OBSERVATIONAL STUDY

Insulin resistance and steatosis in HBV-HCV co-infected patients: Role of PNPLA3 polymorphisms and impact on liver fibrosis progression

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Abstract

AIM: To evaluate steatosis, insulin resistance (IR) and patatin-like phospholipase domain-containing 3 (PNPLA3) and their relation to disease progression in hepatitis B and C viruses (HCV-HBV) co-infected patients.

METHODS: Three hundred and thirty patients with biopsy proven chronic hepatitis were enrolled: 66 had HBV-HCV, 66 HBV and 198 HCV infection. Prevalence of steatosis, IR and PNPLA3 polymorphisms and their relation to anthropometric, biochemical, virological and histological parameters were evaluated.

RESULTS: Prevalence of steatosis in group HBV-HCV was similar to that in HCV (47.0% *vs* 49.5%, respectively); group HBV showed the lowest steatosis (33.3%). Group HBV-HCV had a lesser degree of steatosis than HCV (*P* = 0.016), lower HCV RNA levels (*P* = 0.025) and lower prevalence and degree of IR (*P* = 0.01). PNPLA3 polymorphisms were associated with steatosis. Group HBV-HCV showed higher levels of liver fibrosis than group HCV (*P* = 0.001), but similar to that observed in HBV group. In HBV-HCV group, liver fibrosis was not associated with steatosis, IR or PNPLA3. HBV infection was the independent predictor of advanced liver fibrosis.

CONCLUSION: HBV-HCV co-infected patients have lower degree of hepatic steatosis, IR and HCV RNA than HCV mono-infected; co-infected patients showed a more rapid liver fibrosis progression that seems to be due to the double infection and/or HBV dominance.

Core tip: We evaluated the prevalence and role of steatosis, insulin resistance and patatin-like phospholipase domain-containing 3 (PNPLA3) polymorphisms on disease progression in hepatitis B and C viruses (HCV-HBV) co-infected patients. The data showed that HBV-HCV patients have lower levels of liver steatosis and insulin resistance than HCV mono-infected patients. HBV seems to interact with HCV reducing HCV replication and HCV-related metabolic features. Thus, the influence of HCV-related steatosis and insulin resistance as well as PNPLA3 polymorphism do not significantly impact liver fibrosis progression in HBV-HCV patients. The more rapid progression of liver fibrosis observed in HBV-HCV co-infected patients seems to be mostly associated with HBV infection.

INTRODUCTION

Liver steatosis is a feature of chronic hepatitis C virus (HCV) infection[1-3]. HCV genotype 3 directly induces the highest degree and prevalence of steatosis (up to 80%), whereas HCV-related steatosis in non-3 genotypes is mainly associated with metabolic conditions[4]. A close association between steatosis and insulin resistance (IR) has been reported in HCV non-3 genotype-infected patients, but, normally, insulin resistance is not a feature of genotype-3 infection[2]. In HCV infection, IR precedes the development of steatosis and modulates fatty liver deposition through several, non-mutually exclusive, mechanisms; the appearance of steatosis, in turn, worsens IR[2,5]. Furthermore, it has been reported that in genotype-1 infection, IR correlates with the serum level of HCV RNA[6-7]. Both steatosis and IR are associated with a more rapid progression of liver fibrosis[1,8]. In chronic hepatitis B virus (HBV) infection, hepatic steatosis has been reported with a lower prevalence[9,10] than that observed in HCV infection, although one report[11] showed a high prevalence of steatosis in HBV-infected patients. Furthermore, in HBV infection, steatosis seems to be related to metabolic factors and does not seem to correlate with histological hepatic damage[9,12-14]. Recently, the single nucleotide polymorphism (SNP) of the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene, involved in the lipid metabolism, has been associated with liver steatosis in chronic hepatitis[15-20].

Chronic HBV-HCV co-infection is infrequent, but it is associated with a more severe clinical presentation[21-25] and with a more rapid progression to liver cirrhosis and hepatocellular carcinoma[26-28]. There are no direct data on liver steatosis and IR in patients with HBV-HCV co-infection, nor on their impact on the progression of the liver disease. During HBV-HCV co-infection, a reciprocal inhibition of the viral genomes has been reported[29-31] and this condition, especially in HCV-genotype-1 infection, could influence the development of IR and steatosis. Thus, at present, it remains unclear whether HBV infection affects the prevalence and level of steatosis and IR in HBV-HCV co-infected patients and their impact on liver disease progression.

Accordingly, the aim of this study was to evaluate the prevalence and degree of liver steatosis and IR and their role in the progression of liver disease in a cohort of HBV-HCV co-infected patients as compared with a cohort of HBV and HCV mono-infected patients. The role of the viral and host metabolic and genetic factors, such as PNPLA3 polymorphisms, was also evaluated.

MATERIALS AND METHODS

Patients

Three hundred and thirty Caucasian patients with histology proven chronic hepatitis were enrolled in the study. Sixty-six were HBV-HCV co-infected patients, 66 HBV mono-infected (ratio 1:1) and 198 HCV mono-infected (ratio 1:3). HBV-HCV co-infected patients were age-, gender-, and HCV genotype-matched with control mono-infected groups. The study was conducted from 2009 to 2013. However, considering the low prevalence of HBV-HCV co-infected patients, all HBV-HCV co-infected patients recorded in the data base from 2006 were enrolled if there was a serum sample stored at -30 ℃ at the time of the liver biopsy and if there was a sample available for genetic purposes.

Patients were recruited from four Liver Units (see author's affiliations) of the Second University of Naples, Italy. The patients were considered co-infected and enrolled in the study if they were HBs Ag positive/HCV-Ab positive/HBV-DNA and HCV-RNA positive; all patients HBV and HCV mono-infected were HBV-DNA positive and HCV-RNA positive, respectively. All patients included were anti-HIV- and anti-HDV-negative, naive for antiviral therapy and reported no active intravenous drug addiction or daily alcohol intake over 30 g. The possible source of infection was identified only in the minority of the enrolled patients; in fact, anamnestic blood transfusion was present in 8%, previous surgery in 4%, a family history of hepatitis infection in 4%, and a past history of drug abuse in 1.8%. All patients underwent complete physical examination, full liver function tests, fasting glucose, triglycerides, cholesterol, blood cell counts, viral markers (HBV, HCV, HDV, HIV) and liver ultrasound scan. The body mass index (BMI: kg/m^2) and waist circumference were recorded for all patients. Visceral obesity was defined as waist circumference > 102 cm in male and > 88 in female. An anamnestic estimation of possible duration of infection was made. All laboratory data presented in this study refer to the values at the time

of the liver biopsy. All blood samples were withdrawn at the time of liver biopsy and serum were stored at -30 ℃ within two hours from collection.

Serum insulin and homeostasis model assessement-insulin resistance

Serum insulin was evaluated using human insulin immunoassay (Insulin Cobas, Roche Diagnostics, Indianapolis, IN, United States). IR was determined by homeostasis model assessement-insulin resistance (HOMA-IR) using the following formula: fasting plasma glucose (mmol/dL) x fasting serum insulin (IU/mL)]/22.5. To establish the cut-off level of IR in our population, HOMA-IR was evaluated in 130 healthy subjects and the cut-off value was set at the 75th percentile of the HOMA-IR value in our mono-infected control groups[32] that was 2.60. In the three groups evaluated the determination of levels of insulinemia and glycaemia were done at the same time using the stored serum and samples from the three groups were analysed in parallel.

Liver histology

All patients gave their informed consent for liver biopsy. Liver specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin and Masson's trichrome stain and evaluated in a blinded way by the pathologist. The Ishak scores to grade necro-inflammation- and fibrosis were used[33]. Steatosis was scored as follows: 0, if less than 5% of hepatocytes had fatty deposition; (1) from 5% to 29%; (2) from 30% to 59%; and (3) > 60%.

All the evaluations were conducted in accordance with good clinical practice and with the Helsinki Declaration. The local Ethics Committee approved the study.

Serological determinations

Serum markers for HBV, HCV, HDV and HIV infection were sought in serum using commercially available immune-enzymatic assays (Abbott Laboratories, North Chicago, IL and Ortho Diagnostic Systems, Raritan, NJ).

HBV and HCV genotypes and viral load

Hepatitis B virus genotypes were determined by phylogenetic analysis of sequences of 400 nt of the S region, as previously described[34]. HCV genotypes were determined using immunoblotting HCV genotype assay Lipa (VERSANT HCV Genotype 2.0 Assay (LIPA), Siemens, Erlangen, Germany) following the manufacturer's instructions. HBV DNA and HCV RNA viral load were assessed by real-time PCR using commercial kits (COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0, COBAS® TaqMan® HCV Test v2.0; Roche diagnostics, S.p.A. Monza, Italy).

PNPLA3 polymorphism study

Genomic DNA was extracted from whole blood by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and analyzed for the PNPLA3 polymorphism. All patients were genotyped for the PNPLA3 rs738409 C to G variant underlying the I148M substitution. The following prim-

ers were used, F: 5'-GCCCTGCTCACTTGGAGAAA-3' and R: 5'-TGAAAGGCAGTGAGGCATGG-3'. The FokI restriction enzyme, as previously described, was used to identify the variant, since the G allele eliminates a FokI restriction site. Random samples were confirmed by direct genotyping, which provided concordant results in all cases[35].

Statistical analysis

The data are expressed as mean or median. Differences between the groups were evaluated by Student's *t* test for parametric data and by the Mann-Whitney *U* test for non-parametric data. Spearman's correlation test was used to identify factors significantly associated. The Chi-square test was used to evaluate differences in the prevalence. The analysis of variance was used to evaluate the different distribution of steatosis in the genetic polymorphisms. Logistic regression analysis was used to evaluate the independent factors associated with advanced liver fibrosis. The data were analysed using SPSS 13.5, and *P* < 0.05 was assumed to denote significance.

RESULTS

General characteristics of patients

The general characteristics of the study population are shown in Table 1. The three groups were comparable for demographic and anthropometric parameters. The approximate duration of the disease and the lipid profile were similar in the three groups. Waist circumference was similar in groups HCV-HBV and HCV (91.7 ± 9.8 and 90.2 ± 10, respectively). The serum glucose levels were lower, albeit not significantly, in group HBV-HCV than in group HCV (*P* = 0.09). The AST values were significantly lower in the co-infected patients than in the HBV mono-infected (*P* = 0.02), while the ALT values were significantly lower in the co-infected than in the HBV and HCV mono-infected (*P* = 0.001).

Virological characteristics of patients

The median values of the HBV DNA and HCV RNA levels are shown in Table 1. HBV-HCV co-infected patients showed lower levels of HBV DNA and HCV RNA than those observed in the HBV and HCV mono-infected patients (*P* = 0.0001 and *P* = 0.025, respectively). The majority (93%) of the HCV patients were infected by genotype non-3 (Table 1) and 98% of HBV patients had genotype D.

Steatosis and IR

Table 2 shows the prevalence and degree of steatosis in the three groups. There was no difference in the prevalence of liver steatosis between group HBV-HCV and group HCV (47.0% *vs* 49.5%), but a lower prevalence of steatosis was observed in group HBV (34%). An analysis of the degree of steatosis showed higher levels (scores 2-3) in group HCV than in group HBV-HCV (*P* = 0.016, Table 2). The above results did not change when the

Table 1 General characteristics of the 330 patients include in the study

	HBV-HCV group	HBV group	HCV group	P
No. of patients	66	66	198	
Medianage (range)	48.5 (25-67)	47 (23-65)	50 (22-65)	NS
Males	60.60%	63.60%	55.50%	NS
Disease duration (yr ± SD)	22.3 ± 9.7	21 ± 7.6	23.2 ± 8.4	NS
BMI (mean ± SD)	25.7 ± 3	26 ± 4.5	26.7 ± 4	NS
Glycaemia (mean ± SD) mg/dL	90 ± 13.4	85.8 ± 14.4[a]	95 ± 20[b]	0.09 (a vs b)
HOMA	2.48 ± 2.65[c]	2.0 ± 1.17	3.63 ± 4.5[d]	0.042 (c vs d)
Cholesterol (mean ± SD) mg/dL	182 ± 34	182 ± 31	182 ± 41	NS
Triglycerides (mean ± SD) mg/dL	109 ± 55	85 ± 29	103 ± 53	NS
AST (mean ± SD) IU/L	55 ± 39[e]	83 ± 84[f]	65 ± 52	0.02 (e vs f)
ALT (mean ± SD), IU/L	44 ± 62.5[g]	124.95 ± 92[h]	90 ± 74[i]	0.001 (g vs h) 0.001 (g vs i)
				0.01 (h vs i)
Median HBV DNA (range) IU/mL	1.9×10^3 (1500-10 × 10^7)	2×10^5 (3000-1× 108)		0.0001
Median HCV RNA (range) IU/mL	1.15×10^5 (120- 6.4 × 10^5)		6.98×10^5 (2818-8 × 10^6)	0.025
HCV genotype:				
3	7%		8.7%	NS
Non-3	93%		91.3%	
HAI score (mean ± SD)	5.9 ± 2.9	6.2 ± 3.4	6.3 ± 3.6	NS
Fibrosis score (mean ± SD)	3.32 ± 0.45[l]	3.46 ± 0.48[m]	2.9 ± 0.30[n]	0.001 (l vs n)
				0.001 (m vs n)

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HOMA: Homeostasis model assessement; AST: Aspartate transferase; ALT: Alanine transferase; NS: No significant.

Table 2 Steatosis prevalence and distribution in the different groups

	Steatosis prevalence	Steatosis grade 1	Steatosis grade 2-3
HBV-HCV group (n = 66)	47.0%	43.6%	3.4%
HBV group (n = 66)	33.3%	21.2%	12.1%
HCV group (n = 198)	49.5%	23.2%	26.3%[a]

[a]P = 0.016 vs group HBV-HCV. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Table 3 Distribution of patatin-like phospholipase domain-containing 3 polymorphisms and their relation to steatosis n (%)

PNPLA3	Steatosis no	Steatosis yes	Steatosis score 1	Steatosis score 2-3
Overall				
p.148I/I	83 (55)	65 (36)	44%	23%
p.148I/M	62 (41)	82 (46)	49%	40%
p.148M/M	6 (4)	32 (18)[b]	7%	37%
HBV/HBV-HCV groups				
p.148I/I	32 (61)	36 (45.6)	55%	12.5%
p.148I/M	20 (38)	33 (41.8)	42%	37.5%
p.148M/M	1 (1)	10 (12.6)[d]	3%	50.0%
HCV group				
p.148I/I	53 (54)	44 (44)	60%	17%
p.148I/M,	43 (44)	31 (31)	30%	33%
p.148M/M	2 (2)	25 (25)[f]	10%	50%

[b]P = 0.0001; [d]P = 0.0001; [f]P = 0.0001 vs Steatosis no group. PNPLA3: Patatin-like phospholipase domain-containing 3; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

analysis was done excluding patients with HCV genotype 3, but considering the low number of patients with genotype 3 the results deserve further evaluation. In group HBV, a higher degree of steatosis (score 2-3) was closely associated with obesity (BMI > 30).

Figure 1 shows the mean serum levels of HOMA-IR in the three groups studied. HBV-HCV co-infected patients showed an intermediate value of HOMA-IR, i.e., between the highest level in group HCV and the lowest in group HBV, however, such value was significantly lower than that observed in HCV but not significantly higher than that observed in group HBV. Similarly, the prevalence of IR (HOMA-IR cut-off > 2.60) in the HBV-HCV co-infected patients was lower than that observed in group HCV (21% vs 54%, P = 0.005), but not significantly different from that observed in group HBV (23%).

The relation between IR and steatosis was evaluated and, as expected, in group HCV a correlation between the levels of IR and steatosis was observed (r = 0.27; P = 0.006), whereas, such a correlation was not seen in the HBV-HCV co-infected group (data not shown).

PNPLA3 polymorphisms and steatosis

Table 3 shows the distribution of the PNPLA3 polymor-

phisms. In accordance with our recently published data[19], the results of the present study showed that the PNPLA3 I148M polymorphism was associated with a more severe degree of steatosis both in groups HCV and HBV-HCV (P =0.003). An analysis of the overall study population confirmed that the PNPLA3 I148M polymorphism caused a predisposition to liver steatosis (P = 0.001). In Table 3, the data have been showed aggregate (HBV and HBV-HCV groups) considering that similar results have been obtained.

Liver fibrosis progression

The data given in Table 1 show that HBV-HCV co-infected patients had similar levels of liver fibrosis to those observed in group HBV, but significantly higher than those observed in group HCV (P = 0.001). This higher
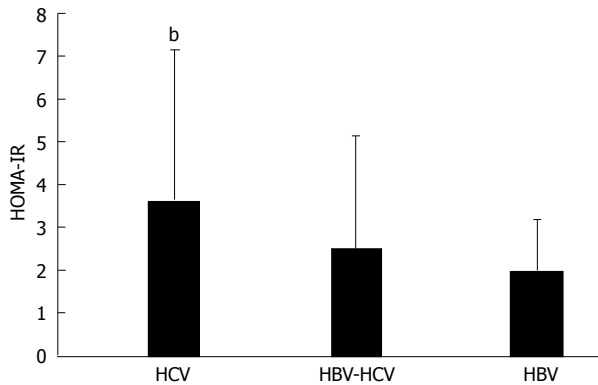
Figure 1 Homeostasis model assessement-insulin resistance in the three groups of patients. [b]$P < 0.001$, HCV *vs* HBV-HCV and HBV groups. HOMA-IR: Homeostasis model assessement-insulin resistance; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

degree of fibrosis in HBV-HCV group was independent of necro-inflammatory activity, because the HAI was similar in the three groups (Table 1), and, in addition, was not independently associated with liver steatosis, IR or PNPLA3 polymorphisms.

Factors associated with liver fibrosis

An overall evaluation, including all groups, on the factors associated with advanced liver fibrosis showed that the presence of HBV ($P = 0.0001$), age ($P = 0.031$), liver necro-inflammation ($P = 0.02$), and liver steatosis ($P = 0.047$) were the factors associated at univariate analysis with liver fibrosis. Regression analysis showed that HBV was the only independent factor associated with advanced fibrosis (coefficient B, 0.214; standard error of B, 0.055; 95%CI, lower: 0.104 - higher: 0.323; $P = 0.0001$).

DISCUSSION

In the present study, we explored the prevalence and possible role of liver steatosis and IR on liver disease progression in patients with chronic HBV-HCV co-infection. The data show that HBV-HCV co-infection does not influence the well-known capacity of HCV to induce steatosis; HBV-HCV co-infected patients showed a lesser amount of liver fat accumulation in comparison with HCV-infected patients. In addition, the results of this study demonstrate that HBV-HCV co-infected patients had lower serum levels of HCV RNA, a lower prevalence and degree of IR, and despite a similar duration of the disease, HBV-HCV patients showed higher levels of liver fibrosis than those observed in HCV mono-infected patients. The data suggest that HBV may interact with HCV and change some HCV metabolic characteristics. The mechanisms implicated in such interaction are not known, but some hypotheses can be made based on the results of this study.

Hepatic steatosis in chronic HCV infection is associated with alterations in the lipid and glucose metabolism[36,37]. IR in HCV infection has been reported in up to 80% of cases[38]. A close association between steatosis

and IR has been observed in HCV genotype non-3-infected patients, but IR is not generally a feature of genotype-3 infection[39,40]. HCV genotype-1-infected patients have higher prevalence of impaired glucose metabolism, and IR is correlated with the level of viral replication[6,7]. In HCV infection, IR precedes the development of steatosis and modulates fatty liver deposition[41,42]. The data of the present study show that HBV-HCV co-infected patients had lower levels of HCV RNA, IR and glucose. A fluctuating virological profile related to mutual HBV-HCV interference and the effect of this biological process on the clinical presentation and treatment strategy have been described[29,43]. It is possible that viral interference between HBV and HCV in hepatocytes might control or modulate the interaction between HCV and the lipid and glucose metabolism. However, a recent *in vitro* study[44] supports the hypothesis that HBV and HCV can replicate in the same cell without evidence of direct interference and that the *in vivo* effects may depend on the host immune response. However, the extensive virological and molecular interactions between the two viruses in co-infected patients are not well understood. Evidence seem to indicate that an in verse relationship occurs in the replication levels of the two viruses, suggesting direct or indirect viral interference[44,45]. Studies *in vitro* showed that the HCV core protein suppresses HBV replication[29,46,47]. On the other hand, an inhibition of HCV replication in patients with chronic hepatitis C who were super-infected with HBV have also been demonstrated[21,48]. Thus, the type of interaction between these two viruses in patients who are co-infected may be influenced by which virus infection is experienced first[24]. On these bases, our results seem to confirm that HBV "interference" induces lower levels of HCV replication, which may not support a significant development of IR and, in turn, not favor high amounts of liver fat deposition. Future experimental studies analyzing the effects of HBV replication on the development of IR and steatosis in HBV-HCV co-infected cells could produce interesting results.

It is well known that metabolic factors, in particular high levels of steatosis and IR are associated with a decreased likelihood of achieving a sustained virological response with interferon-based treatment[49-51], but little information is available for protease-inhibitor regimens[52]. Thus, determining the metabolic profile in HBV-HCV patients could prove useful to predict the outcome of treatment for these patients, but specific studies are necessary.

In accordance with the data available on the correlation between the PNPLA3 I148M variant and liver steatosis in NAFLD and in chronic HCV and HBV infection[15-20], the data of this study confirm the independent role of the PNPLA3 polymorphisms in inducing high degree of steatosis.

It has been well established that in chronic HCV infection, IR, a high degree of steatosis (greater than 20%-30%) and higher levels of glucose are associated with a more rapid progression of liver fibrosis[1,39]. Although the data from this study showed that HBV-HCV

co-infected patients had a more "favorable" anti-fibrotic metabolic profile, these patients had higher levels of liver fibrosis than those observed in HCV-infected patients. These data seem to indicate a prominent "direct" viral effect of the two viruses, rather than HCV-related metabolic factors, in the progression of liver fibrosis. Alternatively, considering that the levels of fibrosis in HBV-HCV co-infected patients are similar to those observed in the HBV mono-infected, and that HBV is the independent factor associated with advanced fibrosis, it is possible that HBV infection plays a dominant role in the progression of liver fibrosis.

It is necessary to underline that this study has some limitations; first, it is a cross-sectional study conducted in one geographic area; second, the very low number of HCV genotype 3 enrolled do not permit to draw conclusion about the role of genotype; third, due to the very low frequency of occurrence of double infection, a relative low number of patients have been included in the HBV-HCV co-infected group. However, despite these limitations, this study represents the essential basis for a future larger multicenter study evaluating the interaction between HBV and HCV infection.

In conclusion, the results of this study demonstrate that in HBV-HCV co-infected patients a high degree of liver steatosis is uncommon, possibly due to reciprocal viral interference causing lower levels of HCV replication and subsequently lower levels of IR. However, despite the "anti-fibrotic" metabolic profile observed, HBV-HCV co-infected patients had a higher degree of fibrosis, probably due to the dual infection and/or HBV dominance.Thus, in the unstandardized complex therapeutic managements of HBV-HCV co-infected patients an early control of HBV infection could be of importance to avoid the rapid progression of liver fibrosis.

COMMENTS

Background
Liver steatosis and insulin resistance (IR) are closely associated with chronic hepatitis C infection. The pathogenic link between steatosis, IR and chronic hepatitis C virus (HCV) infection is complex and it is associated with both viral and host factors. A host genetic factor, such as the polymorphism of the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene, involved in the lipid metabolism, is associated with liver steatosis in chronic hepatitis of different etiology. Both liver steatosis and IR are associated with a more rapid progression to liver cirrhosis. In chronic hepatitis B virus (HBV) infection, hepatic steatosis and IR have been reported with a lower prevalence than that observed in HCV infection. Chronic HBV-HCV co-infection is associated with a more rapid progression to liver cirrhosis. During HBV-HCV co-infection, a reciprocal inhibition of the viral genomes has been reported that could influence both steatosis and IR. There are no direct data on prevalence and pathogenic role of liver steatosis and IR in patients with HBV-HCV co-infection.

Research frontiers
At present, it remains unclear whether HBV infection affects the prevalence and level of steatosis and IR as well as the role of PNPLA3 in HBV-HCV co-infected patients and their impact on liver disease progression. The role of insulin resistance as promoting factor for liver steatosis and of this latter in promoting liver fibrosis has been extensively demonstrated in non-alcoholic fatty liver disease and in HCV related chronic hepatitis.

Innovations and breakthroughs
The study explores the unknown area of interaction between HBV with HCV on development of IR and liver steatosis, the role of *PNPLA3* gene polymorphisms, and their impact on the progression of liver disease. The results seem to indicate that HBV interacts with HCV reducing HCV replication and HCV-related metabolic features. Thus, steatosis and IR as well as PNPLA3 polymorphism do not significantly impact liver fibrosis progression in HBV-HCV patients. The more rapid progression of liver fibrosis observed in HBV-HCV co-infected patients seems to be mostly associated with HBV infection.

Applications
The knowledge of factors that influence the liver disease progression can improve therapeutic strategy in HBV-HCV co-infected patients.

Terminology
Liver steatosis is considered as a burden greater than 5% of triglycerides and other fats inside liver cells; it is the hepatic manifestation of the metabolic syndrome and contributes to progression of liver disease. Insulin resistance is a reduced ability of body tissues to respond to insulin, thus larger quantities of insulin are needed to maintain normal blood levels of glucose. It contributes to serious health problems including type 2 diabete and metabolic syndrome. The *PNPLA3* is a gene, involved in the lipid metabolism and has been associated with liver steatosis.

Peer review
The authors reported that a close association between steatosis and IR has been reported in HCV non-3 genotype-infected patients. The pathogenetic link between IR and chronic HCV infection is complex and is associated with HCV genotype.

REFERENCES

1   **Adinolfi LE**, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364 [PMID: 11391523]

2   **Adinolfi LE**, Restivo L, Marrone A. The predictive value of steatosis in hepatitis C virus infection. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 205-213 [PMID: 23445230 DOI: 10.1586/egh.13.7]

3   **Powell EE**, Jonsson JR, Clouston AD. Steatosis: co-factor in other liver diseases. *Hepatology* 2005; **42**: 5-13 [PMID: 15962320]

4   **Lonardo A**, Loria P, Adinolfi LE, Carulli N, Ruggiero G. Hepatitis C and steatosis: a reappraisal. *J Viral Hepat* 2006; **13**: 73-80 [PMID: 16436124 DOI: 10.1111/j.1365-2893.2005.00669.x]

5   **Vidali M**, Tripodi MF, Ivaldi A, Zampino R, Occhino G, Restivo L, Sutti S, Marrone A, Ruggiero G, Albano E, Adinolfi LE. Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. *J Hepatol* 2008; **48**: 399-406 [PMID: 18164507 DOI: 10.1016/j.jhep.2007.10.011]

6   **Adinolfi LE**, Zampino R, Restivo L, Guerrera B, Santoro A, Ciervo A, Rinaldi L, Scialdone VR, Ferrara M, Ruggiero G.HCV infection impairs glucose metabolism which promotes a more aggressive course of liver disease and metabolic syndrome. *J Hepatol* 2010; **52** (Suppl 1): S401-S402

7   **Dai CY**, Yeh ML, Huang CF, Hou CH, Hsieh MY, Huang JF, Lin IL, Lin ZY, Chen SC, Wang LY, Chuang WL, Yu ML, Tung HD. Chronic hepatitis C infection is associated with insulin resistance and lipid profiles. *J Gastroenterol Hepatol* 2013 Jun 28; Epub ahead of print [PMID: 23808794 DOI: 10.1111/jgh.12313]

8   **Petta S**, Cammà C, Di Marco V, Alessi N, Cabibi D, Caldarella R, Licata A, Massenti F, Tarantino G, Marchesini G, Craxì A. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. *Am J Gastroenterol* 2008; **103**: 1136-1144 [PMID: 18477344 DOI: 10.1111/j.1572-0241.2008.01813.x]

9   **Machado MV**, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. *J Gastroenterol Hepatol* 2011; **26**: 1361-1367 [PMID: 21649726

DOI: 10.1111/j.1440-1746.2011.06801.x]

10 **Bugianesi E**, Salamone F, Negro F. The interaction of metabolic factors with HCV infection: does it matter? *J Hepatol* 2012; **56** Suppl 1: S56-S65 [PMID: 22300466 DOI: 10.1016/S0168-8278(12)60007-5]

11 **Petta S**, Tripodo C, Grimaudo S, Cabibi D, Cammà C, Di Cristina A, Di Marco V, Di Vita G, Ingrao S, Mazzola A, Marchesini G, Pipitone R, Craxì A. High liver RBP4 protein content is associated with histological features in patients with genotype 1 chronic hepatitis C and with nonalcoholic steatohepatitis. *Dig Liver Dis* 2011; **43**: 404-410 [PMID: 21324757 DOI: 10.1016/j.dld.2010.12.013]

12 **Wong VW**, Chu WC, Wong GL, Chan RS, Chim AM, Ong A, Yeung DK, Yiu KK, Chu SH, Woo J, Chan FK, Chan HL. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut* 2012; **61**: 409-415 [PMID: 21846782 DOI: 10.1136/gutjnl-2011-300342]

13 **Rastogi A**, Sakhuja P, Kumar A, Hissar S, Jain A, Gondal R, Sarin SK. Steatosis in chronic hepatitis B: prevalence and correlation with biochemical, histologic, viral, and metabolic parameters. *Indian J Pathol Microbiol* 2011; **54**: 454-459 [PMID: 21934202 DOI: 10.4103/0377-4929.85074]

14 **Zheng RD**, Chen JN, Zhuang QY, Lu YH, Chen J, Chen BF. Clinical and virological characteristics of chronic hepatitis B patients with hepatic steatosis. *Int J Med Sci* 2013; **10**: 641-646 [PMID: 23569427 DOI: 10.7150/ijms.5649]

15 **Valenti L**, Nobili V, Al-Serri A, Rametta R, Leathart JB, Zappa MA, Dongiovanni P, Fracanzani AL, Alterio A, Roviaro G, Daly AK, Fargion S, Day CP. The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. *J Hepatol* 2011; **55**: 1409-1414 [PMID: 21777557 DOI: 10.1016/j.jhep.2011.03.035]

16 **Valenti L**, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, Dongiovanni P, Maggioni M, Fracanzani AL, Rametta R, Colombo M, Fargion S. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011; **53**: 791-799 [PMID: 21319195 DOI: 10.1002/hep.24123]

17 **Trépo E**, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, Lemmers A, Berthillon P, Amininejad L, Chevallier M, Schlué J, Kreipe H, Devière J, Manns M, Trépo C, Sninsky J, Wedemeyer H, Franchimont D, Moreno C. Impact of patatin-like phospholipase-3 (rs738409 C> G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 2011; **54**: 60-69 [PMID: 21488075 DOI: 10.1002/hep.24350]

18 **Müller T**, Buch S, Berg T, Hampe J, Stickel F. Distinct, alcohol-modulated effects of PNPLA3 genotype on progression of chronic hepatitis C. *J Hepatol* 2011; **55**: 732-733 [PMID: 21316406 DOI: 10.1016/j.jhep.2011.01.025]

19 **Zampino R**, Coppola N, Cirillo G, Boemio A, Pisaturo M, Marrone A, Macera M, Sagnelli E, Perrone L, Adinolfi LE, Miraglia del Giudice E. Abdominal fat interacts with PNPLA3 I148M, but not with the APOC3 variant in the pathogenesis of liver steatosis in chronic hepatitis C. *J Viral Hepat* 2013; **20**: 517-523 [PMID: 23808989 DOI: 10.1111/jvh.12053]

20 **Viganò M**, Valenti L, Lampertico P, Facchetti F, Motta BM, D'Ambrosio R, Romagnoli S, Dongiovanni P, Donati B, Fargion S, Colombo M. Patatin-like phospholipase domain-containing 3 I148M affects liver steatosis in patients with chronic hepatitis B. *Hepatology* 2013; **58**: 1245-1252 [PMID: 23564580 DOI: 10.1002/hep.26445]

21 **Sagnelli E**, Pasquale G, Coppola N, Scarano F, Marrocco C, Scolastico C, Santantonio T, Gentile A, Piccinino F. Influence of chronic coinfection with hepatitis B and C virus on liver

histology. *Infection* 2004; **32**: 144-148 [PMID: 15188073]

22 **Coppola N**, Pisapia R, Tonziello G, Martini S, Imparato M, Piai G, Stanzione M, Sagnelli C, Filippini P, Piccinino F, Sagnelli E. Virological pattern in plasma, peripheral blood mononuclear cells and liver tissue and clinical outcome in chronic hepatitis B and C virus coinfection. *Antivir Ther* 2008; **13**: 307-318 [PMID: 18505182]

23 **Sagnelli E**, Coppola N, Pisaturo M, Masiello A, Tonziello G, Sagnelli C, Messina V, Filippini P. HBV superinfection in HCV chronic carriers: a disease that is frequently severe but associated with the eradication of HCV. *Hepatology* 2009; **49**: 1090-1097 [PMID: 19263473 DOI: 10.1002/hep.22794]

24 **Coppola N**, Stanzione M, Messina V, Pisaturo M, De Pascalis S, Macera M, Tonziello G, Fiore M, Sagnelli C, Pasquale G, Sagnelli E. Current Concepts of HBV/HCV Coinfection: Coexistence, but Not Necessarily in Harmony. *Curr Hepat Rep* 2010; **9**: 260-269 [PMID: 21258658]

25 **Coppola N**, Stanzione M, Messina V, Pisaturo M, De Pascalis S, Macera M, Tonziello G, Fiore M, Sagnelli C, Pasquale G, Sagnelli E. Tolerability and efficacy of anti-HBV nucleos(t)ide analogues in HBV-DNA-positive cirrhotic patients with HBV/HCV dual infection. *J Viral Hepat* 2012; **19**: 890-896 [PMID: 23121368 DOI: 10.1111/j.1365-2893.2012.01627.x]

26 **Donato F**, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int J Cancer* 1998; **75**: 347-354 [PMID: 9455792]

27 **Kew MC**, Yu MC, Kedda MA, Coppin A, Sarkin A, Hodkinson J. The relative roles of hepatitis B and C viruses in the etiology of hepatocellular carcinoma in southern African blacks. *Gastroenterology* 1997; **112**: 184-187 [PMID: 8978357]

28 **Amin J**, Law MG, Bartlett M, Kaldor JM, Dore GJ. Causes of death after diagnosis of hepatitis B or hepatitis C infection: a large community-based linkage study. *Lancet* 2006; **368**: 938-945 [PMID: 16962883]

29 **Raimondo G**, Brunetto MR, Pontisso P, Smedile A, Maina AM, Saitta C, Squadrito G, Tono N. Longitudinal evaluation reveals a complex spectrum of virological profiles in hepatitis B virus/hepatitis C virus-coinfected patients. *Hepatology* 2006; **43**: 100-107 [PMID: 16323213]

30 **Squadrito G**, Orlando ME, Pollicino T, Raffa G, Restuccia T, Cacciola I, Di Marco V, Picciotto A, Colucci G, Craxì A, Raimondo G. Virological profiles in patients with chronic hepatitis C and overt or occult HBV infection. *Am J Gastroenterol* 2002; **97**: 1518-1523 [PMID: 12094876]

31 **Coppola N**, Marrone A, Pisaturo M, Starace M, Signoriello G, Gentile I, Adinolfi LE, Sagnelli E, Zampino R. Role of interleukin 28-B in the spontaneous and treatment-related clearance of HCV infection in patients with chronic HBV/HCV dual infection. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 559-567 [PMID: 24081499 DOI: 10.1007/s10096-013-1985-7]

32 **Gayoso-Diz P**, Otero-González A, Rodriguez-Alvarez MX, Gude F, García F, De Francisco A, Quintela AG. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* 2013; **13**: 47 [PMID: 24131857 DOI: 10.1186/1472-6823-13-47]

33 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864]

34 **Coppola N**, Potenza N, Pisaturo M, Mosca N, Tonziello G, Signoriello G, Messina V, Sagnelli C, Russo A, Sagnelli E. Liver microRNA hsa-miR-125a-5p in HBV chronic infection: correlation with HBV replication and disease progression. *PLoS One* 2013; **8**: e65336 [PMID: 23843939 DOI: 10.1371/journal.pone.0065336]

35 **Giudice EM**, Grandone A, Cirillo G, Santoro N, Amato A, Brienza C, Savarese P, Marzuillo P, Perrone L. The association of PNPLA3 variants with liver enzymes in childhood

obesity is driven by the interaction with abdominal fat. *PLoS One* 2011; **6**: e27933 [PMID: 22140488 DOI: 10.1371/journal.pone.0027933]

36 **Syed GH**, Amako Y, Siddiqui A. Hepatitis C virus hijacks host lipid metabolism. *Trends Endocrinol Metab* 2010; **21**: 33-40 [PMID: 19854061 DOI: 10.1016/j.tem.2009.07.005]

37 **Felmlee DJ**, Hafirassou ML, Lefevre M, Baumert TF, Schuster C. Hepatitis C virus, cholesterol and lipoproteins--impact for the viral life cycle and pathogenesis of liver disease. *Viruses* 2013; **5**: 1292-1324 [PMID: 23698400]

38 **Adinolfi LE**, Restivo L, Zampino R, Lonardo A, Loria P. Metabolic alterations and chronic hepatitis C: treatment strategies. *Expert Opin Pharmacother* 2011; **12**: 2215-2234 [PMID: 21883025 DOI: 10.1517/14656566.2011.597742]

39 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704 [PMID: 14724822]

40 **Hwang SJ**, Lee SD. Hepatic steatosis and hepatitis C: Still unhappy bedfellows? *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 96-101 [PMID: 21199519 DOI: 10.1111/j.1440-1746.2010.06542.x]

41 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848 [PMID: 14988838]

42 **Fartoux L**, Poujol-Robert A, Guéchot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 2005; **54**: 1003-1008 [PMID: 15951550]

43 **Zarski JP**, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, Tran van Nhieu J, Seigneurin JM, Buffet C, Dhumeaux D. Characteristics of patients with dual infection by hepatitis B and C viruses. *J Hepatol* 1998; **28**: 27-33 [PMID: 9537860]

44 **Bellecave P**, Gouttenoire J, Gajer M, Brass V, Koutsoudakis G, Blum HE, Bartenschlager R, Nassal M, Moradpour D. Hepatitis B and C virus coinfection: a novel model system reveals the absence of direct viral interference. *Hepatology* 2009; **50**: 46-55 [PMID: 19333911 DOI: 10.1002/hep.22951]

45 **Raimondo G**, Saitta C. Treatment of the hepatitis B virus and hepatitis C virus co-infection: still a challenge for the hepatologist. *J Hepatol* 2008; **49**: 677-679 [PMID: 18804888 DOI: 10.1016/j.jhep.2008.08.003]

46 **Schüttler CG**, Fiedler N, Schmidt K, Repp R, Gerlich WH, Schaefer S. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein. *J Hepatol* 2002; **37**: 855-862 [PMID: 12445429]

47 **Chen SY**, Kao CF, Chen CM, Shih CM, Hsu MJ, Chao CH, Wang SH, You LR, Lee YH. Mechanisms for inhibition of hepatitis B virus gene expression and replication by hepatitis C virus core protein. *J Biol Chem* 2003; **278**: 591-607 [PMID: 12401801]

48 **Sagnelli E**, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, Scolastico C, Filippini P. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002; **36**: 1285-1291 [PMID: 12395342]

49 **European Association for Study of Liver.** EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]

50 **Romero-Gómez M**, Del Mar Viloria M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez ML, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636-641 [PMID: 15765399]

51 **Grasso A**, Malfatti F, De Leo P, Martines H, Fabris P, Toscanini F, Anselmo M, Menardo G. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. *J Hepatol* 2009; **51**: 984-990 [PMID: 19695729 DOI: 10.1016/j.jhep.2009.07.008]

52 **Serfaty L**, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, Drenth JP, Lonjon-Domanec I, DeMasi R, Picchio G, Beumont M, Marcellin P. Insulin resistance and response to telaprevir plus peginterferon α and ribavirin in treatment-naive patients infected with HCV genotype 1. *Gut* 2012; **61**: 1473-1480 [PMID: 22387529 DOI: 10.1136/gutjnl-2011-300749]

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