Rassegna

Review

Is it possible to predict HCV-related liver cirrhosis through routine laboratory parameters?

É possibile predire la cirrosi epatica HCV-correlata attraverso parametri laboratoristici di routine?

Ivan Gentile, Antonio Riccardo Buonomo, Emanuela Zappulo, Guglielmo Borgia

Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy

Hepatitis C virus (HCV) is estimated to chronically infect about 160,000,000 people in the world and to be responsible for more than 360,000 deaths a year [1, 2].

HCV is a non-stable virus. In fact it is currently classified in 7 major genotypes (identified with numbers 1 to 7) and in 67 subtypes [3]. Finally, HCV is detected in a single host as a mix of closely related viral populations called quasi-species. The biological explanation for this huge genomic instability lies in the lack of a proof-correction activity of the viral RNA-dependent RNA polymerase.

HCV is acquired through blood transfusion before 1990s, use of glass syringes, unsafe sexual intercourse, invasive medical procedures, tattooing, and intravenous drug use [4-11]. After its entry in the human host, HCV produces an acute infection that becomes chronic in a high percentage of cases (54-86%), likely due to the high viral heterogeneity that allows its escape from host immune system [12-14]. Once hepatitis becomes chronic, it entails a high risk of developing liver cirrhosis. In fact, 15-56% of patients with chronic hepatitis C develop a liver cirrhosis. This wide range of disease progression is due to the presence or absence of factors that contribute to a higher and quicker progression such as alcohol intake, iron overload, steatosis, HBV or HIV co-infection [2, 12, 14-20]. Actually, the incidence of new infections is declining at-least in developed countries [11, 21-23]. However, due to the large amount of people infected in past decades, it is expected that the number of patients with an advanced disease will increase in the next years [9].

With the exception of extrahepatic diseases (such as mixed cryoglobulinemia, Sjögren syndrome, non-Hodgkin lymphoma [14, 24-28]), all the severe consequences of HCV infection and the total burden of death occur only in presence of liver cirrhosis. Complications include hepatocellular carcinoma development, ascites, variceal bleeding, jaundice and end-stage liver disease. Therefore the presence of cirrhosis is a threshold with a high negative prognostic value [29].

It is demonstrated that the natural history of the disease can be halted or even reversed in case of a successful viral eradication by antiviral therapy [30-36].

Antiviral therapy currently consists in the combination of pegylated-interferon and ribavirin. In case of patients infected with HCV genotype 1, a third drug (namely, a protease inhibitor boceprevir or telaprevir) is often added to increase the rate of viral clearance [37-42]. Viral clearance is obtained if HCV-RNA persists undetectable for 6 months after therapy withdrawal. This is called Sustained Virological Response (SVR) and represents the surrogate endpoint in clinical trials concerning anti-HCV drugs [30]. In fact, patients who maintain an

Corresponding author
Ivan Gentile
E-mail: ivan.gentile@unina.it

undetectable level of HCV-RNA 6 months after the completion of therapy have a life-long viral clearance [43]. Recently, it has been suggested that a shorter period (12 weeks) is sufficient to assess a durable viral clearance as nearly all patients that have undetectable levels of HCV-RNA 12 weeks after therapy withdrawal (SVR12) maintain this status at the classical 6-month time point [44].

The mean SVR rate achieved with interferon-based combination in treatment-naïve patients is about 80% for those with genotype 2 or 3 (with PEG-IFN + ribavirin) and 70% for those with genotype 1 (with PEG-IFN + ribavirin + a protease inhibitor).

However, this mean value is higher for patients with positive predictive factors (white race, absence of liver cirrhosis or advanced fibrosis, presence of the CC polymorphism of interleukin-28B gene, low pre-treatment levels of viral load, ferritin or homocysteine, and high baseline values of vitamin D [45-52]). Moreover, it is not possible to treat all patients mainly because interferon-based combinations are

Table 1 - Scores used to predict cirrhosis based on noninvasive, routinely-available parameters.

Test	*Formula*	*Cut-off value*	*SE*	*SP*	*PPV*	*NPV*	*DA*	*PLR*	*NLR*
AAR [74]	AST/ALT	>1	53.2	100	100	80.7	84.2	NC	0.47
APRI [75]	([AST/ULN]/PLT [10^9/L])×100	≤1 >1	89.3	75	37.9	97.6	77.1	3.57	0.14
		≤2 >2	57.1	92.7	57.1	92.7	87.5	7.81	0.46
CISCUN [76]	Age+AST+PLT+ Prothrombin Activity (Range, 0-4) Age: ≤40 years = 0; >40 years = 1 AST: ≤2 ULN = 0; >2 ULN = 1 Platelet count (109/L): ≥160=0; <160=1 Prothrombin activity: ≥100% = 0; <100% = 1	≤1 >1	94.1	50.5	32.9	97.1	59.4	1.90	0.12
		<4 ≥4	37.3	98.5	86.4	85.9	85.9	24.59	0.64
GUCI [71]	Normalized ASTxINRx100/platelet count (10^9/L)	>1	80	78	31.4	96.9	78.2	3.63	0.26
Fibrosis index [73]	8-0.001 x Platelet count (10^9/L) - Albumin (g/dL)	≥3.3	67.7	97.9	75	97.1	95.4	32.61	0.33
Lok [70]	Log odds = -5.56 -0.0089 x platelet count (10^3/mm^3) + 1.26 x AST/ALT ratio + 5.27 x INR Formula to calculate final probability: exp (logodds)/ (1 exp(logodds))	≤0.2	92.2	30	46.2	85.5	54.5	1.32	0.26
		>0.5	54	85.2	70.5	74	72.9	3.66	0.54
Platelet count [72]		<150 (x 10^9/L)	77	88	56	95	NA	6.42	0.26

AAR: AST/ALT ratio; APRI: AST to platelet ratio index; CISCUN: Cirrhosis Score University of Naples; GUCI: Göteborg University cirrhosis index; ULN: Upper Limit of Normal range; SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value; DA: diagnostic accuracy (rate of correctly-classified patients); PLR: positive likelihood ratio; NLR: negative likelihood ratio; NA: non-available. Modified from (29).

paradoxically contraindicated in the most severe stages of liver cirrhosis (e.g. in presence of ascites) and in patients with severe impairment of other vital organs [42, 53].
This kind of therapy is also poorly tolerated and costly [54, 55]. Finally, due to these limitations, several direct antiviral agents active against HCV are in an advanced phase of clinical development [56-58]. In some cases, these new drugs will allow to use interferon-free combinations and therefore to overcome the said limitations of the currently available treatment (all but the cost that will increase!) [57-61].
Given the above, the clinician that cures a patient with chronic HCV infection, has to answer two main questions regarding therapy:

1) to treat or not to treat the patient;
2) in case of affirmative answer, to treat now or wait for more active and better tolerated drugs. In both questions, the presence of cirrhosis is crucial as it is a strong indication for a prompt antiviral treatment [62].

Finally, in presence of liver cirrhosis it is mandatory to perform periodical screening for the presence of esophageal varices and HCC by the mean of periodical upper endoscopy and ultrasound examination.
Therefore it is crucial to know whether the patients has cirrhosis for prognostic, therapeutic and screening purposes. If we exclude the cases of decompensated liver cirrhosis that are diagnosed clinically, the best way to diagnose liver cirrhosis in HCV-positive patients is histological examination obtained through liver biopsy.
However, percutaneous liver biopsy is an invasive technique and it is associated with a low but not null rate of complications (0.3-0.8%) and death (0.01-0.3%) [63-66]. Consequently, several authors have devised different non-invasive tests to diagnose liver cirrhosis [67].
The most used ones are based on the measurement of liver stiffness through a dedicated machine (fibroscan) [68], on a panel of blood tests and a proprietary algorithm (fibrotest) [69] or on routinely-available parameters (e.g., aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio, GUCI, CISCUN, platelet count (PLT), APRI, the Lok score, the Fibrosis index and the King's College score) [70-76].
This review focuses on non-invasive scores based on routinely available parameters. The formulae to calculate the main non-invasive scores in cirrhosis prediction as well as their diagnostic performance are reported in Table 1 [29]. The scores listed in Table 1 can be divided in two groups:

1) the first group characterized by high values of positive predictive value (PPV) and therefore a positive result indicates a high chance of cirrhosis;
2) the second group characterized by a high value of negative predictive value (NPV) and therefore a negative result indicates a low probability of having cirrhosis.

The AST/ALT ratio belongs to the first group as in the original paper it yields a PPV as high as 100% [74]. However, the high value of PPV for this score has not been confirmed by other studies [76, 77]. Therefore its usefulness in predicting cirrhosis is low, whereas it is often included in noninvasive scores to predict gastroesophageal varices in patients with liver disease [10, 78].
With respect to the second group, it is noteworthy that a good screening test should have a very high rate of NPV, close to 100%. Only in this case the rate of false negative is close to zero. Unfortunately none of the proposed scores reaches a NPV of 100%.
It is noteworthy that 3 scores (CISCUN, Lok and APRI) have two different cut-offs, one to diagnose cirrhosis and another to rule out it [70, 75, 76].
In other words, patients with a very positive result of the test (≥4 for CISCUN, >0.5 for Lok and >2 for APRI), are diagnosed to have cirrhosis. Patients with a very negative result of the test (≤1 for CISCUN, ≤0.2 for Lok and ≤1 for APRI) can also avoid to perform a liver biopsy due to a low risk of cirrhosis. Patients in the grey zone have to perform liver biopsy (CISCUN=2 or 3, APRI between 1 and 2 and Lok between 0.2 and 0.5). The determinant factor in these scores is the proportion of patients in the 2 tails compared to the total amount of patients and therefore the rate of those who can safely avoid the liver biopsy.
Moreover, some scores are very easy to obtain (CISCUN, AST/ALT ratio, platelet count), others need a calculator (as Lok score, GUCI, APRI and fibrosis index).
Two limitations make the comparison of these results not possible:

1) very few studies have compared the different scores in the same population. It is not justified to compare data obtained from different cohorts with different rates of cirrho-

sis and different techniques to obtain the liver tissue (e.g. length and size of the needle);
2) in all these studies, each score was compared to the percutaneous liver biopsy that constituted the gold diagnostic standard. However, this technique is not perfect as it entails a relatively high rate of false negative for the diagnosis of cirrhosis (up to 20%) when compared to surgical biopsy [79-81]. Moreover, histological examination of longer and thicker tissue samples provides a better estimate of liver fibrosis than short and thin ones [82, 83]. The biological explanation for this phenomenon is that the distribution of fibrosis is not uniform but patchy, and a liver biopsy sample constitutes only from one hundred-thousandth to one thirty thousandth of the liver.

Finally, the advantage of liver biopsy is that it provides additional information that any non-invasive score cannot provide (e.g. iron stain, steatosis evaluation, necroinflammatory activity). In conclusion, several authors devised scores based on non-invasive parameters for prediction of liver cirrhosis, but none was able to provide the same diagnostic performance of liver biopsy.

Some scores show promising results in terms of both high negative and positive predictive values (as Lok score, CISCUN or Fibrosis index). However, their diagnostic performance should be compared with the results of a rigorous and well-performed histologic evaluation and of the fibroscan or the fibrotest in independent cohorts.

Keywords: cirrhosis, HCV, liver biopsy, CISCUN, APRI, fibroscan.

SUMMARY

It is estimated that hepatitis C virus (HCV) chronically infects about 160 million people worldwide. Between 15 and 56% of these chronic carriers will evolve towards liver cirrhosis during their lifetimes. In managing subjects with HCV chronic infection, it is crucial to perform accurate staging of the disease and specifically to ascertain whether or not they have liver cirrhosis for at least three reasons:

1) the presence of cirrhosis has prognostic relevance as it entails a relatively high risk of developing decompensation (e.g., ascites, encephalopathy, jaundice, oesophageal variceal bleeding) or evolution toward hepatocellular carcinoma (HCC);
2) the presence of liver cirrhosis is the main indication for urgent treatment and paradoxically a factor predicting a poor response to currently available therapies. The timing of therapy is particular important considering that the new era of interferon-free antivirals will be a reality in a few years;
3) finally, in the presence of liver cirrhosis current guidelines recommend periodical screening for the presence of oesophageal varices and HCC.

With the exception of the most advanced stages, the diagnosis of liver cirrhosis is obtained by performing a percutaneous liver biopsy, which is an invasive technique and therefore is associated with a low but non-negligible rate of complications and even death. Finally, it has a non-null rate of false negative for the diagnosis of liver cirrhosis when compared with surgical biopsy. For these reasons several authors have devised non-invasive scores to predict cirrhosis using different means. The most useful are based on liver stiffness (fibroscan), on a panel of blood tests and a proprietary algorithm (fibrotest) or on routinely available parameters. This review focuses on the different scores based on routine parameters that differ in their ease of calculation, in their diagnostic power and in the information provided. Further studies are required to compare the diagnostic performance of different non-invasive scores with the histologic evaluation and other non-invasive methods (fibroscan or fibrotest) on independent cohorts of patients.

RIASSUNTO

Si stima che circa 160 milioni di persone nel mondo siano cronicamente infette con il virus dell'epatite C (HCV). Una percentuale variabile tra il 15 e il 56% di tali portatori cronici evolve verso la cirrosi epatica durante la loro vita. Nella gestione dei pazienti con infezione cronica da HCV è essenziale effettuare una stadiazione accurata della malattia e, in particolare, accertare l'eventuale presenza di cirrosi epatica per almeno tre ragioni:

1) *la presenza di cirrosi epatica ha una forte rilevanza prognostica poiché è associata ad un rischio relativamente alto di scompenso (come ascite, encefalopatia, ittero, sanguinamento da varici esofagee) o evoluzione verso l'epatocarcinoma (HCC);*
2) *la presenza di cirrosi è una forte indicazione per un trattamento antivirale urgente e paradossalmente un fattore che predice una scarsa risposta alle attuali terapie. La scelta del momento del trattamento è cruciale considerando che è alle porte la nuova era delle terapie antivirali interferon-free;*
3) *infine, in presenza di cirrosi le linee guida internazionali raccomandano uno* screening *periodico per la ricerca di varici esofagee ed HCC.*

Con l'eccezione degli stadi più avanzati della malattia, la diagnosi di cirrosi è effettuata attraverso l'esecuzione di una biopsia epatica percutanea che è una metodica invasiva e come tale è associata ad un rischio basso ma non nullo di complicanze e anche di morte. Infine, la biopsia epatica presenta un tasso non nullo di falsi negativi per la diagnosi di cirrosi se comparato alla biopsia chirurgica. Per questi motivi molti autori hanno proposto metodi non-invasivi per diagnosticare la cirrosi epatica. I più usati sono basati sulla misurazione della stiffness *(fibroscan), su un pannello di test ematici con un algoritmo brevettato (fibrotest) o su parametri di routine. Questa rassegna è focalizzata sui differenti score basati sui parametri di routine che differiscono tra loro nella facilità di calcolo, nel potere diagnostico e nel tipo di informazioni in grado di fornire. Studi volti a comparare la performance diagnostica dei diversi score con la biopsia epatica e altri sistemi non-invasivi (fibroscan o fibrotest) su differenti coorti di pazienti sono necessari.*

REFERENCES

[1] Perz J.F., Armstrong G.L., Farrington L.A., Hutin Y.J., Bell B.P. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J. Hepatol.* 45, 529-538, 2006.

[2] Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin. Microbiol. Infect.* 17, 107-115, 2011.

[3] Smith D.B., Bukh J., Kuiken C., et al. Expanded classification of hepatitis C Virus into 7 genotypes and 67 Subtypes: updated criteria and assignment web resource. *Hepatology* 1, 318-327, 2013.

[4] Gentile I., Di Flumeri G., Scarica S., et al. Acute hepatitis C in patients undergoing hemodialysis: experience with high-dose interferon therapy. *Minerva Urol. Nefrol.* 65, 83-84, 2013.

[5] Gentile I., De Stefano A., Di Flumeri G., et al. Concomitant interferon-alpha and chemotherapy in hepatitis C and colorectal cancer: a case report. *In Vivo* 27, 527-529, 2013.

[6] Carney K., Dhalla S., Aytaman A., Tenner C.T., Francois F. Association of tattooing and hepatitis C virus infection: a multicenter case-control study. *Hepatology* 57, 2117-2123, 2013.

[7] Coppola N., Pisapia R., Tonziello G., et al. Improvement in the aetiological diagnosis of acute hepatitis C: a diagnostic protocol based on the anti-HCV-IgM titre and IgG Avidity Index. *J. Clin. Virol.* 46, 222-229, 2009.

[8] Coppola N., Pisapia R., Marrocco C., et al. Anti-HCV IgG avidity index in acute hepatitis C. *J. Clin. Virol.* 40, 110-115, 2007.

[9] Davis G.L., Alter M.J., El-Serag H., Poynard T., Jennings L.W. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 138, 513-521, 2010.

[10] Gentile I., Viola C., Graf M., et al. A simple non-invasive score predicts gastroesophageal varices in patients with chronic viral hepatitis. *J. Clin. Gastroenterol.* 43, 81-87, 2009.

[11] Petruzziello A., Coppola N., Diodato A.M., et al. Age and gender distribution of hepatitis C virus genotypes in the metropolitan area of Naples. *Intervirology* 56, 206-212, 2013.

[12] Hoofnagle J.H. Hepatitis C: the clinical spectrum of disease. *Hepatology* 26, 15S-20S, 1997.

[13] Piazza M. Epatite Virale Acuta e Cronica. IX ed. 2009 Naples, Ghedini s.a.s.

[14] Maasoumy B., Wedemeyer H. Natural history of acute and chronic hepatitis C. *Best Pract. Res. Clin. Gastroenterol.* 26, 401-412, 2012.

[15] Sagnelli E., Pisaturo M., Stanzione M., et al. Clinical presentation, outcome, and response to therapy among patients with acute exacerbation of chronic hepatitis C. *Clin. Gastroenterol. Hepatol.* 11, 1174-1180, 2013.

[16] Coppola N., Gentile I., Pasquale G., et al. Anti-HBc positivity was associated with histological cirrhosis in patients with chronic hepatitis C. *Ann. Hepatol.* 13, 20-26, 2014.

[17] Fortunato G., Calcagno G., Bresciamorra V., et al. Multiple sclerosis and hepatitis C virus infection are associated with single nucleotide polymorphisms in interferon pathway genes. *J. Interferon Cytokine Res.* 28, 141-152, 2008.

[18] Fortunato G., Castaldo G., Oriani G., et al. Multivariate discriminant function based on six biochemical markers in blood can predict the cirrhotic evolution of chronic hepatitis. *Clin. Chem.* 47, 1696-1700, 2001.
[19] Gentile I., Meola M., Buonomo A.R., et al. Human platelet antigen-3 genotype predicts platelet count in patients with HCV infection. *In Vivo* 27, 773-777, 2013.
[20] Coppola N., Zampino R., Bellini G., et al. Association between a polymorphism in cannabinoid receptor 2 and severe necroinflammation in patients with chronic hepatitis C. *Clin. Gastroenterol. Hepatol.* 12, 334-340, 2014.
[21] Sagnelli E., Stroffolini T., Mele A., et al. The importance of HCV on the burden of chronic liver disease in Italy: a multicenter prevalence study of 9,997 cases. *J. Med. Virol.* 75, 522-527, 2005.
[22] Guadagnino V., Stroffolini T., Caroleo B., et al. Hepatitis C virus infection in an endemic area of Southern Italy 14 years later: evidence for a vanishing infection. *Dig. Liver Dis.* 45, 403-407, 2013.
[23] Guadagnino V., Stroffolini T., Rapicetta M., et al. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. *Hepatology* 26, 1006-1011, 1997.
[24] Ferrari S.M., Fallahi P., Mancusi C., et al. HCV-related autoimmune disorders in HCV chronic infection. *Clin. Ter.* 164, e305-e312, 2013.
[25] Coppola N., Pisaturo M., Guastafierro S., et al. Increased hepatitis C viral load and reactivation of liver disease in HCV RNA-positive patients with onco-haematological disease undergoing chemotherapy. *Dig. Liver Dis.* 44, 49-54, 2012.
[26] Zampino R., Coppola N., Cirillo G. et al. Abdominal fat interacts with PNPLA3 I148M, but not with the APOC3 variant in the pathogenesis of liver steatosis in chronic hepatitis C. *J. Viral Hepat.* 20, 8, 517-523, 2013.
[27] Coppola N., Pascalis S.D., Pisaturo M., et al. Sustained virological response to antiviral treatment in chronic hepatitis C patients may be predictable by HCV-RNA clearance in peripheral blood mononuclear cells. *J. Clin. Virol.* S1386-S1653, 2013.
[28] Fusco F., D'Anzeo G., Rossi A., et al. Erectile dysfunction in patients with chronic viral hepatitis: a systematic review of the literature. *Expert Opin. Pharmacother.* 14, 2533-2544, 2013.
[29] Gentile I., Borgia G. Prediction of liver cirrhosis in patients with HCV chronic infection through routine laboratory parameters: myth or reality? *J. Mol. Genet. Med.* 7, 76, 2013.
[30] Gentile I., Borgia G. Surrogate endpoints and non-inferiority trials in chronic viral hepatitis. *J. Hepatol.* 52, 778, 2010.
[31] Braks R.E., Ganne-Carrie N., Fontaine H., et al. Effect of sustained virological response on long-term clinical outcome in 113 patients with compensated hepatitis C-related cirrhosis treated by interferon alpha and ribavirin. *World J. Gastroenterol.* 13, 5648-5653, 2007.
[32] Tsuda N., Yuki N., Mochizuki K., et al. Long-term clinical and virological outcomes of chronic hepatitis C after successful interferon therapy. *J. Med. Virol.* 74, 406-413, 2004.
[33] Bruno S., Stroffolini T., Colombo M., et al. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 45, 579-587, 2007.
[34] Di Marco V., Almasio P.L., Ferraro D., et al. Peg-interferon alone or combined with ribavirin in HCV cirrhosis with portal hypertension: a randomized controlled trial. *J. Hepatol.* 47, 484-491, 2007.
[35] Hermine O., Lefrere F., Bronowicki J.P., et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N. Engl. J. Med.* 347, 89-94, 2002.
[36] Takahashi K., Nishida N., Kawabata H., Haga H., Chiba T. Regression of Hodgkin lymphoma in response to antiviral therapy for hepatitis C virus infection. *Intern. Med.* 51, 2745-2747, 2012.
[37] Venkatraman S., Bogen S.L., Arasappan A., et al. Discovery of (1R,5S)-N-[3-amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]- 3-[2(S)-[[[(1,1-dimethylethyl)-amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]- 6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(S)-carboxamide (SCH 503034), a selective, potent, orally bioavailable hepatitis C virus NS3 protease inhibitor: a potential therapeutic agent for the treatment of hepatitis C infection. *J. Med. Chem.* 49, 6074-6086, 2006.
[38] Tong X., Chase R., Skelton A., Chen T., Wright-Minogue J., Malcolm B.A. Identification and analysis of fitness of resistance mutations against the HCV protease inhibitor SCH 503034. *Antiviral Res.* 70, 28-38, 2006.
[39] Lin C., Kwong A.D., Perni R.B. Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect. Disord. Drug Targets* 6, 3-16, 2006.
[40] Gentile I., Viola C., Borgia F., Castaldo G., Borgia G. Telaprevir: a promising protease inhibitor for the treatment of hepatitis C virus infection. *Curr. Med. Chem.* 16, 1115-1121, 2009.
[41] Coppola N., Pisaturo M., Tonziello G., Sagnelli C., Sagnelli E., Angelillo I.F. Efficacy of Pegylated interferon alpha-2a and alpha-2b in patients with genotype 1 chronic hepatitis C: a meta-analysis. *BMC Infect. Dis.* 12, 1471-2334, 2012.
[42] Ghany M.G., Strader D.B., Thomas D.L., Seeff L.B. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 49, 1335-1374, 2009.
[43] Morisco F., Granata R., Stroffolini T., et al. Sustained virological response: A milestone in the treatment of chronic hepatitis C. *World J. Gastroenterol.* 19, 2793-2798, 2013.
[44] Chen J., Florian J., Carter W., et al. Earlier sustained virologic response end points for regulatory approval and dose selection of hepatitis C therapies. *Gastroenterology* 144, 1450-1455, 2013.

[45] Lange C.M., Bojunga J., Ramos-Lopez E., et al. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. *J. Hepatol.* 54, 887-893, 2011.
[46] Ismail M.H. Prediction of sustained virologic responses to combination therapy of pegylated interferon-alpha and ribavirin in patients with chronic hepatitis C infection. *J. Family Community Med.* 20, 35-40, 2013.
[47] Borgia G., Gentile I., Fortunato G., et al. Homocysteine levels and sustained virological response to pegylated-interferon alpha2b plus ribavirin therapy for chronic hepatitis C: a prospective study. *Liver Int.* 29, 248-252, 2009.
[48] Distante S., Bjoro K., Hellum K.B., et al. Raised serum ferritin predicts non-response to interferon and ribavirin treatment in patients with chronic hepatitis C infection. *Liver* 22, 269-275, 2002.
[49] Gentile I., Viola C., Paesano L., et al. Iron depletion before HCV antiviral therapy: a pilot, randomized, controlled trial. *J. Clin. Apher.* 24, 190-196, 2009.
[50] Abu-Mouch S., Fireman Z., Jarchovsky J., Zeina A.R., Assy N. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naive patients. *World J. Gastroenterol.* 17, 5184-5190, 2011.
[51] Nimer A., Mouch A. Vitamin D improves viral response in hepatitis C genotype 2-3 naive patients. *World J. Gastroenterol.* 18, 800-805, 2012.
[52] Coppola N., Marrone A., Pisaturo M., et al. Role of interleukin 28-B in the spontaneous and treatment-related clearance of HCV infection in patients with chronic HBV/HCV dual infection. *Eur. J. Clin. Microbiol. Infect. Dis.* 33, 559-567, 2014.
[53] Ghany M.G., Nelson D.R., Strader D.B., Thomas D.L., Seeff L.B. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 54, 1433-1444, 2011.
[54] Gentile I., Viola C., Reynaud L., et al. Hemolytic anemia during pegylated IFN-alpha2b plus ribavirin treatment for chronic hepatitis C: ribavirin is not always the culprit. *J. Interferon Cytokine Res.* 25, 283-285, 2005.
[55] Tosone G., Borgia G., Gentile I., et al. A case of pegylated interferon alpha-related diabetic ketoacidosis: can this complication be avoided? *Acta Diabetol.* 44, 167-169, 2007.
[56] Gentile I., Borgia F., Coppola N., Buonomo A.R., Castaldo G., Borgia G. Daclatasvir: the first of a new class of drugs targeted against Hepatitis C Virus NS5A. *Curr. Med. Chem.* In press. PMID: 24372205
[57] Gentile I., Borgia F., Buonomo A.R., Castaldo G., Borgia G. A novel promising therapeutic option against hepatitis C virus: an oral nucleotide NS5B polymerase inhibitor sofosbuvir. *Curr. Med. Chem.* 20, 3733-3742, 2013.
[58] Sarrazin C., Hezode C., Zeuzem S., Pawlotsky J.M. Antiviral strategies in hepatitis C virus infection. *J. Hepatol.* 56, S88-S100, 2012.
[59] Gentile I., Borgia F., Zappulo E., et al. Efficacy and safety of sofosbuvir in treatment of chronic hepatitis C: the dawn of the a new era. *Rev. Recent Clin. Trials* In press. PMID: 23859195
[60] Stedman C.A. Current prospects for interferon-free treatment of hepatitis C in 2012. *J. Gastroenterol. Hepatol.* 28, 38-45, 2013.
[61] Gentile I., Buonomo A.R., Borgia F., Castaldo G., Borgia G. Ledipasvir: a novel synthetic antiviral for the treatment of HCV Infection. *Expert Opin. Investig. Drugs.* 23, 561-571, 2014.
[62] Tillmann H.L. Hepatitis C infection and presence of advanced fibrosis: wait or treat? Why wait? There is no time to lose, is there? *J. Hepatol.* 58, 412-414, 2013.
[63] Cadranel J.F., Rufat P., Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 32, 477-481, 2000.
[64] Perrault J., McGill D.B., Ott B.J., Taylor W.F. Liver biopsy: complications in 1000 inpatients and outpatients. *Gastroenterology* 74, 103-106, 1978.
[65] Sagnelli E., Sagnelli C., Pisaturo M.A., Coppola N., Pasquale G., Piccinino F. Liver biopsy in chronic hepatitis C: the experience of 15 Italian wards of infectious diseases. *Infez. Med.* 20, 31-36, 2012.
[66] Janes C.H., Lindor K.D. Outcome of patients hospitalized for complications after outpatient liver biopsy. *Ann. Intern. Med.* 118, 96-98, 1993.
[67] Martinez S.M., Crespo G., Navasa M., Forns X. Noninvasive assessment of liver fibrosis. *Hepatology* 53, 325-335, 2011.
[68] Ziol M., Handra-Luca A., Kettaneh A., et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 41, 48-54, 2005.
[69] Imbert-Bismut F., Ratziu V., Pieroni L., Charlotte F., Benhamou Y., Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 357, 1069-1075, 2001.
[70] Lok A.S., Ghany M.G., Goodman Z.D., et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 42, 282-292, 2005.
[71] Islam S., Antonsson L., Westin J., Lagging M. Cirrhosis in hepatitis C virus-infected patients can be excluded using an index of standard biochemical serum markers. *Scand. J. Gastroenterol.* 40, 867-872, 2005.
[72] Lackner C., Struber G., Liegl B., et al. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology* 41, 1376-1382, 2005.
[73] Ohta T., Sakaguchi K., Fujiwara A., et al. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. *Acta Med. Okayama* 60, 77-84, 2006.

[74] Sheth S.G., Flamm S.L., Gordon F.D., Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am. J. Gastroenterol.* 93, 44-48, 1998.
[75] Wai C.T., Greenson J.K., Fontana R.J., et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 38, 518-526, 2003.
[76] Gentile I., Coppola N., Pasquale G., et al. A simple noninvasive score based on routine parameters can predict liver cirrhosis in patients with chronic Hepatitis C. *Hepatitis Monthly* 13, e8352, 2013.
[77] Reedy D.W., Loo A.T., Levine R.A. AST/ALT ratio > or = 1 is not diagnostic of cirrhosis in patients with chronic hepatitis C. *Dig. Dis. Sci.* 43, 2156-2159, 1998.
[78] Gentile I., Thabut D. Noninvasive prediction of oesophageal varices: as simple as blood count? *Liver Int.* 30, 1091-1093, 2010.
[79] Poynard T., de Ledinghen V., Zarski J.P., et al. Relative performances of fibrotest, fibroscan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. *J. Hepatol.* 56, 541-548, 2012.
[80] Bedossa P., Dargere D., Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 38, 1449-1457, 2003.
[81] Pagliaro L., Rinaldi F., Craxi A., et al. Percutaneous blind biopsy versus laparoscopy with guided biopsy in diagnosis of cirrhosis. A prospective, randomized trial. *Dig. Dis. Sci.* 28, 39-43, 1983.
[82] Colloredo G., Guido M., Sonzogni A., Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J. Hepatol.* 39, 239-244, 2003.
[83] Scheuer P.J. Liver biopsy size matters in chronic hepatitis: bigger is better. *Hepatology* 38, 1356-1358, 2003.