors. This case is an example of how complex it can be to manage these patients, taking into account the underlying disease and the frequent associated comorbidities. It is desirable that more evidence be collected to refine the therapeutic options in these patients. Furthermore, the routine dosage of the concentrations of DOACs during adverse events provides useful information in the subsequent management of the patient.

PO120

EVOLOCUMAB-RESISTANT HYPERCHOLESTEROLEMIA IN A PATIENT WITH PRIMARY BILIARY CHOLANGITIS. A CASE REPORT

V. Liberati, E. Lotti, F. Crudele, E. Sticchi, B. Giusti, R. De Cairo, A. Rogolino, R. Marcucci

Dipartimento medicina sperimentale e clinica, Università di Firenze, Malattie Aterotrombotiche, AOU Careggi, Italy

Background: Hypercholesterolemia is a common finding in almost 75% of patients with primary biliary cholangitis (PBC). Hypercholesterolemia is a well-known modifiable risk factor for cardiovascular disease in the general population, however in PBC's patients the role of hypercholesterolemia in cardiovascular disease remains controversial. Most of the studies had not found a statistically significant increase of atherosclerosis incidence in patients with PBC and hypercholesterolemia compared with age and sex-matched controls. Among factors contributing to possible decrease atherogenesis in patients with hypercholesterolemia and PBC there is LipoproteinX (LpX), an atypical lipoprotein rich in phospholipid and nonesterified cholesterol with a similar density to LDL, found in cholestasis states (*e.g.* PBC) as a result of biliary lipids reflux into the plasma. LpX seems to delay atherosclerosis process by preventing LDL-oxydation and the adverse effects of oxidated-LDL on the endothelium; therefore it has been hypothesized that hypercholesterolemia in PBC might even have a cardiovascular protective effect. LpX does not contain apolipoprotein B (ApoB), so it can't be recaptured by LDL receptor (LDLR); this could explain a possible failure in reducing cholesterol concentration with PCSK9 inhibitors in PBC's patients. **Case Report**: This paper reports a case of hypercholesterolemia refractory to the PCSK9 inhibitor Evolocumab in a 58 years-old woman with a recent diagnosis of PBC. She was admitted in our Centre for marked hypercholesterolemia. Lipid panel showed: total cholesterol 375 mg/dl, LDL cholesterol 260 mg/dl and HDL cholesterol 81 mg/dl. She did not tolerate statins, fibrates, ezetimibe and cholestyramine. Evolocumab wasn't effective on reducing LDL-C levels. Genetic analysis with NGS for familial hypercolesterolemia (FH) did not show genetic alterations of the PCSK9, ApoB and LDRLR genes. In December 2021 she started complaining

asthenia and itch. Blood tests showed an important increase of cholestasis indices (FA, GGT, bilirubin, biliary acids) and transaminases (AST, ALT). Anti-nuclear antibody (ANA), anti-mitochondrial antibody (AMA) and anti-mitochondrial M2 antibody were positive. Fibroscan showed a fibrosis score of F3. The patient was diagnosed with primary biliary cholangitis and a medical therapy with ursodeoxycholic acid (UDCA) was started. Assuming that high LDL-C in PBC is primarily due to the presence of LpX, which could explain the lack of response with Evolocumab, we detect indirectly the presence of LpX by measuring ApoB levels. Patients with elevated Lp-X typically have low Apo B. ApoB levels were not extremely high (160 mg/dl), not excluding our hypothesis. **Conclusions**: The only explanation for PCSK9-resistance in our patient is a LpX-hypercholesterolemia. A limitation of our report is that the measure of ApoB levels is not an accurate method for detecting LpX. Electrophoresis techniques, which show directly the presence of LpX comigrating with LDL, are more sensitive, but they are not available in all laboratories. Since studies emphasize that marked hypercholesterolemia in PBC is not associated with an increased cardiovascular risk and the patient has not further cardiovascular risk factors and is refractory to lipid-lowering therapy, we consider not necessary to ahead lipoprotein apharesis in primary prevention.

PO121

DEALING WITH PEDIATRIC CANCER ASSOCIATED THROMBOSIS: A MONOCENTRIC COHORT STUDY

E. Campello[1], D. Tormene[1], A. Francavilla[2], S. Sartori[3], S. Sebellin[3], M.F. Pelizza[3], M. Nosadini[3], J.N. Pin[3], G. Lorenzoni[2], A. Biffi[3], D. Gregori[2], L. Spiezia[1], M. Martinato[2], P. Simioni[1]

[1]Department of Medicine, University of Padova, Italy; [2]Unit of Biostatistics, Epidemiology and Public Health Department of Cardiac Thoracic Vascular Sciences and Public Health, University of Padova, Italy; [3]Department of Women's and Children's Health, University Hospital of Padova, Italy

Background and Aims: Little is known about cancer-associated thrombosis (CAT) in children. We aim to report a pediatric case of CAT successfully treated with rivaroxaban and to describe clinical presentation, management and outcomes of pediatric CAT. **Methods**: All consecutive patients admitted at the Pediatric Department of the Padova University Hospital between January 2007 and December 2021 with a thrombotic event were retrospectively enrolled. Inclusion criteria were age >28 days, confirmed acute venous or arterial thrombosis, cancer among risk factors. Clinical outcomes including thrombosis-related death, recurrence and bleeding complications were recorded. **Results**: Case: A 13-year-old girl undergoing systemic chemotherapy via central vein catheter (CVC) for a right frontal bone Ewing sarcoma, developed symptomatic right internal jugular vein occlusion (May 2021), thus enoxaparin 8000 UI twice/die was started followed by CVC removal and reduction to 8000 UI/die after 1 month (weight 81 kg). In July chemotherapy was stopped for disease remission. A follow-up CT scan in August showed occluding thrombosis of the internal jugular, subclavian and anonymous right veins extended to the superior vena cava with an additional non-occluding thrombosis to the cavo-atrial junction. Enoxaparin 8000 UI twice/die was re-started followed by rivaroxaban 20 mg for 3 months. In November a CT scan showed initial recanalization of the anonymous, subclavian and jugular veins, reduction of cavo-atrial thrombosis. Rivaroxaban is still ongoing without any hemorrhagic complications. Cohort study: 59 pediatric patients with CAT were considered (Table 1). The most involved neoplasms were hematological malignancies. 24 events (40.6%) were cerebral arterial thrombosis; 35 (59%) were venous thrombosis (VTE, 8 cerebral vein, 25 systemic vein thrombosis and 2 pulmonary embolism). Of 27 VTE, 15 (55%) were CVC-related, 19 (70%) were symptomatic. Main sign/symptoms were 4 catheter malfunction, 2 dyspnea and 13 limb edema and pain. Antithrombotic therapy was administered in 51/59 (86%) of patients, mainly heparin (98%) (low molecular weight heparin [mean dosage 162.25 U/kg/die] or unfractionated heparin [mean dosage 19.7 U/kg/h]). One patient was treated with warfarin (2%). As for the outcomes, 2 patients died of CAT-related death (3.4%), 6 patients (10.1%) underwent CAT recurrence after a mean period of 540 weeks [IQR 428-652]. Recurrence events were 3 cerebral vein and 3 systemic vein thrombosis. As for bleeding complications only 3 minor events were reported (5%). **Conclusions**: Pediatric CAT characteristics and outcome resemble those of adult CAT. Particularly, a high incidence of both arterial and venous events was reported; hematological neoplasms and CVC are the major risk factors; heparin the only used therapeutic option; a high incidence rate of recurrence was detected.

Table 1. Characteristics of the study population.

	Pediatric CAT n. 59
Age - years	11.3 [1.1-20]
Gender – M	34 (58%)
Type of cancer - n(%)	
Leukemia	39 (66%)
Lymphoma	8 (14%)
Brain	4 (7%)
Solid non-brain neoplasm	4 (7%)
Myeloproliferative syndromes	1 (1%)
Other	3 (5%)
Type of thrombosis -n (%)	
arterial thrombosis	24 (41%)
venous thrombosis	35 (59%)
Site of thrombosis - n (%)	
cerebral arterial	24 (41%)
cerebral vein	8 (13.5%)
systemic vein thrombosis	25 (42%)
pulmonary embolism	2 (3.3%)
Presence of CVC	15 (25.4%)
Symptoms of venous thrombosis - n (%)	
asymptomatic	8 (30%)
symptomatic	19 (70%)
Therapy – n (%)	51 (86%)
heparin	50 (98%)
warfarin	1 (2%)
Outcomes – n(%)	
CAT-related death	2 (3.4%)
Recurrence	6 (10.1%)
Major bleeding	0

PO122

VENOUS THROMBOEMBOLISM PROPHYLAXIS IN PEDIATRIC TRAUMA: A MANAGEMENT ASSESSMENT

A.M. Pizzini[1], M. Silingardi[1], C. Fantoni[1], C. Coniglio[2], C. Ghizzi[3]

[1]Department of Internal Medicine, Maggiore Hospital Carlo Alberto Pizzardi, Bologna, Italy; [2]Department of Anesthesiology, Intensive Care and Pre-Hospital Emergency Services, Maggiore Hospital Carlo Alberto Pizzardi, Bologna, Italy; [3]Pediatric Unit, Maggiore Hospital Carlo Alberto Pizzardi, Bologna, Italy

Background and Aims: Venous Thromboembolism (VTE) is rare in healthy children (incidence around 1%), but in hospitalized patients the incidence has increased over the last decades, due to improved medical care and radiographic detection methods. Trauma is an independent VTE risk factor in adults, with a reported incidence from less than 1% to 7.6%. In pediatric trauma patients the incidence is lower (0.1-1.7%) but in the subset of pediatric patients with hospital-associated VTE, trauma is on of the most frequent diagnoses. **Methods**: We evalued a case series of twenty injured children related to the Trauma Center of our hospital over a period of about two years. The patients were stratified according to the risk assessment algorithms proposed by JK. Petty in "Seminars in Pediatric Surgery 2017". This work describes the patients characteristics and the efficacy and safety outcomes related to heparin prophylaxis. **Results**: The results are summarized in the Table 1.

Table 1. Results.

	[illegible]	[illegible]
Middle age	12.6 years (6-17) (5/10 under 13 years)	9.9 years (17 m-16y) (3/10 over 13 years)
Puberty	5/10	2/10
ISS ≥ 16	9/10	2/10
Surgery	7/10 (6 orthopedic surgery, 1 neurosurgery)	5/10 (2 orthopedic surgery, 2 neurosurgery, 1 vascular surgery)
ICU	8/10	5/10
CVC	8/10	5/10
Other risk factors	5/10 (2 obesity, 1 hormonal therapy, 1 hereditary thrombophilia 1 plaster)	1/10 obesity
Anti-Xa Level	0.29 UI/ml (0.40-0.12)	No
Thrombosis	None	-1 right distal vein thrombosis in a 16-year old adolescent patient undergoing ankle osteosynthesis (ISS13, no ICU) -1 superficial chemical phlebitis
Hemorrage	1 isolated episode of hematemesis in erosive gastropathy (NSAID use)	None

Prophylaxis is performed more frequently in older children and in puberty, confirming this group at greater risk of VTE. More than two risk factors regardless of trauma, are present in children in prophylaxis, even in those under 13 years. The trauma severity (Injury Severity Score ISS ≥16), surgery and vascular device are the main risk factors for thrombosis. Anti-Xa activity was founded in the normal range (0.2-0.4 UI/ml) in nine children and in one it was 0.12 UI/ml, but the heparin dosage was not modified for concomitant antiplatelet theraphy. The distal venous thrombosis is likely due to incorrect risk stratificatin while the superficial phlebitis was small and did not request heparin therapy. Clinically relevant non major bleeding was favored by concomitant anti-inflammatory theraphy. **Conclusions**: These data suggest that critically injured adolescent are at meaningful risk of VTE and represent an important group for prophylaxis. Risk stratification as poposed by the algoritm can be considered a simple tool to identify patients at high risk of VTE who may benefit from heparin prophylaxis. However further studies are needed for its validation.

PO123

RECURRENT FAILURES IN ASSISTED REPRODUCTIVE TECHNIQUES: THE FIRST REGISTRY

E. Grandone[1,2], D. Colaizzo[1], A. De Laurenzo[1], P. Totaro[3], T. Bartolotti[4], E. Bucherini[5], M. Margaglione[6], on behalf of the SSC Subcommittee on Women's Health Issues in Thrombosis and Hemostasis

[1]Thrombosis and Haemostasis Unit, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo (Foggia); [2]Ob/Gyn Department, University of Foggia, Foggia; [3]Santa Maria Hospital, Bari; [4]Demetra Artebios, Lugo di Romagna, Ravenna; [5]Angiologia e Medicina Vascolare, AUSL Romagna, Lugo di Romagna, Ravenna; [6]Medical Genetics, University of Foggia, Foggia, Italy

Background: Clinical pregnancy rate among women undergoing embryo transfer (ET) after Assisted Reproductive Techniques (ART) varies from 20% to 35% per transfer and the higher is the number of attempts, the lower the chance of a live birth (Harlin J, *et al.* Hum Reprod 2002). Although the role of thrombophilia screening is very controversial in this setting (Di Nisio M, Blood. 2011), nevertheless several Fertility Clinics test women for inherited and acquired thrombophilia before ART. The benefit of LMWH in improving live-births after ART has been shown irrespective of thrombophilia status (Potdar N, *et al.* Hum Reprod Update 2013; Grandone E, *et al.* PLOS One 2014). Because of their antithrombotic and vasodilatory properties, many studies have investigated the effects of low-dose aspirin or low-molecular-weight heparin (LMWH) to improve ART outcomes. Two systematic reviews and meta-analyses assessing the effect of low dose aspirin and LMWH in women undergoing ART showed that low dose aspirin was not associated with a higher rate of live birth, whereas LMWH could be effective in increasing the rate of live births (Helmerhorst FM, BMJ 2004; Dentali F. J Thromb Haemost 2011). Despite increasing knowledge on reproductive failures over the past decades, there are still several aspects of clinical management, treatment and prognosis with uncertainties that need to be addressed. We have set up the multi -centre prospective registry "FIRST" (recurrent Failures in assIsted

Reproductive Techniques), NCT 02685800. The main objective of the registry is to investigate factors affecting reproductive outcomes and assess the best clinical management strategies of women with recurrent otherwise unexplained implantation/pregnancy failures. **Methods**: We are recruiting women approaching ART cycle with 2 or more implantation failures/losses of clinical pregnancies after ART (*i.e.*: First, second and third level techniques). Exclusion criteria are: Uterine abnormalities, Hydrosalpinx, Chromosomal abnormalities in parents, Known hemorrhagic diathesis, Previous inclusion in the study. We estimated the inclusion of 624 women; this estimate is calculated on the basis of (1) the probability of observing live births after ART procedures (20-30%), (2) the estimate of an absolute increase of 5-10% in the number of live births after a prophylaxis of LMWHs (statistical power: 80%, significance level: 0.05). Baseline characteristics, past and current obstetric history are obtained during routine clinical follow-up or telephone interviews and recorded into a dedicated database. All women are followed until 4 weeks after the delivery or pregnancy test after ART procedure. Approval of local Ethics Committees from 6 Centers (Fertility Clinics and Thrombosis and Haemostasis Centers) was obtained. **Results**: So far, 6 Centers have recruited 493 women. Preliminary findings are attached. Overall, 35/86 (40.7%) live births were observed after any prophylaxis (LMWH and or ASA) and 64/341 (18.8%) in absence of prophylaxis (p: 0.000, OR: 2, 9, 95% CI 1.7 - 4.8) (Table 1). **Conclusions**: Preliminary data suggest that use of prophylaxis with ASA and/or LMWH can improve fetal outcomes in women undergoing ART.

Table 1.

ART Cycle N (%) (n= 493)	No treatment N (%)	ASA N (%)	LMWH N (%)	ASA+LMWH N (%)	Live births N (%) (n=99)
FVL heteroz 27 (5.5)	6 (22.2)	1 (3.7)	17 (63)	3 (11.1)	10 (37)
PT heteroz 29 (5.9)	11 (37.9)	2 (6.9)	14 (48.3)	2 (6.9)	9 (31)
Severe thrombophilia 9 (1.8)	3 (33.3)	1 (11.1)	5 (55.6)	0 (0)	2 (22.2)
No thrombophilia or not performed 428 (86.8)	350 (81.8)	24 (5.6)	46 (10.7)	8 (1.9)	78 (18.2)

PO124

DON'T BE AFRAID OF THROMBOTIC RISK OF HORMONES: THE IMPORTANCE OF PERSONALIZED MEDICINE FOR A SAFE PRESCRIPTION

E. Grandone[1], F. Marongiu[2], D. Barcellona[3]

[1]Fondazione IRCCS Casa Sollievo della Sofferenza, Thrombosis and Haemostasis, San Giovanni Rotondo (FG), Italy; [2]The First I.M. Sechenov Moscow State Medical University, Obstetrics & Gynaecology, Moscow, Russian Federation; [3]University of Foggia; University of Cagliari, Italy

This narrative review is focused on coagulation and fibrinolysis changes in users of estrogens and progestins at different ages of life. Indeed, the risk of venous or arterial thrombosis widely changes according to age and different risk factors throughout life. The aim should be avoiding inflexible or fearful behavior encouraging clinicians to find the best solution for the individual woman after a careful counseling. Search methods - MEDLINE and EMBASE databases were searched up to November 2021 with the following key-words: "estrogens" OR "progestogens" OR "combined contraceptives" OR "pill" OR "contraception" OR "patch" OR "vaginal ring" OR "IUD" OR "hormonal replacement therapy" AND "venous thromboembolism", OR "deep vein thrombosis" OR "pulmonary embolism" OR "arterial thrombosis". Articles were excluded if they: 1) are not closely related to the topic of the review 2) are not published on peer reviewed journals, 3) are not in English language. Estrogens and progestins can variably induce a hypercoagulable state (1,2). Furthermore, they can induce a hyper-fibrinolytic state counterbalancing the activation of blood coagulation (3). From a clinical perspective, compared to non-users, women in child-bearing age using second generation pill have a two-threefold higher risk of venous thromboembolism (VTE) while those using third / four generation oral contraceptives have a six to sevenfold increased risk (4,5). In postmenopausal women venous thrombotic risk increases with almost all combined oral contraceptives while transdermal hormonal replacement therapy and tibolone seem to be the safest treatments (6,7). As regard to arterial thrombosis in child-bearing women age, the relative risk decreases by lowering the estrogens dose, with no significant differences with regard to progestogens in third / fourth generation pill compared to those in second generation ones. Robust data show that cyproterone acetate and drospirenone do not increase thrombotic risk in combined oral contraceptives formulations (8). Oral progestogens and levonorgestrel Intra Uterine Device (IUD) are the safest products, since they do not increase neither the venous thromboembolic nor the arterial thrombotic risk (9,10). In women within 10 years of menopause onset, the use of low dose of oral or transdermal hormonal therapy seems to be safe with regard to the risk of cardiovascular death and stroke (7, 11). Recently, two large clinical trials (12,13) have shown that the risk of venous and arterial thrombosis is similar to that of second generation pill, by replacing ethinylestradiol with valerate estradiol or 17 B-estradiol. This narrative review could be of some help in the daily clinical practice, since it gives a comprehensive view of the balance risk/benefit profile for available products. Finally, it will give some practical suggestions on the choice of the best hormonal therapy in defined situations. Age, obesity, smoking, family history of thrombosis and known thrombophilia are common conditions in women who plan to take hormonal therapies. Although ISTH has classified the

estrogen therapy as a minor transient risk factor for thrombosis, the synergistic effect of individual risk factors might heavily affect the risk of venous and arterial thrombosis (14). In these situations, choosing the most suitable hormonal therapy for the individual patient can significantly reduce the absolute thrombotic risk.

INDEX OF AUTHORS

C

D

N

W

Z

Official Journal of
SISET
Società Italiana per lo Studio dell'Emostasi e della Trombosi