Research Article

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Relationship between uric acid levels and cardiometabolic findings in a large cohort of β-thalassemia major patients

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Aim: to evaluate the relationship between uric acid (UA), hepatic and cardiac iron overload (T2*-MRI), ferritin, endocrinological diseases and cardiac complications in a large thalassemia major (TM) cohort. **Methods:** A total of 369 TM patients (187 men; 33 ± 6 years) were retrospectively studied, from the myocardial iron overload in thalassemia (MIOT) electronic databank. **Results:** Multiple regression model identified male sex ($p < 0.001$), BMI ($p < 0.001$) and T2* ($p \leq 0.001$) as UA independent correlates. Moreover, UA and derivatives of reactive oxygen species (an oxidative index; $r = -0.3$; $p \leq 0.05$) are inversely correlated. Conversely, the multivariate logistic analysis identified low UA (NANHES-III criteria) as one independent predictor for low global heart T2* ($p < 0.5$) together with liver iron concentrations (>3 mg/g/dw), heart failure, endocrinopathies, ferritin (>2000 ng/l), alanine transaminase (>40 UI/l) and/or aspartate transaminase (>35 UI/l) and/or glutamyl transferase (>64 UI/l). **Discussion:** UA appears directly associated to T2* and inversely with derivatives of reactive oxygen species, and as such reduced according to increased oxidative stress and cardiac iron overload in TM patients.

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Keywords: iron overload • MIOT study • oxidative stress • thalassemia major • uric acid

Although iron chelation therapy has improved prognosis and survival in β-thalassemia major (TM), these patients are subjected to oxidative damage in different organs by secondary iron overload [1]. Heart is particularly susceptible to oxidative stress damage that can lead to heart failure, which is one of the main causes of thalassemic complications, together with hepatic and endocrinological complications [1,2]. Antioxidants represent an effective protective barrier against oxidative damage [3–5]. In particular, uric acid (UA) is a powerful endogenous antioxidant, responsible for the majority of blood antioxidant power (contribution estimated around 60/80%), which has been also associated with cardiovascular (CV) risk [6–8].

MRI represents the elective strategy to assess iron overload in different organs, including liver and heart [2,9]. In particular, CV magnetic resonance provides the opportunity to quantify biventricular function parameters with excellent reproducibility [10] and the late gadolinium enhancement (LGE) CV magnetic resonance is a noninvasive technique validated to detect necrosis/fibrosis [11,12]. Although UA has been alternatively found associated or not

to levels of ferritin in TM, there are no data focused on the association between UA levels and MRI parameters of iron overload in such patients [3,5,13].

Thus, the aim of the present study was to study the relationship between UA levels and iron status, assessed by means of serum ferritin levels and MRI-liver iron concentrations (LIC) and iron heart concentration assessed using MRI-T2* in a large cohort of TM patients. In addition, the association of UA with endocrinological features and CV complications in such patients was also evaluated.

Materials & methods

Study population

We analyzed 369 TM patients (187 men; mean age 33 ± 6 years) consecutively enrolled in the myocardial iron overload in thalassemia (MIOT) study [14]. The MIOT project is a network of nine MRI centers and 68 thalassemia centers wherein MRI examinations are performed using homogeneous, standardized and validated procedures and wherein patients' demographic, clinical, laboratory, instrumental and follow-up data are collected in a centralized database via the world wide web [14].

All patients had been regularly transfused since early childhood and began chelation therapy from the mid-to-late 1970s, although patients born after the seventies received chelation therapy from early childhood.

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the study. The project was approved by the institutional ethics committee.

Biochemical analysis

All clinical and laboratory investigations were carried out at the thalassemia centers wherein the patients were treated.

Biochemical analysis included analysis of UA, ferritin, fasting glucose, creatinine, alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (GGT), by routine clinical chemistry analyzers. Standardized protocols were adopted by all clinical laboratories, which meet the standard for blood withdrawal, taken at morning after a night's fasting, samples centrifugation at 2500 × *g*, for 10 min and immediate analytical evaluation. For UA, External Quality Assessment programs in Laboratory Medicine, conducted by the spin-off QualiMedlab-CNR (Pisa, Italy) in co-operation with ProBioQual (Lyon, France), evidenced that intra-assay and inter-assays coefficients of variation were below 5% [15].

Severe iron status was defined when ferritin resulted >2000 ng/ml [16,17].

In a subgroup of patients, data on another parameter of oxidative stress was obtained. In brief, levels of derivatives of reactive oxygen species (d-ROMs) were evaluated by using the d-ROMs test (Diacron, Italy), as previously described [18]. The results are expressed as arbitrary units (AU; d-ROM reference value corresponds to <320 AU).

MRI exam

MRI was performed using a 1.5-T scanner (GE Signa/Excite, WI, USA).

For iron overload assessment, T2* gradient-echo multiecho sequences were acquired. For the heart a multislice approach was used: basal, medium and apical slices were acquired [19,20]. A mid-hepatic slice was obtained [21]. T2* image analysis was performed using a custom written, previously validated software (HIPPOMIOT®) [22]. The software provided the T2* value for all the 16 segments of the left ventricle (LV). Global heart T2* value was obtained by averaging all segmental values. Hepatic T2* values were calculated in a circular region of interest [23] and were converted into LIC using the Wood's calibration curve [24,25].

Steady-state free procession cine images were acquired during 8 s breath-holds in sequential 8 mm short-axis slices from the atrioventricular ring to the apex to assess biventricular function parameters quantitatively in a standard way, using MASS software (Medis, Leiden, The Netherlands) [10].

LGE images were acquired in short axis views from 10 to 18 min, after intravenous administration of gadobutrol (Gadovist; Bayer Schering Pharma; Berlin, Germany) (0.2 mmol/kg), using a fast gradient-echo inversion recovery sequence. Depending on the left ventricle size, 10–14 short axis views were acquired. Also, vertical, horizontal and oblique long axis views were acquired. Inversion times were adjusted to null for the normal myocardium. The LGE was evaluated visually by experienced observers using a two-point scale, considered present if visualized in two different views [12]. LGE images were not acquired in patients with a glomerular filtration rate less than 30 ml/min/1.73 m^2 and in patients who refused the contrast medium administration.

Table 1. Demographic, clinical and laboratory characteristics of thalassemia major patients.

Variables	All	Women	Men	p-value
Demographic, clinical and instrumental parameters				
Age (years)	33 ± 6	34 ± 6	33 ± 6	ns
LIC (mg/g dw)	8 ± 9	8 ± 8	8 ± 10	ns
Global heart T2* (ms)	29 ± 12	29 ± 12	30 ± 12	ns
Cardiac dysfunction n (%)	148 (40)	54 (30)	94 (50)	<0.001
Heart failure n (%)	57 (15)	18 (10)	39 (20)	<0.001
Arrhythmias n (%)	31 (8)	10 (5)	21 (11)	<0.05
Smoking habit n (%)	90 (24)	34 (19)	56 (30)	<0.05
BMI (kg/m^2)	23 ± 3	22 ± 3	23 ± 3	<0.05
Endocrinological diseases				
Type 2 diabetes n (%)	42 (11)	26 (13)	16 (8)	ns
Hypogonadism n (%)	184 (50)	98 (54)	86 (46)	ns
Hypoparathyroidism n (%)	44 (12)	23 (13)	21 (11)	ns
Hypothyroidism n (%)	94 (25)	49 (27)	45 (24)	ns
Biochemical parameters				
UA (mg/dl)	4.4 ± 1.2	4.0 ± 1.0	4.7 ± 1.3	<0.001
Ferritin (ng/l)	1238 ± 1213	1314 ± 1380	1161 ± 1021	ns
Glycemia (mg/dl)	100 ± 36	100 ± 38	101 ± 35	ns
Creatinine (mg/dl)	0.8 ± 0.9	0.8 ± 1.0	0.8 ± 0.6	ns
ALT (UI/L)	45 ± 41	40 ± 41	53 ± 40	<0.001
AST (UI/L)	41 ± 33	36 ± 31	47 ± 34	<0.001
GGT (UI/L)	34 ± 33	27 ± 29	40 ± 38	<0.001

LIC: Liver iron concentration; ns: Not significant; UA: Uric acid.

Statistical analysis

Analyses were performed using the statistical package Statview, version 5.0.1 (SAS Institute, Abacus Concept, Inc., CA, USA). All continuous variables were expressed as mean ± standard deviation. Categorical variables were expressed as frequencies and percentages.

Comparisons between groups were made by independent samples t-test or Mann–Whitney test. χ^2 test was performed for categorical variables, while regression analysis was performed to assess the relationship between continuous variables.

Owing to skewness, log transformation of ferritin, glycemia, ALT, AST and GGT were used for statistical analyses. Log-transformed values were then back-transformed for data presentation.

All variables in Table 1 were evaluated as determinants for UA levels in TM patients, and variables with univariate association of $p \leq 0.05$ were included into a multivariate regression to estimate independent factors for elevated UA.

Variables with univariate association of $p \leq 0.05$ were entered into a logistic multivariate analysis to estimate independent predictors for global heart T2* after adjusting for risk factors.

A two-tailed probability value of 0.05 was considered statistically significant.

Diagnostic criteria for cardiac complications

Heart dysfunction was diagnosed in the presence of left ventricular and/or right ventricular ejection fractions <2 standard deviation from the mean values normalized to age and [26] gender.

Heart failure was identified based on symptoms, signs, biomarkers and instrumental parameters, according to the AHA/ACC [27] guidelines.

Arrhythmias were diagnosed and classified according to the AHA/ACC guidelines [28].

Results

Characteristics of patients

Demographic and clinical characteristics of the enrolled TM patients are shown in Table 1.

Table 2. Predictors for global heart T2* <20 ms in logistic univariate and multivariate models.

Variables	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Age <38 years	2.2	1.2–4.1	<0.05	–	–	–
LIC >3 mg/g/dw	5.4	2.9–9.8	<0.001	4.0	1.9–8.5	<0.001
Heart failure	4.2	2.3–7.5	<0.001	4.1	1.8–9	<0.001
Smoking habit	1.9	1.1–3.2	<0.05	–	–	–
endocrinopathies	2.6	1.5–4.5	<0.001	3.6	1.8–7.2	<0.001
UA (<7.0 mg/dl in men and <5.7 mg/dl in women)	6.2	0.8–47	≤0.05	8.8	1.1–74	<0.05
Ferritin >2000 ng/l	6	3.4–10.6	<0.001	3.0	1.4–6.4	<0.01
ALT >40 UI/l and/or AST >35 UI/l and/or GGT >64 UI/l	3	1.6–5.4	<0.001	2.6	1.2–5.3	≤0.01

LIC: Liver iron concentration; OR: Odd ratio; UA: Uric acid.

There were 91 patients (25%; 43 men) with significant cardiac iron (global heart T2* value <20 ms). The contrast medium was not administrated in 47 patients. No patient showed an ischemic pattern of myocardial fibrosis. Among the patients with LGE areas, the 56.1% had two or more foci of fibrosis, involving the septum more frequently.

Heart dysfunction, heart failure and arrhythmias (supraventricular in the 93.5% of the cases) were significantly more frequent in males.

UA & its correlates in TM patients

UA levels ranged from 1.5 to 8.7 mg/dl (skewness: 0.7; kurtosis: 0.4) in the whole population.

As expected, UA levels were significantly higher in male than in female patients (4.8 ± 1.3 vs 4.0 ± 1.0 mg/dl; $p < 0.001$). Hyperuricemia, defined as a UA level >7.0 mg/dl in men and >5.7 mg/dl in women according to the US National Health and Nutrition Examination Survey (NHANES-III) laboratory definition, was observed in 10 (5%) males and 9 (5%) females, respectively.

UA did not correlate with ferritin or LIC values. No association between UA and endocrinological diseases or cardiac complications was observed. Other than sex, among all the variables reported in Table 1, UA directly correlated with global heart T2* ($r = 0.2$; $p < 0.001$), BMI ($r = 0.25$; $p < 0.001$) and GGT ($r = 0.11$; $p < 0.05$). The multiple regression model identified male sex (T-value = 4.9; $p < 0.001$), BMI (3.8; $p < 0.001$) and global heart T2* (3.3; $p \leq 0.001$) as independent correlates of UA levels.

Data on alcohol consumption were available in 367 subjects; subjects who consumed alcohol (male: >60 g/day; female: >40 g/day) had higher UA levels (4.4 ± 1.3 vs 3.6 ± 0.8 mg/dl; $p \leq 0.01$). When this variable was added to the multivariate analysis, it remained as an independent factor (T-value = 2.2; $p < 0.05$) for UA together with male sex, BMI and heart T2*.

Moreover, in a small subgroup of patients (n = 68), we obtained data on d-ROMs, and mean values were 309 ± 60 AU, with 33% of patient showing values above 320 AU (reference range). An indirect relationship was observed between UA and d-ROMs ($r = -0.3$; $p \leq 0.05$).

Global heart T2* predictors

Patients with T2* global heart ≥20 ms showed higher levels of UA in respect to those with global heart T2* <20 ms (4.5 ± 1.3 vs 4.1 ± 1.1 mg/dl; $p < 0.01$).

At the univariate analysis, age <38 years (75th percentile), LIC (>3 mg/g/dw), heart failure or endocrinopathies (Type 2 diabetes, or hypogonadism, hypoparathyroidism, hypothyroidism), smoking habit, lower UA (according to NANHES-III criteria), ferritin (>2000 ng/ml) and elevation of liver enzymes resulted in significant predictors for low global heart T2* (<20 ms) (Table 2). The multivariate logistic analysis confirmed low UA as one significant independent predictor for low global heart T2* (Odds ratio: 8.8; CI: 1.1–74; $p < 0.5$), together with LIC (>3 mg/g/dw), heart failure, endocrinopathies, ferritin (>2000 ng/l), ALT (>40 UI/l) and/or AST (>35 UI/l) and/or GGT (>64 UI/l) (Table 2).

Discussion

Our data suggest that UA levels did not correlate with ferritin or MRI-LIC, nor with extent of endocrinological diseases or cardiac complications, but it was independently correlated with global heart T2* values (so lower whether higher cardiac overload) in TM patients.

Accordingly, UA resulted in an interesting biomarker in the cardiological field, considered with suspicion due to its frequent correlation with CV established risk factors, outcomes and overall mortality [6–8]. Nonetheless, as elevated UA is associated with CV risk and events, in different studies this power is lost after adjustment for potential confounders, thus suggesting that UA is not independent from other risk factors [6,29]. Accordingly, the role of UA as a CV causative factor appeared uncertain in different recent meta-analyses [30,31].

Now at the core of the discussion about UA is whether this parameter represents an independent risk factor for CV events with a direct and causal role or whether it is just a marker for an adverse risk profile [6]. We recently evaluated UA significance as predictor for hard events (HE; mortality and nonfatal myocardial infarction) in a large cohort of patients referred for coronary angiography [29]. High UA results associated with HE only in women, although this relationship loses its significance after multivariate adjustment [29]. Moreover, being that UA is correlated with the ferric reducing ability of plasma (an index of antioxidant capacity), its elevation appears more likely compensatory than causative for HE [29].

In TM patients, although recurrent transfusions may favor oxidative stress, data concerning the antioxidant status are quite contradictory, because the total antioxidant capacity or single different antioxidants have been generally found enhanced in TM patients in respect to control subjects, and interpreted as a reaction to increased oxidant status [32–36]. As higher UA may be associated with severity of ineffective erythropoiesis and as a result of destruction of nucleated red cells. Previous data generally show higher UA levels in TM patients compared with control subjects, although in one case hyperuricosuria without hyperuricemia was observed in a group 37 children with TM [4–5,37]. This point is important because, beyond its antioxidant capacity, experimental data suggested that UA at high levels may act as pro-oxidant adverse factor, as it directly induces cardiomyocyte growth and heart interstitial fibrosis, affecting growth and profibrotic signaling pathways, and inducing superoxide production and endothelin-1 protein and mRNA expression [38,39]. In this context, although none report the percentage of hyperuremic patients, it is important to evidence that although UA resulted higher in TM in respect to control subjects, mean level found in such studies were not high at all (in the first study 5.5 vs 4.7 mg/dl, in the other one 4.11 vs 3.27 mg/dl; patients vs controls) [4,5]. Accordingly, in our population UA mean corresponded to 4.4 mg/dl (well lower than the reference limit of the NHANES-III criteria), likely not so high to reach values such as to induce pro-oxidizing effects, with a percentage of hyperuricemic patients that resulted low (5%). Moreover, although the odds ratio and CI (index of the accuracy of the estimate) for UA are quite wide, UA results significantly related inversely to cardiac iron overload (being UA directly related to global heart T2* values: higher T2*, lower cardiac iron). Moreover, there is an inverse relationship between UA and d-ROMs (index of oxidative stress). Thus, UA appeared reduced according to increased oxidative stress and cardiac iron overload in TM patients. In this context, it is important to remind that increased UA may be related to higher intake of purine or fructose and alcohol, information not reported in the available studies on TM patients [6,40]. Unfortunately, also in the MIOT database a food frequency questionnaire is not available, although TM subjects enrolled followed dietary habits that were characteristic of the Mediterranean diet. Instead, alcohol consumption that we evaluated in a subgroup of TM patients, emerges as an independent predictor for UA levels.

It is known that UA is related to renal function. In our population there was not a significant correlation between UA and creatinine, index of kidney function. This result may be due to the fact that in the overall population the majority of patients had conserved renal function, as only 19 subjects (5%) had glomerular filtration rate <60 ml/min/1.73 m^2.

We observed that UA elevation is closely associated with BMI, and also directly with triglycerides and inversely with high density lipoproteins ($n = 368$; $r = 0.3$; $p < 0.001$ and $n = 364$; $r = -0.27$; $p < 0.001$, logTG and high density lipoproteins, respectively; data not shown), being possibly involved in the development of the cluster of CV risk factors associated with metabolic syndrome in our TM population. Now, there are increasing data which evidence the relationship between UA and metabolic syndrome and its components, although there are no data on the relationship between UA and metabolic risk factors in TM [41–43]. Previous findings, obtained with different other antioxidants, especially levels of catalase activity and reduced glutathione, found a relationship between higher oxidative stress and metabolic parameters in β-thalassemia patients [32]. Thus, it remains to be

evaluated if the direct correlation of UA with BMI in TM patients may reflect a compensative antioxidant response to overweight/obesity-induced oxidative stress.

We did not observe a significant relationship between UA and ferritin levels, an association which has been alternatively observed or not found in other TM cohorts [3,5,13]. Instead, we confirmed the close association between serum ferritin and liver enzymes (AST: $r = 0.4$; $p < 0.001$, ALT: $r = 0.33$; $p < 0.001$ and GGT: $r = 0.12$; $p < 0.05$; data not shown), which suggests that hepatocellular injury and liver dysfunction are related to iron overload [44].

Interestingly, female patients which presented cardiac fibrosis also had higher levels of UA with respect to female patients without cardiac fibrosis (4.4 ± 1.3 vs 3.9 ± 1 mg/dl; $p < 0.05$). This result, observed only in TM females, could be interesting in view of the fact that TM females survive longer and develop less cardiac complications (heart failure or arrhythmias), at parity of accumulated heart iron [10,45]. Moreover, as myocardial fibrosis and iron overload represented two independent events in TM [46], these findings may suggest a different relationship between cardiac fibrosis, iron overload and oxidative stress in TM patients belonging to different sexes. Interestingly, in the CV field, increasing data suggest a stronger association between UA and cardiac ischemic disease in women, compared with a lesser degree in men [6,29]. However, now it is difficult to advance hypothesis and identify the mechanisms at the basis of sex specific differences associated to these events.

Conclusion

UA results directly related to global heart T2* values and inversely with d-ROMs, and as such it appeared reduced according to increased oxidative stress and cardiac iron overload in TM patients.

In addition, UA did not correlate with iron status assessed by means of serum ferritin levels and MRI-LICs, neither with endocrinological features and CV complications.

Summary points

- The relationship between uric acid (UA), hepatic and cardiac iron overload (T2*-MRI), ferritin, endocrinological diseases and cardiac complications was evaluated in a large thalassemia major cohort.
- Multiple regression model identified male sex, BMI and T2* as UA independent correlates.
- UA and derivatives of reactive oxygen species (an oxidative index) are inversely correlated.
- The multivariate logistic analysis identify low UA (NANHES-III criteria) as one independent predictor for low global heart T2* together with liver iron concentration (>3 mg/g/dw), heart failure, endocrinopathies, ferritin (>2000 ng/l), alanine transaminase (>40 UI/l) and/or aspartate transaminase (>35 UI/l) and/or glutamyl transferase (>64 UI/l).
- UA appears to be directly associated to T2* and inversely with derivatives of reactive oxygen species, so reduced with increased oxidative stress and cardiac iron overload in thalassemia major patients.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the study. The project was approved by the institutional ethics committee.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Berdoukas V, Coates TD, Cabantchik ZI. Iron and oxidative stress in cardiomyopathy in thalassemia. *Free Radic. Biol. Med.* 88(Pt A), 3–9 (2015).
2. Pepe A, Meloni A, Rossi G *et al.* Cardiac complications and diabetes in thalassaemia major: a large historical multicentre study. *Br. J. Haematol.* 163(4), 520–527 (2013).

3. Naithani R, Chandra J, Bhattacharjee J, Verma P, Narayan S. Peroxidative stress and antioxidant enzymes in children with beta-thalassemia major. *Pediatr. Blood Cancer* 46(7), 780–785 (2006).
4. Livrea MA, Tesoriere L, Pintaudi AM *et al.* Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood* 88(9), 3608–3614 (1996).
5. Bazvand F, Shams S, Borji Esfahani M *et al.* Total antioxidant status in patients with major beta-thalassemia. *Iran J. Pediatr.* 21(2), 159–165 (2011).
6. Vassalle C, Mazzone A, Sabatino L, Carpeggiani C. Uric acid for cardiovascular risk: Dr. Jekyll or Mr. Hide? *Diseases* 4, 12 (2016).

• **Uric acid (UA) and its relationship with cardiovascular risk.**

7. Gagliardi AC, Miname MH, Santos RD. Uric acid: a marker of increased cardiovascular risk. *Atherosclerosis* 202(1), 11–17 (2009).

•• **UA and cardiovascular risk.**

8. Kanbay M, Segal M, Afsar B, Kang DH, Rodriguez-Iturbe B, Johnson RJ. The role of uric acid in the pathogenesis of human cardiovascular disease. *Heart* 99(11), 759–766 (2013).
9. Borgna-Pignatti C, Meloni A, Guerrini G *et al.* Myocardial iron overload in thalassaemia major. How early to check? *Br. J. Haematol.* 164(4), 579–585 (2014).
10. Marsella M, Borgna-Pignatti C, Meloni A *et al.* Cardiac iron and cardiac disease in males and females with transfusion-dependent thalassemia major: a T2* magnetic resonance imaging study. *Haematologica* 96(4), 515–520 (2011).

• **Gender-related differences in cardiac iron overload in thalassemia major patients.**

11. Meloni A, Pepe A, Positano V *et al.* Influence of myocardial fibrosis and blood oxygenation on heart T2* values in thalassemia patients. *J. Magn. Reson. Imaging* 29(4), 832–837 (2009).
12. Pepe A, Positano V, Capra M *et al.* Myocardial scarring by delayed enhancement cardiovascular magnetic resonance in thalassaemia major. *Heart* 95, 1688–1693 (2009).
13. Rasool M, Malik A, Jabbar U *et al.* Effect of iron overload on renal functions and oxidative stress in beta thalassemia patients. *Saudi Med. J.* 37(11), 1239–1242 (2016).
14. Meloni A, Ramazzotti A, Positano V *et al.* Evaluation of a web-based network for reproducible T2* MRI assessment of iron overload in thalassemia. *Int. J. Med. Inform.* 78(8), 503–512 (2009).
15. Qualimedlab. www.qualimedlab.it
16. Casale M, Meloni A, Filosa A *et al.* Multiparametric cardiac magnetic resonance survey in children with thalassemia major: a multicenter study. *Circ. Cardiovasc. Imaging* 8(8), e003230 (2015).
17. De Sanctis V, Elsedfy H, Soliman AT *et al.* Acquired hypogonadotropic hypogonadism (AHH) in thalassaemia major patients: an underdiagnosed condition? *Mediterr. J. Hematol. Infect. Dis.* 8(1), e2016001 (2016).
18. Vassalle C. An easy and reliable automated method to estimate oxidative stress in the clinical setting. *Methods Mol. Biol.* 477, 31–39 (2008).
19. Pepe A, Positano V, Santarelli F *et al.* Multislice multiecho T2* cardiovascular magnetic resonance for detection of the heterogeneous distribution of myocardial iron overload. *J. Magn. Reson. Imaging* 23(5), 662–668 (2006).
20. Meloni A, Positano V, Pepe A *et al.* Preferential patterns of myocardial iron overload by multislice multiecho T*2 CMR in thalassemia major patients. *Magn. Reson. Med.* 64(1), 211–219 (2010).
21. Positano V, Salani B, Pepe A *et al.* Improved T2* assessment in liver iron overload by magnetic resonance imaging. *Magn. Reson. Imaging* 27(2), 188–197 (2009).
22. Positano V, Pepe A, Santarelli MF *et al.* Standardized T2* map of normal human heart in vivo to correct T2* segmental artefacts. *NMR Biomed.* 20(6), 578–590 (2007).
23. Meloni A, Luciani A, Positano V *et al.* Single region of interest versus multislice T2* MRI approach for the quantification of hepatic iron overload. *J. Magn. Reson. Imaging* 33(2), 348–355 (2011).
24. Wood JC, Enriquez C, Ghugre N *et al.* MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 106(4), 1460–1465 (2005).
25. Meloni A, Rienhoff Hy JR, Jones A, Pepe A, Lombardi M, Wood JC. The use of appropriate calibration curves corrects for systematic differences in liver R2* values measured using different software packages. *Br. J. Haematol.* 161(6), 888–891 (2013).
26. Meloni A, Dell'amico M, Favilli B *et al.* Left ventricular volumes, mass and function normalized to the body surface area, age and gender from CMR in a large cohort of well-treated thalassemia major patients without myocardial iron overload. *J. Cardiovasc. Magn. Reson.* 13(Suppl. 1), P305 (2011).
27. Jessup M, Abraham WT, Casey DE *et al.* 2009 focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. *Circulation* 119(14), 1977–2016 (2009).

28. Buxton AE, Calkins H, Callans DJ *et al.* ACC/AHA/HRS 2006 key data elements and definitions for electrophysiological studies and procedures: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (ACC/AHA/HRS Writing Committee to Develop Data Standards on Electrophysiology). *Circulation* 114(23), 2534–2570 (2006).
29. Vassalle C, Chatzianagnostou K, Vannucci A *et al.* Gender differences for uric acid as predictor of hard events in patients referred for coronary angiography. *Biomark. Med.* 10(4), 349–355 (2016).
30. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. *Arthritis Care Res.* 62(2), 170–180 (2010).

•• **Hyperuricemia and risk of coronary heart disease events, also by a gender point of view.**

31. Wheeler JG, Juzwishin KD, Eiriksdottir G, Gudnason V, Danesh J. Serum uric acid and coronary heart disease in 9458 incident cases and 155,084 controls: prospective study and meta-analysis. *PLoS Med.* 2(3), e76 (2005).
32. Boudrahem-Addour N, Izem-Meziane M, Bouguerra K *et al.* Oxidative status and plasma lipid profile in beta-thalassemia patients. *Hemoglobin* 39(1), 36–41 (2015).

• **Relationship between oxidative stress and lipid profile in β-thalassemia patients.**

33. Hamed EA, Elmelegy NT. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. *Ital. J. Pediatr.* 36, 39 (2010).
34. Ozdemir ZC, Koc A, Aycicek A, Kocyigit A. N-Acetylcysteine supplementation reduces oxidative stress and DNA damage in children with beta-thalassemia. *Hemoglobin* 38(5), 359–364 (2010).
35. Cakmak A, Soker M, Koc A, Aksoy N. Prolidase activity and oxidative status in patients with thalassemia major. *J. Clin. Lab. Anal.* 24(1), 6–11 (2010).
36. Shazia Q, Mohammad ZH, Rahman T, Shekhar HU. Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in Beta thalassemia major patients: a review of the literature. *Anemia* 2012, 270923 (2012).
37. Smolkin V, Halevy R, Levin C *et al.* Renal function in children with beta-thalassemia major and thalassemia intermedia. *Pediatr. Nephrol.* 23(10), 1847–1851 (2008).
38. Chen CC, Hsu YJ, Lee TM. Impact of elevated uric acid on ventricular remodeling in infarcted rats with experimental hyperuricemia. *Am. J. Physiol. Heart Circ. Physiol.* 301(3), H1107–H1117 (2011).
39. Jia G, Habibi J, Bostick BP *et al.* Uric acid promotes left ventricular diastolic dysfunction in mice fed a western diet. *Hypertension* 65(3), 531–539 (2015).
40. Manafikhi H, Drummen G, Palmery M, Peluso I. Total antioxidant capacity in beta-thalassemia: a systematic review and meta-analysis of case–control studies. *Crit. Rev. Oncol. Hematol.* 110, 35–42 (2017).
41. Khichar S, Choudhary S, Singh VB, Tater P, Arvinda RV, Ujjawal V. Serum uric acid level as a determinant of the metabolic syndrome: a case control study. *Diabetes Metab. Syndr.* 11(1), 19–23 (2016).

•• **Relationship between UA and metabolic syndrome.**

42. Abbasian M, Ebrahimi H, Delvarianzadeh M, Norouzi P, Fazli M. Association between serum uric acid (SUA) levels and metabolic syndrome (MetS) components in personnel of Shahroud University of Medical Sciences. *Diabetes Metab. Syndr.* 10(3), 132–136 (2016).
43. Bonakdaran S, Kharaqani B. Association of serum uric acid and metabolic syndrome in Type 2 diabetes. *Curr. Diabetes Rev.* 10(2), 113–117 (2014).
44. Wang M, Liu R, Liang Y *et al.* Iron overload correlates with serum liver fibrotic markers and liver dysfunction: potential new methods to predict iron overload-related liver fibrosis in thalassemia patients. *United Eur. Gastroenterol. J.* 5(1), 94–103 (2017).
45. Vassalle C, Meloni A, Pistoia L, Pepe A. Liver-pancreas- heart triangle and HCV in thalassemia: expanding the horizon through biomarker networks. *Int. J. Hematol. Ther.* 3(1), 1–6 (2017).

• **Role of biomarkers in thalassemia major complexity.**

46. Sanyal SK, Johnson W, Jayalakshmamma B, Green AA. Fatal "iron heart" in an adolescent: biochemical and ultrastructural aspects of the heart. *Pediatrics* 55(3), 336–341 (1975).