

Hepatology highlights

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PNPLA3 rs738409 causes steatosis according to viral & IL28B genotypes in hepatitis C

Ampuero J, et al. Liver steatosis is commonly seen in patients with chronic hepatitis C virus (HCV), and has been associated with worsening fibrosis¹ as well as reduced sustained virologic response (SVR) to treatment.² A combination of host and viral factors influence the development of steatosis, with the metabolic syndrome and associated insulin resistance as key players.³ PNPLA3 is a patatin-like phospholipase family 3 protein that has been found to be associated with significant steatosis compared to other candidate genes,⁴ except in HCV genotype 3.

In the cross sectional study by Ampuero, *et al.*, the aim was to assess the role of PNPLA3 allele-G on steatosis in a real world population, as well as *in vitro*. 474 consecutive patients with HCV treated with standard protocol peginterferon (PEG-IFN) and ribavirin (RBV) were included. All individuals had baseline HCV viral load, genotyping, and liver biopsy. PNPLA3 and IL28B genotyping were performed by PCR of single nucleotide polymorphisms (SNP) rs738409 and rs12979860, respectively. *In vitro* cell cultures of Huh7.5 (rs12979860 genotype CT) and Huh7 (rs12979860 genotype CC) were infected with HCV genotypes 1 and 3, with subsequent gene expression analysis.

The majority of patients in the study were HCV genotype 1 (68.8%), and the lowest representation was from genotype 2 (2.5%). Baseline fibrosis favo-

red lower grades (F1 54.6%, F2 25.7%) compared to advanced fibrosis (F3 9.1%, F4 6.5%). Steatosis was seen in 46.4%.

The PNPLA3 allele-G was associated with a significant difference in steatosis in IL28B-CT/TT (57.7%) than in non-allele G (37.1%, $p < 0.001$). No difference was seen in IL28B CC (G-allele 47.8%, non-G-allele 42%, $p = 0.442$). When looking at HCV genotypes, allele-G was associated with steatosis in all genotypes except for 3 (61.9 *vs.* 62%, $p = 0.993$). Multivariate analysis revealed independent variables for steatosis to be PNPLA3 allele-G (O.R. 1.84; $p = 0.007$), age (O.R. 1.04, $p = 0.017$), HCV genotype 3 (O.R. 2.46, $p = 0.006$) and HOMA > 4 (O.R. 2.72, $p = 0.010$).

From the gene expression analysis, Huh7.5 cells (CT genotype) had decreased expression of PNPLA3 (fold inhibition = 3.2 ± 0.2), whereas Huh 7.0 cells (CC genotype) had increased PNPLA3 expression (fold induction = 1.5 ± 0.2).

This study confirms that PNPLA3 allele-G is an independent risk factor for steatosis. From the analysis of IL28b and PNPLA3, differential expression of PNPLA3 seen in the CT genotype *vs.* CC genotype may help to explain the reduced steatosis and improved treatment outcomes in CC. Additionally, it also supports that the mechanism of steatosis development in HCV genotype 3 is virus dependent rather than through host mechanisms seen in the other genotypes. This may help to advance our knowledge in the direct antiviral age given the difficulties seen with treatment outcomes in genotype 3.

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Long term nucleotide and nucleoside analogs treatment in chronic hepatitis B HBeAg negative genotype D patients and risk for hepatocellular carcinoma

Pellicelli A, et al. The role of therapy for chronic hepatitis B (CHB) has focused on short term goals of normalization of liver biochemistry as well as suppression of viral replication. Long term benefits have included reversal of fibrosis^{5,6} and reduction of hepatocellular carcinoma (HCC).⁷ CHB genotypes vary in distribution, with genotype B and C predominantly in Asia and D found worldwide.⁸ Clinically, CHB genotyping is used to assess response to interferon, where genotypes A and B have greater response than genotypes C and D.^{9,10} Many of the natural studies have occurred in Asian populations.¹¹ The role of genotype on risk of HCC in those on therapy is not well characterized.

In the study by Pellicelli, *et al.*, the aim was to assess the impact of nucleoside and nucleotide analogs on fibrosis and HCC risk in chronic hepatitis B genotype D. This prospective study spanned over 14 years (1998-2012), where 306 treatment naïve HBeAg negative genotype D were studied from the CLEO database. Lamivudine (LAM) was the only available agent until 2003, with the introduction of Adefovir, and first line therapy from 2007 onwards was either entecavir or tenofovir. Patients with decompensated cirrhosis and HCC diagnosed within 18 months were excluded. 113 patients had compensated cirrhosis (37%) and the remainder had CHB (193, 63%). Entecavir or lamivudine was used in the majority (126, 111). 60% (67/111) of those treated with lamivudine required rescue therapy due to resistance

(41/67 or partial virologic response (26/67), defined as HBV DNA decrease of $> 1 \log_{10}$ IU/mL, but detectable HBV DNA after at least 6 months of therapy in treatment-compliant patients. Virologic response (VR), defined as undetectable HBV DNA during therapy, was seen in 96 and 89% of CHB and cirrhosis respectively.

HCC developed in 8.2% of patients (25/306), with 60% developing after 48 months of therapy. The cumulative risk was significantly higher at 10 years with cirrhosis (43%) than CHB (0.5%). Univariate analysis showed age > 60 years, platelet count < 100 , cirrhosis and need for rescue therapy to be independent risk factors. Multivariate analysis however revealed age > 60 years and cirrhosis to be the only independent variables for HCC (OR 7.5 and 7.6, respectively). Multivariate analysis amongst cirrhotics in particular revealed that age > 60 years was the only statistically significant risk factor (OR 9.8, $p < 0.003$), and maintenance of VR, treatment with LAM or need for rescue therapy were not. Ten year survival rates were 93% in CHB and 86% with cirrhosis.

This study confirms and reinforces the importance of age and cirrhosis as important risk factors for HCC. This study did not show that maintenance of virologic response in cirrhotics altered risk for HCC. This study did not directly assess histologic changes on therapy or compare to a non-treated population to conclude that therapy altered the natural history of CHB in this specific genotype. This may underestimate the benefit of therapy by reducing the number of individuals that would progress to cirrhosis from the CHB group and subsequent risk of HCC. The ten year survival rates further the rationale that therapy for CHB is associated with longevity.

Preoperative transcatheter arterial chemoembolization for resectable HCC: a single center analysis

Jianyong, et al. Hepatocellular carcinoma (HCC) is a major health burden worldwide and a leading cause of cancer-related death. With effective screening of high risk populations it is possible to detect lesions that are small and amenable to curative treatment with locoregional therapy. Surgical resection is also a therapeutic option, particularly in patients without significant liver dysfunction or portal hypertension. However, recurrence after hepatic resection is common and 5-year survival rates are reported lower than 40%.¹² Furthermore, patients

will present with large lesions that are not amenable to surgical resection. Neoadjuvant treatment with transarterial chemoembolization has been evaluated to improve the rate of recurrence of HCC or to downstage unresectable to resectable lesions. These have not shown a survival advantage of neoadjuvant TACE.¹³ However, these studies are limited by their small sample size and the heterogeneity of the study population.

In this issue, Jianyong, *et al.* studied the impact of preoperative TACE in a large single center cohort of patients with initially resectable HCC. They retrospectively identified a cohort of patients with histologically proven HCC and preserved liver function who underwent an R0 resection and who did not

have any evidence of metastatic disease. This cohort was divided in two groups depending on whether preoperative TACE was performed. Patients with recurrent HCC, other preoperative treatments and rupture HCC were excluded. The cohort included 588 patients, 405 in the liver resection group (LR) and 183 who received preoperative TACE (TACE-LR), the majority with HBV as an underlying liver disease. Baseline demographic and clinical characteristics were similar between the two groups. No difference in the post-operative complications was observed. Analysis of long-term outcomes didn't show any difference in 1-,3- and 5- years overall survival (83.7, 68.9, 57.5 *vs.* 80.9, 65 and 54.1% in LR and TACE-LR groups, respectively) and recurrence free-survival (79.6, 61.7, 49.6% *vs.* 76.0, 55.7, 43.7% in LR and TACE-LR groups, respectively). Multivariate analysis showed that and AFP level > 400 ng/mL and macro-vascular invasion were the most important predictors of survival af-

ter resection and that macro-vascular invasion was the most important predictor of recurrence-free survival. Increased procedural cost was the main difference observed between the two groups, but only for TACE therapy that followed the initial TACE treatment.

The results of this study suggest that neoadjuvant TACE does not reduce the risk of haematogenous spread and has no impact on overall and tumour free survival in initially resectable HCC. It increases cost of treatment and causes unnecessary delays. These results, however can not be generalized to the situation of using TACE as a method to downstage an initially unresectable tumour. Whether the results of this study can also be generalized to HCC cases in non-HBV disease remains to be validated. Furthermore, patients will present with large lesions that are not amenable to surgical resection who will need some form of locoregional therapy.

Long term changes in liver histology following treatment of chronic hepatitis C virus

Schiffman M, et al. Eradication of HCV infection is associated with a reduction in the risk of developing hepatic decompensation and hepatocellular cancer.^{14,15} The decline in inflammation that is seen with sustained virological response (SVR) is associated with an improved in fibrosis score in patients treated with peginterferon based therapy. However, it is unclear if peginterferon will have a positive effect on fibrosis in patients who do not achieve SVR. Studies showing a beneficial effect in non-responders are limited by a short follow-up period and a more recent study has even shown rapid progression of fibrosis after failed interferon based therapy.¹⁶ In the current issue, Schiffman, *et al.* evaluated the long-term impact of interferon based therapy on fibrosis scores in patients who achieved or did not achieve SVR.

Patients were recruited if they had chronic HCV with no other cause of liver disease except for benign steatosis, mild-moderate non-alcoholic fatty liver disease. Patients were subsequently treated with peginterferon based therapy with or without ribavirin or deferred treatment and underwent a liver biopsy at least 5 years after the initial biopsy. Patients were further excluded if during follow-up they started treatment or were retreated with peginterferon or any other agent, relapse after having

achieved SVR 24 or developed decompensated cirrhosis. Recruitment took place between 1991 and 2004. Patients were divided into three groups, those who deferred treatment (n = 47), those who failed to achieve SVR (n = 189) and patients who achieved SVR with the exception of those who had no fibrosis in the initial liver biopsy (n = 122). Inflammation was scored with Knodell method and fibrosis with Knodell prior to 2000 and Knodell and Ishak thereafter.

Patients who deferred treatment and those who did not achieve SVR had a progression of their inflammation and fibrosis score. However, there was no difference in the inflammation score changes between patients who deferred treatment and those who did not achieve SVR (1.9 ± 2.7 *vs.* 1.3 ± 2.9 , respectively). Similarly, there was no difference between the changes in fibrosis score (0.7 ± 1.1 *vs.* 0.4 ± 1.3 , respectively) and rate of change per year. When comparing patients with no fibrosis at baseline, no differences in the proportion that progressed to portal fibrosis, bridging fibrosis or cirrhosis was identified. Patients who achieved SVR saw their inflammation and fibrosis score improved. The inflammation score declined from 6.7 ± 2.4 to 2.2 ± 2.2 and the fibrosis score from 3.3 ± 1.3 to 1.8 ± 1.9 . Among the 20 patients who have cirrhosis at the baseline biopsy, 10 had no longer cirrhosis 5 years after achieving SVR.

The results of this study suggest that increases in inflammation are associated with increases in fibrosis. However, long term benefits of interferon therapy are only observed in patients who achieve SVR.

Future therapy for HCV will in all likelihood exclude interferon based regimens, but we could expect the regression of fibrosis in patients who achieve SVR with the new combinations of anti-viral agents.

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