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# OUTCOMES VIROLOGICI E CLINICI CON LA TERAPIA ANTIVIRALE ORALE IN PAZIENTI CON CIRROSI HCV-CORRELATA

# VIROLOGICAL AND CLINICAL OUTCOMES WITH ORAL ANTIVIRAL THERAPY IN HCV-RELATED CIRRHOSIS

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**VIROLOGICAL AND CLINICAL OUTCOMES WITH ORAL ANTIVIRAL THERAPY IN HCV-RELATED CIRRHOSIS**

**Abstract:** The hepatitis C virus (HCV) treatment landscape has rapidly changed over recent years. The development of direct-acting antiviral (DAA) agents that specifically target various steps in the HCV lifecycle has revolutionized therapeutic options for patients with HCV, with the development of highly effective and well-tolerated oral interferon-free (IFN-free) regimens. There are several DAAs that have been approved and others that are currently in development, targeting different nonstructural HCV or host proteins that are essential for HCV replication. Data on virological response in different HCV genotypes and patients subgroups are well established in the literature, while results on clinical outcome are much more limited.

In this thesis, I will describe real-life virological and clinical outcome with DAA therapy in a large cohort of patients with HCV treated in clinical practice in the Veneto Region. For this purpose, we have used the NAVIGATORE web-platform that is recording and monitoring all HCV-DAA based treatments in the Veneto region and we have assessed virological and clinical outcomes of DAAs treatment in 3 subgroups of patients: 1) HCV infected cirrhotics (both compensated and decompensated) to clarify drugs efficacy and clinical outcomes, including long term benefits of treatment, 2) Patients with extrahepatic manifestations due to HCV and 3) patients with HCV aged > 70 years.

Our analysis has been based on a cohort of 2946 cirrhotic patients. DAAs were safe and efficacious in these patients, including those with advanced, or decompensated cirrhosis. SVR rates for most genotypes were excellent, but the initially used Sofosbuvir plus Ribavirin regimens were suboptimal in HCV-1 and HCV-3 infected patients. Efficacy was not significantly affected by common comorbidities but it was reduced in patients with Child B cirrhosis. Severe adverse event were unusually: tolerability and safety were excellent in patients with Child A cirrhosis, while were reduced in Child B patients, particularly when treated with Ribavirin containing regimens . Patients with decompensated cirrhosis often showed an improvement in Child scores and patients with compensated cirrhosis showed a reduced incidence of complications, compared to the expected rates in the natural history of untreated disease. The "de novo" incidence of HCC was also reduced during and after DAA and the incidence of HCC recurrence not increased during and after DAAs. A subgroup of patients developed an aggressive type of HCC during therapy, and this was more frequent in non-responders, an observation that certainly deserves further evaluation. Extrahepatic manifestations of HCV infection also improved with successful antiviral therapy. Moreover, a sub-analysis in a group of patients over 70 years, the "elderly group", confirmed excellent safety and efficacy of DAAs also in this setting.

**OUTCOMES VIROLOGICI E CLINICI OTTENUTI CON LA TERAPIA ANTIVIRALE ORALE IN PAZIENTI CON CIRROSI HCV-CORRELATA**

**Abstract:** Il panorama del trattamento dell'epatite C si è rapidamente modificato negli ultimi 7 anni. Lo sviluppo di antivirali diretti (direct-acting antiviral = DAA), che hanno come bersaglio diversi punti nel ciclo vitale del virus dell'epatite C (HCV), ha rivoluzionato le opzioni terapeutiche per i pazienti con epatite C, con la disponibilità di regimi orali (non più basati sull'interferone) altamente efficaci e ottimamente tollerati. Ci sono diversi DAAs attualmente in sviluppo o già approvati, che hanno come bersaglio diverse proteine di HCV non-strutturali o altri targets essenziali per la replicazione di HCV. I dati di risposta virologica nei diversi genotipi HCV e in diversi sottogruppi di pazienti sono ben documentati in letteratura, mentre i risultati sugli outcomes clinici sono molto più limitati.

In questa tesi sono descritti gli outcomes "real-life" clinici e virologici, ottenuti con la terapia con DAA in una larga coorte di pazienti con epatopatia HCV correlata, trattati nella pratica clinica nella Regione Veneto. A tal proposito, abbiamo usato la piattaforma NAVIGATORE, che registra e monitorizza tutti i trattamenti per HCV DAA-based in Veneto, ed abbiamo valutato gli outcomes clinici e virologici ottenuti con trattamenti con DAA in 3 gruppi di pazienti: 1) pazienti con cirrosi HCV correlata (sia compensati che scompensati) allo scopo di verificare l'efficacia e gli outcomes clinici, inclusi i benefici a lungo termine del trattamento, in questa categoria di pazienti., 2) pazienti con manifestazioni extraepatiche da HCV e 3) un gruppo di pazienti con età > 70 anni.

La nostra analisi ha considerato una coorte di 2946 pazienti cirrotici. I DAAs si sono dimostrati sicuri ed efficaci in questi pazienti, inclusi quelli con cirrosi avanzata o scompensata. I tassi di SVR per la maggior parte dei genotipi sono stati eccellenti, ma i regimi usati inizialmente con solo sofosbuvir e ribavirina erano subottimali nei pazienti con infezione da genotipo 1 e 3.

L'efficacia non è stata significativamente influenzata dalla presenza delle più comuni comorbidità, ma è risultata ridotta nei pazienti con cirrosi in Child B. Gli effetti collaterali gravi sono rari: la tollerabilità e la sicurezza sono state eccellenti nei pazienti sia con fibrosi F3 che in quelli con cirrosi in Child A, mentre sono risultate ridotte nei pazienti in Child B, particolarmente quando trattati con regimi contenenti ribavirina. I pazienti con cirrosi scompensata hanno spesso presentato un miglioramento nel punteggio di Child ed i pazienti con cirrosi compensata hanno mostrato una ridotta incidenza di complicanze rispetto ai tassi attesi nella storia naturale della malattia non trattata. L'incidenza di HCC "de novo" è risultata ridotta durante e dopo terapia con DAA e l'incidenza della recidiva di HCC non è aumentata durante o dopo terapia con DAA. Un sottogruppo di questi pazienti ha sviluppato un tipo di HCC più aggressivo durante la terapia, e ciò si è verificato più frequentemente nei pazienti non-responder alla terapia, osservazione questa che certo merita ulteriori valutazioni. Anche le manifestazioni extraepatiche HCV correlate sono migliorate in relazione al successo terapeutico. Inoltre una sub-analisi in un gruppo di pazienti ultra-settantenni ("gruppo Anziani") ha confermato una sicurezza ed una efficacia della terapia con DAA eccellenti anche in questo setting.

## INTRODUCTION

Infection with the hepatitis C virus (HCV) remains a significant global health issue. Chronic hepatitis C is a leading cause of cirrhosis and hepatocellular carcinoma and of liver related deaths. Moreover, chronic HCV infection has been shown to contribute to extrahepatic morbidity and mortality, causing lymphoproliferative disorders, vasculitis, and contributing to development of type 2 diabetes and associated cardiovascular disease. Hepatitis C is a slowly progressing liver disease, with cirrhosis occurring about 20–30 years after infection. Cirrhosis cannot be viewed as a single, irreversible end-stage phase of HCV disease but rather as a spectrum characterized by progressive increase in hepatic venous pressure gradient and decrease in liver function, finally leading to hepatic decompensation. The occurrence of varices initiates the second stage of cirrhosis, and portal hypertension progresses as the third stage defined by the development of ascites. Variceal haemorrhage initiates the fourth stage. The occurrence of bacterial infections delineates an additional fifth stage of cirrhosis with high mortality rates. Variceal bleeding, bacterial infections, hepatorenal syndrome, acute on chronic liver failure and hepatocellular carcinoma are the leading causes of death. With the ageing HCV population, there is a growing burden of complications of chronic HCV infection (CHC), including cirrhosis, decompensated liver disease, and hepatocellular carcinoma. These complications are expected to triple over the next 15 years. However, CHC is curable, and viral eradication is associated with improved patient survival and reduced complications of CHC.

For the past decade, the only therapy for HCV has been pegylated-interferon combined with ribavirin (PR). Unfortunately, this therapy was limited by the significant toxicity, inconvenience of administration via injection, extended treatment duration (up to 48 weeks) and suboptimal response rates (overall response rates 54%–56%). Furthermore, not all patients were eligible for PR therapy. Thus, there was an urgent need for more efficacious and better-tolerated treatments for HCV.

In 2010 the first proof of concept study showed that an IFN-free using a combination of direct-acting antivirals (DAAs) agents was possible and highly effective. The development of a cell culture system for HCV and subsequent detailed characterization of the HCV lifecycle have allowed the development of new therapies that directly target steps in the HCV replication cycle, so called direct-acting antivirals. The development of these DAAs has rapidly evolved and has radically changed the HCV treatment paradigm to allow IFN-free regimens. Interferon (IFN)-free treatments are now the treatment of choice for

patients with chronic hepatitis C. Previously difficult to treat patients by IFN-containing treatments can now be treated safely by IFN-free therapies (1,2).

## HCV lifecycle

HCV is a small enveloped single-stranded RNA virus with a 9,600 nucleotide genome. HCV gains entry into hepatocytes through a receptor complex. After endocytosis, uncoating occurs and the HCV genomic RNA is released from the nucleocapsid into the cytoplasm. Translation into a single large polyprotein occurs in the endoplasmic reticulum, which is cleaved by viral and host proteases into ten mature HCV proteins, including structural proteins (HCV core protein and envelope proteins E1 and E2) and nonstructural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). These viral and host proteins form a membrane-bound replication complex and transcription takes place, which is dependent upon the RNA-dependent RNA polymerase (NS5B polymerase), with the positive-strand RNA serving as the template for transcription. Mature virions are formed, leading to virion budding and release. Each step of the HCV lifecycle could be a potential target for DAAs (Figure 1). The NS3/4A protease, NS5A protein, and NS5B polymerase are key enzymes in the HCV replication cycle. Post-translational cleavage of the polyprotein is dependent on the NS3 protease. The NS5B polymerase is required for viral replication. The NS5A protein plays a necessary role in viral replication and assembly (2). Inhibition of these enzymes are attractive targets for anti-HCV therapy and are currently the most advanced available agents (table 1) (3-10).

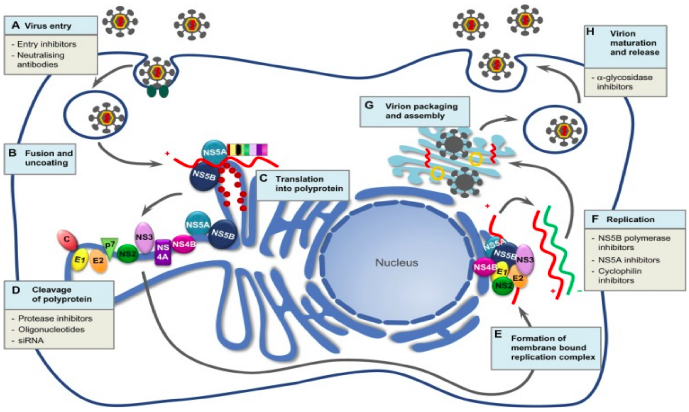


*Figure 1 Hepatitis C virus (HCV) lifecycle and potential targets for direct-acting antivirals (DAAs).*

*(A) The virus gains entry by receptor-mediated endocytosis. (B) Fusion and uncoating occur and the HCV genomic RNA is released from the nucleocapsid into the cytoplasm. (C) Translation into a single large polyprotein occurs in the endoplasmic reticulum. (D) This polyprotein is then cleaved by viral and host proteases into 10 mature HCV proteins, including structural proteins (HCV core protein and envelope proteins E1 and E2) and non-structural proteins (P7,NS3, NS4A, NS4B, NS5A, and NS5B). (E) These viral and host proteins form a membrane bound replication-complex. (F) Transcription takes place, dependent upon the RNA helicase (RNA-dependent RNA polymerase or NS5B polymerase) where the positive-strand RNA serves as a template for transcription. (G) Virion assembly occurs in the Golgi apparatus when viral glycoproteins combine with newly produced RNA. (H) Virion maturation, budding and release from the hepatocyte occurs. The site of action of current DAAs are listed at each step in the HCV life cycle.*

| *DRUG CLASS* | *POTENCY* | *GENOTYPE* | *GENETIC BARRIER* | *DDI* | *SAFETY IN DECOMPENSATED* |
|---|---|---|---|---|---|
| ***ProteaseInhibitors*** | | | | | |
| Simeprevir | + | HCV-1,4 | low | ++ | no |
| Paritaprevir | ++ | HCV-1,4 | low | ++ | no |
| Grazoprevir | ++ | HCV-1,4 | low | ± | no |
| ***NS 5A Inhibitors*** | | | | | |
| Daclatasvir | ++ | pangeno | low | + | yes |
| Ledipasvir | ++ | HCV-1,3,4 | low | + | yes |
| Ombitasvir | ++ | HCV-1,4 | low | ++ | ? |
| Elbasvir | ++ | HCV-1,4 | low | ± | no |
| Velpatasvir | ++ | pangeno | low | ± | no |
| ***NS5B Polymerase inhibitors*** | | | | | |
| **NNUC** | | | | | |
| Dasabuvir | +/- | HCV-1 | low | ++ | no |
| **NUC** | | | | | |
| Sofosbuvir | ++ | pangeno | high | ± | yes |

*Table 1: The various classes of agents developed to treat HCV (DAAs).*

*Available DAAs target proteins involved with the replication of HCV and include NS5B nucleotide polymerase inhibitors, NS5B non-nucleoside polymerase inhibitors, and NS5A replication complex inhibitors and protease inhibitors.*

## Direct-acting antiviral agents

The ideal DAA has a high barrier to resistance, pangenotypic activity, picomolar potency, few drug–drug interactions (DDIs), minimal toxicity, and a pharmacokinetic profile that allows once daily dosing. The genetic barrier to resistance is the number of amino acid substitutions required to confer resistance to a DAA. DAAs with a low barrier to resistance only require 1–2 substitutions in order to render the drug ineffective, whereas a DAA with a high genetic barrier to resistance requires 3 or more substitutions, and hence drugs with a high barrier to resistance are preferred as the backbone of many IFN-free DAA combinations. In addition, the replication fitness of resistance-associated variants (RAVs) needs to be considered, as RAVs with poor replication fitness may not emerge to be dominant under DAA selection pressure (e.g., S282T RAV with sofosbuvir), whereas RAVs with preserved replication fitness emerge rapidly to become dominant (e.g., Q80K in the context of simeprevir)(2).

**NS3/4A protease inhibitors**

NS3/4A protease inhibitors (PIs) block the catalytic site of the NS3/4A protease, resulting in failure of polyprotein cleaving and processing. The first DAAs to be developed and licensed for the treatment of HCV genotype 1 (HCV-1) were the NS3/4A PIs, telaprevir, and boceprevir. While offering a significant improvement in sustained virological response rates (SVRs) for HCV-1, these agents required combination with PR and had significant additional toxicity to PR, a large daily pill burden, and significant DDIs. Simeprevir (SMV), a second-wave first-generation NS3/4A PI with a more favourable pharmacokinetic profile allowing once daily dosing, with less additional toxicity on top of PR therapy and improved genotype coverage, has since been developed and has rapidly replaced telaprevir and boceprevir. Second-generation NS3/4A PIs offer the advantages of higher potency and improved adverse event (AE) profiles, while still allowing daily dosing. (see paritaprevir PTV, grazoprevir GZR...)(2).

**NS5B polymerase inhibitors**

The NS5B polymerase is highly conserved across all HCV genotypes, and thus is an ideal target for DAA development. NS5B polymerase inhibitors are divided into two types: nucleos(t)ide NS5B inhibitors and non-nucleos(t)ide NS5Binhibitors.

Nucleos(t)ide NS5B inhibitors: Nucleos(t)ide analogs of the NS5B polymerase act as chain terminators within the catalytic site of the NS5B polymerase (nucleotide inhibitors [NIs]). These agents provide a high genetic barrier to resistance, have pangenotypic activity (as the NSB5 polymerase is highly conserved), high potency, and limited DDIs, and offer daily dosing. SOF is the only NI that has reached the market to date.

Nonnucleos(t)ide NS5B inhibitors: These agents bind to different allosteric sites of the NS5B polymerase, which result in conformational changes, rendering the polymerase ineffective. These domains are highly variable between genotypes, and hence these agents only have activity against specific genotypes. This class of agents also has a low barrier to resistance (2).

**NS5A inhibitors**

The NS5A protein has multiple functions and is important in viral replication and assembly. Inhibitors of the NS5A protein have been shown to be potent antivirals, although the exact mechanism by which these agents interact with the NS5A protein and inhibit HCV replication remains unclear. Several NS5A inhibitors have now been licensed, including daclatasvir (DCV), ledipasvir (LDV), ombitasvir (OBV), elbasvir (EBR), velpatasvir (VEL)(2).

Therapy of chronic hepatitis C has been revolutionized by the DAAs. These drugs, given orally in different combinations, are safe and highly effective in eradicating HCV in all the different categories of HCV infected patients, including those with advanced, or decompensated, cirrhosis, who were not suitable candidates for IFN-containing treatments due to contraindications. The combinations of DAAs recommended by guidelines are evolving, and those proposed by the European Association for the Study of the Liver are described in the table, with schedules and regimens proposed for cirrhotic patients with compensated disease (11).

**Table 7. Treatment recommendations for HCV-monoinfected or HCV/HIV coinfected patients with chronic hepatitis C with compensated (Child-Pugh A) cirrhosis, including treatment-naïve patients and patients who failed on a treatment based on pegylated IFN-α and ribavirin (treatment-experienced, DAA-naïve patients).**

| Patients | Treatment-naïve or -experienced | Sofosbuvir/ ledipasvir | Sofosbuvir/ velpatasvir | Ombitasvir/ paritaprevir/ ritonavir and dasabuvir | Ombitasvir/ paritaprevir/ ritonavir | Grazoprevir/ elbasvir | Sofosbuvir and daclatasvir | Sofosbuvir and simeprevir |
|---|---|---|---|---|---|---|---|---|
| **Genotype 1a** | Treatment-naïve | 12 wk, no ribavirin | 12 wk, no ribavirin | 24 wk with ribavirin | No | 12 wk, no ribavirin if HCV RNA ≤800,000 (5.9 log) IU/ml or 16 wk with ribavirin if HCV RNA >800,000 (5.9 log) IU/ml[b] | 12 wk, no ribavirin | No |
| | Treatment-experienced | 12 wk with ribavirin[a] or 24 wk, no ribavirin | | | | | 12 wk with ribavirin[a] or 24 wk, no ribavirin | |
| **Genotype 1b** | Treatment-naïve | 12 wk, no ribavirin | 12 wk, no ribavirin | 12 wk, no ribavirin | No | 12 wk, no ribavirin | 12 wk, no ribavirin | No |
| | Treatment-experienced | | | | | | | |
| **Genotype 2** | Both | No | 12 wk, no ribavirin | No | No | No | 12 wk, no ribavirin | No |
| **Genotype 3** | Treatment-naïve | No | 12 wk with ribavirin[c] or 24 wk, no ribavirin | No | No | No | 24 wk with ribavirin | No |
| | Treatment-experienced | | | | | | | |
| **Genotype 4** | Treatment-naïve | 12 wk, no ribavirin | 12 wk, no ribavirin | No | 12 wk with ribavirin | 12 wk, no ribavirin | 12 wk, no ribavirin | 12 wk, no ribavirin |
| | Treatment-experienced | 12 wk with ribavirin or 24 wk, no ribavirin | | | | 12 wk, no ribavirin if HCV RNA ≤800,000 (5.9 log) IU/ml or 16 wk with ribavirin if HCV RNA >800,000 (5.9 log) IU/ml | 12 wk with ribavirin or 24 wk, no ribavirin | 12 wk with ribavirin or 24 wk, no ribavirin |
| **Genotype 5 or 6** | Treatment-naïve | 12 wk, no ribavirin | 12 wk, no ribavirin | No | No | No | 12 wk, no ribavirin | No |
| | Treatment-experienced | 12 wk with ribavirin or 24 wk, no ribavirin | | | | | 12 wk with ribavirin or 24 wk, no ribavirin | |

[a]Add ribavirin only in patients with RASs that confer high-level resistance to NS5A inhibitors at baseline if RAS testing available.
[b]Prolong to 16 weeks and add ribavirin only in patients with RASs that confer resistance to elbasvir at baseline if RAS testing available.
[c]Add ribavirin only in patients with NS5A RAS Y93H at baseline if RAS testing available.

*Table 2: Treatment recommendations for HCV patients with chronic hepatitis C with compensated (Child-Pug A) cirrhosis (from EASL recommendations 2016)*

**Eradication of hepatitis C virus infection in patients with cirrhosis reduces risk of liver and non-liver complications**

Data on clinical outcomes after HCV infection eradication have recently started to become available. Nahon et al (12), analysing the French Cir-Vir cohort, have confirmed a significant reduction in clinical events, both liver-related or non-liver related, in a group of patients achieving SVR with IFN-based therapy and also in a smaller subgroup of IFN-free treated cirrhotics. They found that achievement of SVR reduces overall mortality. This author clearly showed that achieving SVR in HCV-infected cirrhotic patients leads to improved prognosis: SVR influenced the incidence of liver complications, including HCC and mortality, and had also a positive impact on the occurrence of extrahepatic manifestations. But they also concluded that the follow-up after SVR in DAA-treated patients is still too short to allow drawing any definite conclusion on this point.

In general, patients with decompensated cirrhosis (Child-Pugh B/C) have lower response rates than patients with compensated cirrhosis (Child-Pugh A), may be for reduced drug delivery due to shunting leading to HCV reservoirs, altered drug metabolism and uptake (due to impaired liver synthetic function) or impaired immune responses which are common in severe cirrhotic patients. Obviously, among patients with decompensated cirrhosis, the IFN-free regimens are far better tolerated as compared to the PegIFN and RBV combination therapy. However, as more real-world data are emerging, some safety issues regarding the use of DAAs among those patients with the most advanced liver disease have also been arisen. Some patients with hepatic decompensation developed severe drug-induced liver injury leading to death and liver transplantation. Lactate acidosis have described among patients treated with sofosbuvir-based regimens, with or without the addition of RBV. Occurrence of hepatic decompensation during antiviral treatment has been reported for some DAA regimens, leading the FDA to discourage the use of dasabuvir, ombitasvir, and paritaprevir/ritonavir for patients with decompensated liver disease. Other protease inhibitors may also not represent an ideal class of DAAs for patients with the most severe cirrhosis, considering safety issues which were encountered with the first-generation protease inhibitors telaprevir and boceprevir among patients with cirrhosis and low platelets or low albumin levels (13). Because of the beneficial safety profile of the DAAs, our experience with antiviral therapy in patients with chronic HCV infection and decompensated cirrhosis is increasing rapidly. Still, because these therapeutic options have just surfaced, studies with sufficient follow-up to assess the true clinical impact of IFN-free therapy among the patients with the most advanced liver disease have to be awaited. In the meantime, several interesting observations in the short

term have been presented, which focus on the Model for End-Stage Liver Disease (MELD) score. Deterding et al. treated 34 HCV-infected patients with Child-Pugh B/C with various IFN-free regimens. At 12 weeks post-treatment, the MELD score improved in 68%, remained stable in 23%, and worsened in 10% of patients. Based on the first experiences in England, Foster et al. reported the change in MELD score 4 weeks after the cessation of DAA therapy. Of the 220 patients, 47.7% had no significant change in MELD score; in 41.8% patients, MELD score improved by ≥2 points; and in 10.5% patients, MELD score worsened by ≥2 points. Additional analyses indicated that the MELD score more frequently improved rather than declined among younger patients (<65 years) and patients with a high albumin level (>35 g/L) .Still, MELD improvement was mostly moderate, so the important question of whether liver transplantation can really be averted remains. If not, the slight improvement in MELD score may actually negatively impact the patient's chances on the waiting list (13, 29).

Liver-related morbidity and mortality. Most of the Western studies that assessed the association between HCV eradication with IFN-based therapy and hepatic decompensation or HCC have solely included patients with advanced liver disease, who are most at risk for these cirrhosis-related complications. However, as we have previously depended on IFN-based treatment regimens, most available studies predominantly included cirrhotic patients with relatively favourable characteristics. These studies showed that the incidence in liver failure was markedly reduced among patients with chronic HCV infection and advanced liver fibrosis who attained SVR. Interestingly, this beneficial outcome became apparent rather soon after HCV clearance. Hereafter, larger studies with longer follow-up duration not only confirmed these results but also showed a significant association between SVR and a reduced occurrence of HCC, with strong hazard ratios (HRs) adjusted for many potential confounders. In a recent meta-analysis, in which the results of all available cohort studies among patients with advanced liver disease were pooled, the results indicated that within this population the HR of SVR for the occurrence of HCC was 0.23. The combination of several studies that included patients with all stages of fibrosis resulted in a pooled HR of 0.24 regarding SVR and HCC occurrence. Considering the potential benefits of SVR in these cirrhosis-related morbidities, it is not surprising that patients with cirrhosis who clear their chronic HCV infection have a reduced liver-related mortality (24). Still, it is worthy to note that patients with advanced liver disease are not free from cirrhosis-related complications following HCV eradication. A combined cohort including 1000 patients with advanced liver fibrosis and IFN-induced SVR showed that the annual risk of HCC remains at about 1% following HCV eradication

in patients with cirrhosis. The risk of HCC depends on age, the presence of diabetes mellitus, and laboratory markers of liver disease severity. One may thus expect still a significant incidence of HCC following successful antiviral therapy in the era of DAAs, which will enable older patients with more advanced liver disease to attain SVR (13).

Extrahepatic consequences. While a large number of studies indicated potential liver-related benefits of SVR, more recent efforts have focused on the association between antiviral therapy and extrahepatic disease. With respect to patient-reported outcomes, eradication of HCV infection decreases both the frequency and the severity of fatigue. Even a few years ago, it was reported that the risk of diabetes mellitus is about three times lower among patients with SVR as compared to patients without SVR. The deteriorating consequences of diabetes mellitus are diverse but surely include renal failure and cardiovascular events. A reduced incidence of both of these solid endpoints in cases of antiviral therapy use was recently shown in a nationwide cohort study from Taiwan. The cumulative 8-year incidences of end-stage renal disease, acute coronary syndrome, and ischemic stroke, were significantly lower among treated as compared to untreated patients ($p<0.05$ for all), and the effect of antiviral therapy remained statistically significant in multivariate analysis. Gragnani et al. showed that HCV eradication led to the disappearance of cryoglobulinemia and resolved manifestations of the mixed cryoglobulinemia syndrome in nearly all patients. Other potential extrahepatic benefits of SVR that have been presented within recent years include a reduced occurrence of malignant lymphomas. Through these effects on extrahepatic morbidity, patients with SVR have not only a reduced liver-related mortality but also a reduced liver-unrelated mortality (13).

**Clinical outcomes with DAA-Based SVR: HCC reccurrence and "de novo" HCC**

If the high rate of SVR seems constant among several clinical trials testing DAA combinations , the impact on complications of cirrhosis is still unknown. We can only speculate from the results of observational studies among compensated cirrhotic patients treated by IFN regimens. In these patients, SVR were obtained mostly by the combination IFN and ribavirin treatments and decreased the occurrence of liver decompensation, of hepatocellular carcinoma and of death.

The rate of hepatocellular carcinoma (HCC) occurrence is strongly decreased after SVR but not abolished with an incidence of HCC ranging from 0.4 to 2% per year after viral eradication with IFN based therapies. In contrast, the evidence of decreased liver related

complications after viral clearance using DAA is still scarce (14). An intense and controversial debate has been recently raised on which is the residual risk of developing hepatocellular carcinoma in cirrhotic patients treated with DAAs. This debate has been triggered by some reports suggesting that such risk might not be reduced, as it was in cirrhotic patients achieving SVR with IFN-based therapy, and could be even increased, expecially in cirrhotics who had already experienced HCC, having been successfully treated for it, with complete radiological response, before receiving DAAs treatment. These findings prompted other Authors to review and publish their data in similar clinical settings, generating a rather heterogeneous and controversial scenario that remains yet to be fully completed and understood.

HCC recurrence

Published data on HCC recurrence in HCV patients treated with DAAs after complete response to HCC cure have shown rates of HCC recurrence quite variable reflecting heterogeneity in study design, type of patients included and follow-up. The debate was generated by the report of Reig and coworkers (15). These Authors had initially observed a number of individual patients with previous, successfully cured HCC, who developed tumour recurrence after receiving antiviral therapy with DAAs, despite successful HCV eradication, and were impressed by the temporal relation between antiviral therapy and HCC recurrence. They decided to perform a systematic review of the cases treated with DAAs in four Referral Hospitals in Spain and were able to describe 58 patients with treated HCC (ablation, resection, or chemoembolization) with a complete radiological response, who were then given all-oral DAA combination therapy. The vast majority of patients achieved SVR 12. However, during a median follow-up period of 5.7 months after starting DAAs, 16 patients (27.6%) developed radiological evidence of HCC recurrence with a median time from DAAs start to tumour recurrence of only 3.5 months (range 1.1-8 months). The observed HCC recurrence was thought by the Authors to be “surprisingly high” compared to the expected incidence in the natural history of successfully treated HCC, considering that the vast majority of the included HCC could be classified at low risk of recurrence (15). This conclusion was also supported by comparison with HCC recurrence observed by the Authors in a prospective study after surgical resection in another prospective series (unpublished) after ablation (2.45% at 4 months and 27.6% at 12 months) and in the double blind placebo controlled STORM trial. The highest rate of HCC recurrence (41.17%) was indeed observed in patients with a short time frame (<4 months) between HCC treatment and last assessment of complete response by imaging. This observation was confirmed in the analysis of Cammà et al.(16) who extracted the

Kaplan–Meier HCC recurrence estimation curves from Reig's paper and showed that the probability of HCC recurrence during the first 6 months after starting DAAs was more than double (approaching 50%) in patients with a time frame between HCC treatment and last assessment of HCC complete response <6 months compared to the probability (<15%) estimated in patients with a longer time frame. This may suggest that the high rate of early tumour recurrence described by the Spanish Authors could have been driven mainly by individual cases, including those initially observed, initiated on DAAs shortly after HCC treatment. Data on early recurrence of HCC in cirrhotics patients treated with DAAs have also been reported by Conti et al. (17) These Authors described 59 patients with a history of previous HCC, treated with surgical resection, RFA, TACE, PEI and with a combination of procedures. All patients had a complete tumour response and were treated with approved oral DAA combinations after a median interval of 376 days (range 45-2706 days). 53 of the 59 patients (89.8%) achieved SVR. However, during a 24 week post-treatment follow-up 17 (28.8%-95% CI 17.76-42.07%) showed HCC recurrence, that was associated with younger age and with more severe liver fibrosis. The crude rate of HCC recurrence was almost identical to that reported by Reig et al. (28.8% vs 27.6%), but follow-up after DAAs initiation was longer leading to a different (lower) time-based incidence. Also in this study, it was evident that patients with a previous HCC remain at risk of HCC recurrence after treatment with DAAs, even when HCV is successfully eradicated, but the design of study did not allow to define whether the observed recurrence rate was increased, reduced or unmodified compared to what is expected in the natural history of the disease. Another study that has contributed to raise concern on HCC recurrence in patients treated with DAAs came from the Majo Clinic in the US where Yang et al. (18) described their experience in HCV patients undergoing liver transplant with HCC and reported higher HCC recurrence (27.8%) in the patients who had received DAAs pre-transplant. Other studies, have not confirmed increased incidence of HCC recurrence in patients treated with DAAs. The French report on the different ANRS collaborative prospective studies of HCV patients (19) described recurrence of HCC in three distinct cohorts. (I) In the CO22 HEPATER Cohort, including 267 patients with an history of cured HCC, the rate of HCC recurrence was 12.7% (8.76×100 patients/year) in 189 patients who had received DAAs treatment compared to 20.5% (7.92×100 patients/year) of the 78 untreated patients. (II) In the CO12 CirVir cohort, incidences of HCC recurrence were 13.32×100 patients/year in 13 DAAs treated patients and 20.76×100 patients/year among 66 untreated patients. (III) In the CO23 CUPILT cohort, including 314 patients undergoing liver transplant for HCV related HCC, HCC recurrence was seen in only 2.2% of the cases during a follow-up of 7±3 months after antiviral

therapy. The Authors concluded that there was no evidence of an increased risk of HCC recurrence in patients treated with DAAs. A rather similar low rate of HCC recurrence in cirrhotic patients treated with DAAs has been reported also by Zavaglia et al. (20) and by Cheung et al. (21) with incidence of 3.2% over a mean 8 months of follow-up and of 6.29% over a mean 15 months follow-up. The heterogeneity of all these results most likely reflects the heterogeneity of the clinical setting they refer to, with a number of variables that could have heavily affected HCC recurrence rates. Absence in many studies of matched untreated controls further complicates interpretation and do not allow to draw firm conclusions. Cabibbo et al. (22) have recently conducted a meta-analysis of published studies of HCV patients with adequate follow-up after curative treatment of HCC, not receiving any type of antiviral therapy. The Authors analysed 11 such studies inclusive of 701 patients: the estimated probability of HCC recurrence was 7.4% at 6 months, of 20% at 12 months and of 47% after 24 months. There were rather large confidence intervals together with significant heterogeneity, largely expected, in relation to baseline patients and tumour characteristics and type of HCC treatment. These recurrence rates may be useful to design and stratify prospective observational studies able to assess more adequately the effect of DAA therapy on HCC recurrence. On this line, Petta et al. (23) have recently tried to compare retrospectively HCC recurrence rates in untreated patients with persistent HCV replication, and in patients achieving SVR with IFN-based therapy or with DAAs oral treatment. The results were suggestive for reduce risk of HCC recurrence in patients with SVR, without any difference between IFN-based and IFN-free (DAAs based) therapy.

"De novo" HCC in patients treated with antiviral therapy

Natural history studies indicate that in HCV patients the risk of developing HCC is dependent on patient age and gender, stage of liver disease and comorbidities and cofactors such as type 2 diabetes, alcohol and HBV coinfection. In patients treated with IFN-based regimens, the residual risk of developing HCC was reduced (HR ranging from 0.24 to 0.40), although not fully abolished in patients with SVR (residual risk ranging from 0.33%/year to 1.40-2.00/year depending on patient age and stage of liver disease) (24, 25). Recently, Nahon et al. (12) have reported data from a prospective study inclusive of 1323 patients with compensated cirrhosis treated with antiviral therapy (mainly IFN based). A total of 668 patients achieved SVR that was associated with a decreased incidence of hepatocellular carcinoma compared to patients not achieving SVR (hazard ratio 0.29, 95% CI: 0.19-0.43). Residual risk of developing HCC after SVR was associated with presence of metabolic syndrome and of type 2 diabetes. Incidence of “de novo” HCC

after DAAs therapy has been also investigated by Cheung et al. (21) in patients with advanced, decompensated cirrhosis. These Authors described 377 cirrhotic patients (Child B in 72.7%, Child C in 10.1%) without a previous diagnosis of HCC, treated with approved DAAs. HCC developed in 15 (3.9%) during the first 6 months after initiation of DAAs (notably five of these within the first 3 months) and in additional 10 (2.6%) at longer follow-up (6-15 months after initiation of antivirals). Patients with SVR had lower rates of HCC incidence compared to those without SVR and to untreated patients. Interestingly, a trend towards progressive reduction of the risk was observed over time in patients with SVR. In another retrospective study of 285 HCV patients with advanced liver disease (10% Child B) treated with DAAs in the absence of previous diagnosis of HCC, “de novo” occurrence of a liver tumour was seen in 3.16% (95% C.I.: 1.45-5.90) during the 24 weeks after antiviral treatment (17). This crude rate translates in an annual rate after initiation of DAAs not exceeding 4.2% and most likely lower, considering that patients were treated for 12-24 weeks (most of them likely for 24 weeks) and then followed up for 24 weeks. Overall, 91.6% of patients achieved SVR and HCC occurred in 8.3% of patients without SVR and in 2.7% of those with SVR. A somehow high rate of “de novo” HCC after DAAs has been reported by Cardoso et al., (26) with tumour incidence of 7.4% among 54 patients with cirrhosis (67% with Child A) treated with DAAs in Spain. All patients developing HCC had SVR12. In contrast, in the French prospective series of 167 cirrhotic patients achieving SVR with DAAs, only 1 (0.6%) was found to develop HCC during follow-up (12).

## AIM AND MATERIALS AND METHODS

Aim. Aim of this project was to assess virological and clinical outcomes of DAAs treatment in HCV infected cirrhotics (both compensated and decompensated) by studying a large cohort of patients treated with DAAs in the Veneto region. Furthermore we intended to assess virological responses and clinical outcomes in HCV patients treated with DAAs at an “elderly stage” i.e. > 70 years of age, as well as in patients with severe extra-hepatic manifestations of HCV, taking advantage from the fact that DAA treatment was allowed in Italy since the very beginning in these patients subgroups.

The project was conducted by assembling and analysing a database using the NAVIGATORE web-platform, that – since January 2015- is recording and monitoring all HCV-DAA based treatments in the Veneto region (and more recently also in the Alto-Adige

and Lombardia regions), in a network of Clinical Units coordinated by the Department of Molecular Medicine of the University of Padua.

NAVIGATORE is an acronym and means: **N**uovi **A**nti**VI**rali per HCV: **G**estione **AT**travers**O** **R**egistro degli **E**venti.

We have intended to assess virological and clinical outcomes of DAAs treatment in HCV infected cirrhotics (both compensated and decompensated), in patients > 70 years and in cases of severe extrahepatic manifestations in a “real-life setting” with the aim to clarify:

1. virus kinetics of response and SVR rates
2. clinical outcomes in relation to virological response
3. long term benefits of treatment.

To do this, we have collected prospective data from a large cohort of patients treated with DAAs in the Veneto region. This was made possible by analysing the data on the NAVIGATORE web-platform coordinated by our Department (DMM).

The main end points of our analysis were:

1) Evaluation of virological outcomes and efficacy of DAA therapy:

- rates of viral eradication and virological response in the different subgroups of patients analysed in relation to stage, HCV genotype and treatment schedule (SVR 12 = sustained virological response at week 12 after the end of treatment = EOT)

2) Evaluation of clinical outcomes including:

- changes in CHILD-PUGH and MELD scores,
- variation in the incidence of clinical events (hepatocellular carcinoma, portal hypertension, ascites, variceal bleeding, liver transplantation),
- safety and clinical outcome in patients with HCV and aged > 70 years,
- assessment of clinical outcomes in patients with extra-hepatic HCV related manifestations and disease (vasculitis, B-cell Lymphoma, cryoglobulinaemia).

Study design. The results were obtained during an ongoing prospective observational study which includes all consecutive HCV patients treated since January 1$^{st}$ 2015 with oral DAAs in 27 Clinical Centers in Veneto-Italy. The study is conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and obtained approval by the Ethics Committee. Individual data are prospectively entered on the NAVIGATORE Platform at monthly intervals during antiviral therapy, 12 and 24 weeks after end of therapy and at 48 week interval thereafter for a planned follow-up of 5 years. Data entry was monitored by several and standard quality control parameters, and by periodical audit meetings with the responsible clinicians.

Patient selection. All consecutive HCV patients entered from January 1st 2015 to 24th May 2017 in the NAVIGATORE data-base were evaluated. In the 3 months before initiation of antiviral therapy all patients underwent complete laboratory and clinical evaluation and abdomen ultrasound to define stage of liver disease and to assess baseline features guiding the treatment approach. If a suspected focal lesion was identified in the liver, the diagnostic work up was completed with another imaging technique, either computerized tomography (CT) scan or magnetic resonance imaging (MRI).

Antiviral therapy and follow-up. All patients received DAAs treatment according to available DAAs and national and regional guidelines, that were defined and updated periodically and implemented in specific algorithms on the NAVIGATORE platform to optimise and to made homogeneous the regimens used by the participating Centers. . From January 2015 to the end of February 2015 patients were treated with the combination of Sofosbuvir and Ribavirin, since the last week of February the treatment with Sofosbuvir plus Simeprevir. became available and during May 2015 the other combinations of Sofosbuvir/Ledipasvir, Sofosbuvir/Daclatasvir and then Ombitasvir plus Paritaprevir plus Ritonavir and Dasabuvir. were added to the Platform options. Patients were treated for 12 or 24 weeks, with or without addition of Ribavirin, according to guidelines and treater's decision. The assigned schedule and any modifications during treatment were recorded on the NAVIGATORE Platform. Patients were seen (for visit and blood tests) monthly during therapy, and 12 and 24 weeks after termination of treatment and then at 6 months intervals. During follow-up patients with F3 or cirrhosis at entry continued to periodical (every 3 to 6 months) surveillance for HCC.

Sustained Virological response (SVR) to therapy was defined as undetectable HCV-RNA by a quantitative real-time PCR with a limit of detection of 12 IU/ml. SVR was assessed at week 12 after EOT

Statistical analysis. Results are presented as median for continuous variables and as frequency for categorical data. HCC characteristics were compared between patients with SVR and without SVR using the Student t test or the Wilcoxon rank-sum test for continuous variables. Categorical variables were compared using the Chi-squared test or the Fisher exact test if necessary. Baseline features associated with risk of complications and death were tested using univariate and multivariate Cox models. We estimated the incidence of HCC occurrence as cases per 100-patient-years with its 95% confidence limits, and described by the Kaplan-Meier method. The suspected influencing factors were tested by the Mantel test. We developed a proportional hazards multivariate Cox's model, with the forward stepwise option, to select the most relevant factors for HCC development,

and estimated their independent Hazard Ratios, with 95% confidence limits. A p value less than 0.05 was considered statistically significant.

## RESULTS

At the time of this analysis, May 2017, 4764 HCV treated patients have been included.

### - Demographic Data

Among the 4764 treated patients 62,4% are males and mean age is 58.18+/-10.8 years (range 21-90 years). The distribution of genotypes is as follows: 62,4% HCV-1 (41.3% HCV-1b, 19,2% HCV-1a), 13,3% HCV-2, 16,6% HCV-3, and 7.2% HCV-4.
62% had cirrhosis (87% CHILD A ,10% CHILD B and 4% CHILD C), and 25,7% had F3 fibrosis at the time DAA were initiated.
Many patients (53%) had comorbidities with type 2 diabetes in 16,8%, cardiovascular disease in 30% obesity in 8,2% and nephropathy in 2,8%. Extrahepatic disease (lymphoma, vasculitis or cryoglobulinemic syndrome) was recorded in 3%.

| Treated population 4764 pts | | % |
|---|---|---|
| Males/Females | 2973/1791 patients | 62,4 / 37,6 |
| Mean Age +/- ds (years) | 58,18+/-10,8 (range 21-90) | |
| HCV-genotype | 1a | 19,2 |
| | 1b | 41,3 |
| | 2 | 13,3 |
| | 3 | 16,6 |
| | 4 | 7,2 |
| Stage of liver disease | F3 (pre-cirrhosis) | 25,7 |
| | F4 (cirrhosis) | 62 |
| Comorbidities | extra-hepatic disease | 3 |
| | type 2 diabetes | 16,8 |
| | cardiovascolar disease | 30 |
| | obesity | 8,2% |
| | nephropathy | 2,8% |

*Table 3: Demographic Data*

**- Efficacy and Safety data in F3 and in Cirrhotic Patients**

Patients were treated according to the implemented algorythms that I developed according to national and regional guidelines and were then monitored monthly during therapy and every 3 months thereafter.

At the time of this analysis patients had a mean follow-up of 398.8±115 days after the date DAAs were initiated and SVR12 rates were of 97.2% in F3, 92.7% in Child A cirrhotics and 80% in Child B cirrhotics. Virus eradication with SVR12 was observed in 94,8% with HCV-1a, in 98% with HCV-1b, in 95% with HCV-2, in 85,7% with HCV-3 and in 95,4% with HCV-4. Premature therapy discontinuation occurred in 53 patients including: 12 who died for hepatic causes, 11 who died for non-liver related causes, 4 patients who developed HCC and 21 patients who developed side-effects that were deemed severe and possibly or certainly drug-related by the treating physician. Mean follow-up after end of therapy was 338.2±768.6 days for patients who had reached EOT, and mean follow-up after SVR12 was 254.3+684.5 days for patients with SVR12 at the time of this analysis.

Efficacy was excellent in most groups, not being significantly affected by comorbidities but it was reduced by around 10% in patients with Child B cirrhosis.

Tolerability and safety were excellent in patients with F3 and with Child A cirrhosis, while were reduced in Child B patients, particularly when treated with Ribavirin containing regimens (Table 4).

| ***SAFETY*** | ***F3*** | | ***F 4*** | | | |
|---|---|---|---|---|---|---|
| | | | ***Child A*** | | ***Child B*** | |
| | ***RBV yes*** | ***RBV no*** | ***RBV yes*** | ***RBV no*** | ***RBV yes*** | ***RBV no*** |
| *% Early Discontinuation* | *0* | *0,7* | *1,2* | *1,1* | *2,7* | *3,3* |
| *% Clinically Significant Side Effects* | *3,6* | *0* | *3,9* | *1,8* | *7,6* | *0,9* |
| *% Ascites* | *0* | *0* | *1,3* | *1,8* | *10,9* | *4,9* |

*Table 4: Safety of DAA according to stage liver disease and ribavirin (RBV) use.*

**Efficacy and Clinical Outcomes in Cirrhotic Patients**

We have considered for these analysis 2946 cirrhotic patients.

Global SVR, according to genotype, was:

HCV-1a (524 patients): 94,8%

HCV-1b (1182 patients): 98%

HCV-2 (380 patients): 95%

HCV-3 (555 patients): 85,7%

HCV-4 (232 patients): 95,4%.

More detailed SVR rates, stratified by HCV genotypes and regimens, are presented in the following tables.

| **Drug regimens in HCV 1a** | Patient number | SVR (%) |
|---|---|---|
| SOF/LDV 12 wks | 21/524 | 100 |
| SOF/LDV + rbv12 wks | 101/524 | 97 |
| SOF/LDV 24 wks | 85/524 | 92,7 |
| SOF/LDV + rbv 24 wks | 97/524 | 98,3 |
| 3 D + rbv 12 wks | 1/524 | 100 simple size bias |
| 3 D + rbv 24 wks | 110/524 | 100 |
| 3 D 24 wks | 10/524 | 100 simple size bias |
| 2 D+rbv 12/24 wks | 1/11 /524 | 100 simple size bias |
| SOF/SMV +/- rbv | 55/524 | 87 |
| SOF/RBV 24 wks | 10/524 | 33,3 |
| SOF/DAC 12wks, 24 wks | 1/3/524 | 100 simple size bias |
| SOF/DAC +rbv 24 wks | 3/524 | 100 simple size bias |
| ELB/GRZ | 10/524 | ongoing |

*Table 5: % of SVR in genotype 1a patients according to regimens.*

| **Drug regimens in HCV 1b** | Patient number | SVR (%) |
|---|---|---|
| SOF/LDV 12 wks | 51/1182 | 94,7 |
| SOF/LDV + rbv12 wks | 114/1182 | 95,2 |
| SOF/LDV 24 wks | 233/1182 | 97,6 |
| SOF/LDV + rbv 24 wks | 145/1182 | 98,1 |
| 3 D + rbv 12 wks | 259/1182 | 98,6 |
| 3 D + rbv 24 wks | 4/1182 | 66,7 simple size bias |
| 3 D 24 wks | 5/1182 | 100 simple size bias |
| 3 D 12 wks | 88/1182 | 91,2 |
| 2 D+rbv 12/24 wks | 30/2/1182 | 100/50 simple sze bias |
| SOF/SMV +/- rbv | 163/1182 | 92 |
| SOF/RBV 24 wks | 27/1182 | 39 |
| SOF/DAC 12wks, 24 wks, 24 wks+rbv | 5/1182 | 100 simple size bias |
| ELB/GRZ | 42/1182 | ongoing |

*Table 6: % of SVR in genotype 1b patients according to regimens.*

| **Drug regimens in HCV 2** | Patient number | SVR (%) |
|---|---|---|
| SOF/DAC 12 wks | 80/380 | 93 |
| SOF/RBV 12 wks | 28/380 | 100 |
| SOF/RBV 16- to 24 wks | 55/380 | 94,9 |
| SOF/RBV 24 wks | 211/380 | 94,8 |

*Table 7: % of SVR in genotype 2 patients according to regimens.*

| **Drug regimens in HCV 3** | Patient number | SVR (%) |
|---|---|---|
| SOF/DAC24 wks | 84/555 | 94,6 |
| SOF/DAC + rbv 24 wks | 329/555 | 94,2 |
| SOF/DAC/RBV 12 wks | 12/555 | 100 simple size bias |
| SOF/RBV 24 wks | 89/555 | 64,2 |
| SOF/LDV + rbv 24 wks | 12/555 | 75 simple size bias |

*Table 8: % of SVR in genotype 3 patients according to regimens.*

| **Drug regimens in HCV 4** | Patient number | SVR (%) |
|---|---|---|
| SOF/SIM +/- RBV 12wks | 35/232 | 90,3 |
| SOF/LED/RBV 12wks | | 100 |
| SOF/LED 24wks | | 87,5 |
| 2D 12 wks | | 100 |
| 2D 24 wks | | 98,5 |
| ELB/GRZ | | ongoing |

*Table 9: % of SVR in genotype 4 patients according to regimens.*

**- Outcomes in CHILD A and CHILD B cirrhosis**

Child–Pugh and MELD scores are two scores with similar prognostic values, widely used for the assessment of prognosis in liver cirrhosis. Child–Pugh score was firstly proposed by Child and Turcotte and include: ascites, hepatic encephalopathy, total bilirubin, INR and albumin. Child–Pugh score is mainly used to assess the severity of liver dysfunction in clinical work. Model for end-stage liver disease (MELD) score incorporate only 3 objective variables, including total bilirubin, creatinine, and INR. Currently, it is used to rank the priority of liver transplantation candidates. These scores are used to predict the clinical outcomes in cirrhotic patients. In our analysis, we have defined patients with compensated cirrhosis as “Child A” and those with decompensated cirrhosis as “Child B and C”.

In our population, the most part of patients are compensated (92,3% of cirrhosis are in Child A).

| CHILD PUGH | % PATIENTS |
|---|---|
| CHILD A | 92,3 |
| CHILD B | 7 |
| CHILD C | 0,2 |
| UNKNOWN | 0,5 |

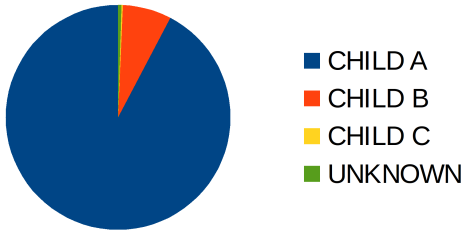


*Figure 2: distribution of compensated and decompensated cirrhosis*

Child A patients:

When we analyzed the clinical outcomes in compensated cirrhotic patients during the first year after initiation of DAA therapy, we could demonstrate a reduction in the incidence of major clinical complications in DAA treated patients, compared to the expected incidence rates in untreated patients, as show in the following table, that compares treated patients (in the first column data from NAVIGATORE) versus untreated patients (in the second column data from natural history studies, as summarized by D'Amico et al. 2014) (30).

| | TREATED PATIENTS | UNTREATED PATIENTS |
|---|---|---|
| GASTROINTESTINAL BLEEDING | 0.51%/year | 1.5%/year |
| ASCITES | 1,6%/year | 3%/year |
| LIVER DECOMPENSATION | 2,1%/year | 4,2%/year |
| HCC | 1,6%/year | 3%/year |
| LIVER RELATED DEATH | 0,39%/year | 1-3%/year |

*Table 10: incidence %/year of complications in treated versus untreated cirrhotic patients*

Child B and C patients:

In our cirrhotic patients treated in the decompensated phase, we could analyse changes in Child score during or after DAAs treatment: Most patients (78.5%) showed improvement in Child-Pugh score, while 16% remain stable and only 5.8% progressed in their decompensation score, despite being cured with DAAs. Some of these patients required liver transplantation, that could be performed in a virus free setting. On the other hand, delisting was possible in around 25% of patients showing CHILD and MELD score improvement with DAAs therapy.

| | | | |
|---|---|---|---|
| **CHILD PUGH** | | | |
| ***IMPROVED*** | **78,5%** | | |
| | | - 1 POINT | 23,2% |
| | | - 2 POINTS | 29,7% |
| | | - 3 POINTS | 23,2% |
| | | - 4 POINTS | 2,2% |
| ***STABLE*** | **16,0%** | | |
| **WORSENED** | **5,8%** | | |
| | | - 1 POINT | 2,2% |
| | | - 2 POINTS | 2,9% |
| | | - 4 POINTS | 0,7% |

*Table 11: changes in Child-Pugh score in cirrhotic decompensated patients during or after therapy*

**- Recurrence of Hepatocellular Carcinoma in DAA Treated Patients in the NAVIGATORE Database**

There were 154 patients with an history of HCC, successfully treated with surgery or locoregional strategies before initiation of DAA treatment. 73,4% were males and 26,6% females, with a mean age of 64,8 years (range 45-87 years). The distribution of HCV genotypes in these patients was as follows: 22 % HCV-1a, 44% HCV-1b, 12%HCV-2, 13% HCV-3 and 7% HCV-4.
An HCC recurrence during or after DAA therapy was observed in 29/154 patients (18,8%).

**- Incidence of “de novo” Hepatocellular Carcinoma in DAA Treated Patients in the NAVIGATORE Database**

All patients were seen monthly during therapy, and 12 and 24 weeks after termination of treatment and then at 6 months intervals. During follow-up patients underwent periodical (every 3 to 6 months) surveillance for HCC by ultrasound. Patients developing any suspicious liver lesions were further assessed with CT scan or MRI to confirm or exclude HCC. Serum-alfafetoprotein levels were measured and a guided liver biopsy was performed when indicated, according to the AASLD recommendations. HCC diagnosis was then established by histological examination or by validated non-invasive imaging techniques and by alfa-fetoprotein.
2279 HCV patients (66.6% males, 85.7% with cirrhosis, of whom 91% Child A and 9% Child B, 62% HCV-1, 11.2% HCV-2, 18.5% HCV-3, 8.45% HCV-4, ) were included. We excluded patients with history of HCC, of liver transplant or a follow-up of less of 4 weeks after initiation of DAAs.
During follow-up, 52 patients were diagnosed with hepatocellular carcinoma, including 36 male and 16 female patients with a mean age of 59.15+/- 9.9 years.

The overall HCC incidence was of 1.64 x 100 patients/year (95% CI : 1.14-2.28).

When patients were stratified according to the stage of liver disease at baseline, HCC incidence rates were:

0.23 x 100patients/year (95% CI 0,01-1,27) in F3,

1.55 x 100patients/year (95% CI 1.18-2.01) in Child A cirrhosis and

3,68 x 100 patients/year (95% CI : 1.92-6.41) in Child B cirrhosis.

The Kaplan Meyer curves of HCC occurrence in these 3 subgroups of patients are described in Figure 3. There was a statistically significant difference in the probability of developing HCC between pre-cirrhotic F3 patients and cirrhotic patients (p = 0,0012) as well as between patient with compensated (C-P class A) and decompensated (C-P class B) cirrhosis (p = 0,013).

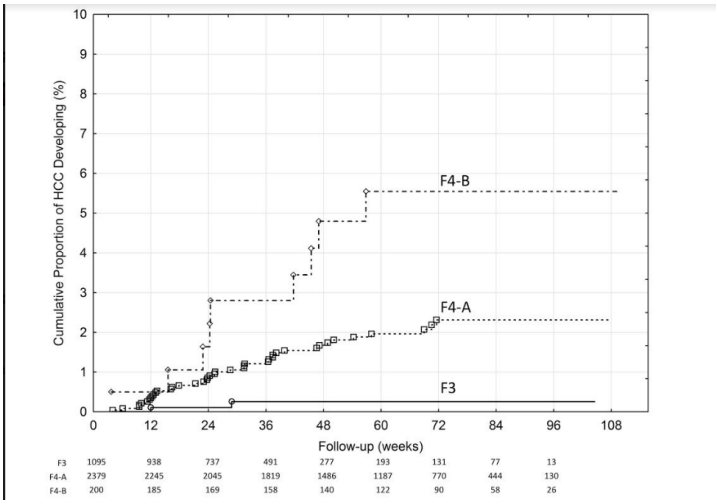


*Figure 3: HCC occurrence in F3, Child A and Child B patients (Kaplan Meyer curves)*

There were no statistically significant differences as to age, gender, HCV genotype, DAA schedule received. However, patients with HCV-3 showed a trend towards higher risk (HR: 1.69) compared to other genotypes. Among different baseline laboratory parameters analysed, an APRI value above 2.5 was found associated with statistically significant higher risk of developing HCC compared to a lower value (HR: 2.5 , 95% CI 1.21-5.14 p 0.013). Other variables, such as liver stiffness measured by FIBROSCAN at baseline and prevalence of comorbidities such as diabetes, obesity and/or cardiovascular disease, were not found to influenced the HCC risk.

When patients with and without SVR were compared, the risk of developing HCC was markedly and significantly higher in the latter (incidence in NO-SVR: 8.73 x 100patients/year, 95% CI: 5.47-13.26) compared to the former cases (incidence in SVR: 1.30 x 100patients/year, 95% CI : 0.91-1.81 – HR 0.13 – 95% CI: 0.07-0.23 p= 0.001 ). By

multivariate analysis, two baseline variables were statistically associated with HCC, i.e. an APRI score > 2.5 (HR 1.88, 95% CI: 1.03-3.42 p= 0.038) and Child B cirrhosis (HR 2.11 with 95% CI: 1.00-4.46 p= 0.050). The multivariate analysis after DAA treatment, confirms a risk for patients with an APRI score > 2.5 (HR 1.83, 95% CI: 0.89-3.75 p= 0.099) and demonstrates the benefit of SVR (HR 0.20, 95% CI: 0.09-0.41 p= 0.001), as showed in the table 12.

| **BASELINE** | HR | 95% CI | p | **AFTER DAA TREATMENT** | HR | 95% CI | p |
|---|---|---|---|---|---|---|---|
| **APRI score ≥ 2.5** | 1.88 | 1.03-3.42 | 0.038 | **APRI score ≥ 2.5** | 1.83 | 0.89-3.75 | 0.099 |
| **Child B** | 2.11 | 1.00-4.46 | 0.050 | **SVR-12** | 0.20 | 0.09-0.41 | 0.001 |

*Table 12: Multivariate Cox's regression (Forward stepwise selection)*

Next table (table 13) described in detail HCC incidence rates in our cirrhotic patients in relation to different variables.

| SOLO CIRROTICI | | n | FW-T_d | Person-years observed | HCC | % | Incidense (% person-years) | 95% CL | HR (Univariate Cox regr.) | 95% CL | p= |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | Female | 921 | 360450 | 1001.3 | 16 | 1.7 | 1.60 | 1.01-2.43 | 1 | | 0.684 |
| | Male | 1660 | 679293 | 1886.9 | 34 | 2.0 | 1.80 | 1.36-2.40 | 1.13 | 0.62-2.05 | |
| | | | | | | | | | | | |
| Age | <65y | 1804 | 742853 | 2063.5 | 34 | 1.9 | 1.65 | 1.21-2.19 | 1 | | 0.596 |
| | >=65y | 773 | 294424 | 817.8 | 16 | 2.1 | 1.96 | 1.23-2.97 | 1.17 | 0.65-2.13 | |
| | | | | | | | | | | | |
| HCV genotype | 1 | 1561 | 631084 | 1753.0 | 27 | 1.7 | 1.54 | 1.09-2.12 | 1 | | 0.607 |
| | 2 | 318 | 122584 | 340.5 | 5 | 1.6 | 1.47 | 0.58-3.01 | 0.99 | 0.38-2.57 | |
| | 3 | 485 | 200163 | 556.0 | 14 | 2.9 | 2.52 | 1.52-3.94 | 1.69 | 0.89-3.22 | |
| | 4 | 214 | 85914 | 238.7 | 4 | 1.9 | 1.68 | 0.57-3.83 | 1.08 | 0.38-3.10 | |
| | | | | | | | | | | | |
| APRI Score (if available) | <2.5 | 1442 | 581720 | 1615.9 | 23 | 1.6 | 1.42 | 0.97-2.02 | 1 | | 0.015 |
| | >=2.5 | 645 | 274292 | 761.9 | 22 | 3.4 | 2.89 | 1.95-4.12 | 2.07 | 1.15-3.72 | |
| | | | | | | | | | | | |
| Child-Pugh (if available) | A | 2377 | 950331 | 2639.8 | 41 | 1.7 | 1.55 | 1.18-2.01 | 1 | | 0.013 |
| | B | 200 | 88152 | 244.9 | 9 | 4.5 | 3.68 | 1.92-6.41 | 2.5 | 1.21-5.14 | |
| | | | | | | | | | | | |
| Therapy | 2D-3D | 578 | 220089 | 611.4 | 9 | 1.6 | 1.47 | 0.77-2.57 | 1 | | 0.273 |
| | DacSof | 441 | 144764 | 402.1 | 8 | 1.8 | 1.99 | 0.99-3.59 | 1.35 | 0.52-3.49 | |
| | LedSof | 851 | 284705 | 790.8 | 12 | 1.4 | 1.52 | 0.87-2.46 | 1.02 | 0.43-2.41 | |
| | SimSof | 260 | 159964 | 444.3 | 5 | 1.9 | 1.13 | 0.44-2.37 | 0.95 | 0.32-2.84 | |
| | SofRBV | 453 | 231487 | 643.0 | 16 | 3.5 | 2.49 | 1.56-3.78 | 2.05 | 0.90-4.65 | |
| | | | | | | | | | | | |
| SVR-12 (if available) | no | 121 | 65973 | 183.3 | 16 | 13.2 | 8.73 | 5.47-13.26 | 1 | | 0.001 |
| | yes | 1459 | 717957 | 1994.3 | 26 | 1.8 | 1.30 | 0.91-1.81 | 0.13 | 0.07-0.25 | |
| | | | | | | | | | | | |
| | All | 2583 | 1041009 | 2891.7 | 50 | 1.9 | 1.73 | 1.35-2.19 | | | |

*Table 13: Risk of developing HCC in cirrhotic patients according to different variables.*

The 52 patients who developed HCC during the observation period are described individually in Table 14.

| Patient | Age (years) | Liver Disease | HCV genotype | DAA treatment | SVR 12 | wks from start DAA to diagnosis HCC | HCC Pattern |
|---|---|---|---|---|---|---|---|
| 1 | 60 | CHILD A | 1a | SOF/LDV | no | 4 | 3 |
| 2 | 51 | CHILD B | 3 | SOF/DCV | no | 4 | 3 |
| 3 | 53 | CHILD A | 3 | SOF + RBV | no | 6 | 3 |
| 4 | 66 | CHILD A | 2 | SOF + RBV | yes | 10 | 1 |
| 5 | 57 | CHILD A | 3 | SOF/DCV + RBV | no | 10 | 3 |
| 6 | 49 | CHILD A | 3 | SOF + RBV | no | 10 | 1 |
| 7 | 54 | CHILD A | 1b | SOF + RBV | no | 11 | 3 |
| 8 | 66 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | yes | 12 | 2 |
| 9 | 55 | F3 | 4 | SOF/LDV | no | 12 | 1 |
| 10 | 56 | CHILD A | 1a | SOF/LDV + RBV | yes | 12 | 1 |
| 11 | 50 | CHILD A | 1a | SOF/LDV | no | 12 | 2 |
| 12 | 61 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | yes | 13 | 2 |
| 13 | 44 | CHILD A | 1a | SOF/Peg-IFN + RBV | no | 13 | 3 |
| 14 | 51 | CHILD A | 1b | SOF/LDV + RBV | yes | 13 | 1 |
| 15 | 75 | CHILD A | 1b | SOF/LDV + RBV | no | 14 | 3 |
| 16 | 79 | CHILD A | 2 | SOF + RBV | no | 16 | 2 |
| 17 | 56 | CHILD A | 4 | SOF/LDV + RBV | yes | 16 | 1 |
| 18 | 58 | CHILD A | 3 | SOF + RBV | no | 18 | 3 |
| 19 | 65 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | yes | 21 | 1 |
| 20 | 67 | CHILD B | 1b | SOF/LDV | no | 23 | 3 |
| 21 | 52 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | no | 23 | 1 |
| 22 | 75 | CHILD A | 1b | SOF/LDV + RBV | no | 24 | 1 |
| 23 | 73 | CHILD A | 1b | SOF/LDV | yes | 24 | 1 |
| 24 | 59 | CHILD B | 1b | SOF + RBV | no | 24 | 1 |
| 25 | 51 | CHILD A | 1b | SOF + RBV | no | 24 | 3 |
| 26 | 69 | CHILD B | 1b | OBV/PTV/r/DSV + RBV | yes | 24 | 2 |
| 27 | 52 | CHILD A | 4 | SOF/SMV + RBV | no | 25 | 1 |
| 28 | 50 | CHILD A | 1b | SOF/SMV + RBV | yes | 25 | 3 |
| 29 | 56 | CHILD A | 3 | SOF + RBV | no | 29 | 1 |
| 30 | 82 | F3 | 2 | SOF + RBV | no | 29 | 1 |
| 31 | 56 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | yes | 31 | 2 |
| 32 | 51 | CHILD A | 3 | SOF/DCV + RBV | yes | 31 | 1 |
| 33 | 50 | CHILD A | 3 | SOF + RBV | no | 32 | 1 |
| 34 | 59 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | yes | 36 | 3 |
| 35 | 75 | CHILD A | 1a | SOF/LDV | yes | 37 | 1 |
| 36 | 51 | CHILD A | 3 | SOF/DCV + RBV | no | 37 | 3 |
| 37 | 73 | CHILD A | 1b | SOF/LDV | yes | 37 | 3 |
| 38 | 47 | CHILD A | 4 | OBV/PTV/r/ + RBV | yes | 38 | 1 |
| 39 | 69 | CHILD A | 1b | SOF/SMV + RBV | yes | 40 | 1 |
| 40 | 57 | CHILD B | 3 | SOF/DCV + RBV | yes | 42 | 1 |
| 41 | 62 | CHILD B | 4 | SOF + RBV | yes | 45 | 1 |
| 42 | 57 | CHILD A | 1b | SOF/SMV + RBV | yes | 47 | 2 |
| 43 | 77 | CHILD B | 2 | SOF + RBV | yes | 47 | 1 |
| 44 | 66 | CHILD A | 2 | SOF + RBV | yes | 47 | 1 |
| 45 | 54 | CHILD A | 3 | SOF/DCV + RBV | no | 49 | 2 |
| 46 | 53 | CHILD A | 3 | SOF + RBV | no | 50 | 3 |
| 47 | 51 | CHILD A | 3 | SOF/DCV + RBV | yes | 54 | 1 |
| 48 | 40 | CHILD B | 1b | SOF/LDV + RBV | yes | 57 | 2 |
| 49 | 52 | CHILD A | 1b | OBV/PTV/r/DSV + RBV | yes | 58 | 3 |
| 50 | 71 | CHILD A | 2 | SOF + RBV | yes | 69 | 1 |
| 51 | 47 | CHILD A | 1a | SOF/SMV + RBV | no | 71 | 1 |
| 52 | 66 | CHILD A | 3 | SOF/DCV + RBV | no | 72 | 1 |

*Table 14: desciption of 52 patients who developed HCC.*

As we have already described, they included 36 male and 16 female patients with a mean age of 59,15 + 9.9 years (range 40 to 82 years) All major HCV genotypes were represented (6 HCV-1a 11.5% 21 HCV-1b 40.4%, 6 HCV-2 11.5%, 14 HCV-3 26.9% 5 HCV-4 9.6%). SVR12 was obtained in 26 patients while the remaining 26 either interrupted treatment prematurely or did not achieved SVR12. Time from initiation of DAAs and HCC diagnosis ranged from 4 to 72 weeks (mean 29 + 17.8 weeks ). 20 patients developed HCC while treated with DAAs while in the remaining 32 the tumor was diagnosed after the end of treatment.

When the pattern of HCC seen in our patients was considered, 27 patients (52%) presented with a single HCC lesion, and 9 (17.3%) with 2-3 small HCC nodules < 3 cm in diameter. A more aggressive type of tumor was seen in the remaining 16 patients with multiple nodules of variable size (8 cases- 15.4%) or an infiltrative diffuse HCC (8 cases : 15.4%) with portal thrombosis in 6 (11.5%) and extrahepatic metastasis in 4 (7.7%).

Figure 4 described the distribution of different patterns of HCC in relation to timing after initiation of DAAs. The more aggressive pattern prevailed in the early period of observation and often occurred during therapy, while the single HCC nodular pattern was predominant at longer follow-up. It should be underlined, however, that the majority of aggressive tumor (9 out of 10 with infiltrative pattern) were diagnosed following abrupt onset of clinical symptoms and/or abnormal biochemical or alfa-fetoprotein values, while all other cases were diagnosed at the time of periodical US examination of the liver, and this may have resulted in delayed detection of the latter compared to the former tumors.

Figure 5 describe the frequency of these different HCC patterns in relation to virological outcome. In this analysis patients who discontinued therapy were classified as non responders. The more aggressive pattern of HCC was seen somehow more frequently (46,2%) in patients without SVR compared to those with SVR (15,5%) in which the single nodule pattern prevailed (61,5%).

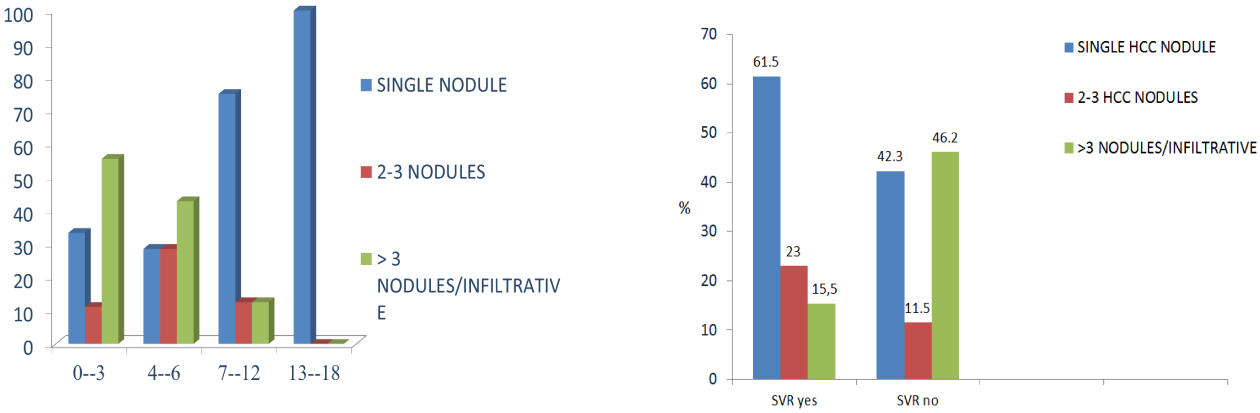


*Figure 4 and 5: different patterns of HCC in relation to time of they occurred (4) and in relation to virological outcomes (5).*

Our prospective study, based on a large cohort of consecutive patients treated with DAAs, indicates that the risk of HCC during/after treatment with DAAs is comparable, or possibly lower, than that expected in the natural history of the disease, being strictly dependent on stage of liver disease. In patients with Child A cirrhosis, our data base has recorded, during the first year after initiation of DAAs, an HCC rate of 1.55 x 100 patients/year with 95% CI between 1.18 and 2.01, compared to an annual incidence of 2.8% and 3.9% observed in 2 historical cohorts of untreated patients from the same geographic region. As regards patients with Child B cirrhosis treated with DAAs, the rate of HCC we observed was of 3.68 x 100 patients/year with 95% CI between 1.92 and 6.41, compared to an annual incidence of 5.7 % in an historical untreated cohort followed-up in our Institution between 2002 and 2014 (unpublished). A striking difference in our study was in the risk of HCC in relation to the response to antiviral therapy. Although HCC was observed in 26 patients with SVR, the tumor incidence was significantly higher in patients without HCV eradication (figure 5). The incidence of HCC was not increased in our patients treated with DAAs compared to what is expected in the natural course in HCV patients with advanced liver disease, but the pattern of HCC was somehow unusual in a subgroup of patients who developed an aggressive type of tumor, that was multifocal or infiltrative at diagnosis. This pattern was observed in 16 patients, i.e. in <0.5% of the whole cohort of 3682 patients, and prevailed in the early phase of observation, being diagnosed either during (7 cases) or immediately after (3 cases) antiviral therapy.

**- Clinical Outcomes in Extrahepatic Manifestations**

Among our HCV infected patients, we have recorded 287 patients with extrahepatic disease HCV-related. In particular, 76 patients with non-Hodgkin lymphoma (NHL-B), 106 with clinically relevant vasculitis and 105 with cryoglobulinemia (mixed cryoglobulinemia syndrome with cryocrit value higher than 2%). Mean age was 60,7+/-13,2 years and genotype 1b was the most represented: 53% was genotype 1 (11% HCV-1a, 42% HCV-1b) and 30% was genotype 2. Most of these patients had a mild form of liver disease and only 10% had a diagnosis of cirrhosis (F4), see table 15.

| **Cohort Extrahepatic Disease** | **287 (n. patients)** | |
|---|---|---|
| Non-Hodgkin lymphoma | 76 | |
| Vasculitis | 106 | |
| Cryoglobulinemia | 105 | |
| Mean age (years) | 60,7 +/- 13,2 | |

| | | |
|---|---|---|
| Genotype 1a | 11% | |
| Genotype 1b | 42% | |
| Genotype 2 | 30% | |
| | | |
| Liver fibrosis stage | F0 | 8.6% |
| | F1 | 42,4 |
| | F2 | 30 |
| | F3 | 19 |
| | F4 | 10 |

*Table 15: Features description in extrahepatic manifestations cohort*

Among the 76 patients with NHL-B, we have recorded a complete remission in 28,6%, a partial remission in 44,1%, unchanged or advanced disease in 10% and unknown outcome in 17,3%.

Among the 106 patients with significant vasculitis, we have recorded a complete remission in 17,4%, a partial remission in 45,3%, unchanged or advanced disease in 5,8% and unknown outcome in 31,5%.

Among the 105 patients with cryoglobulinemia, we have recorded a complete remission in 49,5%, a partial remission in 25% and unknown outcome in 25,5%.

| | | | |
|---|---|---|---|
| **Non-Hodgkin lymphoma** | 76 patients | complete remission | 28,6% |
| | | partial remission | 44,1% |
| | | unchanged or advanced | 10% |
| | | unknown | 17,3% |
| **Vasculitis** | 106 patients | complete remission | 17,4% |
| | | partial remission | 45,3% |
| | | unchanged or advanced | 5,8% |
| | | unknown | 31,5% |
| **Cryoglobulinemia** | 105 patients | complete remission | 49,5% |
| | | partial remission | 25% |
| | | unknow | 25,5% |

*Table 16: Outcomes in patients with extrahepatic manifestations.*

**- Efficacy and Tolerability of DAA Treatment in the Elderly ( > 70 yrs)**

Our data base has allowed us to analysed efficacy and safety in a subgroup of patients that has not been investigated in registration trials, i.e. an "elderly" subgroup aged > 70 years. 1681 HCV patients aged > 70 years have been recorded on the NAVIGATORE

platform and 942 of these have been treated with DAAs. Compared to younger patients recorded, these older 70 years had significantly higher prevalence of females (63% vs 35%), naives (77% vs 65%), cirrhotics (49% vs 39%), previous HCC (3,8% vs 1,4%), HCV-1b (53% vs 34%) and HCV-2 (26% vs 7%), diabetes (12% vs 6%) and CVD (29% vs 9%) and lower prevalence of HCV-1a (2% vs 22%) and HCV-3 (1% vs 19%).

| Recorded patients | **<70 years** | **>70 years** |
|---|---|---|
| **Patient number** | **6676** | **1681** |
| % Females | 35 | 63 |
| % Naives | 65 | 77 |
| % Cirrhotics | 39 | 49 |
| % Previous HCC | 1,4 | 3,8 |
| % HCV-1a | 22 | 2 |
| % HCV-1b | 34 | 53 |
| % HCV-2 | 7 | 26 |
| % HCV-3 | 19 | 1 |
| % Diabetes/CVD | 6/9 | 12/29 |

*Table 17: Features of < 70 years people relation to > 70 years people in our population.*

Patients were treated with approved DAAs according to guidelines and without significant differences in regimen choice with respect to patients < 70 years. Efficacy and safety were similar between patients < and > 70yrs without any statistically significant differences in SVR rates with different HCV genotypes. The elderly patients had more frequently an issue of possible drug-drug interactions related to the polytherapies they were on for several comorbidities, but mortality and clinically significant adverse events were not numerically different compared to younger patients.

| **HCV** | **<70 years** | **>70 years** | |
|---|---|---|---|
| 1a | 426/452 (94%) | 11/13 (85%) | |
| 1b | 723/767 (94%) | 287/279 (96%) | |
| 2 | 151/164 (92%) | 147/158 (93%) | p = ns |
| 3 | 336/385 (87%) | 6/8 (75%) | |
| 4 | 179/188 (95%) | 3/3 (100/%) | |

Table 18: SVR rates according to age and genotype.

| | **<70 years** | **>70 years** | |
|---|---|---|---|
| Cases | 3428 | 942 | |
| Gastro-Intestinal bleeding | 0.5 % | 0.1 % | |
| Ascites | 2.4 % | 2.0 % | |
| HCC | 2.3 % | 2.7 % | |
| Liver related death | 0.5 % | 0.5 % | p = ns |
| Other causes death | 0.3 % | 0.3 % | |

Table 19: Safety data according to age.

## DISCUSSION

In this thesis we have assessed virological and clinical outcomes in a large cohort of patients with hepatitis C treated with oral DAAs combinations in clinical practise with particular focus on 3 subgroups of patients: a) patients with advanced liver fibrosis or cirrhosis, both compensated and decompensated, b) patients with severe extrahepatic manifestations due to HCV, and c) patients with an age > 70 years. For this purpose I have participated since the very beginning to the design and development of the NAVIGATORE platform, a web-based platform that is monitoring patients with HCV as they are diagnosed, referred and treated with antiviral therapy in 27 Clinical Centers in our Region, in Veneto. The NAVIGATORE platform is used to monitor patients flows and treatment planning, while representing also a network that allows drugs use optimisation and homogeneity in clinical management. It permits also prospective recording of virological and clinical outcomes and of safety data. We considered a database records of 4764 DAAs treated patients in Veneto Region from 1st January 2015 to 24th May 2017.

DAAs were safe and highly effective in eradicating HCV in all the different categories of HCV infected patients, including advanced or decompensated, cirrhosis as well as patients aged > 70 years Among the 4764 treated patients, 62% (2946 patients) had cirrhosis (87% CHILD A ,10% CHILD B and 4% CHILD C) and many patients had comorbidities, but efficacy was excellent in most groups. As expected, SVR rates were somehow reduced in patients with Child B cirrhosis and with HCV-3 genotype. Tolerability and safety were also excellent in patients with F3 and with Child A cirrhosis, while were reduced in Child B patients, particularly when treated with Ribavirin containing regimens.

We were able to demonstrate a clear reduction in the incidence of major clinical complications in DAA treated patients, compared to the expected rates in untreated patients. Among decompensated cirrhotic patients, many improved in Child-Pugh score during and after therapy, particularly when achieving SVR. Cirrhotic patients showed a reduced incidence of complications with DAA therapy, compared to what was expected according to the natural course of the disease.

Our analysis was particularly focused on the residual risk of developing HCC after HCV eradication by DAAs in patients with advanced fibrosis and cirrhosis.

In the past, evidence was obtained indicating that the risk of developing HCC in cirrhotic HCV patients was significant reduced, although not completely abolished, following HCV

eradication with IFN-based therapy. It was therefore surprising and at the same time alarming when Reig et al reported “an unexpected high rate” of HCC recurrence early during or after DAAs in patients with a previous history of cured HCC and when Conti et al. seems to confirm that. These and some following reports (in most part retrospective and often including small series of patients with significant heterogeneity in baseline features and available follow-up) described quite variable results, not allowing to draw any firm conclusion on what has become a major issue for the treatment of cirrhotic patients with DAAs, i.e. the residual risk of hepatocellular carcinoma during and after treatment. We have analysed in details the incidence of HCC recurrence and that of “de novo” occurrence in patients with cirrhosis treated with DAAs in our database. The results of this analysis indicate that the risk of developing HCC during/after DAAs is not modified compared to what is expected in the natural course of the disease, being largely dependent on stage of liver disease. The risk of developing HCC was markedly and significantly higher in patients with more advanced liver disease and without SVR. Considering that most of our patients developed HCC early in the course of antiviral therapy, a relation of cause and effect between HCC and virological failure rather than the opposite appears likely, and might reflect persistence in tumour cells of HCV virions less accessible/susceptible to antivirals, in line with other studies also. Although the incidence of HCC was not increased in our patients treated with DAAs compared to what is expected in the natural course in HCV patients with advanced liver disease, the pattern of HCC was somehow unusual in a subgroup of patients who developed an aggressive type of tumour, that was multi-focal or infiltrative at diagnosis. While 52% of them developed a single small HCC nodule and 17% developed 2-3 small HCC nodules, the remaining 31% were diagnosed with larger or aggressive, often infiltrative, tumours. This was more frequent during rather than after DAA treatment and in patients who did not achieved SVR.

These tumours were most likely present already at baseline being still microscopic or unrecognisable and it cannot be excluded that they might have been boosted in growth and spread by the profound immunological and molecular changes that are known to occur in the liver micro-environment following abrupt interruption of HCV replication by DAAs. Meanwhile our data, while clearly indicate that DAA treatment doesn’t increase HCC risk in cirrhotics, reinforce the recommendation that cirrhotic patients should be carefully investigated at baseline to exclude HCC and then continue to be monitored during and after therapy independently of virological outcome. DAAs treatment is no pharmacological prevention of HCC even with successful antiviral therapy, and at least during the first 12 months after initiation of treatment, when microscopic and therefore

initially invisible HCC foci might be present and emerge during or after antiviral therapy even with an aggressive pattern of growth (27). Further data and analysis on the molecular, pathological and clinical characteristics of incident HCCs occurring during/after DAAs treatment are needed to better clarify these issues. This might be true both for “de novo” occurrence and for recurrence of HCC, this latter event being in our patients again not more frequent than expected in the natural history of the disease.
As other Authors have demonstrated, viral eradication was not only associated with a reduction of liver-related consequences of HCV infection, but also with a reduction of relevant extrahepatic disease manifestations. SVR obtained with IFN-based therapy was associated with an improved overall survival and with an improved quality of life (28).

We were able to analyse clinical outcomes in 287 patients with extrahepatic manifestations linked to HCV. In our preliminary analysis of disease outcomes, most of these patients showed a remission, partial or complete, of the extrahepatic syndrome, including those diagnosed with a B cell lymphoma, or with vasculitis. Furthermore, patients with detectable cryglobulins at baseline, often showed disappearance of circulating cryo during and after DAA therapy. These results need to be now consolidated and confirmed by longer follow-up to assess the precise role and benefits of antiviral therapy in these specific clinical settings.

Finally, considering that in our Region, as in most parts of Italy, the HCV-infected population is ageing and the proportion of patients with advanced liver disease aged > 70 years and presenting with several other comorbidities is particularly high, we decided to perform a sub-analysis of in a special population. Accordingly, we have been able to describe efficacy, safety and clinical outcomes of DAA therapy in almost 1000 patients with HCV aged > 70 years. Our findings indicate excellent results of oral antiviral therapy in this clinical settings, behaving in a very similar way compared to younger patients, without any specific age related problematic issues. This was true also for patients > 70 years with advanced liver disease, or cirrhosis.

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