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Review

"Immune activation, aging and gender" and progression of liver disease

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Abstract. Hepatitis C is the predominant cause of liver disease in the HIV-positive population and the most important of the non-AIDS-related causes of death. HCV disease tends to become chronic more frequently in HIV-positive subjects, and to evolve more rapidly into cirrhosis of the liver. The rapidity of the evolution varies considerably from one individual to the next and, if in HIV-negative subjects cirrhosis manifests itself after approx. 40-50 years of disease, in HIV-positive subjects it emerges 10-15 years earlier (1, 2). The severity of the fibrosis is not a gradual event and can be worsened by many factors. Age, sex, duration of the infection and assumption of alcohol are the most well-known variables; obesity, diabetes, steatosis and metabolic disorders are equally important factors that affect the progression of liver disease (3). The severity of the liver disease is very different in men compared to women. Being male is undoubtedly one of the factors most closely related to the gravity of fibrosis (4). In HCV mono-infected women, cirrhosis appears from the age of 60 onwards. With the onset of the menopause, in fact, the progression of liver disease accelerates and the risk of developing cirrhosis or cancer of the liver becomes particularly significant in women over 50. The conditions of menopause or of amenorrhea, irrespective of age, are therefore correlated with the progression of liver disease (5). This evidence led researchers to theorize on the possible anti-fibrogenic role of estrogens. In fact, estrogens in physiological doses in the plasma of women in fertile age contribute to controlling the progression of liver disease through antioxidant mechanisms and lipid peroxidation control mechanisms (6). The reduction of estrogens during the menopause is closely linked to the increase of metabolic disorders. During the menopause, steatosis and cardiovascular diseases increase in parallel with the increase of atherogenic lipoproteins, the accumulation of intra-abdominal fat and the onset of insulin resistance (3, 7). Recent works have demonstrated how the concentration of HCV viremia in plasma correlates with the degree of insulin resistance and with the concentration of circulating triglycerides, demonstrating how the role of HCV in altering the hepatic lipid and glucide metabolism is functional to its replicative capacity. The correlation between the HCV viral load and the metabolic set-up is still unclear in the mono-infection and has been rarely studied in the HIV/HCV co-infection, where the picture is further altered by the metabolic impact of certain antiretroviral therapies. Over recent years, most of the HIV/HCV co-infected population, belonging – as is common knowledge – to the old intravenous drug user risk categories, have reached the age of 50 and have 20-25 years' history of liver disease. The women are reaching the menopause and are frequently characterized by prolonged states of amenorrhea. This conditions exposes HIV-positive women and the co-infected population, currently in care in our centres, to a sudden and rapid worsening of the liver condition. Over the last few years, we are also witnessing the launching on the market of new anti-HCV molecules which must, of necessity, find an outlet as new drug applications for HIV/HCV co-infected patients, as well as being used to the best advantage in populations with particular complications. (www.actabiomedica.it)

Key words: HIV/HCV co-infection, progression of liver disease, aging

Introduction

The natural course of Hepatitis C in HIV-positive patients is more rapid than in HCV mono-infected patients, both in terms of fibrogenesis and in terms of carcinogenesis.

The HIV-positive patient model is undoubtedly of great interest because it groups together factors of immune activation, metabolic alterations and aging, that are not so well identifiable in other chronic inflammatory diseases of the liver.

The HIV-positive, HCV co-infected woman, in both the pre- and post-menopause phases, provides further data useful for the understanding of the inflammatory condition which, from minimum, becomes maximum during the onset of the menopause, due to the loss of estrogens which exert a protective function.

For women in pre-menopausal period or with prolonged amenorrhea, and for patients aging with HIV/HCV co-infection, treatment with peginterferon and ribavirin, the current standard of care in anti-HCV treatment, faces greater difficulties in achieving success both in patients with chronic hepatitis C (CHC) sustained by easy-to-treat genotypes, and in those sustained by difficult-to-treat genotypes. New generations of antiviral drugs specific to HCV will soon be available for HCV mono-infected patients, but for HIV/HCV co-infected patients more time is going to be required.

While awaiting generations of anti-HCV drugs suitable for the co-infected population – which is complex and is likely to present higher risks of resistance and low adherence, to those encountered in mono-infected subjects – antiretroviral treatment is the only possibility for combating the progression of the liver disease. A cautious approach is essential when choosing these drugs in order not to cause acceleration, due to long-term pro-steatogenic and pro-fibrogenic adverse effects.

Immunomodulating drugs, such as CCR5 inhibitors, could play a certain role in controlling hepatic fibrogenesis. Immunomodulation could prove to be particularly interesting for patients presenting with more marked immune activation characteristics, such as particular hormonal conditions, residual HIV viremia, co-infection with other hepatic viruses and higher levels of microbial translocation.

The aim of this brief review is to develop the above-mentioned issues on the basis of the new suggestions emerging from the literature of this last year and to develop the theme of the progression of liver disease in pre- and post-menopausal women.

HCV replication and metabolic profile

The association between HCV virus plasma levels and the metabolic profile of patients with CHC has aroused great interest and is the object of studies which aim to throw light on the relationship between insulin resistance, triglyceride levels, cholesterol, circulating lipoproteins in plasma and degree of steatosis and hepatic fibrosis. In particular, the aspects relative to the close relationship between the replication mechanisms of intrahepatic HCV and the capacity to alter liponeogenesis and sequester fatty acids in the hepatocytes in order to produce lipoviroproteins, would enable researchers to understand which mechanisms are used by the metabolic set-up to accelerate the progression of the liver disease.

In a recent retrospective analysis conducted to evaluate the factors correlated with HCV viremia in a group of 669 patients with CHC, a correlation was shown to exist between HCV viremia genotype 1 and the level of plasma triglycerides and HbA1c. HCV viremia genotype 2, on the other hand, was correlated with the level of Low Density Lipoproteins-LDL and the level of platelets (8).

In patients with CHC, steatosis and fibrosis seem to be inversely associated with the level of total cholesterol and with the level of LDL cholesterol (9). This observation correlates well with the virus's capacity to block the production of lipoproteins, particularly the Very Low Density Lipoproteins-VLDL and to sequester fatty acids in the hepatocytes; the greater and more prolonged the fibrotic or steatosic liver damage, the higher the capacity. The extent to which this data is correlated with the replication capacity of HCV, the fitness of the virus and the level of circulating HCV-RNA is not known.

In particular, the role of circulating triglycerides in the plasma of HCV patients is unclear.

In patients enrolled in the Virahep-C study (Study of Viral Resistance to Antiviral Therapy) a linear association was found to exist between the level of triglycerides and the level of HCV-RNA (p=0.0034) and steatosis (p<0.0001) (9).

The increase in plasma triglycerides is strictly correlated with the degree of insulin resistance induced by a series of mechanisms directly caused by the virus and indirectly induced by the alteration in the mitochondrial functions of the hepatocyte, leading to an increase in the free radicals of Oxygen (ROS), which have the power to trigger steatohepatitis and worsen insulin resistance.

Insulin resistance is the first cause of the increase in circulating triglycerides, so the two parameters could be interpreted univocally.

High levels of HOMA-IR have been proved to be correlated with high HCV-RNA values in HCV mono-infected patients (10, 11). Recently, an association between high levels of HOMA-IR and triglycerides with high levels of circulating HCV-RNA has been found also in HIV/HCV co-infected patients (11).

Among the theories that are seeking to understand how and why insulin resistance and triglycerides are correlated with HCV viremia, there is a recent study that assessed the variables correlated with HCV viremia, associated or otherwise with LPV. This demonstration is of considerable importance because it is well-known that in the animal model and in cell cultures, LPVs associated with LDL–apoB are much more infectious than LPV bound to HDL. So in 51 patients with CHC sustained by genotype 1 the variables correlated with the LPV concentration were analysed. HCV viremia, either associated or not associated with the LPVs, was assessed through real time PCR. The relationship between viremia associated and not associated with LPV was calculated using the formula LPV/LPV+non LPV. The mean of the ratio was 0.241 with extremely pronounced variation intervals (0.029-0.74). The multivariate analysis demonstrated how insulin resistance, calculated with the HOMA-IR index and the TGD/HDL cholesterol ratio was a major determinant of HCV viremia correlated with LPVs (p 0.037- p 0.019). A higher concentration of HCV viremia associated with LPV was also correlated with the non-achievement of the sustained virological response (SVR) after therapy with Peginterferon and ribavirin (p 0.037) and with higher liver stiffness (p 0.001). The authors' conclusions emphasize how the correlation between HOMA-IR and HCV viremia correlated with LPVs could provide a useful explanation for understanding why and how HOMA-IR is such an important determinant of the outcome of anti-HCV treatment, and help clarify with which mechanisms insulin resistance contributes to liver damage (12).

Immune activation and progression of fibrosis and steatosis

The pathogenesis of liver disease in HIV/HCV co-infected patients is characterized by the concomitant presence of an immunodepressive condition and of constant immune activation, which leads to an increase in the inflammatory and oxidative disease of the hepatic parenchyma. In inflammatory and oxidative liver disease, the accumulation of leukocytes and macrophages – particularly of Kupffer's cells which migrate, building up in the areas most affected by the inflammatory and oxidative attack – is mediated by proinflammatory cytokines, such as IL-8 and by proteins with chemotactic properties for macrophages, such as MCP-1.

Macrophages and monocytes have the ability to release, at the site of inflammation, proinflammatory cytokines such as TNF-alpha and IL-1-beta which perpetuate the inflammatory damage to the liver.

The inflammatory phase is associated with the appearance of myofibroblast-like cells responsible for the production of ECM (Extra Cellular Matrix) and these are the main effectors of the fibrogenic process. During the fibrotic process, the Stellate Cells (HSCs) and mesenchymal cells also acquire a myofibroblast-like phenotype with functional, biochemical and structural changes that make these cells more suitable for tissue repair (13). When they are activated, the HSCs express receptors for soluble mediators of the fibrogenesis such as PDGF (Platelet derived growth factor), TGF- β (Transforming Growth Factor β) and produce cytoskeletal proteins such as alpha-SMA (Al-

pha-smooth muscle actin) (14). The hepatocytes, the cholangiocytes and the damaged Kupffer cells release factors with the power to activate the HSCs such as: TG β, PDGF, CTGF (Connective Tissue Growth Factor), Leptin, EGF (Epidermal Growth Factor), Angiotensin II, products associated with oxidative stress, MCP-1 (Monocyte chemoattractant protein-1), IL-8.

Once the HSCs have been activated, they increase the production of fibrillar collagens of metalloproteinase inhibitors, fundamental for the degradation of fibrillar ECM (TIMP-Tissue Inhibitors of Metalloproteinase*),* MMP-2 and 3 which degrade the normal hepatocyte matrix. Additionally, proliferation and cell survival increases leading to the expansion of the fibrogenic cells, particularly in the most active remodelling areas, such as the margin of the fibrous septa in chronic hepatitis. PDGF, EGF and angiotensin II modulate the mitogenic processes and migratory capacities which enable the fibrogenic cells to move towards areas of active fibrogenesis, following the chemotactic signals. Migration is a factor involved in the localization of the fibrotic process in areas other than the hepatic lobule. The presence of proinflammatory cytochines such as IL-1, TNF or IFN-g (15) enable the up-regulation of chemoattractive factors such as MCP-1 and IL8 and the acquisition of a contractile phenotype when exposed to vasoactive agents such as angiotensin, endothelin-1 and thrombin (16). Due to the proximity of these cells to the sinusoids and to the newly formed vessels, their contractile action is thought to be involved in the portal hypertension mechanism.

An important inflammatory stimulus that is thought to affect the progression of hepatic fibrosis in HIV/HCV co-infected patients is microbial translocation.

Microbial translocation is connected with the depletion of the CD4 lymphocytes of the intestinal mucosa, leading to a reduction in its integrity and greater permeability to bacterial products, such as lipopolysaccharides (LPS) (17-22).

The Kupffer cells in the liver are directly activated by the microbial translocation and the LPSs are captured by the hepatic macrophages through the LBP (circulating LPS binding protein) and through the CD14 receptor. The membranes bind the LPSs through the Toll-like receptor 4 (TLR4) and, as a result, intracellular transcription factors are activated, leading to the triggering of the cytokine cascade which, in turn, activates intrahepatic fibrogenesis (20-22).

In HIV-positive subjects, plasma concentrations of LPS and of immune activation markers are clearly higher compared to healthy controls. The LPSs were proved to be correlated with the low number of CD4+ T lymphocytes and with the gravity of the liver disease (23). It is not yet clear whether the increase in circulating LPSs and the greater expression level of the circulating immune activation markers bound directly to them, can lead to a depletion of CD4+ T lymphocytes and the progression of the liver disease, or whether they are simply a consequence of the immunodepression and cirrhosis (24)

In our experience, patients with a difficult-to-treat genotype and with more advanced liver disease show levels of microbial translocation and high levels of lipopolysaccharides correlated with a reduced virological response during therapy with peginterferon and ribavirin (25)

Immune activation and progression of the liver disease: gender differences

Inflammatory liver disease is strongly affected by sexual hormones to such an extent that this accounts for the gender-related differences in the progression of the liver disease. Men affected by chronic hepatitis C progress towards cirrhosis more rapidly than women, at least until the onset of the menopause. After the menopause, in fact, there is a more rapid progression towards advanced stages of fibrosis and cirrhosis (26) and a weaker response to therapy with peginterferon and ribavirin (27). The reduction in the concentration of estradiol during the menopause is associated with an increase in TNF-alpha and IL-1 beta (28). This variation implies a marked increase in O2 free radicals (Reactive Oxygen Species -ROS) (28) which cause oxidative damage to the parenchyma. Estrogens reduce the transduction of the intracellular signal mediated by NF-κB, involved in the activation of genes that are essential for the inflammatory response (29-31).

In a study conducted on hepatocyte cell cultures of elderly males and women in the pre-menopausal period, the production of proinflammatory cytokines was inhibited by the administration of estrogens, while it was stimulated by progesterone supplements (32, 33).

The reduction in ovarian function in women coincides with a spontaneous increase of TNF-α, IL-1β, IL-8, and MCP-1, and it has been demonstrated that the physiological concentrations of estrogens have the power to reduce the spontaneous secretion of cytokines in peripheral blood (34). The variation in proinflammatory cytokines changes with the fluctuation of sex hormones and, notwithstanding the existence of conflicting data, it is interesting to observe how the levels of TNF alpha are higher during the luteal phase – when the concentrations of progesterone are higher – compared to the follicular phase which is dedicated to the production of estrogens.(35)

Preliminary data on the use of CCR5 inhibitors and hepatic stiffness

The extent to which immune activation factors are important in generating the progression of liver disease, both in fibrogenic and in steatogenic terms, has been widely demonstrated. The possibility to use immunomodulating drugs able to slow down intrahepatic immune activation could be a useful strategy in the control of fibrogenesis, especially in patients with marked immune activation characteristics (HIV co-infection, marked microbial translocation etc.).

It has been demonstrated that HSCs express the receptor CCR5 on their membrane. This is a receptor for chemokines, that has a serpentine structure of 7 transmembrane domains with an alpha helical structure and an N-terminal extracellular segment, involved in the binding with chemokines in the transduction of the intracellular signal and activation of the G protein. The cells that express the CCR5 receptor on their membrane are blood-derived dendritic cells, macrophages, lymphocytes, endothelial cells, pancreatic beta cells and hepatic stellate cells. CCR5 mediates the binding with CCL3/MIP1-α, CCL4/MIP 1-β, CCL5/RANTES. CCR5, and the chemokines that bind to it play a fundamental role in the differentiation of the type Th1 or Th2 responses of TCD4+ lymphocytes (36, 37). In HIV/HCV co-infected patients, the progression of hepatic fibrosis is more rapid; one of the possible explanations is that the HSCs are activated by transfection with HIV, but also by simple exposure to gp 120 through the binding to the receptor CCR5. HIV can, therefore, trigger fibrogenic mechanisms autonomously, through receptor CCR5 (38).

In patients with deletion Δ32 for CCR5 and chronic HCV hepatitis, a reduction in portal and periportal necro-inflammatory activity was found, but not a reduction in fibrosis, as though the decreased expression of CCR5 reduced the immune-induced necroinflammatory damage but did not improve the progression of the fibrosis induced by HCV, due, perhaps, to the reduced expression of the Th1immune response (39, 40). It has, moreover, been demonstrated on the animal model, how inhibition of the CCR5 receptor is able to reduce inflammatory damage and fibrosis in mice with experimentally induced biliary cirrhosis .(41) Studies are currently underway to assess whether the CCR5 inhibitor (maraviroc) can affect the inflammatory state of the liver in HIV/HCV co-infected patients.

The preliminary analysis of the pilot study MAICOL (MAraviroc In HIV/ HCV Co-infection and Liver fibrosis), currently underway at the Institute of Infectious and Tropical Diseases of the University of Brescia, has highlighted the potential for the use of CCR5 inhibitors in the context of a "liver friendly" antiretroviral strategy.

The study in progress targets HIV/HCV co-infected patients, with non-advanced chronic hepatopathy, under treatment with atazanavir/ritonavir 300/100 mg in association with tenofovir/emtricitabine and undetectable HIV viremia.

The patients enrolled in the study were randomized to the antiretroviral treatment in course – maraviroc at a dosage of 150 mg/bid. – or to the control group.

The main aim of the study was to assess the variation in stiffness, in the fibrotest and in the plasma concentrations of hyaluronic acid after 24, 48 and 96 weeks compared to baseline in the two groups of patients enrolled.

The secondary aim was to assess the safety of maraviroc therapy in co-infected patients without cirrhosis of the liver and, in particular, to assess the variation in the level of circulating HCV-RNA which could, presumably, change under the effect of the inhibition of one of the main receptors – such as CCR5 – involved in the cell-mediated response.

The preliminary analysis on the first 24 patients that had completed the 6th month of treatment showed how the characteristics at baseline were superimposable in the two groups of patients (Tab. 1). It also showed excellent tolerability and immunovirological safety, without significant variations in HCV viremia, HIV viremia and the T CD4+ lymphocyte count, in the patients treated, after 6 months of therapy. No variations in the metabolic set-up or in liver or kidney function were observed (Tab. 2).

Of interest was the variation in liver stiffness, measured with Fibroscan® from baseline to the 6th month of treatment, with a reduction in the stiffness

Table 1. Characteristics at baseline of patients enrolled in the MAICOL study to September 2010

Parameters N(%), Median (IQR) 20	All (n:59)	Arm A Control (n:30)	Arm B: + MVC (n:29)	p
Age (yrs)	46 (43-48)	45 (43-46)	45 (43-47)	0,9
Female gender	12 (20,3%)	7 (23,3%)	10 (25,6%)	0,4
Body mass index	23,1 (20-25,8)	23,9 (22-25)	22,1 (20-25)	0,6
ARV exposure (yrs)	12 (9-14)	12 (8-14)	13 (10-14)	0,8
HCV Genotype, 1-4 2-3	 55 (93,2%) 4 (6,7%)	 27 (90,3%) 3 (10%)	 28 (96,5) (4,5)	 0,3
HCV RNA load ($\log_{10}$ IU/mL)	5,8 (5,4-6,2)	5,9 (5,4-6,3)	5,7 (5,3-6,0)	0,3
CD4 cell count (cell/mm3)	486 (405-654)	514 (385-735)	486 (413-612)	0,4
AST (IU/ml)	43 (31-59)	43 (33-59)	42 (30-58)	0,6
ALT(IU/ml)	68 (45-92)	66 (44-91)	74 (49-92)	0,8
Stiffness Kpa	7,1 (5,4-9,4)	6,8 (5,6-8,1)	7,2 (5,4-10,1)	0,1
HOMA-IR	2,9 (1,6-5,2)	2,5 (1,5-3,8)	3,4 (1,9-6,1)	0,3

Table 2. Safety and tolerability at week 24 in both groups of patients

Parameters N(%), Median (IQR)		All (n:24)	Arm A Control (n:12)	Arm B: + MVC (n:12)	p (Cont vs MVC)
HCV RNA load ($\log_{10}$ IU/mL)	BL	5,8 (5,3-6,2)	5,9 (5,5-6,3)	5,7 (5,3-6,0)	0,09
	W24	5,9 (5,2-6,6	6,1 (5,6-6,2)	5,8 (4,9-6,3)	0,2
	p	0,1	0,9	0,7	
CD4 cell count (cell/mm3)	BL	561 (409-746)	577 (406-771)	545 (409-706)	0,4
	W24	585 (407-755)	631 (435-862)	573 (379-658)	0,2
	p	0,6	0,4	0,8	
AST (IU/ml)	BL	38 (30-58)	41 (34-59)	38 (27-58)	0,6
	W 24	35 (29-60)	38 (29-48)	33 (28-63)	0,6
	p	0,6	0,4	0,8	
ALT(IU/ml)	BL	60 (45-105)	66 (48-109)	55 (41-101)	0,08
	W24	62 (44-84)	63 (46-80)	50 (42-85)	0,7
	p	0,1	0,4	0,9	
SAE		2	0	2 (pneumonia)	

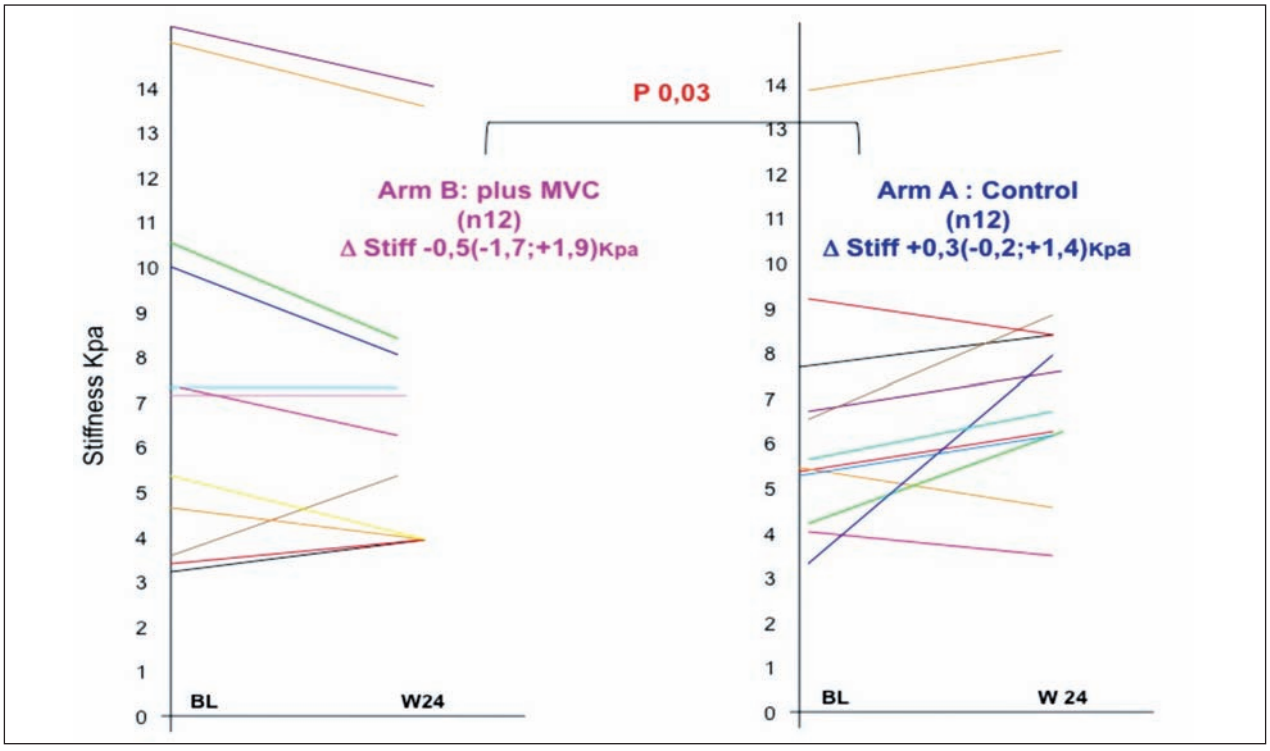

Figure 1. Variation of liver stiffness from baseline to week 24 in the two groups of patients in the study

value of -0.5(-0.7, +1.3) KPa in patients administered maraviroc, compared to an increase of +0.35 (0.2;+1.4) in patients who had not received this drug (p0.03) (Fig. 1).

Three of the 4 patients on maraviroc who experienced a change in the stiffness stage, showed an improvement, passing from Grade III to Grade II. In contrast, all three patients not receiving maraviroc, with a change in the stiffness stage, worsened, passing from Grade I to Grade II (42).

The preliminary analysis of this pilot study made it possible to continue with the enrolments and analyses in order to confirm the liver stiffness improvement trend. Evaluations of the fibrotest and hyaluronic acid variations are presently underway, in addition to a long-term assessment of the outcome of these patients, the real end point for understanding whether an approach with special therapeutic strategies – also with an immunomodulating action – can impact the natural course of HCV in co-infected patients naïve to Peginterferon and ribavirin treatment.

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