CORRESPONDENCE

Serum M30 Levels: a Potential Biomarker of Severe Liver Disease in Nonalcoholic Fatty Liver Disease and Normal Aminotransferase Levels

To the Editor:

We congratulate Fracanzani and colleagues[1] on their insightful article identifying factors associated with severe liver disease in patients with nonalcoholic fatty liver disease (NAFLD) and normal aminotransferase levels. First, the authors have convincingly demonstrated that normal alanine aminotransferase (ALT) is not a valuable criterion to exclude patients with NAFLD from liver biopsy. More importantly, they have shown that increased insulin resistance as assessed by the homeostasis model assessment of insulin resistance (HOMA-IR) is a statistically significant and independent predictor of the presence of both nonalcoholic steatohepatitis (NASH) and severe fibrosis among subjects with NAFLD. The authors concluded that determination of insulin resistance in subjects with normal ALT levels may be clinically relevant in the selection of NAFLD cases for histological assessment of disease severity and progression.[1]

The field of biomarkers is an area of fast growing interest in the setting of NAFLD. A neoepitope in cytokeratin 18, termed M30 antigen, becomes available at an early caspase cleavage event during apoptosis and has been regarded as a biochemical marker of liver injury.[2,3] Thus, we sought to determine whether serum M30 levels may be associated with the presence of NASH among NAFLD cases with normal ALT levels. Eighteen patients (four males and 14 females), mean age of 51.0 $\pm$ 7.6 years, with liver biopsy-confirmed NAFLD and normal ALT levels ($<$ 40 U/L) were investigated. The diagnosis of NASH was based on the Kleiner criteria.[4] M30 levels were detected by enzyme-linked immunosorbent assay (M30 Apoptosense ELISA kit; Peviva AB, Bromma, Sweden). Compared with patients with NAFLD without NASH (n = 12), mean serum M30 levels were significantly raised in the six patients with normal ALT and NASH levels (198.2 $\pm$ 37.2 IU/L versus 80.7 $\pm$ 19.2 IU/L, $P < 0.01$). In multivariate analysis, M30 levels and HOMA-IR were the only independent predictors of the presence of NASH in patients with NAFLD and normal ALT levels.

Besides confirming the elegant proof by Fracanzani and coworkers that determining HOMA-IR is clinically useful for identifying patients with NAFLD who are candidates for histological assessment of disease severity,[1] our pilot results allow us to postulate that M30 levels may be an additional good index of the presence of NASH in this patient group. Further studies in larger samples are warranted to confirm our pilot data.

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Reply:

We thank Dr. Yilmaz et al. for comments on our article.[1] The results of the pilot study Yilmaz and colleagues conducted in patients with nonalcoholic fatty liver disease (NAFLD) and normal alanine aminotransferase (ALT) levels confirm our finding that the severity of insulin resistance is an independent predictor of liver damage in these patients, and suggest that serum levels of M30, a neoepitope of cytokeratin 18 (CK-18) released during the early phases of apoptosis, may prove useful to discriminate patients with nonalcoholic steatohepatitis (NASH) from those with simple steatosis. There is growing interest in evaluating apoptosis markers as potential predictors of liver damage in NAFLD, and preliminary studies in patients with increased ALT levels have provided encouraging results.[2] Recent data showed that cytokeratin CK-18 (M65 antigen) and caspase-cleaved CK-18 (M30 antigen), two soluble forms of extracellular cytokeratin 18, have both a good sensitivity and specificity, with M65 having a higher predictive value to diagnose NASH.[3] Larger prospective cooperative studies are needed to test the performance of these new apoptosis markers in discriminating patients with NASH and potentially progressive liver disease from those with benign disease, even before severe fibrosis ensues, and to compare these tests with other noninvasive risk scores.[4] Based on the present results, and the preliminary data reported by Yilmaz, we think that future research should evaluate the combined role of genetic, apoptotic, and serological markers of insulin resistance in the noninvasive diagnosis of NASH including patients with or without increased ALT.

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Hepatitis B Virus Viral Load and Treatment Decision

To the Editor:

Tong et al. recently described the failure of several hepatitis B virus (HBV) treatment guidelines to identify treatment eligibility for a significant proportion of 67 of their patients who went on to develop hepatocellular carcinoma or died of liver-related complications, probably due to disease progression as a result of inadequate viral suppression.[1] However, three of these guidelines were published[2-4] prior to 2006, when the importance of HBV viral burden was less clearly established in terms of HBV-associated disease progression.[5,6] The article by Tong and colleagues is very timely, given that recently published Dutch guidelines as well as the most recent algorithm by Keefe et al. still reinforce the HBV DNA threshold for treatment initiation at 10^5 copies/mL for treatment initiation for all patients,[7] or for patients who are hepatitis B e antigen (HBeAg)-positive,[8] respectively. Using a higher viral load threshold for patients who are HBeAg-positive seems questionable, given that HBeAg has been identified as an independent risk factor for disease progression. In contrast, withholding treatment in patients who are HBeAg-negative with viral load up to 19,999 IU/mL (10^5 copies/mL) and persistently normal alanine aminotransferase (ALT) values seems justifiable.[9]

It would be interesting to learn from the authors if all patients, who progressed, would have qualified for treatment following the German guidelines (as summarized in figure 1), which were developed following the criteria according to the workshop of the scientific medical professional societies.[10]

In short, these guidelines have a hierarchical approach: (1) All patients with evidence for advanced fibrosis are eligible for antiviral therapy with an agent with a high genetic barrier to resistance (tenofovir or entecavir at present) in the presence of detectable HBV DNA; if no virus is detectable, patients should be re-evaluated every 3-6 months; (2) all patients with a viral load $\geq 10^4$ copies/mL would qualify for therapy if ALT is (a) $>2\times$ upper limit of normal or (b) histology shows $\geq$F2 fibrosis or (c) high risk for hepatocellular carcinoma development (male gender, age, family history, or aflatoxin exposure in the past).

If viral load is less than 10^4 copies/mL, patients should be re-evaluated every 6-12 months. These guidelines for HBV DNA and ALT thresholds apply to both HBeAg-positive and HBeAg-negative patients. Furthermore, the incorporation of histology into the decision-making process allows for the identification of a greater proportion of patients at risk of disease progression, and thus eligible to receive appropriate antiviral therapy.

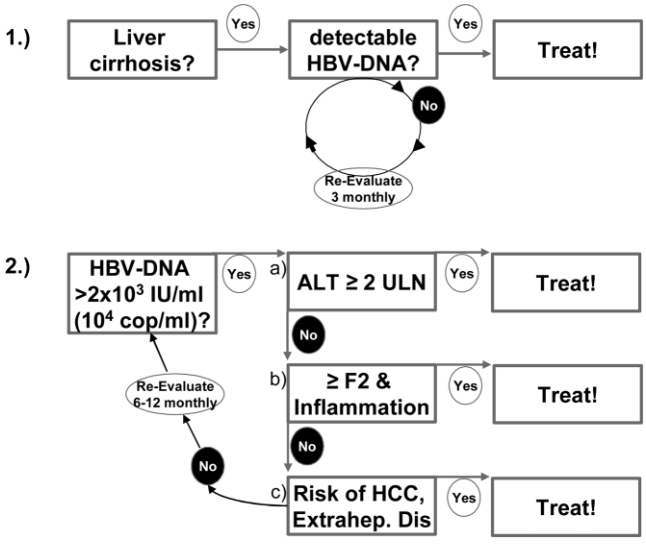

Fig. 1. Treatment Algorithm According to the German Treatment Guidelines for HBV.

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Revisiting the Treatment Recommendations for Chronic Hepatitis B

To the Editor:

I read with great interest the work by Tong et al.[1] on the evaluation of current treatment recommendations based on a longitudinal cohort of 369 chronic hepatitis B patients followed for a mean of 84 months. Under the published guidelines, in 2003-2007, 40% to 80% of patients who developed hepatocellular carcinoma (HCC) or died of liver-related problems did not fall into the criteria for antiviral treatment. The authors have proposed adding low platelet count and low serum albumin to the guidelines so that up to 100% of patients who develop HCC or die can be identified. I have a different angle of interpretation on the results of this study.

Low platelet count and low serum albumin are surrogate markers of portal hypertension and hepatic dysfunction, respectively. Patients who have these laboratory findings are likely suffering from early liver cirrhosis. In the report by Tong et al.,[1] five of nine patients who developed HCC were diagnosed as having chronic hepatitis by histology and therefore did not fulfill the recommended treatment criteria. These patients probably had normal alanine aminotransferase (ALT) and/or intermediate hepatitis B virus (HBV) DNA levels (between 10,000 and 100,000 copies/mL). This observation revealed the insufficiency of liver biopsy in determining the true liver histologic staging, particularly when the biopsy core is not long enough.[2] It would be interesting to know the length and number of portal tracts of the liver biopsies among patients with histologic chronic hepatitis in this report. Furthermore, 5% to 18% of hepatitis B e antigen–negative chronic hepatitis B patients with normal ALT (<30 IU/L in men and <19 IU/L in women) might already have developed liver cirrhosis.[3]

In the current report,[1] 7 of 15 patients with cirrhosis who developed HCC could not be identified by the treatment recommendations. The reason for missing these patients was most likely their low HBV DNA levels (lower than 10,000 copies/mL). Previous large-scale longitudinal studies have confirmed that HBV DNA and liver cirrhosis are independent risk factors of HCC.[4,5] In other words, patients with cirrhosis have a significant risk of developing HCC even when their HBV DNA levels are not high. In the cohort studied by Tong et al.,[1] two-thirds of the patients who had HBV DNA levels lower than 10,000 copies/mL in fact had HBV DNA levels lower than 1000 copies/mL. The number of patients with cirrhosis who had undetectable HBV DNA but developed HCC on subsequent follow-up was not reported. It remains dubious whether antiviral treatment can reduce the risk of liver-related complications in patients with cirrhosis with very low viremia.

In summary, to improve current treatment recommendations, high-quality liver biopsy of adequate length should be encouraged when there is clinical suspicion of liver cirrhosis (e.g., middle-age patients with low platelet count and/or low serum albumin), regardless of the ALT and HBV DNA levels. Antiviral treatment should be recommended for all patients with cirrhosis with detectable HBV DNA in order to maximize the possible benefit of treatment.

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Do Guidelines Preclude Hepatitis B Patients from Receiving Treatment?

To the Editor:

We read with great interest the article by Tong et al.[1] in the October 2008 issue of HEPATOLOGY.

In this article, the authors determined whether the four published guidelines/algorithm (European Association for the Study of the Liver, American Association for the Study of Liver Diseases, Asian Pacific Association for the Study of the Liver, and US Panel) on hepatitis B treatment correctly identified for treatment patients who subsequently developed hepatocellular carcinoma (HCC) or suffered non-HCC liver-related deaths. Tong et al. retrospectively analyzed the outcome of 369 hepatitis B surface antigen (HBsAg)–positive patients followed for a mean of 7 years, and they reported that if the criteria for antiviral therapy stated in the current treatment guidelines/algorithm had been used, only 20% to 60% of the patients who developed HCC and 27% to 70% of the patients who suffered non-HCC liver-related deaths would have been identified for antiviral therapy. The authors found that the addition of baseline serum albumin, platelet count, and presence of basal core promoter and precore mutations would improve the accuracy of the identification of these patients to 90% to 100%. The authors concluded that if current guidelines were used, a significant proportion of HBsAg-positive patients would be deprived of the possible benefits of antiviral therapy.

However, the results of this and other studies on this topic[2] need to be interpreted carefully. First, all guidelines recommend that patients who are not initiated on treatment should continue to be monitored and that treatment should be initiated later when the indications arise.[3] In this study, the authors focused on laboratory test results at the first visit and did not consider the option of treatment after an initial period of observation. As the authors indicated, alanine aminotransferase (ALT) levels fluctuated during follow-up. Thus, many of the patients would have met treatment criteria at some point during the course of follow-up. Second, 78.5% of the patients were Asians and likely acquired hepatitis B virus (HBV) infection perinatally or during early childhood. The mean age of the patients at recruitment was 48 years,

indicating that most of the patients had been infected for more than 4 decades. In light of recent findings that persistently high serum HBV DNA is a predictor of clinical outcomes and significant liver disease may be present in patients with normal ALT, the 2007 American Association for the Study of Liver Diseases practice guidelines recommended that lower HBV DNA and ALT cutoffs be applied to patients older than 40 years and liver biopsies be performed in patients with HBV DNA and ALT values in the grey zone.[3] Finally, the authors assumed that had antiviral treatment been administered at presentation, all cases of HCC and liver-related mortality would have been prevented. Clinical studies have shown that antiviral therapy is not effective in improving survival for patients with advanced liver failure.[4] Moreover, antiviral therapy decreases but does not completely prevent the risk of HCC.[5,6]

We congratulate the authors for conducting this study. However, we caution readers that decisions on hepatitis B treatment should not be based solely on laboratory test results at the first visit. Given the fluctuating nature of chronic HBV infection, all patients should be monitored for life so treatment can be initiated later when the indication arises.

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Reply:

We thank Tilmann et al.,[1] Chan,[2] and Degertekin and Lok[3] for their thoughtful comments and questions concerning our article.[4] As they clearly point out, the decision to treat a patient with chronic hepatitis B according to guidelines remains controversial, and ultimately, the final choice to treat an individual patient should be based on inclusion in the guideline criteria along with the clinical judgment of the physician. As many of the guideline criteria are now being reevaluated, the letters by Chan and Tilmann et al. aptly raise the question of lowering the hepatitis B virus (HBV) DNA criteria in patients with cirrhosis to allow treatment in those patients who have detectable HBV DNA concentrations of $\leq 10^4$ copies/mL. The clinical outcomes of the 129 patients with cirrhosis in our database by qualitative HBV DNA categories (negative, $\leq 10^4$, and $>10^4$) are shown in Table 1. As shown in the table, six patients with cirrhosis who either died from a non–hepatocellular carcinoma (HCC) liver-related event or who developed HCC had detectable baseline HBV DNA concentrations that were $\leq 10^4$ copies/mL. However, 11 patients had HBV DNA levels below the limit of detection and either died from a liver-related event or developed HCC. Thus, modification of the treatment guidelines to include patients who have detectable baseline HBV DNA concentrations of $\leq 10^4$ copies/mL would have captured patients from our database who ultimately had a poor outcome but would not have addressed those patients who presented with HBV DNA concentrations below the limit of detection. It is important to note, as the three letters point out and as we mentioned in the discussion section of our original article, that further prospective treatments trials are required to determine if antiviral therapies will have any impact on the incidence of HCC, especially in patients with less active liver disease [i.e., low or undetectable levels of viremia and normal or nearly normal alanine aminotransferase (ALT) levels].

Table 1. Distribution of Clinical Outcomes by the HBV DNA Category for Patients with Cirrhosis (n = 129)

HBV DNA Category	Total: n (%)	Liver-Related Death: n (%)	Developed HCC: n (%)	Alive: n (%)
Negative	21 (16.3)	6 (16.2)	5 (23.8)	10 (14)
$\leq 10^4$	12 (9.3)	4 (10.8)	2 (9.5)	6 (8.5)
$>10^4$	96 (74.4)	27 (73)	14 (66.7)	55 (77.5)

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

The letter by Tilmann et al.[1] also suggests that the use of more recently developed guidelines, such as those published by Cornberg et al.,[5] may have captured additional patients with poor prognosis who were not recommended for treatment by the original four guidelines assessed in our article.[4] To address this question, we conducted an analysis of patients in our database using the criteria established by Cornberg et al. The initial recommendation by Cornberg et al. is to treat all patients with cirrhosis who have detectable HBV DNA. As shown in Table 1, use of these criteria would have captured the six patients who had a poor outcome and presented with baseline HBV DNA levels of $\geq 10^4$ but would have excluded the 11 patients with a poor prognosis who presented with HBV DNA levels below the detectable limits. Although we agree that the utility of treatment of patients with cirrhosis and serum HBV DNA levels below the detectable limits remains unknown, it is important to point out that patients who have serum HBV DNA levels below the detectable limit of the assay still may have underlying HBV DNA replication at low levels and may benefit from antiviral therapy.

The application of Cornberg et al.'s criteria[5] to the population of patients without cirrhosis in our database would have captured the nine patients who developed HCC and were not identified by the original European Association for the Study of the Liver, US Panel, Asian Pacific Panel, or American Association for the Study of Liver Diseases guidelines as candidates for therapy. In our database,[4] nine patients without cirrhosis who were excluded from antiviral therapy by at least one of the guidelines eventually developed HCC. Cornberg et al.'s criteria initially assess patients without cirrhosis by baseline serum HBV DNA concentrations and recommend therapy in those patients who have serum HBV DNA concentrations of $>10^4$ copies/mL and who also meet subsequent ALT, liver histology, and HCC risk factor criteria. All nine patients who were noncirrhotic and developed HCC and who were excluded from treatment by at least one guideline had a baseline serum HBV DNA concentration of $>10^4$ copies/mL. Of the

nine, only two met Cornberg et al.'s criteria for ALT cutoff (>2 times the upper limit of normal). The remaining seven patients were recommended for treatment on the basis of Cornberg et al.'s guidelines as they met the requirement for HCC risk factors (all were male and >40 years old). Thus, Cornberg et al.'s criteria captured our patients without cirrhosis who were excluded from treatment by the European Association for the Study of the Liver, US Panel, Asian Pacific Panel, or American Association for the Study of Liver Diseases guidelines.

Finally, the letter by Degertekin and Lok[3] addresses the issue of use of serum ALT as criteria for treatment decisions. In our database, the ALT cutoff was the most frequent reason for exclusion from treatment by the four treatment guidelines in those patients who ultimately died from a liver-related event or developed HCC. Degertekin and Lok suggest that continued monitoring of ALT would have identified those patients who were excluded from treatment on the basis of the ALT criteria but who eventually died from a liver-related event or developed HCC. Although we agree that continued monitoring of patients with chronic hepatitis B would have identified some of these patients, it is important to point out that, as shown in Fig. 1B of our original article,[4] the ALT levels fluctuated significantly over time, and this makes ALT an unreliable prognostic indicator at any time point. Additionally, patients who did not develop liver-related complications also had fluctuating ALT levels during follow-up. In contrast, the cutoffs that were established for platelet and serum albumin concentrations (≤130,000 mm^3 and ≤3.5 g/dL, respectively) were significantly more reliable than ALT monitoring because in no case did a patient who presented with platelet and serum albumin concentrations that met the cutoff criteria have a subsequent value outside the range of the cutoff. Therefore, utilization of the platelet and serum albumin concentration cutoffs defined in our article could allow for a treatment decision to be made earlier with less variable data in comparison with ongoing monitoring of serum ALT concentrations.

At present, there are no studies that validate whether the current treatment guidelines actually include patients who may die from liver-related complications or develop HCC. The goals of treatment, including hepatitis B e antigen seroconversion, normalization of ALT, and reduced HBV DNA levels in patients who meet treatment guidelines, may only be a short-term fix, and continual evaluation of criteria for including patients who develop complications must be an ongoing process.

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Potential conflict of interest: Nothing to report.

The Optimal Ribavirin Dose for Patients Infected with Hepatitis C Virus Genotype 3: Should We Utilize More?

To the Editor:

The article by Ferenci et al.[1] investigating the efficacy of combination therapy with a low dose of ribavirin (RBV) in patients with chronic hepatitis C virus (HCV) genotype 2 or 3, suggests that 400 mg/day of RBV suffices in patients infected with HCV genotype 3 (HCV-3) to achieve as high sustained virological response (SVR) rates as those attained by the standard 800 mg/day dosing (SVR: 63.9% versus 67.5%), whereas the same results could not be replicated in patients with HCV-2. In the latter patients, in fact, the SVR rates following low-dose RBV were significantly lower than those attained with a standard dose of RBV (55.6% versus 77.8%). At variance with the author comments, we believe that the 67% SVR rate obtained in patients with HCV-3 by the standard-of-care RBV regimen is less than satisfactory, too. In fact, it negatively compares with the majority of other reports, especially studies assessing a weight-based dosing of RBV.[2-4] In fact, when the efficacy is analyzed of a 24-week treatment with pegylated interferon-α2a given at 180 μg/week plus RBV at 800 mg/day in 34 consecutive patients with HCV-3 treated at our center in the last 2 years, and the response rates are stratified by RBV dosing, a ≥11.5 mg/kg/day RBV dose led to higher SVR rates and lower relapse rates, reaching statistical significance by per-protocol analysis (Table 1). Although we acknowledge that the sample size of our study was too small to draw definite conclusions, it should be noted that the prevalence of moderators of treatment outcome like cirrhosis and baseline

Table 1

Type of Response	RBV ≥ 11.5 mg/kg/day		RBV < 11.5 mg/kg/day		*P* Value
	N	%	N	%	
Intention-to-treat					
ETR	13/14	(93)	17/20	(85)	0.67
SVR	12/14	(86)	10/20	(50)	0.06
RR	1/13	(8)	7/17	(41)	0.09
Per-protocol					
ETR	13/13	(100)	17/18	(95)	1.0
SVR	12/13	(92)	10/18	(56)	0.04
RR	1/13	(8)	7/17	(41)	0.09

ETR, end of treatment response; RR, relapse rate.

viremia >800,000 IU/mL was equally balanced between patients given ≤11.5 mg/kg/day RBV and ≥11.5 mg/kg/day RBV (14% versus 20% and 45% versus 64%, respectively). We wonder whether body weight–based dosing of RBV matters also in the Ferenci study, where in fact relapsers were heavier than sustained responders (mean body weight: 78.8 kg versus 70.4 kg). We think that a controlled study would help understanding whether weight-based RBV can improve the 24-week treatment outcome of patients with HCV-3 who are given pegylated interferon-α2a at 180 μg/week.

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Reply:

We thank Dr. Anghemo and his colleagues for their comments regarding our randomized controlled trial that compared ribavirin dosages of 400 and 800 mg/day in combination with peginterferon alfa-2a given for 24 weeks in treatment-naive patients with hepatitis C virus (HCV) genotype 2 or 3 infection.[1] As Anghemo and his colleagues correctly pointed out, no conclusions can be drawn from the few data shown in their letter. This is also unnecessary because, in addition to our study, two large randomized multicenter trials have compared high and low ribavirin dosage regimens in treatment-naive genotype 2/3 patients.[2,3]

The seminal study by Hadziyannis et al.[2] clearly showed that there was no statistically significant difference in sustained virologic response (SVR) rates between genotype 2/3 patients treated for 24 weeks with peginterferon alfa-2a in combination with a high (1000 or 1200 mg/day) or low (800 mg/day) ribavirin dosage (81% versus 84%, respectively). Importantly, the incidence of treatment-related serious adverse events, ribavirin dosage reductions, and decreases in the hemoglobin concentration to less than 100 g/L was higher in patients randomized to the high ribavirin dosage regimen.[2]

Jacobson et al.[3] subsequently confirmed these results in a large randomized multicenter trial. There was no significant difference in SVR rates between patients treated with ribavirin at a dosage of 800 to 1400 mg/day (67.7%) or 800 mg/day (65.0%) in combination with peginterferon alfa-2b for 24 weeks.[3]

Thus, our study was the third randomized multicenter study of ribavirin dosage in genotype 2/3 patients treated for 24 weeks. Once again, there was no significant difference in SVR rates between the two dosage regimens. Moreover, the individual dose of ribavirin (mg/kg/day) had no impact on SVR (Fig. 1).

On the basis of three randomized trials, we see no need to use ribavirin doses greater than 800 mg/day in treatment-naïve patients with genotype 3, provided that the planned duration of treatment is 24 weeks.

In addition to ribavirin dose, treatment duration is a key determinant of SVR. In a follow-up study, we investigated shortening the duration of peginterferon alfa-2a treatment to 16 weeks in combination with ribavirin at a dosage of either 400 or 800 mg/day. This study had to be stopped for ethical reasons after 100 patients had been recruited because the relapse rate in the low-dose group was 50%. The ACCELERATE trial subsequently demonstrated convincingly that an abbreviated 16-week regimen of peginterferon alfa-2a plus ribavirin 800 mg/day is less effective than a 24-week regimen.[4] It is not clear that the use of higher dosages of ribavirin compensate for the inadequacy of treatment durations less than 24 weeks.[5]

A small proportion of genotype 3 patients appear to be treatment-resistant, as evidenced by detectable HCV RNA levels at week 4 of treatment. These individuals have higher relapse rates and lower SVR rates than patients who clear HCV RNA by week 4. At present, it is not possible to identify these patients before treatment is initiated. How best to manage these individuals is an as yet unanswered question. An ongoing randomized trial will determine whether prolonging treatment to 48 weeks is the key to curing HCV infection in these individuals.

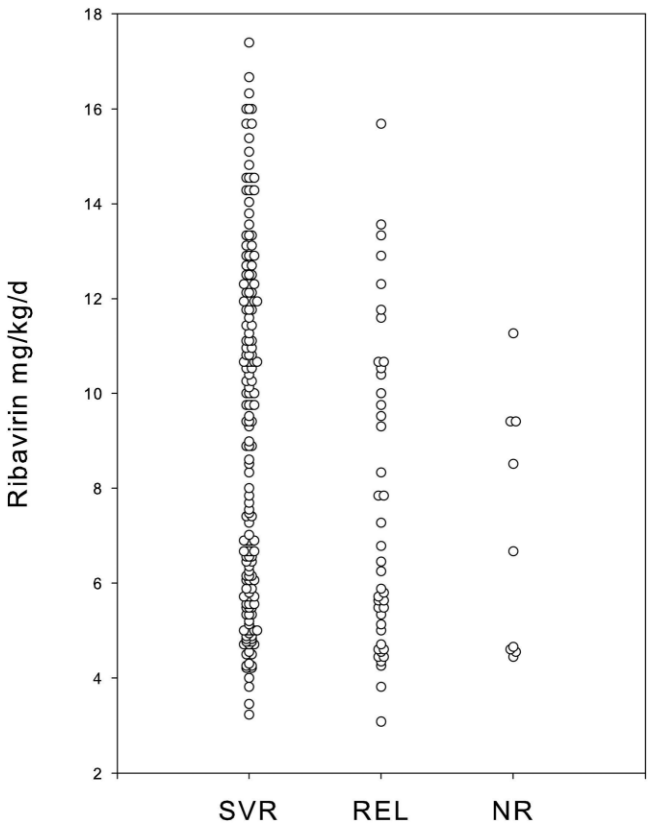

Fig. 1. Individual dose of ribavirin (mg/kg/day) versus the sustained virologic response (SVR), relapse (REL), and nonresponse (NR) rates.

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Hepatitis B e Antigen–Negative Patients with Persistently Normal Alanine Aminotransferase Levels and Hepatitis B Virus DNA > 2000 IU/mL

To the Editor:

We read with great interest the article by Papatheodoridis et al. published in HEPATOLOGY.[1] The authors enrolled 434 hepatitis B e antigen (HBeAg)–negative patients and reported higher proportions of histological indication for treatment (grading score ≥ 7 and/or stage ≥ 2 in Ishak's classification) in chronic hepatitis B patients with higher serum hepatitis B virus (HBV) DNA levels. The authors concluded that HBeAg-negative chronic HBV patients with increased alanine aminotransferase (ALT) and HBV DNA ≥ 20,000 IU/mL almost always require therapeutic intervention, but histological indications for treatment are also present in the majority of such patients with HBV DNA < 20,000 and even HBV DNA < 2000 IU/mL. In contrast, the majority of HBeAg-negative patients with persistently normal alanine aminotransferase (PNALT) and HBV DNA > 2000 IU/mL have minimal histological lesions, and they may not require immediate liver biopsy and treatment. Some concerns have to be addressed.

First, the authors defined the 35 HBeAg-negative patients with PNALT and HBV DNA > 2000 IU/mL as inactive carriers in this study. This is different from the diagnostic criteria for the inactive carrier state in the practice guideline of the American Association for the Study of Liver Diseases, which uses HBV DNA < 2000 IU/mL.[2] Second, the 35 patients defined as inactive carriers all had HBV DNA levels not only >2000 IU/mL but also <20,000 IU/mL. Kumar et al.[3] recently reported that in HBeAg-negative patients with PNALT, the median (range) HBV DNA was 4.29 (2.78-9.20) $\log_{10}$ copies/mL; 55.3% (64/116) and 35.3% (41/116) had HBV DNA ≥ 4 and 5 $\log_{10}$ copies/mL, respectively. Previous studies from France[4] (85 cases) and Taiwan[5] (414 cases) on HBeAg-negative patients with PNALT showed that 18.8% and 55.8% had a level > 4 $\log_{10}$ copies/mL, and 2.4% and 31.6% had an HBV DNA level > 5 $\log_{10}$ copies/mL. Accordingly, 64.1%,[3] 12.5%,[4] and 56.7%[5] of HBeAg-negative patients with PNALT and an HBV DNA level > 4 $\log_{10}$ copies/mL had an HBV DNA level > 5 $\log_{10}$ copies/mL. The lack of patients with PNALT and HBV DNA ≥ 20,000 IU/mL in the study[1] raises the concern of possible selection bias. Third, a histological indication for treatment according to the definition of Papatheodoridis et al.[1] was present in 17% (6/35) of their inactive carriers by the presence of stage 2 fibrosis but without active necroinflammation (grading score of 2-4 in all six cases).[1] Kumar et al. reported that 41.4% (24/58) of patients with PNALT had active liver disease with significant activity (histologic activity index score ≥ 3) and/or significant fibrosis (stages 2-4). They reported that 32.4% (11/34) of patients with histologically inactive disease and 75% (18/24) of patients with active disease had HBV DNA > 5 $\log_{10}$ copies/mL. In other words, 20.7% (6/29) of patients with PNALT and HBV DNA < 5 $\log_{10}$ copies/mL had active liver disease.[3] This implies that the histological characteristics of these patients are a heterogeneous group. Lastly, Feld et al.[6] reported in their follow-up study that 10.7% and 20% of HBeAg-negative patients with normal ALT and HBV DNA from 4 to 5 $\log_{10}$ copies/mL experienced a rise in ALT by 6 and 12 months of follow-up, respectively. Additionally, Zacharakis et al.[7] reported that four (2.1%) patients who had PNALT and serum HBV DNA levels > 2000 IU/mL exhibited HBV reactivation with increased ALT levels; there was a yearly incidence of 0.4% in their follow-up study. Considering all this, we completely agree with the authors that HBeAg-negative patients with persistently or transiently increased ALT and HBV DNA ≥ 20,000 IU/mL always require therapeutic intervention, as suggested by the recommendation of the guidelines of the American Association for the Study of Liver Diseases,[2] and we appreciate and are sympathetic to contributions from Papatheodoridis et al. on strengthening this concept from the histological point of view. Nevertheless, we think the conclusion that HBeAg-negative patients with PNALT and HBV DNA > 2000 IU/mL may not require immediate liver biopsy and treatment requires a more careful reappraisal because of the limitations of the study. HBeAg-negative patients with normal aminotransferase levels and HBV DNA > 2000 IU/mL seem to be one of the patient groups currently overlooked in recent guidelines,[2,8] so more efforts to develop strategies for optimizing treatment outcomes for this group of patents are mandatory.

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Reply:

We thank Dr. Dai et al. for their interest in our article and for supporting our findings that liver biopsy does not usually affect the decision for treatment in hepatitis B e antigen (HBeAg)-negative patients with abnormal alanine aminotransferase (ALT) and hepatitis B virus (HBV) DNA ≥ 20,000 IU/mL, although it offers valuable information for patients receiving long-term therapies. There is also no disagreement on the need for biopsy in HBeAg-negative patients with persistently or transiently abnormal ALT and HBV DNA < 20,000 IU/mL.

The optimal management of HBeAg-negative patients with persistently normal ALT (PNALT) has always been debatable. We intentionally did not perform biopsies in such patients with HBV DNA ≤ 2000 IU/mL, because we believe that only cases with HBV DNA > 2000 IU/mL represent a controversial group with some likelihood of significant histological lesions. There was no selection bias in our study because there was no patient with PNALT and HBV DNA ≥ 20,000 IU/mL during the inclusion period. Such patients are extremely rare in Greece.[1,2] Among the last 300 HBeAg-negative PNALT patients seen at our clinics, <1% had HBV DNA > 20,000 IU/mL (<200,000 IU/mL, minimal necroinflammation and fibrosis in both cases). We have previously commented on the recent definition of "inactive carriers",[3] which does not include PNALT patients with HBV DNA > 2000 IU/mL [however, such patients are not included under any term in recent guidelines[4,5]].

Regarding the conflicting reports on histology and HBV DNA > 20,000 IU/mL in HBeAg-negative PNALT patients, we believe that there are two main reasons. First and most important, not all studies use the same criteria for defining PNALT (a less strict follow-up increases the likelihood of misclassifying patients with ALT fluctuations). We used a strict first-year follow-up with five ALT determinations (every 3 months), while three or fewer ALT determinations (every 6 months) were sufficient for PNALT cases in a study from India (only two ALT values required for inclusion)[6] or the United States,[7] and no relevant information is provided in a study from Taiwan.[8] Second, differences in viral (e.g., genotypes) or host genetic characteristics might be also responsible for such discrepancies; data different from ours are reported in non-European studies.[6-8] Inactive carriers represented <8.4% of chronic HBV cases in the study from India,[6] whereas they represent a large proportion of chronic HBV European patients. Our data are in agreement with another European (French) study[9] including 85 PNALT patients under strict ALT follow-up; HBV DNA > 20,000 IU/mL was exceptionally rare (2%), necroinflammation score was always ≤6, and Ishak fibrosis score was ≤2 in 91% (score 3-4 in 5 of 58 cases). We also agree with Feld et al. that HBV DNA > 20,000 IU/mL is highly predictive of future ALT elevation,[10] as we have reported an increased risk of future reactivation in HBeAg-negative PNALT patients with HBV DNA levels of even >2000 IU/mL.[2]

In conclusion, we believe that our findings are valid and support the suggestion that HBeAg-negative patients with HBV DNA < 20,000 IU/mL and PNALT under strict follow-up, at least in Europe, will not benefit from liver biopsy, which is not expected to show significant lesions. All such cases should be followed for life, whereas patients with HBV DNA > 2000 IU/mL warrant closer follow-up.

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Profiling MicroRNA Expression: a Snapshot of Nonalcoholic Steatohepatitis and a Recording of its Pathogenesis

To the Editor:

We read with great interest the article by Cheung et al.[1] In this report, the authors analyzed the expression profile of microRNAs in liver from patients affected by nonalcoholic steatohepatitis (NASH), and sought to find differentially expressed microRNAs and their potential role in NASH pathogenesis.

Using microarray technology, microRNA profiles of 15 subjects with biopsy-proven NASH were normalized against 15 control subjects with a normal liver histology. Out of a total of 474 microRNAs analyzed, 46 were found differentially expressed in NASH patients. In particular, 23 were up-regulated (i.e., miR-34a and miR-146b), while 23 were down-regulated (i.e., miR-122) and a selection of differentially expressed microRNAs were further validated using quantitative real-time polymerase chain reaction (qRT-PCR). As expected, the predicted targets of these microRNAs include proteins belonging to complex intracellular pathways, which have been previously reported altered in NASH.[2] In fact, the most recognized model of NASH pathogenesis, which is an advanced form of nonalcoholic fatty liver disease (NAFLD), considers the elevated plasma levels of free fatty acids as a first "hit" able to induce several secondary hits, including insulin resistance, increased oxidative stress on hepatocytes, and induction of proinflammatory cytokines.[3]

Interestingly, the authors demonstrated that miR-122 was significantly decreased (63%) in patients with NASH. The *in vitro* silencing of miR-122 affected the messenger RNA expression levels of predicted targets like FAS, sterol regulatory element binding protein-1c (SREBP-1c), SREBP-2, and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). These proteins, which play roles in the lipid metabolism, have been already associated to NASH both in *in vitro* and *in vivo* models.[4-6]

The authors conclude that human NASH is associated with unbalanced hepatic microRNA expression and that the alteration of lipid metabolism, linked to miR-122 down-regulation, could contribute to development of NASH.

Cheung's work is a clear example of the basic application of microRNA profiling to study a pathology such as NASH. However, although the reported results connected to miR-122 expression are sound, their significance is difficult to interpret because the modulation of this microRNA is not a specific sign of NASH. In fact, down-regulation of miR-122 has been previously described in hepatocarcinoma, suggesting that its function is associated with liver cancer development.[7,8]

As previously underlined, we strongly believe in the power of bioinformatics in determining the interconnections among genes, proteins, and pathways potentially involved in NAFLD/NASH pathogenesis.[9] However, microRNA profiling and validation is only the first step toward the comprehension of NAFLD/NASH pathogenesis. The experimental validation of target gene (putative targets) predictions is not an easy issue. Several software and algorithms based on different rationales have been proposed in the last several years (i.e., MiRanda, TargetScan, Diana-microT, and PicTar) to help find candidate targets regulated by microRNAs.[10] Also the performance of individual programs and their combined use to achieve a common list of targets has been evaluated and reported.[11] The development of such algorithms indeed represents a hot topic in biomedical informatics. Moreover, the scarcity of biological data linking specific microRNA action to the fate of their respective messenger RNA targets so far represents the most serious limitation. In fact, as correctly indicated by Cheung and colleagues, their findings "do not provide direct proof of the involvement of microRNAs, but they serve as a broad blueprint and resource for future hypothesis generation and hypothesis-driven studies of the role of microRNAs in the development of NASH."

Finally, we believe that microRNA technology could provide not only a snapshot of the current condition of NASH, but also a record of early pathogenetic events. Thus, a complete profiling of microRNAs in patients with simple hepatic steatosis normalized against NASH, or animal models that provide the possibility to study the evolution to NASH, might help solve these intricate molecular mechanisms.

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