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Original article

Antiretroviral therapy in HIV/HCV co-infection: immune reconstitution, liver, metabolic damage and immune activation

Paola Nasta
Institute of Infectious and Tropical Diseases, University of Brescia

Summary. 50% of HIV-infected people in Italy are carriers of hepatitis C and 9% of these people have the hepatitis B virus. In these people the first cause of death is liver disease. 14% of this population dies from liver disease. Liver with HIV co-morbidity exposes patients to a higher risk of contracting AIDS, a faster progression towards cirrhosis, a more frequent toxicity and a lower tolerability of the antiretroviral therapy. Moreover, HCV infection contributes to a worsening of immunodepression, neurological, cognitive, renal damage, and induces a higher bone fragility.
The choice of when and how to start an antiretroviral therapy and for what medications it would be better to maintain before, during and after treatment with Interferon and Ribavirin are essential elements to improve the prognosis of the co-infected subjects. However, there are still few antiretroviral drugs with enough safety and efficacy data in those subjects with HIV/hepatitis co-infection, as well as solid studies of pharmacokinetics to allow them to be used in patients with advanced fibrosis or cirrhosis.
The main goal of HAART is immune reconstitution, a necessary but inefficient step, to slow down the liver disease progression. Once the plateau of the T CD4+ lymphocyte cell count has been reached, the metabolic and hepatic toxicity of some antiretroviral drugs is likely to contribute towards inflammatory and fibrogenic processes.
Medications capable of assuring the best immunologic recovery without inducing either transaminitis, or immune reconstitution syndrome, or metabolic damage should be chosen in co-infected subjects.
The evidence related to the activation of stellate cells – mainly responsible for hepatic fibrogenesis, - are induced to investigate the anti-inflammatory and antifibrogenic properties of CCR5 inhibitors. The close examination below tries to broach, through literature revision, the topic regarding the choice of the antiretroviral drugs in the HIV/HCV co-infected patient, considering HAART as a fundamental part of the treatment of both the infections.

Introduction

Hepatitis C Virus co-infection was acknowledged as the most dangerous co-morbidity among those in HIV-infected patients.

With the introduction of the combined antiretroviral therapy (cART), the survival of the seropositive subjects increased so much that it allowed chronic hepatitis C to manifest itself in all its aggressiveness, thus becoming one of the main causes of death.

The estimated percentage of HIV/HCV co-infected subjects is enormous, reaching 25% of the whole seropositive population.

As both infections share transmission routes alike, contracting both viruses is relatively frequent in subjects running the risk of contamination with infected blood or blood derivatives. Active drug addicts who use or have used intravenous drugs and haemophiliacs are the people most affected by the double infection (2).

On the contrary, the sexual transmission – prevailing in the HIV population in the last few years – is a rare infection route for HCV, although small epidemic foci happened in the homosexual communities in the United Kingdom at the same time of outbreaks of syphilis and other sexually-transmitted diseases (3).

The mode of transmission accounts for the geographical differences in the majority of HIV/HCV co-infections. In Eastern Europe (4) and South-East China, ¾ of the HIV-positive population is HIV/HCV co-infected. In most African regions as well as in India, where the most prevailing transmission route is the sexual one, the hepatitis C virus co-infection does not reach 5% of the population HIV-carrier (3).

In the European countries where HIV population is mostly accounted for by homosexuals, the frequency of the co-infection levels off at mean levels: approximately 10% (5).

In the USA, the co-infection majority is approximately 18%, reaching 60% in IVDU (Intravenous Drug User) subjects (6).

As to Europe, the most reliable epidemiological data come from a EuroSIDA study (Figure 1). In the

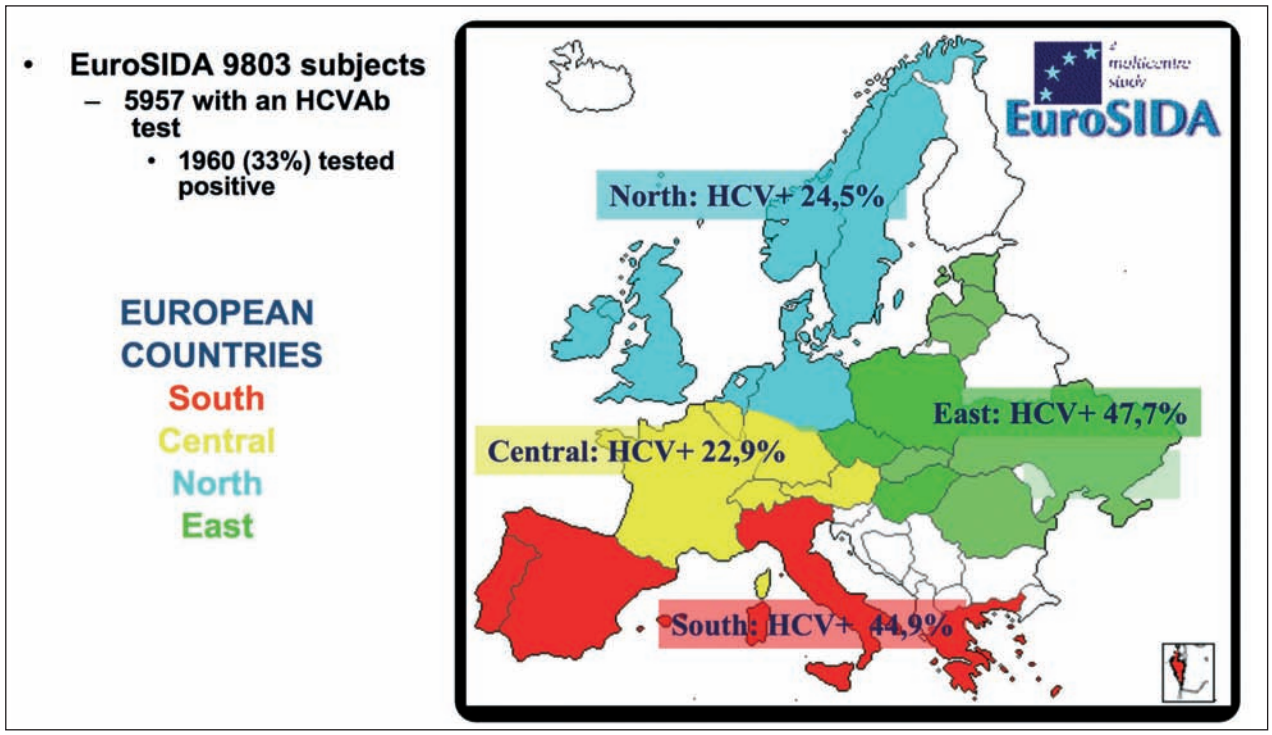

Figure 1. Prevalence of HCV cda co-infection in HIV-positive population in the EuroSIDA cohort

Southern European Countries the prevalence equals 41.4%, in the central ones equals 20.5%, in the Northern ones equals 23.2% and in the Eastern ones it reaches 47% (2).

Geographical differences in the distribution of the viral genotypes were pointed out. HCV genotype 1 is the most widespread in Europe (global prevalence 40-60%), genotype 3 (global prevalence 20-40%) prevails in Eastern Europe, genotype 2 (global prevalence 1-5%) can be found in Northern and central Europe, genotype 4 (global prevalence 5-15%) prevails in North Africa and reaches 20% of infections in Southern Europe (4) (Figure 2).

HCV vertical transmission is uncommon (approximately 5% of HCV-RNA positive women). HIV co-infection increases the risk of foetus exposure (up to 8-20%). Nevertheless, HIV/HCV co-infected pregnant women under effective antiretroviral therapy do not show an increase in the risk of C virus transmission (7). The level of plasma HCV-RNA represents the major predictor of the risk of perinatal transmission. Caesarean section and bottle-feeding are deemed to be protective and are recommended to avoid the transmission of both viruses to the newborn child (8).

HIVAb+ patients with HCV co-infection show important peculiarities compared to subjects with HIV mono-infection. In this population the risk of death due to liver disease and other AIDS-correlated pathologies is higher.

The mortality rate due to liver disease is the second cause of death in HIV/HCV co-infected subjects (Figure 3).

Once the cirrhosis of the liver is developed, HIV/HCV co-infected people show a chance of surviving three years equalling 30% compared to 60% of HCV mono-infected subjects (9).

Antiretroviral therapy may considerably improve the short- and mid-term results of patients with HIV and cirrhosis of the liver, and may slow down fibrosis progression.

However, the hepatic and metabolic toxicity of cART may pose a danger in the co-infected population.

The tolerability of antiretroviral therapy is compromised owing to the loss of working hepatic tissue, and the reduction of the available metabolic routes for antiretroviral drugs may represent a risk factor for the onset of transaminitis or metabolic toxicity.

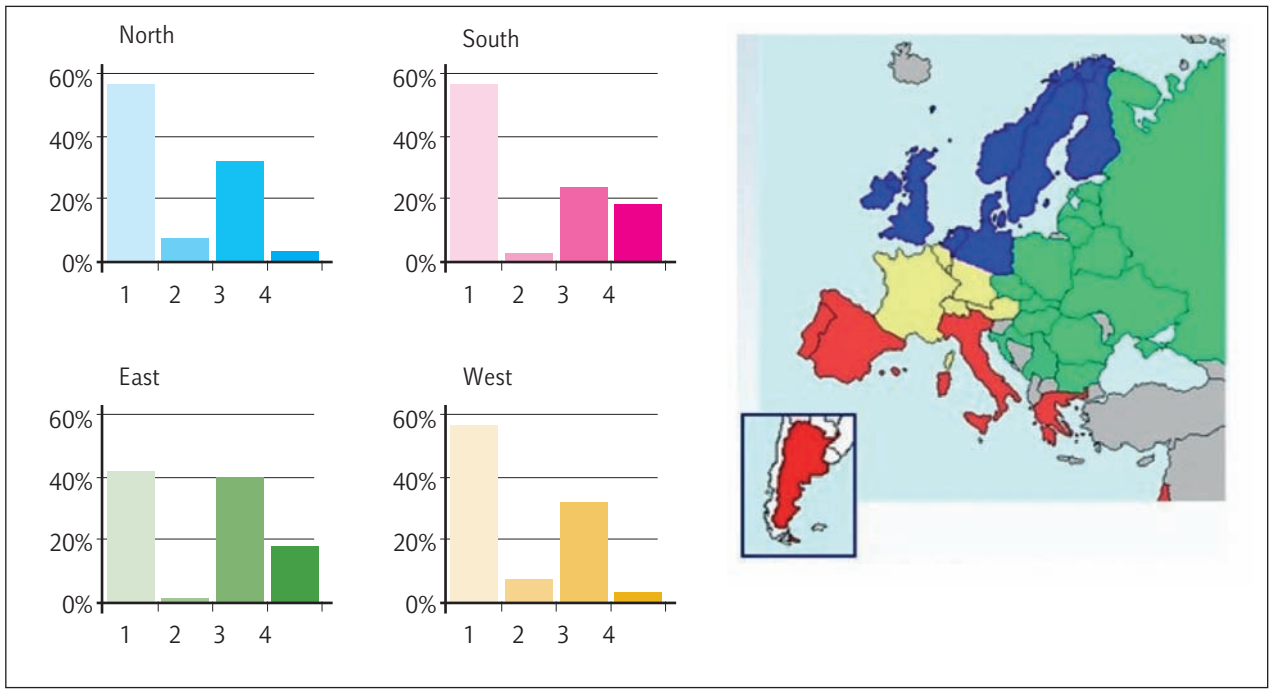

Figure 2. HCV genotype distribution in Europe (V. Soriano et al. J Infect Dis 2008; 198: 1337-44)

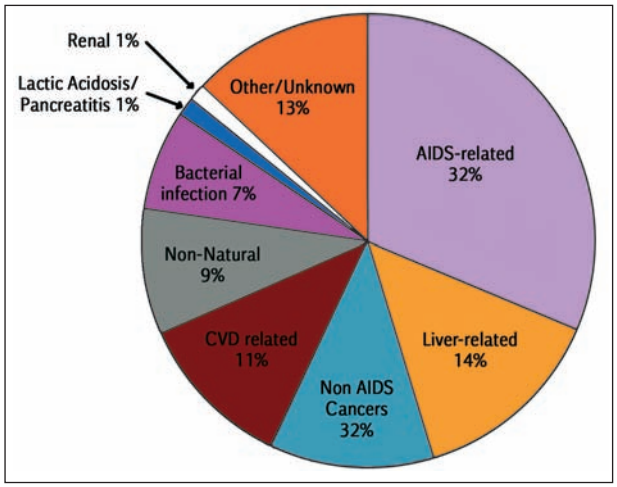

Figure 3. Causes of death in patients with HIV (Smith CJ, #145, 16° CROI 8-11 February 2009)

Changes in the pharmacokinetics of antiretroviral drugs are frequent in the hepatopathic subjects, and the plasma level of drugs should be monitored properly and often (10).

HIV/HCV co-infected subjects must be unequivocally regarded as a particular population to be treated in specialised and dedicated outpatient clinics. **Co-infected subjects have different needs compared to HIV mono-infected population as regards "when to start" and "how to choose" the most suitable antiretroviral therapy. They must choose the most suitable therapy to avoid phenomena of hepatic and metabolic toxicity, to diminish the risk of renal and bone damage – which occurs often in subjects with chronic hepatitis (11), - to ensure the best tolerability in case of any treatment with Peginterferon and Ribavirin. Those treatments should allow, at the same time, the maximum immune recovery, a higher viremic control without exposing the patient to any risks of chronic immune activation or immune reconstitution syndromes.**

The interaction between HIV and HCV and the role played by immune reconstitution

The influence exerted by HCV on HIV-correlated disease progression is not yet entirely clear. Literature suggests different conclusions, showing that HCV does not influence immune reconstitution in patients treated with antiretroviral therapy and that the recovery of T CD4+ lymphocytes seems to be alike in co-infected subjects and in mono-infected patients (12-14), although a possible reduction in the therapy adherence due to diminished tolerability is evident (15).

However, the analysis of some cohorts has recently showed that – with an equal number of T CD4+ lymphocytes – HIV/HCV co-infected subjects, and notably the subjects with cirrhosis, show a higher risk of AIDS defining events (ADI) (ARR 2,6 IC 95% 1,8-3,6); of bacterial infections (ARR 3,1 IC 95% 1,7-5,6); of mycoses (ARR 3,8 95% IC 2,2-6,5) and HIV-correlated pathologies (ARR 2,6 95% IC 1,03-6,9), but not of non-Hodgkin's lymphoma (NHL), which was previously deemed to be the most opportunistic illness subjects with hepatitis C might be exposed to (16).

The influence exerted by the immunodeficiency state on hepatitis C outcome, instead, is pretty clear.

Reduction in T CD4+ lymphocytes exposes to a series of phenomena leading to a reduced clearance of hepatic virus, hence to a higher risk of making an acute infection chronic (17, 18). **Substantial differences in HIV/HCV co-infected subjects, compared to HCV mono-infected subjects, are: higher level of circulating HCV-RNA (19), faster progression towards cirrhosis (20-21) and more frequent possibility of hepatic decompensation with episodes such as bleeding of oesophageal varices and development of ascites (22).**

There are differences in HIV/HCV co-infected subjects compared to the C mono-infected ones even in the manifestations of hepatic decompensation.

There are hepatic decompensation manifestations. In mono-infected subjects, the onset of ascites is more frequent as the first decompensation event. Whilst in HIV/HCV subjects, those events connected with portal hypertension and bleeding of oesophageal varices prevail (24).

A higher risk of developing hepatocellular carcinoma (HCC) was also shown with different characteristics than in mono-infected subjects: tumour progression is faster and multiple lesions appear more often (25).

In short, progression of liver disease from acute to chronic and development of hepatic decompensation are considerably more frequent in HIV/HCV co-infected subjects than in HCV mono-infected subjects (Figure 4).

The most known predictors of the hepatic disease progression are unalterable (advanced age, female gender) or modifiable.

The modifiable factor which is mainly correlated with liver disease progression is the level of T CD4+ lymphocytes, hence the use of antiretroviral therapy (Figure 5).

Studies show how the low level of T CD4+ lymphocytes would expose to a higher risk of progression towards cirrhosis, of hepatic decompensation and of death due to liver disease (26).

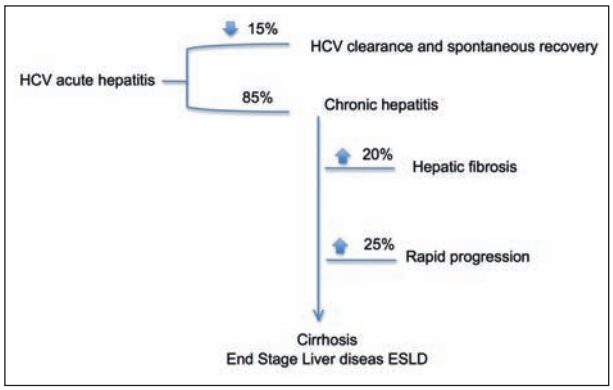

Figure 4. HIV effect on the liver disease progression in subjects with HIV infection

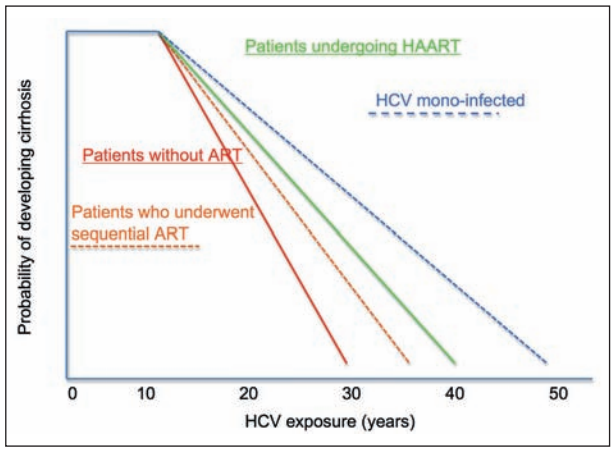

Figure 5. Probability of developing cirrhosis according to cART presence

With HAART introduction, the risk of death due to hepatic events has obviously increased owing to the growth in survival which lets a sufficient time for the liver disease to progress until its final stage. Much literature at the end of the 90s and in the first few years of 2000 showed how the hospitalisations for hepatic decompensation had increased in all the Countries where HAART was widespread (27, 28).

On the other hand, those patients who have never been treated show a speed of cirrhosis onset much faster compared to the subjects undergoing even sequential therapy. **It is evident, however, that combination antiviral therapy, although it slows down cirrhosis development, would never allow equalisation of the onset times of cirrhosis manifestations in HIV positive and HIV negative subjects. The immune damage affects, in any case, the survival of co-infected subjects, who have a higher risk of death due to both AIDS-correlated events and non-AIDS-correlated events than HIV mono-infected people (29).**

The immunologic gain assured by antiretroviral therapy – especially based on protease inhibitors (30) – allows to slow down the hepatic disease progression.

Moreover, the immune system recovery is essential to increase CXCR3/CCR5+ specific CD4 and CD8 lymphocytes migration to the hepatic level and increase the possibility of viral clearance.

It seems like there are no substantial differences between antiretroviral regimens with regard to the recovery of anti-HCV specific CD4 and CD8, but the studies that compare the best combinations of antiretroviral therapy are still very few. A recent research study by A. Perrella and colleagues pointed out how, in naïve subjects treated with Fosamprenavir/RTV, the anti-HCV specific immune response of type 1 was particularly effective (31).

The immunologic recovery is so important in the natural history of co-infection that it induces current American and European guidelines to suggest the possibility of starting HAART first in case of co-infection with hepatitis virus (32, 33).

Antiretroviral therapy has the undeniable advantage of slowing down hepatic progression, yet there are some negative effects that may prevail in the long term, once the immune reconstitution goal has been achieved (Figure 6).

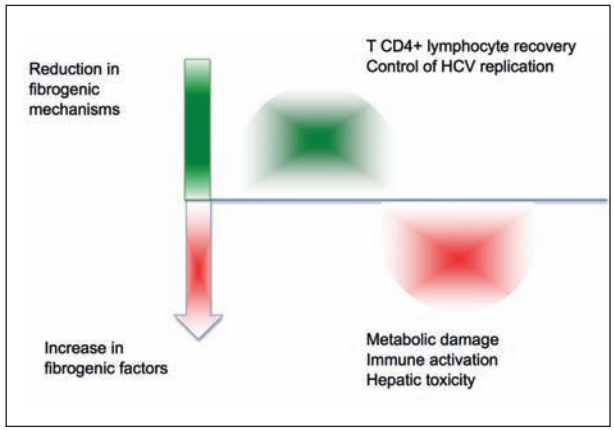

Figure 6. Antiretroviral therapy effect on hepatic fibrosis in time

There are three phenomena which are still currently being studied and are the object of a thorough analysis. The first factor is the hepatic toxicity of some antiretroviral drugs which increase transaminases, hence induce evident necroinfiammation. The second factor is the metabolic toxicity caused by some molecules that might trigger an iatrogenic steatosis more easily than others – an event which is known to contribute to hepatic fibrosis progression to a great extent. There is a third phenomen – whose dynamics are not yet much definite – which might contribute in the long term towards intrahepatic inflammatory state with the consequent activation of fibrogenic mechanisms. This is a condition of chronic immune activation connected with the possible residual replication of HIV in peripheral tissues and organs, with the presence of C virus in hepatic parenchyma and with the possible contribution – more or less important – of the circulating lipopolysaccharide residues reaching the liver from a distended and congested portal circulatory system (microbial translocation).

HAART hepatic toxicity and fibrosis progression

How to choose the best HAART for a co-infected subject?

One of the useful factors in deciding what antiretroviral drugs to use in a subject with chronic hepatic disease is the reduced hepatotoxic impact.

Although a shared hepatotoxicity parameter is not available, a transaminase increase is taken into account compared to its normal value (WHO toxicity scales) or compared to the patient's baseline values (ACTG scales) (Figure 7), a useful factor is to subdivide medications into more, average or less hepatotoxic.

The importance of a transaminase increase is tightly linked to the direct bond between AST and ALT plasma levels and the hepatic necroinflammation state. A constant or occasional high transaminase value is correlated with a faster progression towards cirrhosis (34).

In a study conducted in Brescia, it was shown that in a cohort of 808 subjects observed for 6 years in a row, the mortality rate due to liver disease was connected with an increase > 1,5 times than AST and ALT normal values for at least one year. Their increase could have been continuous or even transitory, but in both cases the risk of death was higher than in those subjects with normal transaminasemia. The analysis, therefore, showed how hepatopatic subjects, who underwent hepatotoxic HAART, had an increased risk of death (35).

The recognised hepatotoxicity mechanisms are: 1) medication's direct hepatic toxicity (mitochondrial toxicity); 2) specific hypersensitivity; 3) immune reconstitution.

Trying to classify medications as more or less hepatotoxic, in terms of induction of transaminitis phenomena, is almost impossible, due to the extreme vari-

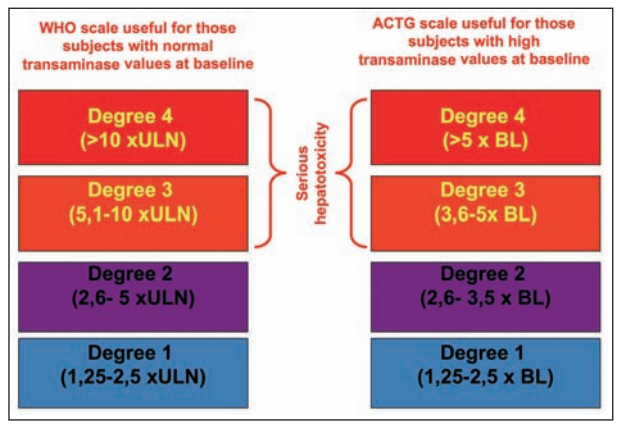

Figure 7. Definition of medication hepatotoxicity

ability of classifications used in studies. Vincent Soriano proposed an extremely concise colorimetric scale which is very significant: it summarises the hepatotoxic characteristics of antiretroviral molecules in coloured boxes (36) (Figure 8).

Studies on the antiretroviral drugs hepatic toxicity highlight the risk of AST/ALT increase in co-infected people from 38 to 54% compared to 1.1-4.8% of the HIV mono-infected subjects (37, 38).

Although hepatic safety represents one of the most important characteristics of medications in general, regulatory trials of all the antiretroviral drugs do not provide sufficient data to define safety in those patients with HIV/hepatitis virus co-infection. In fact, co-infected subjects enrolled in randomised protocols are very few.

Hepatic toxicity linked to the depletion of mitochondrial DNA of the thymidine analogue reverse transcriptase inhibitors (NRTI) (39, 40) is so much well known by now that its use is not recommended in co-infected subjects.

Abacavir, Tenofovir, Lamivudine and Emtricitabine have a reduced toxic mitochondrion potential, hence they proved to be safer during chronic hepatopathy (41).

AST-ALT increase is observed in 4-8% of cases treated with Efavirenz among the reverse transcriptase analogue non-nucleosides as well as in 12-15.6% of subjects being treated with Nevirapine (42, 43).

Ritonavir is recognised as the most hepatotoxic protease inhibitor when used in therapeutic doses.

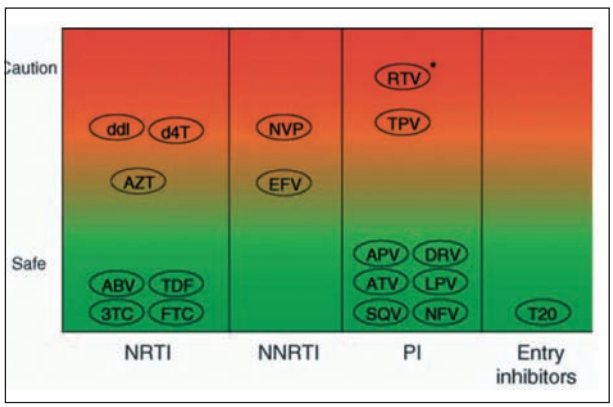

Figure 8. Hepatotoxicity degree of antiretroviral drugs (from Soriano et al AIDS 2008)

Norvir® does not seem to boost transaminases at booster doses of 100 to 200 mg/die (44).

Despite this evidence, the administration of protease inhibitors without Ritonavir is a strategy pursued in co-infected subjects.

The presence of chronic hepatitis at different stages impairs the liver's capacity to metabolise medications (45). Histological damage translates into loss of hepatocytes, hence of working cytochromes. Such anatomical modification induces changes in antiretroviral drugs pharmacokinetics, in particular of protease inhibitors.

The plasma dose of protease inhibitors acquires a great importance, especially in co-infected subjects. The medication plasma dose might underline the necessity of maintaining Ritonavir booster in the event that one had opted to administer a non-boosted protease inhibitor or vice versa to the patient.

Studies conducted on the population of randomised and controlled trials – and confirmed in the population enrolled in the *Expanded Access Programm* (EAP), - show that AST/ALT increase in those subjects treated with Atazanavir is minimum (46, 47).

However, since sporadic cases of transaminitis in subjects without co-infection have been signalled (48), 300 mg/die is the dose of Atazanavir that must be administered in those subjects with functional classification of Child Pugh 7-9, whilst it is not indicated with values of Child-Pugh > 9.

In phase III trials with Lopinavir/RTV an increase in AST/ALT is highlighted, similar to the one observed with Nelfinavir, and definitely lower when compared to Inidinavir and Saquinavir (49). The relative safety of Lopinavir/RTV was confirmed in cohort and even Italian studies (50). The American guidelines invite prudence in using Lopinavir/RTV in subjects with a reduced hepatic function.

Subjects treated with Tipranavir in the RESIST study had a transaminitis frequency of considerably higher $^{3}/_{4}$ degree; HCV or HBV co-infection exposed to a further risk of increase in AST/ALT (51).

The protease inhibitor on which most data on hepatic safety in co-infected subjects come from is Fosamprenavir. Data emerged from regulatory trials show a risk of increase in AST/ALT similar to Nelfinavir (52). The average increment in AST/ALT in

subjects with or without co-infection treated with Fosamprenavir is remarkably reduced after 120 weeks of treatment (53).

A corroboration of the hepatic safety of Fosamprenavir comes from a Spanish trial conducted on 636 subjects, of whom 545 have HIV infection and 5.6% are HBsAg positive. After an average of 6.9 months of therapy (0.4-4.6) in HCV co-infected subjects, ALT increment percentage equalled 0.58%, whilst equalled 2.6% in $HBsAg^+$ subjects (54).

In an analysis conducted on 131 HIV/HCV co-infected subjects, of whom 38.6% have advanced hepatopathy (FIB 4 > 3.25) and 53% have high baseline transaminases, a significant reduction in ALT and AST was seen after 6 months of therapy, and the safety is confirmed in subjects with advanced hepatopathy (55).

Thanks to in-depth pharmacokinetics studies (56-58), Fosamprenavir dosage is also indicated in case of Child Pugh B and C functional classification, always with Ritonavir booster owing to the medication reduced levels in subjects with advanced hepatopathy (Figure 9).

The possibility of correctly dosing the medication and the considerable clinical experience allow consideration for Fosamprenavir/Ritonavir as the choice of inhibitors in HIV/HCV co-infection (59).

Hepatotoxicity of new medications

Darunavir hepatic safety in co-infected subjects is not currently much investigated. An analysis of the increment in transaminases in subjects with and without co-infection, and with and without cirrhosis conducted on patients enrolled in the Italian EAP shows a partial increment in transaminases in co-infected subjects regardless of cirrhosis presence. It is not clear how much highlighted increase in AST/ALT with Darunavir depends on the presence of the 200 mg Ritonavir dose (60). DHHS guidelines do not recom-

Amprenavir (Agenerase®) oral solution	1400 mg PO bid	Not recommended
Atazanavir (Reyataz®)	400 mg PO (treatment-naive patients only) 300 mg + ritonavir 100 mg PO	**Child-Pugh score 7–9:** 300 mg **Child-Pugh score >9:** not recommended
Darunavir (Prezista™)	Darunavir 600 mg + ritonavir 100 mg PO bid	No dosage recommendation; use with caution
Fosamprenavir (Lexiva®)	700 mg + ritonavir 100 mg PO bid 1400 mg PO bid 1400 mg + ritonavir 200 mg PO (treatment-naive patients only)	**Child-Pugh score 5–6:** 700 mg bid 700 mg bid + ritonavir 100 mg **Child-Pugh score 7–9:** 700 mg bid or 450 mg bid + ritonavir 100 mg **Child-Pugh score 10–12:** 350 mg bid
Indinavir (Crixivan®)	800 mg PO q8h	**Mild to moderate hepatic insufficiency because of cirrhosis:** 600 mg q8h
Lopinavir/ritonavir (Kaletra®)	400 mg/100 mg PO bid or 800 mg/200 mg PO (treatment-naive patients only)	Use with caution
Nelfinavir (Viracept®)	1250 mg PO bid	Use with caution
Ritonavir (Norvir®)	600 mg PO bid or 100 mg PO for pharmacokinetic enhancement with another PI	**Mild hepatic impairment:** no dosage adjustment **Moderate to severe impairment:** no data; use with caution
Saquinavir (Invirase®)	1000 mg + ritonavir 100 mg PO bid	Use with caution
Tipranavir (Aptivus®)	500 mg + ritonavir 200 mg PO bid	No dosage recommendation; use with caution **Moderate to severe (Child-Pugh class B or C) impairment:** tipranavir/ritonavir is contraindicated

Figure 9. Dosage of protease inhibitors in patients with hepatopathy (McCabe, Clin Pharmacokinet 2008)

mend the use of Darunavir in case of impaired hepatic function.

Regulatory trials of Raltegravir show an increase in AST/ALT of ¾ degree in 2-3% of treated population. Studies on co-infected populations are not available. A trial was conducted at the Institute of Infectious Diseases in Brescia on 135 subjects treated with integrase inhibitor, of whom 45 patients had HIV/HCV co-infection. It showed how the risk of increase in 1-2 degree transaminases is higher in co-infected subjects (39.5%) compared to HIV mono-infected subjects (9.1%), especially when cholesterol levels equal > 200 mg/dl and when Raltegravir is combined with a protease inhibitor. In subjects with advanced hepatopathy the rise in transaminases does not seem considerably higher (61). However, in cirrhotic subjects with reduced hepatic function, the rapid increase in CD4+ lymphocytes – evident when using Raltegravir – might expose one to immune reconstitution hepatitis with a possible further deterioration of hepatic function (personal data). Even though some molecules of the class of CCR5 inhibitors have been characterised by important hepatic events (62), the MOTIVATE trial showed a considerable hepatic safety of Maraviroc. After 48 weeks the AST/ALT percentage increase of ¾ degree equalled 0.6-3.7% (62).

Metabolic changes and liver disease

Obesity, changes in lipid structure and resistance to insulin occur very frequently in subjects with HIV/HCV co-infection. Insulin resistance is present in half the HIV+ subjects treated with protease inhibitors and type 2 diabetes mellitus (DMT2) represents a second extra-hepatic pathology, after cryoglobulinemia, in HCV+ subjects.

Changes in glucose and fatty acid metabolism contribute to increasing the risk of vascular damage and hasten the progression of hepatic fibrosis in patients with chronic hepatitic C.

Moreover, both hyperinsulinaemia and obesity are considered as negative prognostic factors of the response to therapy with Pegylated Interferon and Ribavirin.

People with HIV/HCV are more prone to developing some pathologies of lipid and glucidic metabolism due to the presence of concomitant factors such as the two viruses and antiretroviral therapy.

In these patients, the metabolic damage treatment takes on a particular significance in order to slow down the progression of steatosis, fibrosis and boost the success of therapy with PegIFN and Ribavirin.

Insulin resistance and diabetes in HIV/HCV co-infection

DMT2 affects 4% of the world population and it is estimated that the prevalence will reach 5.4% within 2025 (63).

The observation of DMT2 higher incidence rate among subjects with chronic viral hepatitis compared to healthy populations dates back to 1994 (64). Since then the evidence of correlation between HCV and DMT2 has become an object of several transverse and longitudinal studies conducted in hepatopathic, diabetic and transplant patients.

Insulin resistance is found more often in patients with cirrhosis compared to stages of slighter fibrosis (62% vs 24%) (65-67), and DMT2 frequency increases as Child Pugh score increases. It gets to the point of being present in 50% of subjects with Child Score equalling 15 (66). But many studies show how the insulin resistance is constant in HCV+ subjects, even with initial fibrosis (68-70) and normal transaminases (71).

The big cohort study conducted by Mheta and colleagues (72) leaves no doubt on the correlation between type 2 diabetes and HCV. The study was conducted on 9481 subjects of whom 8.4% showed a DMT2; 2.1% of them were HCV+ and 0.5% HBsAg+. Patients with HCV prevailed among DMT2 carriers in any age group, but after 40 years of age, the risk of developing diabetes would increase three times as many in HCV+ subjects compared to HCV- ones in absence of high levels of fibrosis.

The risk of developing DMT2 correlates with the observation time: the longer the follow-up the easier it is to find events (73).

After the treatment with Interferon, the metabolic damage reduction can be noticed, thus highlighting the virus' direct role in dysmetabolism genesis (74).

HIV virus is directly involved in metabolic damage.

In the pre-HAART era, the typical change in the lipid structure with hypertriglyceridemia and hypocholesterolemia was described. Antiviral therapy, predisposing to insulin resistance, induces, instead, a different history, characterised by increase in total cholesterol, LDL and VLDL fractions, triglycerides and glycemia. Insulin resistance was found in 50% of the subjects who underwent treatments containing protease inhibitors (75), and the prevalence of DMT2 was signalled in 2-14% (76, 77) of HIV+ subjects in ART with particular frequency in case of lipodystrophy (78).

Pathogenesis of insulin resistance in HIV/HCV co-infection

Insulin resistance is defined as a metabolic condition in which normal glycemic levels can be only ensured by higher insulin levels. The definition of insulin resistance is specified in Figure 10. The three tissue compartments involved in the pathogenic mechanisms that establish and maintain insulin resistance are:

- muscle tissue where myocytes, resistant to the insulin signal, catch less glucose, thus favouring the emergence of plasma hyperglycaemia;
- liver where in the hepatocytes, insensitive to insulin metabolic signal, gluconeogenesis and lipogenesis mechanisms activate, thus helping increase glucose and triglycerides in the plasma and cell;

Prediabetes		Diabete Mellitus
Impaired Fasting Glucose	Impaired Glucose Tolerance	
Fasting glucose 100-125mg/dL	2-hour postloadglucose 140-199 mg/dL during CGTT	Fasting glucose ≥126 mg/dL or 2-hour postload glucose ≥200mg/dL during CGTT or Symptoms of clabates with random glucose of ≥200mg/dL
OGTT, oral glucose tolerance test		

Figure 10. Definition of insulin resistance

- adipose tissue where lipolysis mechanisms activate with the consequent increase in free circulating fatty acids.

Figure 11 represents the cascade of events induced by insulin, once it has been caught by its specific receptor, and the induction mechanisms of insulin resistance attributed to HCV. Insulin promotes tyrosine phosphorylation of IRS1 and IRS2 *receptor substrates* (*Insulin Receptor Subastrates 1 e 2*). The bond activates the phosphorylation of Phosphatidyl inositol 3-Kinase (IP-3) and of Akt protein kinase, which allow the translocation - through the cell membrane – of GLUT-4 (*Glucose Transporter-4),* a specific glucose carrier.

HCV is able to inhibit insulin metabolic action through some mechanisms:

1. Change in insulin receptors

a) It was proved that HCV Core antigen is capable of influencing the regular phosphorylation of tyrosine induced by insulin on IRS1 and IRS2, with consequent receptor degradation (79).
b) HCV core protein (HCc) induces TNFα production, particularly correlated with the hepatic damage progression (80, 81) and capable of changing IRS1 and 2 function, by inducing their phosphorylation on serine (82).
c) TNFα is capable, in adipocytes, of influencing the transcription of genes involved in mechanisms of insulin sensitivity/resistance (83), and a sort of hyper susceptibility to TNFα action in patients with HCV was assumed (84).
d) HCc, through the induction of various *Cytokines such as* TNFα and IL6, is able to activate SOCS-3 (*Suppressor of Cytokines Signalling*-3). SOCS may induce IRS1 and 2 proteasomal degradation through their ubiquitination (85).

2. Accumulation of fatty acids in the hepatocyte and activation of hepatic liponeogenesis and gluconeogenesis

a) HCV stimulates the transduction route of SREBP-1c *(Sterol regulatory element binding protein*) signal as well as of ChREBPS *(Carbohydrate response element-binding protein*). They are the proteins binding to the specific regulation element of sterols and carbohydrates. They are instrumental in the insulin

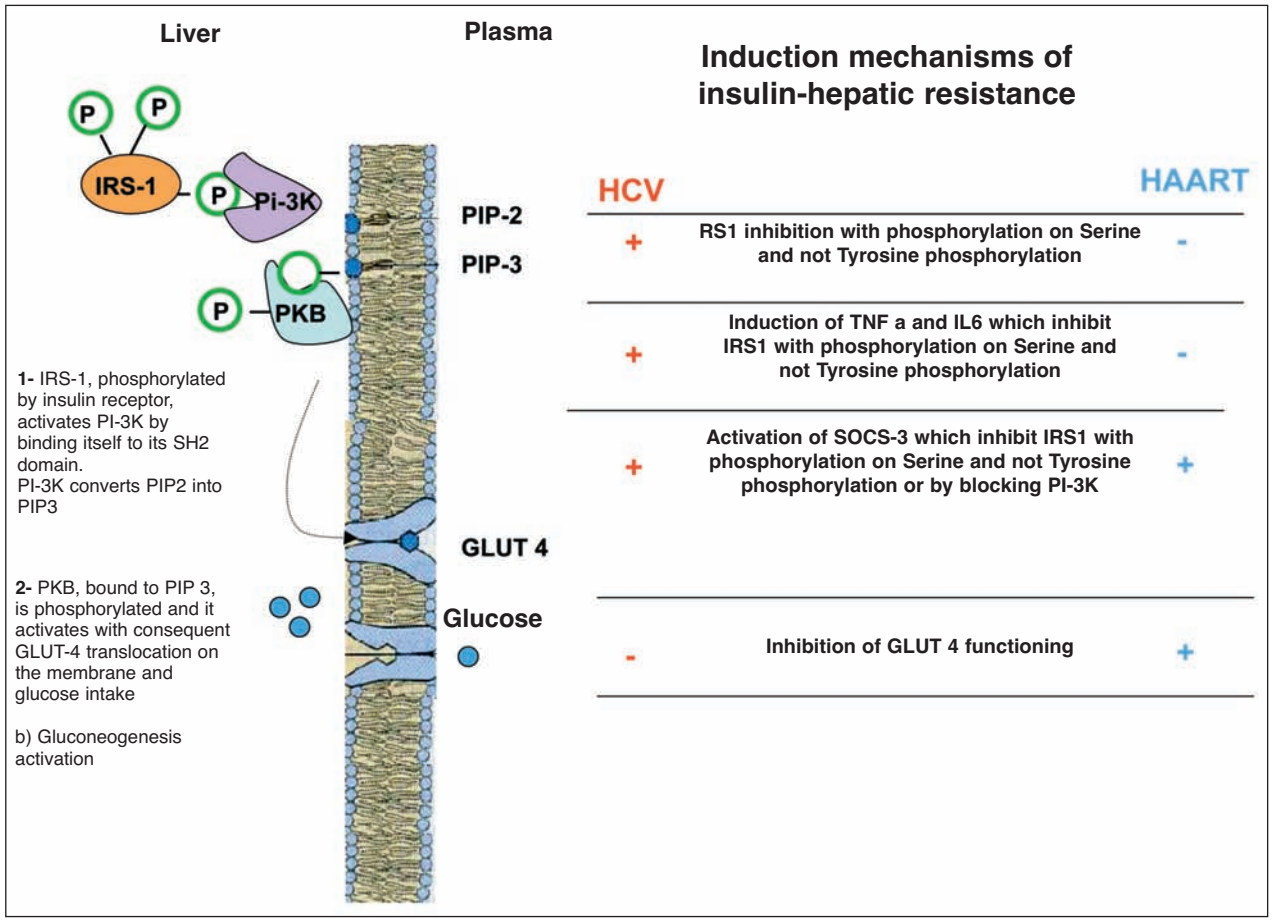

Figure 11. The insulin receptor is a catalytic dimeric receptor with tyrosine kinase activity. Following binding to insulin, its subunits self-phosphorylate with each other on Tyr, thus activating the receptor. The receptor phosphorylates (on Tyr) IRS1-4 *(Insulin Receptor Substrates 1-4)*. IRS1 binds the PI 3-kinase enzyme (PI3K with domain SH2) and activates it. PI3K turns the PIP2 membrane phospholipid into PIP3, which binds PKB by indirectly activating it. The known effects of PKB activation are: a) GLUT4 (*Glucose Transporter-4*) translocation on the membranes, thus favouring glucose intake (adipose tissue and muscle); b) activation of *glycogen synthetase* through phosphorylation, hence inhibition of GSK3 (glycogen synthase kinase 3). The figure describes some mechanisms through which HCV is able to induce insulin resistance, sharing part of such capacities with antiretroviral drugs.

signal translation, and involved in the chronic stimulation of lipogenesis and gluconeogenesis, in the expression of pyruvate kinase and in the reduction of the fatty acid oxidation in liver respectively. It follows that the availability of triglycerides and sugars in hepatocytes is increased (86). SREBP-1c is directly activated by HCV or through SOCS-3 stimulation. Its action predisposes the increase in triglycerides availability inside the hepatocyte, and the final result is a systemic insulin resistance and hepatic steatosis.

b) It was proved that HCc interacts with retinoids X receptor α(RXRα), transcription regulator which controls several cellular functions, including lipid metabolism. HCc is capable of binding itself to RXRα DNA binding domain, by activating it and stimulating lipogenesis (87).

3. Reduced excretion and reduced use of fatty acids in the hepatocyte

a) HCV non-structural proteins (core and NSA5) are also involved in the functionality changes through the membrane breakage of mitochondria, microsomes and peroxisomes (88).

b) Mitochondrial damage may be also induced by the function inhibition of *Peroxisome-proliferator-activated receptors*, (PPAR α and γ) members of the super-family of nuclear receptors involved in the translation of the nutritional and metabolic signals into transcriptional responses influencing the oxidative function of mitochondria, peroxisomes and microsomes (89). A reduced action of PPARα and γ during HCV, especially genotype 3, induces insulin resistance and steatosis through the arrest of beta-oxidation (90).

The role of adipokines

Adipose tissue is quite abundant in the adult and plays three important roles: 1) to store energy, 2) hydrolysis of triglycerides to make free fatty acids available for beta-oxidation, 3) release of adipokines.

The significance of lipid metabolism in the hepatic disease is shown by the rapid progession of cirrhosis in obese people (91). The role played by *non-alcoholic fatty liver disease* (NAFLD) is important *per se*, but obesity worsens chronic viral hepatitis C progression, and it is connected with a higher mortality rate and the development of tumours such as HCC (92, 93).

Adipokines play an important role in the communication between adipose tissue and liver, and they are mainly expressed, but not only, by adipose tissue. All adipose tissue cells (adipocytes, stromal cells, myofibroblasts and medullary cells) contribute towards the production of adipokines. In particular, ectopic fat (subcutaneous or pericardial fat) allows the emergence and maintenance of obesity, since it is more often the seat of chronic inflammatory phenomena (94).

Adipokines are directly involved in the pathogenesis of hepatic damage, since the direct target is the liver, through portal circulation.

Leptin

Leptin, ob gene's product, is mainly synthetised in the adipose tissue. Leptin receptors consist of six isoforms (ObRa - ObRf) expressed in the central nervous system, in several peripheral tissues and in the liver.

ObRb mediates most of leptin biological effects (by activating jak2/Stat3 complex).

ObRe is a soluble receptor that binds itself to the circulating leptin. ObRe increment correlates with a reduced effect of leptin (95).

The circulating leptin is proportional to the fat mass and induces anti-obesity signals, by influencing the sensation of hunger, energy use, protects against the development of hepatic steatosis (96, 97), alcoholic damage, and from acquiring viral and bacterial infections (98, 99).

However, an increase in circulating leptin levels and the presence of hepatic steatosis can be noted in obese patients (95).

This phenomenon is linked to peripheral and intrahepatic leptin resistance.

Some mechanisms inducing leptin resistance in the liver include the intake of some foods, such as: a) fructose (100), which induces hyperleptinaemia and intrahepatic resistance to leptin; b) excessive production of SOCS 3 (*suppressors of cytokines signaling 3*) inhibiting leptin signal (101) and c) the activation of cannabinoid receptors (102).

ObRe production (leptin soluble receptor) is mainly hepatic and induced by the insulin signal. Insulin resistance causes an increase in the soluble receptor production which diminishes leptin biological effect (as the portion linked to the receptor prevails compared to the free one) (103).

Leptin is well known as a powerful profibrogenic agent. At hepatic level (104) it can induce fibrosis by activating Kupffer cells, stellate cells (HSCs) and an increase in TGF-beta (*transforming growth factor beta*) production (105). Leptin action induces the production of free radicals which, in their turn, stimulate proinflammatory mechanisms 106, 107).

Once HSCs are activated, they contribute towards the production of leptin, whilst quiescent HSCs induce high levels of adiponectin with antifibrogenic action (108).

Leptin has been recently connected with HCC development for a direct and/or mediative effect by the induction of angiogenic mechanisms (109-111). Highly undifferentiated human HCC shows high levels of ObR receptors, and it was proved that ObR/Leptin levels are correlated with HCC angiogenesis in vivo (112-113).

Leptin is able to induce HCC proliferation, mi-

gration and invasive character (113), and it increases cholangiocarcinoma metastatic capacity (114). In chronic hepatitis C, leptin increases the possibility of developing HCC by hastening fibrogenesis, angiogenesis and directly acting on the neoplastic cells. However, leptin seems to exert a positive effect in liver protection by triggering the innate immune response (natural killer) capable of reducing the tumour size (115).

Leptin effect is therefore controversial. In particular, we have yet to understand the reason why hepatic resistance to leptin does not protect from the profibrogenic effect of this adipokine.

Leptin levels are constantly high during NASH, regardless of BMI (body mass index), with higher levels in those cases of more advanced disease (116). But these data have not been corroborated by other studies (117, 118). In case of viral hepatitis, some authors, but not others, have correlated leptin levels with viral steatosis and fibrosis progression (119, 120).

Adiponectin

Adiponectin is active in its high molecular weight form, and it binds itself to two specific receptors: AdipoR1, expressed in the skeletal-muscle tissue, and AdipoR2, mainly expressed in the liver (121). Plasma concentration of adiponectin inversely correlates with the fat mass and it is reduced in case of obesity and type 2 diabetes. Adiponectin improves steatosis and exerts an antifibrogenic effect through the partial reduction of TNF-alpha (122).

A saturated fat diet protects from alcoholic damage through the adiponectin hypersecretion (123).

In case of chronic alcoholic hepatopathy, adiponectin levels diminish, whilst homocysteine rises. Betaine reduces homocysteine and increases adiponectin level (124). The adiponectin protective effect at hepatic level seems to be mediated by the increase in PPAR-α activity in the liver (125) and in PPAR-γ activity in adipose tissue (126).

According to this model, PPAR-γ activation in the adipose tissue allows to store and keep triglycerides, thus reducing the circulating amount of free fatty acids. Therefore, it frees both liver and muscles from excessive fats sequestered in their context, thus increasing their sensitivity to insulin. The central adipose tissue expands to allow for this compensation mechanism (126).

In case of hepatic insulin resistance, the effect of adiponectin seems to decrease, and a resistance to adiponectin is established (127). High insulin levels, in fact, diminish the expression of adiponectin receptors, creating resistance to adiponectin.

The effect on the inflammatory cytokines is opposite compared to the one exerted by leptin. Adiponectin, in fact, induces the expression of anti-inflammatory *c*ytokines such as IL 10, and reduces the expression of proinflammatory cytokines (TNF-alpha, IL6, chemo-kine) (128).

Adiponectin is a powerful atherogenic action inhibitor (129); on the contrary, the chronic inflammatory state and lipid peroxidation – which occurs in case of fat accumulation (obesity, steatosis, etc…), - reduces adiponectin levels.

Transgenic ob/ob mice (without leptin) are protected against the onset of hepatitis mediated by T lymphocytes; if adiponectin is removed as well, such a protection disappears (130). Transgenic mice without leptin undergo more serious hepatic damage if subjected to experimental cirrhogenous stimuli. These experiments show how adiponectin is instrumental in counteracting the hepatic damage induced by leptin fibrogenic action (131).

Adiponectin levels are reduced in subjects with NASH regardless of the presence of insulin resistance and of the degree of abdominal circumference.

Reduced adiponectin levels correlate with high degrees of steatosis and necroinflammation, but not with the degree of fibrosis, which is only predicted by insulin resistance (132). Adiponectin levels are inversely correlated with the severity of hepatic damage (133).

On the contrary, the role played by adiponectin in case of viral hepatitis seems to be definitely reduced, if not entirely absent. The absence of any differences between adiponectin levels and mediated hepatic virus, adipokine independent, might account for the most serious degree of insulin resistance during viral hepatitis. Low adiponectin levels were found in case of genotype 3 HCV infection (134, 135), and a diminished response to interferon correlates with low adiponectin levels (136).

Resistin

Resistin is expressed, in the animal model, by adipose tissue, and its plasma concentration depends on both diet and obesity (137). Resistin might represent a connection between obesity and insulin resistance (98). Resistin induces gluconeogenesis and seems to promote both VLDL production and accumulation of fatty acids in the liver (138). In human beings, resistin effect is not clear and it seems to be mainly produced by medullary and inflammatory cells (95). Resistin is produced by activated macrophages and induces the production of proinflammatory cytokines. Resistin expression increases in case of hepatic inflammation and worsens it through the increase in TNF-alpha production (139, 140).

In HIV/HCV subjects a chronic hepatic inflammation can be noticed, mediated by the hepatitis C virus, viral and iatrogenic steatosis and chronic immune activation state (persistency of low HIV copies, microbial translocation). In these subjects the condition of hyperleptinaemia (and of possible leptin resistance), as well as of hypoadiponectinemia might contribute to worsening intrahepatic necro-inflammation, reducing hepatic defensive capacities and inducing profibrogenic mechanisms.

Steatogenesis and fibrogenesis in subjects with HIV/HCV co-infection

Steatosis

Visceral fat is metabolically more active than subcutaneous fat. Hepatic steatosis is connected to visceral obesity, and both correlate with insulin resistance.

The base of the steatosis damage can be understood from the pathogenic mechanisms listed above: the missing sensitivity to insulin signal induces the adipocyte lipolysis with the consequent increase in circulating free fatty acids (FFA). Moreover, through the increase in SREBPs and ChREBP activity, it makes FFA internalisation easier in the hepatocyte and creates the substrate of triglycerides necessary for lipogenesis. At the same time FFAs are neither metabolised – due to the arrest of mitochondrial beta-oxidation – nor externalised through lipoproteins (VLDL, LDL), whose production is inhibited.

In those patients with HCV genotype 3, the virus' role is predominant, and the onset of a real "viral steatosis" can be noticed, unlike the infection with HCV genotype 1, where the steatosis is "metabolic", hence linked to the host's factors.

The reduced assembly capacity of VLDL and LDL lipoproteins containing ApoB (141) is due to MTP (*Microsomal Triglyceride Transfer Protein*) inhibition by the virus. In presence of MTP, both triglycerides and APo B are assembled in VLDL inside the Endoplasmic Reticulum (ER). Following studies show that, more than to block MTP, the virus is able to use it to improve its own replication. Endoplasmic Reticulum (ER) turns into a sort of membraneous support where HCV proteins and NS5A interact with Apo B and triglycerides to create tiny lipid drops containing virion.

LVP (Lipo-viro-particles) are released through exocytosis as small lipid particles of 100 nm (142), capable of circulating in the plasma and infecting other cells by using LDL receptors (143).

Recent studies assumed that MTP inhibition mechanisms – with consequent arrest of LDL-VLDL production – are a strategy used by the virus to diminish the competition on LDL receptors.

On the other hand, fatty acid consumption through beta-oxidation and other mechanisms is linked to PPARα inhibition induced by the virus and described above. The gene transcription of MDR 3 (*Multidrug Resistance Protein*) - involved in the use of fatty acids through the secretion of phospholipids in the bile, - of CPT-1 (Carnitine palmitoyltransferase I gene) and of AOX (Acetyl Co-A Synthetase) depends on PPAR α and γ activity. All of them are necessary to regulate the mitochondrial beta-oxidation, main route for fatty acid consumption (141).

If steatosis recognises the viral pathogenesis for HCV genotype 3 infection, the metabolic origin prevails in the genotype 1 infection, and the host's factors appear completely predomninant.

It is clear how steatotis represents a strategy concocted by the virus to improve its own replication inside the hepatic cell and insure itself a transfection system of the extrahepatic viral sanctuaries. It seems

like it was proved, in fact, that fatty acids contained in the cell may stimulate or inhibit HCV replication according to their saturation (144), and that the entire host's intrahepatic lipid metabolism is disrupted to assure the virus' replication and LPV formation.

It has been known already since 1997 how the patient – especially carrier of HCV genotype 3 – would show low concentrations of cholesterol (145), lipoproteins and ApoB. It was assumed that those patients with HIV/HCV co-infection genotype 3 were protected against hypercholesterolemia due to HAART, given the evidence of low cholesterol levels (146). **But it is actually dangerous to assume a protective effect of HCV genotype 3 from the lipidemic effect of the antiviral therapy, given the proven capacity of the virus to remove free fatty acids from the liver, stop the cholesterol production and boost the steatosis damage.**

Even in the HIV+ person naïve to therapies, a reduced cholesterol concentration is observed; lipid structure seems to be modified by the inflammatory state induced by cytokines (TNFα, IL1, and IL6), capable of inhibiting lipogenesis, inducing lipolysis and apoptosis.

The same pathogenic history can be found in the patient with lipoatrophy induced by HAART (147-150).

The increase in circulating FFAs, therefore, induces insulin resistance, which is typical of patients with HIV and hypertriglyceridemia - linked to the use of some IPs and NRTIs, - and it also increases the lipid layer in the liver, thus triggering steatosis formation.

Fibrosis

Fibrogenesis is a process triggered by the hepatic damage and represents the breaking up of the "repairing" programme during a chronic disease (148).

Epatocyte damage is followed by the activation of resident macrophages (Kupffer cells) and/or by inflammatory cell infiltration. The inflammatory phase is linked to the emergence of myofibroblast-like cells responsible for ECM (*Extra Cellular Matrix)* production, representing the main effectors of fibrogenic process.

In the last 10 years, research has identified *Hepatic Stellate Cells (HSC)* as the main effector cells and responsible ones for ECM placement (151).

HSCs and mesenchymal cells in the fibrotic process acquire a myofibroblast-like phenotype with functional, biochemical and structural changes that make these cells more suitable for tissue repair (152).

Once HSCs are activated, they produce receptors for fibrogenic soluble mediators such as:

1) PDGF (*Platelet derived grow factor*) receptors
2) TGF-β (Transforming growth factor β) receptors
3) cytoskeleton proteins such as alpha-SMA (*Alfa smooth muscle actin*) (153).

Epatocytes, cholangiocytes, damaged Kupffer cells release factors capable of activating HSCs, such as: TGFβ PDGF, CTGF (*Connective Tissue Growth Factor*), Leptin, EGF (*Epidermal Growth Factor*), Angiotensin II, products linked to oxidative stress, MCP-1 (*Monocyte chemoattractant protein-1*), IL-8.

Once HSCs are activated, they show several functional characteristics:

1) Increase in production of fibrillar collagens.
2) Increase in production of metalloproteinase inhibitors, essential for fibrillar ECM degradation (TIMP-*Tissue Inhibitors of Metalloproteinase*).
3) Increase in production of MMP-2 and 3 degrading the normal epatocyte matrix. Both these behaviours are stimulated by TGF-β and CTGF.
4) Increase in proliferation of cell survival, entailing a growth of fibrogenic cells, especially in those areas of more active reshaping, such as fibrous septa edges in chronic hepatitis. This event is mediated by PDGF and EGF as well as angiotensin II modulating mitogenic processes.
5) Increase in migratory capacities allowing fibrogenic cells to move towards areas of active fibrogenesis, following chemotactic signals. Migration is a factor involved in the localisation of fibrotic process in different areas than the hepatic lobule.

PDGF is the biochemical mediator capable of boosting the migratory capacities of activated HSCs.

6) Ability to secrete inflammatory cytokines and chemokines which boost the inflammatory processes and lead to recruiting inflammatory cells.

The presence of proinflammatory cytokines such

as IL-1, TNF or IFN-α (154) allows the *up regulation of chemoattractive factors such as* MCP-1 and IL8.

7) Acquisition of a contractile phenotype when they are exposed to vasoactive agents such as angiotensin, endothelin-1 and thrombin (155). Owing to the proximity of these cells to sinusoids and just formed vessels, their contractile action is thought to be involved in the portal hypertension mechanism.

From steatosis to fibrosis

The correlation between steatosis and advanced degrees of fibrosis is corroborated by various research studies, but the presence of IR *per se* is considered a fibrogenic factor (156).

HOMA (*Homeostasis Model Assessment*) is correlated with the degree and rapidity of fibrosis, regardless of age, alcohol consumption, sex and degree of portal inflammation (157); it increases in patients with serious fibrosis, but it is present even in absence of histological damage. **Insulin, besides its metabolic effects, exerts also important stimulating and mitogenic effects on the cellular replication, whilst hyperglycemia allows the release of profibrogenic cytokines. Hyperinsulinism induces the production of collagen in hepatic stellate cells and hence can be considered an important factor involved in fibrogenesis (157-159).**

An increase in the activity of *Connective Tissue Growth Factor* (CTGF) was assumed, capable of inducing matrix synthesis (160). CTGF and TGF-β (*Transferring growth factor-β)* are expressed at the same time during the phases of skin cicatrisation and in production of keloids. CTGF levels are correlated with the fibrotic degree, regardless of hepatitis aetiology (160). Both insulin and HCV are able to induce the production of CTGF and TGF-β (161).

Adipokines are part of the fibrosis pathogenic mechanisms. Hypoadiponectinemia is connected with progressive degrees of steatosis in patients with NASH (162). Adiponectin improves the sensitivity to insulin and diminishes the hepatic content of triglycerides (163); it also diminishes the damage due to carbon tetrachloride in the experimental model (164). Many studies highlight the ability of antiviral therapy to induce hypoadiponectinemia, which therefore represents an added factor of insulin resistance in HIV/HCV patients in HAART (165).

Leptin, on the other hand, derived from adipocytes, regulates the sensation of hunger and satiety and insulin effects. Leptin-resistance occurs in obese people, hence high circulating levels of leptin are seen. The assumption is that leptin is the key to account for steatosis and fibrosis in obese people (166). Animal models support leptin profibrogenic capacities; in some studies the steatosis degree seems to correlate with the levels of serum leptin, and leptin seems to be an independent factor of steatosis (166).

Antiretroviral drugs, iatrogenic steatosis induction and fibrosis progression

The metabolic effects of some antiretroviral drugs might contribute, in the co-infected subject, to the onset of an iatrogenic steatosis in the long run - cause and effect of insulin resistance and of the increment in leptin plasma levels. A very important contribution to the fibrogenic mechanisms would follow.

NRTIs

Mitochondrial damage attributed to HIV nucleoside-analogue reverse transcriptase inhibitors (NRTI) has been known since 1996 (167). Mitochondrial DNA polymerase inhibition leads to the slow depletion of mitochondrial DNA and the consequent loss of oxidative functions. The disappearance of mitochondrial functionality is tightly correlated with lipoatrophy in adipose tissue, to lactic acidosis and steatosis in the liver, to distal sensory polyneuropathy (DSP) in the peripheral nervous system. The long exposure to NRTIs is correlated with insulin resistance, diabetes and an increase in serum lactates. The exposure to d4T is correlated with the presence of steatosis in HIV-positive subjects (168, 169).

Protease inhibitors

Protease inhibitors, in a different way between each other, induce insulin resistance, increase triglyc-

erides, total cholesterol and peptide C; they reduce HDL cholesterol, increase leptin, and reduce adiponectin (170). The recognised pathogenic mechanisms are GLUT 4 inhibition, mitochondrial toxicity, apoptosis of visceral adipose tissue, SOCS 3 activation, reduction in PPAR-α activity in the liver and PPAR-γ activity in the adipose tissue.

GLUT 4 inhibition

IPs capability to selectively inhibit GLUT-4 was proved.

Their inhibitory action – studied in vitro – seems to be reversible and it is very evident for Indinavir, less for Atazanavir, Saquinavir and Amprenavir, even when connected with a low dose of Ritonavir, disagreeing with Lopinavir/Itonavir (171-176).

Mitochondrial toxicity

New mechanisms of mitochondrial toxicity depending on antiretroviral therapy exposure have been proved lately. Mitochondrial DNA loss in the adipocytes depends on an ROS increase capable of inducing apoptosis through mitochondria-dependent mechanisms. NRTI and PI association and the chronic inflammatory state of adipose tissue induce a mitochondrial disorder (177).

Oxidative damage lies at the heart of insulin resistance induced by Nelfinavir (178).

Lopinavir/RTV, Nelfinavir and Zidovudine, but not Atazanavir, Amprenavir and Abacavir, induce ROS production inside adipocytes (179).

Genes involved in adipocitary stress

Ritonavir is able to modify the expression of proinflammatory cytokines, of response genes to stressor stimuli localised in the adipocyte endoplasmic reticulum, of response genes to oxidative stress, of genes inducing apoptosis and of genes involved in the mechanisms of adhesion and reshaping of extracellular matrix. Moreover, the capacity to inhibit the expression of a new gene involved in the free fatty acid metabolism was highlighted. These changes suggest an activation pattern of genes correlated with stress in adipocytes induced by chronic exposure to Ritonavir (180-182).

Arrest of adipocyte maturation

Exposure to Ritonavir, but not to Atazanavir, reduces preadipocyte maturation both in the omental adipose tissue and in the subcutis (183).

Adipokine expression

Exposure to Ritonavir diminishes adiponectin production (183, 184). Both Ritonavir and Atazanavir modify leptin secretion (183).

The chronic inflammatory state of adipose tissue induced by mitochondrial damage causes a change in adipokine production (177).

Some protease inhibitors seem to be able to modify gene expression in the adipocytes with consequent maturation reduced capacity by adipocytes, and apoptosis mediated by the reduction in mitochondria membrane potential (185, 186).

All protease inhibitors – except Atazanavir, Amprenavir and Abacavir – reduce adipokine expression (179).

Adipocyte apoptosis

Adipocyte apoptosis, lipolysis and lipogenesis inhibition are also consequent to the increase in levels of TNFα, IL6, IL 1, IFNα cytokines, evident in the HIV patient even without HAART (184-188). Ritonavir, Saquinavir and Nelfinavir are capable of inducing lipolysis and reducing lipogenesis, whilst Amprenavir and Inidinavir seem to modify these mechanisms to a lesser extent (189).

Lopinavir/r, Nelfinavir and Zidovudine, but not Atazanavir, Amprenavir and Abacavir, induce MCP-1 and IL 6, TNF and IL 1 beta production (179).

Increase in SOCS expression

SOCS are a well-known inductor of insulin resistance, and in the animal model the capacity to dramatically increase SOCS in the adipose tissue, muscle and liver was proved. SOCS increase induces TNF α

boost and SREBP reduction with a consequent increase in inflammatory stimuli and reduced use of fatty acids. This leads to a condition of hepatic sequestrum of fatty acids and lipid peroxidation induction (190).

Reduction of PPAR α-γ function

PPARs regulate gene transcription in adipose tissue and the liver; they are capable of activating fat lipid peroxidation, and play an important role in the homeostasis of lipogenesis and gluconeogenesis. Protease inhibitors diminish the effect of these enzymes, significantly modifying the metabolism of fatty acids and sugars.

All protease inhibitors, with less evidence for Amprenavir and Atazanavir, reduce adipocyte maturation, induce lipogenesis with a release of circulating fatty acids and a reduction in insulin sensitivity. Leptin increase and insulin resistance trigger fibrogenic stimuli in the liver, thus contributing, in the long term, towards the progression of liver disease (Figure 11).

Metabolic damage and response to therapy with interferon

HOMA score (*Homeostasis Model of Assessmentm*), calculated with insulin before meals (mmol/l) x glycemia before meals (mmol/l) ÷ 22,5, allows identification of the presence of insulin resistance with good approximation.

This score is suitable for identifying the insulin resistance (IR) in population studies. It is necessary to establish the pathological cut-off on the basis of the normal score identified on a sample of the non-diabetic population without any chronic infections or other predisposing pathologies in the same hospital where the research study is carried out.

A study conducted by Romero Gomez and colleagues (191) showed how in HCV + subjects with IR, defined as HOMA > 2, the achievement of the Sustained Virological Response (SVR) was lower than in subjects with HOMA <2. The rates of SVR and Early Viral Response (EVR) are lower in patients with hepatic steatosis (192); this is particularly true for those patients with genotype 1-4 who have a much lesser probability of obtaining EVR compared to patients without steatosis (71% vs 42%) (193).

Hepatic steatosis is a SVR predictive factor independent of age, gender, BMI, viral load, glucose, or fibrosis for the genotypes 1,4,5 and 6, with a long-term virological success percentage of 35% in subjects with steatosis vs 57% (p<0,001) (192). Even those patients with genotype 2 – who usually show SVR high rates – showed significant differences, if steatosis was present (86% vs 96% p 0,04) (194).

The studies devoted to the virological success in obese patients are very few. Bressler and colleagues prove how obese people show a lower SVR rate (OR 0.23) compared to people with a normal weight (195). In a sub-analysis of FRAM (*Fat Redistribution and Metabolic Change*) study conducted on 1119 patients - a subgroup of 247 HIV/HCV co-infected people – a correlation between ALT increase and the presence of visceral adipose tissue (VAT) was noted compared to HIV mono-infected people (196). In the patient with HIV/HCV co-infection, hypertriglyceridemia, insulin resitance and any lipodystrophy, connected with HAART, go with insulin resistance induced by HCV and create a condition particularly adverse to the therapy success with Interferon.

Insulin resistance, defined as HOMA > 2.25 in patients with HIV/HCV co-infection treated with PegIFN alpha 2 at 180 mcgr/week and Ribavirin 1000-1200 mg/die, negatively correlates with RVR, EVR and SVR (197-200).

The high triglyceride level – unlike LDL level (201), - correlates with a rapid and early reduced response (202).

The mechanism of Interferon antiviral activity inhibition induced by insulin resistance was assumed in an in-vitro study.

Peg-interferons alpha-2a and b perform their antiviral activity by binding themselves to a heterodimeric receptor complex (IFNAR1/IFNAR2). Such bond induces JAK-1 (Janus Kinase -1) and Tyk-2 (Tyrosine Kinase-2), which, in turn, activate STAT 1 and STAT2, mechanisms of transduction and activation of the transcriptional signal. The activated STATs turn into multimeric complexes, and are relo-

cated in the nucleus, where they bind with the response element stimulated by *IFN* α -2, promoting transcription of anti-HCV specific genes, whose PKR shows higher antiviral activity (203).

In a recent study that uses the replicon model in HhU-7 cells, IFNa stops HCV replication, but in the presence of hyperinsulinaemia (128 μU/ml), IFN was no longer able to stop HCV replication and PKR was not expressed (204).

Changes in lipid and sugar metabolism in the patient with HIV/HCV co-infection have a multifactorial genesis. The combination of the two viruses and the antiretroviral therapy are the causes that keep insulin resistance directly involved in the process of steatosis and fibrosis. Since insulin resistance is a negative prognostic factor of interferon response, the metabolic syndrome treatment becomes an essential element of anti-HCV therapy, especially for the infections sustained in difficult genotypes. The replacement of antiviral therapy with low-metabolic impact medications might represent an important choice to slow the disease progression and improve the therapy success with PegIFN and Ribavirin.

Immune activation, progression of hepatic fibrosis and role of CCR5 inhibitors

At the intrahepatic level an immune activation condition can be observed, due to the presence of a) one or more chronically infecting hepatitis viruses which stimulate the specific CD8 (CCR5+) response, b) hepatic steatosis of viral or iatrogenic origin that in time turns into steatohepatitis with lipid peroxidation of fatty acids sequestered in the liver, c) phenomena linked to microbial translocation.

Microbial translocation is recognised among the most important mechanisms of chronic immune activation in HIV- positive subjects. The most significant indicator is the plasma concentration of circulating lipopolysaccharide residues (LPS). LPSs induce the activation of monocytes and "*trafficking*" in the central nervous system (205) and liver. The subjects with chronic hepatitis C show an increase in circulating LPS as much higher as the level of T CD4+ lymphocytes is lesser and as the hepatic disease is serious. LPS are considered one of the most involved factors in liver disease in HIV subjects (206).

CCR5 chemokine receptor plays an instrumental role in maintaining immune activation in mediated chronic HIV and HCV.

CCR5: description and function

It is a chemokine receptor; it has a coiled alpha-helix structure with 7 transmembrane domains and an N-terminal involved in the bond with chemokines, in the intracellular signal transduction and in protein G activation. Cells expressing CCR5 receptors on the membrane are the haematic-derived dendritic cells, macrophages, lymphocytes, endothelial cells, pancreatic beta cells and hepatic stellate cells.

CCR5 mediates the bond with CCL3/MIP1-α, CCL4/MIP 1-β CCL5/RANTES chemokines.

CCR5, and chemokines binding to it, play an instrumental role in the response differentiation of type Th1 or Th2 of TCD4+ lymphocytes (207-208).

CCR5, in fact, is expressed by T-helper cells, both by memory T lymphocytes and activated lymphocytes. It is especially expressed by Th1 cells, whilst most Th2 clones do not show the expression of this receptor (209).

CCR5 expression depends on the degree of activation of the immune system, and it is especially mediated by the concentration of circulating IL 2 (210).

Homozygosity for CCR5 gene deletion 32 (CCRΔ32) causes the receptor non-complete expression on the cell, whilst the heterozygous state entails the reduction of cell expression by 20-30%.

CCR2, CCR3, CCR4 and CCR5 are among the recognised receptors capable of binding endothelial inflammation mediators, hence, most likely, at the base of the pathogenic processes of cardiovascular damage. These receptors activate mechanisms mediated by G protein inside the cell with a final effect of leukocyte chemoattraction (211). The inhibition of chemokine receptors was proved to reduce the formation of atheromata in the animal model (212-213).

CCR5Δ32 was proved to be connected to a reduction in cardiovascular damage (myocardial infarction, ictus, transient ischemic attack and reduction in carotid intima media thickness). Homozygosity leads

to a substantial protection, whilst the heterozygous state leads to an intermediate protection.

Moreover, the inflammatory markers, such as PCR, Fibrinogen, alpha 1-Antitrypsin, and Procalcitonin which correlate with the increase in chemotactic activity in the endothelium (215-216), hence with cardiovascular risk (214), are less expressed in patients with CCR5Δ32 compared to the general population (homozygote<heterozygote< wild type).

CCR5 is also expressed on the insulin-pancreatic β cells, and it is directly involved in the immune-mediated pancreatic damage of subjects with type 1 diabetes.

It was proved that CCL3/MIP1-α and CCL5/RANTES, CCR5 ligands, are the products of Th1 type response and their action is deeply detrimental to beta cells. In the animal model, CCR5-/- mice showed a substantial protection towards immune-induced pancreatic damage, leading to the conclusion that, even in type 1 diabetes, CCR5 inhibition might favour organ damage protection (217).

From the literature it emerges that CCR5Δ32 mutation is linked to:

- reduction of HIV infection risk (218);
- reduction of necroinflammation processes during chronic hepatitis C (219-220);
- reduction of inflammatory damage in rheumatoid arthritis (221);
- cardiovascular risk reduction (222);
- pancreatic damage reduction in type 1 diabetes (217).

CCR5 role in the chronic inflammatory state mediated by HCV

Cell immune response plays a key role in HCV infection.

Effectors of induced immune response (CD4+ and CD8+ lymphocytes) and innate immune response (NK and NKT cells) are involved in acute hepatic damage due to virus C, and they are the key factors in Th1 response induction mediated by IFN γ production (223-224).

These cells are capable of reducing hepatic damage induced by the virus just by producing IFN γ and Th1-type immune response (224).

The increase in CD8 in IFN γ production induces HCV *clearance.*

People having a genetic predisposition to a higher NK activity - through a higher IFN γ production, - do not make HCV chronic, if infected, compared to general population.

In the liver of patients with HCV acute infection, CCR5 ligands (MIPα, β RANTES and the same CCR5 receptor) are increased to favour Th1 responses and eradicate the virus. The officially accepted dogma is that a strong Th1 response is linked to virus *clearance during acute HCV infection (225).*

On the other hand, chronic infection is linked to a defective response of CD8+ and NK cells during HCV infection due to consequent diminished IFN γ production (226).

An ineffective response of NK and NKT cells is linked to a longer HCv chronic state (226).

During chronic infection, an intrahepatic accumulation of T CD8+ lymphocytes is observed. They are recalled by the viral infection and migrate from the plasma towards the liver, but they are sequestered and phagocytised by stellate cells with the virus clearance attempt having no effects whatsoever. The intrahepatic accumulation of T CD8+ lymphocytes concurs with a reduction in T CD4+ lymphocytes and NK cells. It follows an inflammatory cytokine increase with consequent implementation of fibrogenic stimuli.

The great quantity of chemokines present in the chronically infected liver by the virus contribute towards the immune-mediated damage by HCV through CCR1 activation, especially expressed by medullary cells (Kupffer cells), and through the prevailing CCR5 activation expressed by hepatic-derived cells (stellate cells).

Testing on the animal model highlighted how the action of chemokines, in particular RANTES – through CCR5 expression – causes the activation, proliferation and migration of stellate cells.

The inhibition of chemokines or CCR5 receptor or CCR1 receptor or, better, of both, considerably reduces the activation of stellate cells and fibrogenesis (227).

T lymphocytes, chemokines and their receptors – notably CCR5 and CCR1 – are essential in both virus clearance – in case of acute hepatitis – and he-

patic fibrosis progression, through an immunemediated chronic damage – in case of chronic hepatitis (228).

One of the mechanisms of virus escape in patients with HCV chronic infection is the reduced CCR5 expression by hepatic cells. In fact, during chronic infection, CCR5 internalisation phenomenon can be observed mediated by the binding of a HCV structural protein – E2 – to one of the virus co-receptors on hepatocytes - CD81. This event causes a reduction of mediated Th1 response with a reduced CD8 (CCR5+) migration from plasma to the liver, which, however, do not cease. Such an event causes a reduction in the strength of mediated Th1 response and predisposes to chronic immune activation perpetuation to which fibrosis progression follows (229).

The efficacy of alpha Interferon in chronic infection was identified in its capacity to upregulate CCR5 expression, whilst Ribavirin is thought to be effective because it stimulates a Th1-type response (230, 231).

The observation that the downregulation of CCR5 expression on TCD8+ lymphocytes would prevent HCV clearance led to the idea that patients with CCR5Δ32 might be more predisposed to flavivirus infection. The frequency of Δ32 mutation was observed to be three times higher in patients with HCV chronic infection compared to the healthy ones (232).

In a group of haemophiliac patients, Δ32 mutation was linked to an increase in HCV infection risk, an increase in HCV-RNA and a reduced infection risk with HIV (232).

In other studies, the highest HCV infection risk and the severity of illness in patients with Δ32 were not confirmed, and the role played by deletion remains controversial (233).

However, it was shown that, in patients with Δ32 and HCV, a reduced portal and peri-portal necroinflammation was observed, but not a fibrosis reduction, as if CCR5 reduced expression would diminish the immune-induced necroinflammatory damage, but would not improve the progression of HCV-induced fibrosis, perhaps due to the reduced expression of Th1 immune response (234, 235).

In conclusion, hepatic fibrosis progression is faster in the patient with HIV/HCV co-infection. HSCs activation causes the pathogenic phenomena inducing hepatic parenchyma fibrosis. Stellate cells are stimulated by the chronic immune activation mediated by CCR5 receptor exposure to HIV gp 120, by the chronic presence of one or more hepatic viruses, by the increased presence of circulating lipopolysaccharides, by the production of mediators by the damaged hepatic cells, by the presence of intrahepatic T CD8+ lymphocytes recalled by chronic infection condition as well as by proinflammatory chemokine self-production.

The activated HSCs produce and respond to chemokines through the expression of receptors, notably CCR5 and CCR1.

CCR5 receptor inhibition correlates with hepatic fibrosis reduction. The subjects, carriers of CCR5Δ32 mutation, show a lesser CCR5 expression; when they are HCV-infected, they have a lower portal inflammation and a reduced progression towards cirrhosis.

HSC activation provides the pathogenic base of hepatic fibrosis; CCR5 inhibition might contribute – to a certain extent – to deactivating these cells, which are essential in the progression of liver disease.

This observation leads us to consider Maraviroc as a possible candidate as an anti-fibrotic medication useful in subjects with non-responder chronic infection or non-eligible to undergo treatment with Peginterferon and Ribavirin.

References

1. Soriano V, Puoti M, Sulkowski M et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 update of recommendation from the HIV/HCV international Panel. AIDS 2007; 21: 1073-89.
2. Rockstroh J, Mocroft A, Soriano V et al. Influence of hepatitis C virus infection on HIV-1 disease progression and response to highly active antiretroviral therapy. J Infect Dis 2005; 192: 992-1002.
3. Modi A, Feld J. Viral hepatitis and HIV in Africa. AIDS rev 2007; 9: 25-39.
4. Soriano V, Mocroft A, Rockstroh J et al. Spontaneous viral clearance, viral load and genotype distribution of hepatitis C virus (HCV) in HIV infected patients with anti-HCV antibodies in Europe. J Infect Dis 2008;198: 1337-44.
5. Danta M, Brown D, Bhagani S et al. Recent epidemic of acute hepatitis C infection in HIV positive men who have sex with men: the Swiss HIV cohort Study. AIDS 2007; 21: 983-91.

6. Weinbaum C, Sabin K, Santibanez S. Hepatitis B, hepatitis C and HIV correctional populations: a review of epidemiology and prevention. AIDS 2005; 19(suppl 3): 41-6.
7. Conte D, Fraquelli M, Prati D et al. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15, 250 pregnant women. Heaptology 2000; 31: 751-5.
8. Airoldi J, Berghella V. Hepatitis C and pregnancy. Obstet Gyencol Surv 2006; 61: 666-72.
9. Bruno R, Sacchi P, Puoti M et al. Natural history of compensated viral cirrhosis in a cohort of patients with HIV infection. T Acquir Immune Defic Syndr 2007(1); 46: 297-303.
10. Wyles D, Gerber J. Antiretroviral drug pharmacokinetics in hepatitis with hepatic dysfunction Clin Infect Dis 2005; 40: 174-81.
11. Wyatt CM, Malvestutto C, Coca SG et al. The impact of hepatitis C virus coinfection on HIV-related kidney disease: a systematic review and meta-analysis. AIDS 2008; 22: 1799-807.
12. Greub G, Lederberger B, Battegay M et al. Clinical progression, survival and immune recovery during antiretroviral therapy in patients with HIV 1 and hepatitis C virus coinfection: the Swiss HIV cohort study. Lancet 2000; 35: 1800-5.
13. De Luca A, Bugarini R, Cozzi Lepri A et al. Coinfection with hepatitis viruses and outcome of initial antiretroviral regimens in previously naive HIV infected subject. Arch Intern Med 2002; 162: 2125-32.
14. Sulkowski M, Moore R, Metha S et al. Hepatitis C and progression of HIV disease. JAMA 2002; 288: 199-206.
15. Braitstein P, Justice A, Bangsberg DR et al. Hepatitis C coinfection is independently associated with decreased adherence to antiretroviral therapy in a population-based HIV cohort. AIDS 2006; 20: 323-31.
16. D'Arminio Monforte A, Cozzi Lepri A, Castagna A et al. Risk of developing specific AIDS Defining Illnesses in patients coinfected with HIV and hepatitis C virus with or without liver cirrhosis. CID 2009; 49: 612-22.
17. Antonucci G, Girardi E, Cozzi-Lepri A et al.Role of hepatitis C virus (HCV) viremia and HCV genotype in the immune recovery from highly active antiretroviral therapy in a cohort of antiretroviral-naive HIV-infected individuals. Clin Infect Dis 2005; 40: e101-9.
18. Sulkowski MS, Moore RD, Mehta SH et al. Hepatitis C and progression of HIV disease. JAMA 2002; 288: 199-206
19. Rauch A, Gaudieri S, Evison J et al. Low current and nadir CD4+ T-cell counts are associated with higher hepatitis C virus RNA levels in the Swiss HIV cohort study. Antivir Ther 2008; 13: 455-60.
20. Macías J, Castellano V, Merchante N et al. Effect of antiretroviral drugs on liver fibrosis in HIV-infected patients with chronic hepatitis C: harmful impact of nevirapine AIDS 2004 26; 18: 767-74.
21. Bonacini M, Louie S, Bzowej N et al. Survival in patients with HIV infection and viral hepatitis B or C: a cohort study. AIDS 2004; 18: 2039-45.
22. Pineda JA, Romero-Gómez M, Díaz-García F et al. HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. Hepatology. 2005; 41: 779-89.
24. Castellares C, Barreiro P,Martin-Carbonero L et al. Liver cirrhosis in HIV-infected patietns: prevalence,aetiology and clinical outcome. J Viral Heapti 2008; 15: 165-72.
25. Puoti M, Bruno R, Soriano V et al. Hepatocellular carcinoma in HIV-infected patients: epidemiological features, clinical presentation and outcome. AIDS 2004; 18: 2285-93.
26. Merchante N, Giron JA, Gonzales-Serrano M et al. Survival and prognostic factors of HIV infected patietens with HCV realted end stage liver disease AIDS 2006; 20: 49-57.
27. Heaptic safety and HAART J Int Assoc Physicians AIDS care 2004; 3: S24-40.
28. Kaplowitz N. Drug induced liver injury. Clin Infect Dis. 2004; 38: S44-S48.
29. Verma S, Wang CH, Govindarajan S et al. Do type and duration of antiretroviral therapy attenuate liver fibrosis in HIV-hepatitis C virus-coinfected patients? Clin Infect Dis 2006; 42: 262-70.
30. Macías J, Mira JA, López-Cortés LF et al. Antiretroviral therapy based on protease inhibitors as a protective factor against liver fibrosis progression in patients with chronic hepatitis C. Antivir Ther 2006; 11: 839-46.
31. Perrella A, Grattacaso S, Gnarini M. Fosamprenavir in HAART schedule induces a rapid virological and biochemical response to HCV coupled to Th1 boosting in HIV/HCV coinfected patients. AASLD 2007, #264.
32. DHHS guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents: - December 1, 2009.
33. European Guidelines for European HIV infected adults –EACS 2009.
34. Söderberg C, Stål P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology (in press).
35. Puoti M, Nasta P, Gatti F et al.HIV-related liver disease: ARV drugs, coinfection, and other risk factors.J Int Assoc Pysicians AIDS Care (Chic Ill). 2009; 8(1): 30-42.
36. Soriano V, Puoti M, Garcia-Gascó P et al. Antiretroviral drugs and liver injury. AIDS 2008; 22: 1-13.
37. Sulkowski MS, Thomas DS, Chaisson RE et al. Hepatotoxicity associated with antiviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. J Am Med Asocc 2000; 283-74-8.
38. Aceti A, Pasquazzi C, Zechini B et al. Heaptotoxicity development during antiretroviral therapy containing protease inhibitor in patients with HIV: the role of B or C virus infection. J Acquir Immun Defic Syndr 2002; 29: 41-8.
39. Miller KD, Cemeron R, Wood LV et al. Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases. Ann Intern Med 2000; 133: 192-6.
40. Moyle G. Mechanism of HIV and nucleoside reverse transcriptase inhibitor injury to mithocondria. Antivir Ther 2005; 10(Suppl 2): M47-52.

41. Braitstein P, Palepu A, Dietrich D et al. Special considerations in the initiation and management of antiretroviral therapy in individuals coinfected with HIV and hepatitis C. AIDS 2004; 18: 2221-34.
43. Martin-Carbonero L, Nunez M, Gonzalez Lahos J, Soriano V. Incidence of liver injury after beginning antiretroviral therapy with efavirenz or nevirapine HIV. Clin Trial 2003; 4: 115-20.
44. Vora S, Michon C, Junet C et al. Switch from indinavir to ritonavir/indinavir regimen in patients treated with highly active antiretroviral therapy coinfected with hepatitis C is not associated with alteration of liver function tests Acquired Immune Defic Syndr 2000; 14: 2795-7.
45. Elbekai RH, Korashy HM, El-Kadi AO et al.The effect of liver cirrhosis on the regulation and expression of drug metabolizing enzymes. Curr Drug Metab 2004; 5: 157-67.
46. Meraviglia P, Schaivini M, Antinori A et al. Liver enzyme elevations in the italian atazanavir expanded access programme. X European AIDS conference November 17-20 Dublin EACS Abstract P6.8.
47. Perez Elias MJ, Gatell JM, Flores J et al. Effect of ritonavir boosted atazanavr in exprienced HIV infected patients regarding chronic hepatitis B/C status. IIIrd IAS conference on HIV pathigenesis and treatment Rio de Janeiro 25-27 July 2005, Abstract YuPe1.1C25.
48. Eholie S, Lacombe K, Serfaty L et al. Acute hepatic cytolisis in an HIV infected patient taking atazanavr. AIDS 2004; 18: 1610-11.
49. Sulkowski MS, Mheta SH, Chaisson RE et al Hepatotoxicity associated with protease inhibitor based antiretroivral regimens with or without concurrent ritonavir AIDS 2004; 18: 2277-84.
50. Chan P. 24-week data from RESIST -2: phase III study of the efficacay and safety of either tipranavir/ritonavir (TPV/r) or an optimized ritonavir (rtv)-boosted standard-of-care comparator PI in a large randomized multicenter trial in treatment-experinced HIV+ patients. Paper presented at 7th international conference on drug therapy in HIV infection 14-18 november 2004 Glasgow-Scotland
51. Rokstroh J, Sulkowski M, Neubacher D et al. 24 week efficacy of tipranavir boosed with ritonavir in hepatitis B or hepatitis C coinfected patients 45th ICAAC, Washinghton DC, december 16-19, 205 Abstract H-525
52. Gathe JC Jr, Ive P, Wood R et al. SOLO: 48-week efficacy and safety comparison of once-daily fasamprenavir/ritonavir versus twice-daily nelfinavir in naive HIV-1-infected patients. AIDS 2004; 18: 1529-37.
53. De Jesus E, Gladysz A, Vera J et al. Safety of Fosamprenavir/ritonavir containing regimen over 120 weeks in HIV 1 infected therapy naive adults with or without hepatitis B and or C coinfection III° IAS Conference on HIV pathogenesis and treatment Rio de Janeiro 24-27 july 2005. Abstract TUPE1.1C03
54. Pineda JA, Perez-Elias MJ, Pena JM Low rate of adverse hepatic events associated with fosamprenavir /ritonavir based antiretroviral therapy. HIV Clinical Trial 2008; 9: 309-12.
55. Nasta P. Gatti F,Cologni G hepatic tolerability of fosamprenavir/rtv in HIV/hepatitis C coinfected subjects with severe hepatic fibrosis (Ninth International Congress on drug therapy in HIV infection 2007 #) HIV and drug therapy.
56. Gatti F, Nasta P, Loregian A et al. Unboosted fosamprenavir is associated with low drug exposure in HIV-infected patients with mild-moderate liver impairment resulting from HCV-related cirrhosis. J Antimicrob Chemother 2009; 63(3): 575-8. Epub 2009 Jan 16.
57. Seminari E, De Bona A, Gentilini G et al. Amprenavir and ritonavir plasma concentrations in HIV-infected patients treated with fosamprenavir/ritonavir with various degrees of liver impairment. J Antimicrob Chemother 2007; 60: 831-6.
58. Spagnuolo V, Gentilini G, De Bona A et al Liver function parameters in HIV/HCV co-infected patients treated with amprenavir and ritonavir and correlation with plasma levels. New Microbiol 2007; 30: 279-82.
59. Esposito V, Chirtianni A Tollerabilità epatica del fosamprenavir in soggetti affetti da infezione da HIV coinfetti con il virus HCV: esperienza clinica. Acta Biomedica 2009; 11-8.
60. McKeage K, Perry CM, Keam SJ et al. Darunavir: a review of its use in the management of HIV infection in adults. Drugs. 2009; 69: 477-503.
61. Nasta P, Gatti F, Borghi F et al. Raltegravir Hepatic tolerability in patients with o without HIV/HCV co-infection. Fifth Workshop on HIV and hepatitis Co-infection. 19-21 June 2009 Lisbon, Portugal, Abs 38.
62. Abel S, Davis JD, Ridgway CE, et al. Pharmacokinetics, safety and tolerability of a single oral dose of maraviroc in HIV-negative subjects with mild and moderate hepatic impairment. Antivir Ther 2009; 14: 831-7.
63. King H, Aubert RDE, Herman WH. Global Burden of diabetes, 1995-2025.Prevalence, numerical estimates and projection. Diabetes Care 1998; 21: 1414-31.
64. Allison MED, Wreighitt T, Palmer CR et al. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. J Hepatol 1994; 21: 1135-39.
65. Mason AL, Lau JYN, Hoang N et al. Association of diabetes mellitus and chronic hepatitis C infection. Hepatology 1999; 30: 1059-63
66. Caronia S, Taylor K, Magliaro L et al. Further evidence for an association between non-insulin dependent diabetes mellitus and chronic hepatitis C virus infection. Hepatology 1999; 30: 1059-63.
67. Thuluvath PJ, John PR. Association between of diabetes mellitus and race. A case control study. Am J Gastroenterol 2003, 98: 438-41.
68. Knobler H, Schilmanter R, Zifroni A et al. Increased risk of type 2 diabetes in non cirrhotic patients with chornic hepatitis C virus infection. Mayo Clin Proc 2000; 75: 355-9.
69. Lecube A, Hernandez C, Genesca J et al. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. Diabetes Care 2004; 27: 1171-5.

70. Antonelli A, Ferri C, Fallai P et al. Hepatitis C virus infection: evidence for an association with type 2 diabetes. Diabetes Care 2005; 28: 2548-50.
71. Mangia A, Schiavone G, Lezzi G et al. HCV and diabetes mellitus: evidence for a negative association. Am J Gastrenterol 1998; 93: 2363-7.
72. Mheta SH, Brancati FL, Strathdee SA et al. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 2000; 133: 592-9.
73. Mheta SH, Brancati SL, Strathdee SA, et al. Hepatitis C Infection and incident type 2 diabetes. Hepatology 2003; 38: 50-6.
74. Baid S, Cosimi AB, Farrell ML et al. Post-transplant diabetes mellitus in liver transplant recipients: risk factors, temporal relationship with hepatitis C virus allograft hepatitis and impact on mortality. Transplantation 2001; 72: 1066-72.
75. The Data collection on Adverse events of anti –HIV drugs (DAD) Study Group Combination Antiretroviral Therapy and the Risk of Myocardial infarction. N Engl J Med 2003; 349: 1993-2003.
76. Beregszaszi M, Dollfus C, Levine M et al. Longitudinal evaluation and risk factors of lipodystrophy and associated metabolic changes in HIV-infected children. J Acquir Immun Def Syndr 2005; 40: 161-8
77. Hommes MJ, Romijin JA, Endert E et al. Insulin sensitivity and Insulin clearance in human immunodeficiency virus infected-men. Metabolism 1991; 40: 651-6.
78. Gan SK, Samaras K, Thompson CH et al. Altered myocellular and abdominal fat partitioning predict disturbances in insulin action in HIV protease inhibitor related lipodystrophy. Daiabetes 2002; 51: 3163-9.
79. Aytung S, Reich D, Shapiro Le et al. Impaired IRS1/PI-3 Kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology 2003; 30: 1384-92.
80. Nelson DR, Lim HL,Marousis CG et al. Activation of tumor necrosis factor alpha system in chronic hepatitis C virus infection Dig Dis Sci 1997; 42: 2487-94.
81. Zybelberg H, Rimaniol AC, Pol S et al. Soluble tumor necrosis factor receptors in chronich heaptis C a correlation with histological fibrosis and activity. J Hepatol 1999; 30: 185-91.
82. Hotamisligil GS, Budavari A, Murray D et al. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of Tumor necrosis factor alpha. J Clin Invest 1994: 1543-9.
83. Ruan H, Hacohen N, Golub TR et al. Tumor necrosis factor suppresses adipocyte-specific genes and activities expression of pre adipocytes genes 3T3-L1 adipocytes: nuclear factor-B activation is obligatory. Diabetes 2005; 51: 1319-36.
84. Knobler H, Schattner A. TNF alfa, chronic hepatitis C and diabetes: a novel triad. QJM 2005; 98: 1-6.
85. Kawaguchi T, Yoshida T, Harada M et al. Hepatitis C virus down regulates insulin receptor substrates 1-2 through up-regulation of suppressor of cytokines signalling -3. Am J Pathol 2004; 165: 1499-508.
86. Donnelly KL, Smith Cl, Schwarzenberg SJ et al. Sources od fatty acid stored in liver and secreted via lipoproteins in patients with nonalcholic fatty liver disease. J Clin Invest 2005; 115: 1343-51.
87. Tsutsumi T, Suzuki T, Shimoike T et al. Interaction of hepatitis C virus core protein with retinoid X receptor alpha modulates its transcriptional activity. Hepatology 2002; 35: 937-47.
88. Okuda M, Li K, Beard MR et al. Mithocondrial injury, oxidative stress and antioxidant gene expression are induced by hepatitis C virus core protein. Gatrenterology 2002; 122: 366-75.
89. Dreyer C, Krey G, Keller H et al. Control of peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 1992; 68: 879-87
90. Dharancy S, Malapel M, Perlemuter G et al. Impaired expression of the peroxisome proliferators-activated receptor alpha during hepatitis C virus infection. Gatroenterol 2005; 128: 334-42.
91. Marchesini G,Moscatiello S, Di Domizio S. Obesity associated liver disease. J Clin Endocrinol Metab 2008; 93: S64-S80.
92. Adinolfi LE, Gambardella M, Andreana A. Steatosis accelerates the progression of liver damage of chronich hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001; 33: 1358-64.
93. Calle EE, Rodriguez C, Walker-Thurmond K et al. Overweight obesity and mortality from cancer in a prospectively studied cohort of US adults. NEJM 2003; 348: 1625-38.
94. Despres JP, Lenieux I. Abdominal obeisty and metabolic syndrome. Nature 2006; 444: 881-7.
95. Mayers MG, Cowley MA, Munzberg H et al. Mechanism of leptin action and leptin resistance. Annu Rev Physiol 2008; 70: 537-56.
96. Andreelli F, Foretz M, Knauf C et al Liver adenosine monophosphate – activated kinase-alpha 2 catalitic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but insulin. Endocrinol 2006; 147: 2432-41.
97. Rabe K, Lehrke M, Parhofer KG et al. Adipokines and insulin resistance. Mol Med 2008; 14: 741-51.
98. Tomita K, Azuma T, Kitamura N et al. Leptin deficiency anhances sensitivity of rats to alcoholic steatohepatitis through suppression of metallothionein. Am J Physiol Gastrointest Liver Physiol 2004; 287: G1078-G85.
99. Balasu Bramaniyan V, Murugaiyan G, Shukla R et al. Leptin downregualtes ethanol induced secretion of proinflammatory cytokines and growth factor. Cytokine 2007; 37: 96-100.
100. Roglans N, Vila L, Farre M et al. Impairment of hepatic STAT-3 activation and reduction of PPAR alpha activity in fructose-fed rats. Hepatology 2007; 41: 753-60.
101. Vila L, Roglans N, Algret M et al. Suppressor of cytokine signaling-3(SOCS-3) and deficit of serine/threonine

(Ser/Thr) phosphoproteins involved in leptin transduction mediate the effect of fructose on rat liver lipid metabolism. Hepatology 2008; 48: 1056-16.
102. Osei-Hyaman D, Liu J, Zhou L et al. Hepatic CB 1 receptor is required for development of diet –induced steatosis, dyslipidemia and insulin and leptin resistance in mice. J Clin Invest 2008; 118: 3160-9.
103. Cohen SE, Kokkotou E, Biddinger SB et al. High circulating leptin receptor with normal leptin sensitivity in liver specific insulin receptor knock –out (LIRKO) mice. J Biol Chem 2007; 282: 23672-8.
104. Ikejima K, Takei Y, Honda H et al. Leptin receptor-mediated signalling regulates hepatic fibrogenesis and remodelling of extracellular matrix in the rat. Gastroenterology 2002; 122: 1399-410.
105. Marra F. Leptin and liver tissue repair: do rodent models provide the answer? J Hepatol 2007; 46: 12-8.
106. De Minicis S, Candelaresi C, Marzioni M et al. Role of endogenous opioids in the modulating HSC activity in vitro and liver fibrosis in vivo. Gut 2008; 57: 352-64
107. Jiang JX, MIkami K, Shah WH et al. Leptin induces phagocytosis of apoptotic bodies by hepatic stellate cells via a RHO-guanosine tryphos-phatase-dependent mechanism. Hepatology 2008; 48: 1497-505.
108. Ding X, Saxena NK, Lin S et al. The roles of leptin and adiponektin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. Am J Pathol 2005; 166: 1655-69.
109. Sierra-Honigmann MR, Nath AK, Murakami C et al. Biological action of leptin as an angiogenic factor. Science 1998; 281: 1683-6.
110. Aleffi S, Petrai I, Bertolani C at al. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. Hepatology 2005; 42: 1339-48.
111. Kitade M, Yoshiji H, Kojima H et al. Leptin – mediated neo-vascularization is a prerequisite for progression of non alcoholic steatohepatitis in rats. Hepatology 2006; 44: 983-91.
112. Ribatti D, Belloni AS, Nico B et al. Leptin-leptin receptor are involved in angiogenesis in human hepatocellular carcinoma. Peptides 2008; 29: 1596-602.
113. Saxena NK, Sharma SK, Ding X et al. Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signalling is involved in leptin mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res 2007; 67: 2497-507.
114. Fava G, Alpini G, Rychlicki C et al. Leptin enhances colangio-carcinoma cell growth. Cancer Res 2008; 68: 6752-61.
115. Ramani K, Yang H, Xia M et al. Leptins mithogenic effect in human liver cancer cells require induction of both methionine adenosyltranferase 2A and 2 Beta. Hepatology 2008; 47: 521-31.
116. Uygun A, Kadayfci A, Yesilova Z et al. Serum leptin levels in patients with non alcoholic steatohepatitis. Am J Gastroenterol 2000; 95: 3584-89.
117. Manco M, Alisi A, Nobili V et al. Risk of severe liver disease in NAFLD with normal ALT levels a pediatric report. Hepatology 2008; 48: 2087-88.
118. Chalasani N, Crabb W, Cummings OW et al. Does leptin paly a role in the pathogenesis of human non alcoholic steatohepatitis ? Am J Gastroenterol 2003; 98: 2771-6.
119. Zografos TA, Rigopulou EI, Liaskos C et al. Alterations of leptin during IFN alfa therapy in patients with chronic viral hepatitis. J Hepatol 2006; 44: 848-55.
120. Giannini E, Ceppa P, Botta F et al. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. Am J Gastroenterol 2000; 95: 3211-7.
121. Kadowaki T, Yamauchi T, Kubota N et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116: 1784-92.
122. Xu A, Wang Y, Keshaw H et al. The fat derived hormone adiponectin alleviates alcoholic and non alcoholic fatty liver disease in mice. J Clin Invest 2003; 112: 91-100.
123. You M, Considine RW, Leone TC et al. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. Hepatology 2005; 47: 867-79.
124. Song Z, Zhou Z, Deaciuc I et al. Inhibition of adiponectin production by omocysteine: a potential mechanism for alcoholic liver disease. Hepatology 2008; 47: 867-79.
125. Masaki T, Chiba S, Tatsukawa H et al. Adiponectin protects LPS-induced liver injury trough modulation of TNF-alpha in KK-Ay obese mice. Heaptology 2004; 40: 177-84.
126. Kim JY, van de Wall E, Laplante M et al. Obesity – associated improvement in metabolic profile through expantion of adipose tissue. J Clin Invest 2007; 117: 2621-37.
127. Lin HW, Kim JY, Pocai A et al. Adiponectin resistance exacerbates insulin resistance in insulin receptor transgenic/knockout mice. Diabetes 2007; 56: 1969-76.
128. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat rev Immunol 2006; 6: 772-83.
129. Hopkins TA, Ouchi N, Shibata R et al. Adiponectin actions in the cardiovascular system. Cardiovasc Res 2007; 74: 11-8.
130. Sennello JA, Fayad R, Morris AM et al. Regulation of T cell mediated hepatic inflammation by adiponectin and leptin. Endocrinology 2005; 146: 2157-64.
131. Kamada Y, Tamura S, Kiso S et al. Enhanced carbon tetracloride-induced liver fibrosis in mice laking adiponectin. Gastreonterol 2003; 125: 1796-07.
132. Lemoine M, Ratziu V, Kim M et al. Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. Liver Int 2009; 29: 1431-8.
133. Targher G, Bertolini L, Rodella S. et al. Associations between plasma adiponectin concentrations and liver histology in patients with non alcoholic fatty liver disease. Clin Endocrinol (Oxf) 2006; 64: 679-83.

134. Durante-Mangoni E, Zampino R, Marrone A et al. Hepatic steatosis and insulin resistance are associated with serum imbalance of adiponectin-tumour necrosis factor-alpha in chronic hepatitis C patients. Aliment Pharmacol Ther 2006; 24: 1349-57.
135. Wang AY, Hickman IJ, Richards AA et al. High molecular weight adiponectin correlates with insulin sensitivity in patients with hepatitis C genotype 3, but not genotype 1 infection. Am J Gastroenterol 2005; 100: 2717-23.
136. Zografos TA, Liaskos C, Rigopoulou EI et al Adiponectin: a new independent predictor of liver steatosis and response to IFN-alpha treatment in chronic hepatitis C. AM J Gastroenterol 2008; 103: 605-14.
137. Steppan CM, Bailey ST, Bhat S et al. The hormone resistin links obesity to diabetes. Nature 2001; 409: 307-12.
138. Singhall NS, Patel RT, Qi Y et al. Loss of resistin ameliorates hyperlipidemia an hepatic steatosis in leptin deficient mice. Am J Physiol Endocrinol Metab 2008; 295: E331-E8.
139. Beier JI, Guo L, von Montfort C et al. New role of resistin in lipopolysaccaride-induced liver damage in mice. J Pharmacol Exp Ther 2008; 325: 801-8.
140. Lin SY, Sheu WH, Chenn WY et al. Stimulated resistin expression in white adipose of rats with bile duct ligation induced live cirrhosis: relationship to cirrhotic hyperinsulinemia and increased tumor necrosis factor alfa. Mol Cell Endocrinol 2005; 232: 1-8.
141. Yamaguchi A, Tazuma S, Nishioka T et al. Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver. Dig Dis Sci 2005; 50: 1361-71.
142. Andre P, Komurian-Pradel F, Deforges S et al. Characterization of low and low-very- density hepatitis C virus RNA-containing particles. J Virol 2002; 76: 6919-28.
143. Favre D, Muellhaupt B. Potential cellular receptors involved in hepatitis C entry into cells. Lipid Dis 2005; 4: 1-6.
144. Chiari FV. Unscrambling hepatitis C virus host interactions. Nature 2005; 1436: 930-2.
145. Barba G, Harper F, Harada T et al. Hepatitis C virus core protein shows a cytoplasmic localization and associate sto cellular lipid storage droplets. Proc Natl Acad Sci USA 1997; 94: 1200-5.
146. Lapadula G,Torti C, Paraninfo G et al. Influence of hepatitis C genotypes on lipid levels in HIV positive patients during highly active antioretroviral therapy Antiv Ther 11: 5121-7.
147. Johnson JA, ALbu JA, Engfelson ES et al. Increased proinflamamtory cytokines activity in HIV infcetd subjects with lipodystrophy. Am J Phsyol Endocrinol Metab 2004; 286: E261-E71.
148. Kannisto K, Sutinen J, Korsheninnikova E et al. Expression of adipogenic transcription factos, peroxisome proliferators activated receptor gamma co-activator, IL6 and CD 45 in subcutaneous adipose tissue in lipodystrophy associated with highly active antiretroviral therapy. AIDS 2003; 17: 1503-11.
149. Mynaricik DC, Combs T, Mc Nurlan MA et al. Adiponectin and leptin levels in HIV infected subjects with insulin resistance and body fat redistribution. J Acquir Immunodef Syndr 2002; 31: 514-20.
150. Christeff N, Melchior JC, de Turchis P et al. Increase serum Interferon alpha in HIV-1 associated lipodistrophy. Eur J Clin Invest 2002;32:43-50
151. Canabay A, Friedman S, Gores GJ Apoptosis: the nexus of liver injury and fibrosis. Hepatology 2004; 39: 273-8.
152. Mann DA, Smart DE.Transcriptional regulation of hepatic stellate cells activation. Gut 2002; 50: 891-6.
153. Cassman D, Roskams T.Beauty is in the eye of the beholder: emergine concepts and pitfails in hepatic stellate cell research. J Hepatol 2002; 37: 397-416.
154. Pinzani M, Marra F. Cytokine receptors and signalling in hepatic stellate cells Semin Liver Dis 2001; 21: 397-416.
155. Marra F. Chemokines in liver inflammation and fibrosis. Fron Biosci 2002; 7: d1899-1914.
156. Kumar D, Farrell GC, Kench J et al. Hepatic steatosis and the risk of hepatocellular carcinoma in chronic C Hepatology 2005; 20: 1395-400.
157. Hui JM, Sud A, Farrell GC et al. Insulin resistance is associated with chornich hepatitis C and virus infection fibrosisi progression. Gastroenterology 2003; 125: 1003-8.
158. Moucari R, Ripault MP, Martinot-Peignoux M, et al.Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. Gut 2009; 58: 1662-9.
159. Merchante N, Rivero A, de Los Santos-Gil I, et al. Insulin resistance is associated with liver stiffness in HIV/HCV co-infected patients. Gut 2009;58:1654-60
160. Rachfal AW, Brigstock DR. Connective tissue growth factor (CTGF/CCN2) in hepatic fibrosis. Hepatol Res 2003; 26: 1-9.
161. Shin JY,Hur W,Wang JS et al. HCV core protein promotes liver fibrogenesis via up-regulation of CTGF with TGF-β1. Exp Mol Med 2005; 37: 138-45.
162. Hui JM, Hodge A, Farrell GC et al. Beyond Insulin resistance in NASH: TNF alpha or adiponectin? Hepatology 2004; 40: 46-54.
163. Yamauchi T, Kamon J, Waki H et al. The fat derived hormone adiponectin reverses insulin resistance associated with lipoatrophy and obesity. Nat Med 2001; 7: 941-6.
164. Kamada Y,Tamura S, Kiso S et al. Enhanced carbon tetrachloride induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003; 125: 1796-807.
165. Lagasthu C, Eustace B, Prot M et al. Some HIV Antiretrovirals increase oxidative stress and alter chemokin, cytokine or adiponectin production in human adipocytes and macrophages, Antiv Ther 2007; 12: 489-500.
166. Chalasani N, Crabb DW, Cummings OW et al. Does leptin play a role in the pathogenesis of human nonalcholic steathoepatitis? Am J Gastroenterology 2003; 98: 2771-76
167. Brinkkman K, Smeitink JA, Romijin JA et al. Mithocondrial toxicity induced by nucleosides-anologue –reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral therapy related lipodystrophy. Lancet 1999; 354: 1112-5.

168. Lo JC, Kazemi MR, Hsue PY et al. The relationship between nuclesoide anologue treatment duration, insulin resistance and fasting arterialized lactate level in patients with chronic HIV infection. Clin Infect Dis 2005; 41: 1335-40.
169. Hadigan C. Insulin resistance among HIV infected patients: unravelling the mechanism. Clinc Infect Dis 2005; 41: 1341-2
170. Carr A, Samaras K, Burtoin S et al. A syndrome of peripheral lipodystrophy, hyperlipidemia and insulin resistance in patients receiving protease inhibitors. AIDS 1998; 12: F51-F58.
171. Murata H, Hruz PW, Mueckler M. The mechanism of Insulin resistance caused by HIV protease inhibitor therapy. J Biol Chem 2000: 275: 20251-4.
172. Noor M, Flint OP, Maa JF et al. Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity: demonstrable differences in vitro and clinically. AIDS 2006; 20: 1813-82.
173. Noor M, Flint OP, Maa JF et al. Effects of atazanavir and lopinavir/ritonavir on insulin sensitivity in HIV seronegative healthy adults. AIDS 2004; 18: 2137-44.
174. Lee GA, Mafong DD, Noor MA et al. HIV protease inhibitor increase adiponectin levels in in HIV negative men. J Acquir Immunodef Syndr 2004; 36: 645-7.
175. Lee G, Rao MN, Schwarts JM et al. A single dose of amprenavir does not induces insulin resistance in healthy normal volunteers. Antiv Ther 2005;10 (Suppl 3)L11(abstract 15).
176. Dube MP, Qian D, Edmondson –Melancon H et al. Prospective intensive study of metabolic changes associated with 48 week of amprenavir based antiretroviral therapy. Clin Infect Dis 2002; 35: 475-81.
177. Villarroya F, Domingo P, Giralt M. Mechanisms of antiretroviral-induced mitochondrial dysfunction in adipocytes and adipose tissue: in-vitro, animal and human adipose tissue studies.Curr Opin HIV AIDS. 2007; 2: 261-7.
178. Ben-Romano R, Rudich A, Etzion S et al. Nelfinavir induces adipocyte insulin resistance through the induction of oxidative stress: differential protective effect of antioxidant agents. Antivir Ther 2006; 11: 1051-60.
179. Lagathu C, Eustace B, Prot M et al. Some HIV antiretroviral increase oxidative stress and alter chemokine, cytokine or adiponectin production in human adipocytes and macrophages Antiviral Ther 2007; 12: 489-500.
180. Adler-Wailes DC, Guiney EL, Koo J, Yanovski JA. Effects of ritonavir on adipocyte gene expression: evidence for a stress-related response. Obesity 2008; 16: 2379-87.
181. Lagathu C Kim M, Maachi M et al. HIV antiretroviral treatment alters adipokine expression and insulin sensitivity of adipose tissue in vitro and in vivo. Biochoimie 2005; 877: 65-71.
182. Lagasthu C, Eustace B, Prot M et al. Some HIV Antiretrovirals increase oxidative stress and alter chemokin, cytokine or adiponectin production in human adipocytes and macrophages. Antiv Ther 2007, 12: 489-500.
183. Jones SP, Waitt C, Sutton R, Back DJ, Pirmohamed M. Effect of atazanavir and ritonavir on the differentiation and adipokine secretion of human subcutaneous and omental preadipocytes. AIDS 2008; 22: 1293-8.
184. Kim RJ, Wilson CG, Wabitsch M, HIV protease inhibitor-specific alterations in human adipocyte differentiation and metabolism. Obesity 2006; 14: 994-1002.
185. Vernochet C, Azoulay S, Duval D et al. Differntial effect of HIV protease inhibition on adipogenesis: intracellular ritonavir is not sufficient to inhibit differentiation. AIDS 2003; 17: 2177-80.
186. Jenneh O, Hoggard PG, Tjia JF et al. Intracellular disposition and methabolism effects of zidovudine, stavudine and four protease inhibitors in culture adypocytes. Antiv Ther 2003; 8: 417-26.
187. Ledru E, Christeff N, Patey O et al. Alteration of tumor necrosis factor alpha T cell homeostasis following potent antiretroviral therapy: contribution to the development of human immunodeficiency virus associated lipodystrophy syndrome Blood 2000; 95: 3191-8.
188. Mynaricik DC, Mc Nurlan MA; Steigbigel RT et al. Association of severe insulin resistance with booth loss of limb fat and elevated serum tumor necrosis factor receptor level in HIV lipodysrtophy. J Acquir Immuniodef Synd 2000; 25: 312-21.
189. Lehard JM, Furfine ES, Jain RG et al HIV protease inhibitors block adipogenesis and increase lipolysis in vitro. Antiviral Research 2000; 47: 121-9.
190. Carper MJ, Cade WT, Cam M, et al HIV-protease inhibitors induce expression of suppressor of cytokine signaling-1 in insulin-sensitive tissues and promote insulin resistance and type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 2008; 294: E558-67.
191. Romero-Gomez M, Viloria MM, Andrade RJ et al.Insulin resistance impairs sustained response rate to peginterferone plus ribavirin in chronic hepatits C patients. Gastroenterology 2005; 128: 636-41.
192. Poynard T, Ratzu V, McHutchinson J et al. Effect of treatment with Peghinterferon of Interferon alpha 2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003; 38: 78-85.
193. Patton HM, Patel K,Behing C et al. The impact steatosis on diseases progression and early and sustained viral response in chornic hepatitis C patients. J Hepatol 2004; 40: 484-90.
194. Piche T, Vandembos F, Abakar-Mahamat A et al. The severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C. J Vir Hepatol 2004; 11: 91-96.
195. Williams J, Lang-Lenton M. Progression of initially mild hepatic fibrosis in patients with chronich hepatitis C infection. J Viral Hepat 2010 (in press).
196. Tein P, Bacchetti P, Kotle D et al. Association between visceral adipose tissue and serum amonotransferase levels in HIV infected individuals with and without hepatitis c virus infection. 3rd International Workshop on HIV and hepatitis co-infection. June 2007, Paris. (Abstract 66).
197. Nasta P, Gatti F, Puoti M et al. Insulin resistance impairs rapid virologic response in HIV/hepatitis C virus coinfec-

ted patients on peginterferon-alfa-2a. AIDS 2008 23; 22: 857-61.
198. Nasta P, Puoti M, Cologni G et al. Insulin resistance impairs rapid and early virologic response in HIV/HCV coinfected patients treated with Pegylated Interferon alpha 2a and Ribavirin. 3rd International Workshop on HIV and hepatitis co-infection. June 2007, Paris. (Abstract 67).
199. Cacoub P, Carrat F, Bédossa P, et al Insulin resistance impairs sustained virological response rate to pegylated interferon plus ribavirin in HIV-hepatitis C virus-coinfected patients: HOMAVIC-ANRS HC02 Study. Antivir Ther. 2009; 14: 839-45.
200. Dai CY, Huang JF, Hsieh MY et al. Insulin resistance predicts response to peginterferon-alpha/ribavirin combination therapy in chronic hepatitis C patients. J Hepatol 2009; 50: 712-8.
201. del Valle J, Mira JA, de los Santos I et al. Baseline serum low-density lipoprotein cholesterol levels predict response to hepatitis C virus therapy in HIV/hepatitis C virus coinfected patients. AIDS. 2008; 22: 923-30.
202. Nasta P, Gatti F, Borghi F. Metabolic profile is a key factor for virologic success of peginterferon alpha a/ribavirin (PEgIFN/RBV) treatmen in HIV/HCV coinfectes patients. Fourth International Workshop on HIV and Hepatitis coinfection 19-21 June 2008 Madrid (abstract 37).
203. Grace MJ, Culter D. pegylated IFNs at HIS-34 improves the in vitro antiviral activity through JAK/STAT pathways. Antiviral Chem Chem 2004; 15: 287-97.
204. Sanjal AJ, Chand N, Comar K et al. Hyperinsulinemia block the inhibition of HCV replication by interferon: A potential mechanism failure of interferon therapy in subjects with HCV and NASH. Hepatology 2004; 40: 179 A.
205. Ancuta P, Kamat A, Kunstman KJ et al. Microbial Translocation Is Associated with Increased Monocyte Activation and Dementia in AIDS Patients PLoS ONE. 2008; 3(6): e2516.
206. Balagopal A, Philp FH, Astemborski J et al. Human Immunodeficiency Virus-related Microbial Translocation and Progression of Hepatitis C Gastroenterology. 2008 July; 135: 226-33.
207. Luther S.A and J. G Cyster. Chemokines as regulators of T cells differentiations. Nat Immunol 2001; 2: 102-7.
208. Zlotnick A and O Yoshie. Chemokines: a new classification system and their role in immunity. Immunity; 2000: 12: 121-7.
209. Lotscher PM, Uguccioni L, Bordali M et al. CCR5 is characteristic of Th1 lymphocytes. Nature 1998; 391: 344-5.
210. Sallusto F, Lenig D, MAckay CR et al Flexible programme of chemokine receptor expression in human polarized T helper 1 and 2 limphocytes J Exp Med 1998; 187: 875-83.
211. Oppermann M. Chemokine receptor CCR5: insides into structure, function and regulation. Cell Signal 2004; 16: 1201-10
212. Bursill CA, Choudhaury RP, Ali Z et al. Brad spectrum CC chemokine blckade by gene transfer inhibits macrophage recruitment and atherosclerotic plaque formation in apoliprotein E knockout mice. Circulation 2004; 110: 2460-6.
213. Puhakka HL, Turunen P, Gruchala M et al. Effects of vaxinia virus anti-inflammatory proteins 35K and TIMP-1 gene transfers on vein graft stenosis in rabbits. In vivo 2005; 19: 5151-21.
214. Avanzas P, Arroyo-Espliguero R, Cosin Sales J et al. Multiple complex stenosis, high neurtophil counts and C reactive protein level in patients with chronic stable angina. Atherosclerosis 2004; 175: 151-7.
215. Han KH, Hong KH, Park JH et al. C reactive protein promotes monocyte chemoattractant protein -1 mediated chemotaxis through upregulating CC chemokine receptor 2 expression in Human monocytes. Circulation 2004; 109: 2566-71.
216. Veillard NR, Kwak B, Pelli G et al. Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice. Circ Rres 2004; 94: 253-61.
217. Pfleger C, Kaas A, Hansen L et al. Relation of circulating concentration of chemokine receptor CCR5 ligands to C peptide, proinsulin and HbA1c and disease progression in type-1 diabetes. Clinical Immunol 2010 (in press).
218. Allkatib G, Combadiere C, Brider C. et al. CC CKR5: a RANTES, MIP-a, MIP-b receptor as a fusion cofactor for macrophage-tropic HIV-1 Science 1996; 272; 1995-8.
219. Wald O, Pappo Z, Azzaria E et al. The CCR5D32 allele is associated with reduced liver inflammation in hepatitis C virus infection. European J Immunogenetics 2004; 31: 249-52.
220. Goulding C, Murphy A, Mac Donald G et al The CCR5 D 32 mutation: impact on disease outcome in individual with hepatitis C infection from a single source. Gut 2005; 54: 1157-61.
221. Zapico I,Coto E,Rodriguez A et al. CCR5(chemokine receptor 5) DNA polimorphism influnces the severity of rheumatoid arthritis.Genes Immun 2000; 1: 288-9.
222. Afzal AR, Kiechl S, Daryani YP et al. Common CCR5 Del 32 frameshift mutation associated with serum levels of inflammatory markers and cardiovascular disease risk in the bruneck population. Stroke 2010; 39 (in press).
223. Taniguchi M, Seino K and Nakayama T. The NKY cell system: bridging innate and acquired immunity. Nat Immunol 2003; 4: 1164-5.
224. Shields P, Morland M, Salmon S et al.Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C infected liver. J Immunol 1999; 163: 6236-43.
225. Boisvert J, KInkel J, Cambpell E et al. Liver-infiltrating limphocytes in end stage hepatitis C virus: subset, activation status and chemokines recpetor phenotypes. J Hepatol 2003; 38: 67-75.
226. Lechner F, Cuero A,Kantzanou M Studies of human antiviral CD8+ limphocytes using class 1 peptides tetramers. Rev Med Virol 2001; 11: 11-22.
227. Seki E, De Minicis S, Gwak GY et al. CCR1 and CCR5 promote hepatic fibrosis in mice. J Clin Invest 2009; 119: 1858-70.

228. Muhanna N, Doron S, Wald O et al. Activation of hepatic stellate cells after phagocytosis of lymphocytes: A novel pathway of fibrogenesis. Hepatology 2008; 48: 963-77.
229. Zeremeski M, Petrovic LM, Talal AH The role of chemokines as inflammatory mediators in chronic hepatitis C virus infection. J Viral hepatol 2007; 14: 675-87.
230. Yang F, Tomura M, Iwasaki S et al. IFN alfa acts on T cell receptor- triggered human peripheral leukocytes to up regulate CCR5 espression on CD4+ and CD8+ T cell. J Clin Immunol 2001; 21: 402-29.
231. Tam R, Pai J, Bard C et al. Ribavirin polarizes human T cells response towards a type 1 cytokine profile. J Hepatol 1999; 30: 376-82.
232. Woitas RP,Ahlenstiel A, Iwan JK et al. Frequency of the HIV protective CC chemokine receptor 5 del 32 /del 32 genotype is increased in hepatits C. Gastroenterology 122: 1721-8.
234. Mangia A, Santoro R, d'Agruma L et al. HCV chronich infection and CCR5 del32/del32. Gastrenterology 2003; 124: 186-869.
235. Kusano F, Tanaka F,Marumo F et al. Expression of C-C chemokine is associated with portal and periportal inflammation in the liver of patients with chronich hepatitis C. Lab Invest 2000; 80: 415-22.