CORRESPONDENCE

Inflammation Does Not Always Kill Hepatocytes During Liver Damage

To the Editor:

We read the article by Horiguchi et al.[1] with great interest. It is a commonly accepted dogma that inflammation induces necrosis and apoptosis of hepatocytes during liver damage. However, clinicians have found that inflammation does not always correlate with hepatocellular damage in chronic liver disease. How to explain the conflict? By using a well-established model of mice with specific deletion of signal transducer and activator of transcription 3 in myeloid cells ($STAT3^{mye-/-}$), Horiguchi and colleagues surprisingly found more inflammatory cells, eg, neutrophils, but less necrosis/apoptosis in the liver of $STAT3^{mye-/-}$ mice than in wild-type mice after carbon tetrachloride (CCl_4) treatment. $STAT3^{mye-/-}$ mice had higher hepatic STAT3 activation and became resistant to hepatic oxidative stress after CCl_4 injection compared with wild-type mice. In contrast to $STAT3^{mye-/-}$ mice, hepatocyte-specific STAT3 knockout ($STAT3^{Hep-/-}$) mice had more liver necrosis/apoptosis but less inflammation after CCl_4 treatment compared with wild-type mice. An additional deletion of hepatocyte STAT3 in $STAT3^{mye-/-}$ mice restored CCl_4-induced hepatic necrosis but reduced liver inflammation. This study suggests that inflammation associated with a predominance of hepatoprotective cytokines may reduce rather than accelerate hepatocellular damage via activation of hepatocyte STAT3 in CCl_4-induced liver damage. The data elucidate a potential mechanism that inflammation does not always correlate with hepatocellular damage.

Interestingly, the same group had also previously investigated inflammation and hepatocellular damage in the same strain of $STAT3^{mye-/-}$ mice treated with concanavalin A (ConA) or ethanol.[2,3] $STAT3^{mye-/-}$ mice had higher inflammation concomitant with more severe hepatocellular damage compared with wild-type mice after ConA or ethanol treatment. The discrepancy between ConA- and CCl_4-induced liver damage in $STAT3^{mye-/-}$ mice could be attributable to the different T helper type 1 (Th1) cytokine (IFN-γ) responses in these two models. In $STAT3^{mye-/-}$ mice, ConA treatment elevated serum IFN-γ levels to more than 2500 pg/mL, whereas CCl_4 treatment only elevated IFN-γ levels to 15 pg/mL. Such high levels of IFN-γ in the ConA model not only directly induce liver damage but also inhibit the hepatoprotective STAT3 signal in the liver, further promoting liver injury.[3] In addition, ethanol consumption significantly inhibited STAT3 activation in $STAT3^{mye-/-}$ mice. Thus, the protective role of STAT3 is inhibited in both models of ConA and ethanol treatment. These data suggest that the etiology of liver disease plays a critical role in determining the interplay between inflammation and hepatocellular damage.

In general, the ratio between proinflammatory and anti-inflammatory factors controls the inflammatory level during liver damage; however, the fate of hepatocytes is determined by the balance between the survival and detrimental factors present within the damaged liver. For example, compared to wild-type mice, $STAT3^{mye-/-}$ mice had increased pro-inflammatory cytokines, eg, IL-6, IL-1, IFN-γ, and chemokines in both the liver and serum.[1] However, proinflammatory factors do not always kill hepatocytes and some of them such as IL-6 protect rather than kill hepatocytes via activation of survival signal STAT3 in hepatocytes. Thus, inflammation is not always a direct killer of hepatocytes.

Besides the dogma that inflammation leads to hepatocyte death, inflammation is also thought as the critical driver for liver fibrogenesis. However, many studies have demonstrated that inflammation does not always correlate with liver fibrosis in patients with chronic liver disease.[4] On the other hand, it is well recognized that degradation of fibrosis needs inflammation.[5] Thus, whether inflammation is a friend or a foe is not a simple question.

Here, we have three questions for the authors: First, in patients with acute liver failure, inflammatory cells, especially monocytes and macrophages, are central to systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and compensation anti-inflammatory response syndrome (CARS).[6] Compared with SIRS patients, patients with MODS have similar levels of proinflammatory cytokines, but higher levels of anti-inflammatory cytokines that suppressed the functions of peripheral and hepatic inflammatory cells. Similarly, in $STAT3^{mye-/-}$ mice, CCl_4 treatment resulted in early elevation of proinflammatory cytokines (at 12 hours after treatment), which remained at the same levels or was decreased at later time points (24 hours). In contrast, the levels of the anti-inflammatory cytokine IL-10 were higher at later time (24 hours) than earlier time (12 hours) points. The situation looks very similar to the early stage of MODS in patients with acute liver failure. It would be very interesting to further investigate the ratio of proinflammatory and anti-inflammatory cytokines/chemokines in $STAT3^{mye-/-}$ mice at 36 hours after CCl_4 treatment. Second, the authors investigated the relationship between inflammation and hepatocellular damage in "chronic" liver disease. However, the maximal CCl_4 treatment time period in the present study was 72 hours, and most of data were obtained from the mice treated with CCl_4 within 24 hours. It would be very interesting to investigate the inflammation and hepatocelluar damage in real "chronic liver disease", eg, mice subjected to 4 weeks of CCl_4-treatment. Finally, we are very interested in the relationship between inflammation and fibrosis in these mice treated chronically with CCl_4.

Anyway, we appreciate Horiguchi and colleagues for providing such fascinating work for further discussion.

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Reply:

We highly appreciate the comments by Weng and colleagues. Our laboratory has been focusing on the role of inflammation in liver injury, fibrosis, and regeneration by using a model of myeloid cell–specific signal transducer and activator of transcription 3 (STAT3) knockout ($STAT3^{mye-/-}$) mice. As it was previously reported, $STAT3^{mye-/-}$ mice are highly susceptible to endotoxin shock with increased production of inflammatory cytokines.[1] We demonstrated that $STAT3^{mye-/-}$ mice were resistant to liver damage after carbon tetrachloride (CCl_4) injection,[2] but more susceptible to concanavalin A–induced and ethanol-induced liver damage, accompanied by more inflammatory cells in the liver.[3,4] We believe, as Weng and colleagues summarized, the balance between pro- and anti-inflammatory cytokines determines the fate of hepatocyte survival or death. These results suggest that the etiology of liver injury determines whether inflammatory cells contribute to attenuating or worsening liver damage.

Weng et al. also raised an important point regarding the effect of inflammation on liver fibrogenesis. Although we have not examined CCl_4-induced chronic liver injury and fibrosis in $STAT3^{mye-/-}$ mice, the effects of inflammation on fibrogenesis in these mice may be complex. Inflammation not only contributes to fibrogenesis, but also plays an important role in the resolution of liver fibrosis.[5] In our study, $STAT3^{mye-/-}$ mice had high levels of liver inflammation associated with higher levels of tumor necrosis factor-α, interleukin-1 (IL-1), and IL-6, which are known to promote liver fibrosis, but also higher levels of IL-10 and interferon-γ, which are known to inhibit liver fibrosis. The balance between these pro- and antifibrogenic cytokines will likely play an important role in determining the progression of liver fibrogenesis in $STAT3^{mye-/-}$ mice after chronic CCl_4 treatment.

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Screening for Biliary Atresia: Swiss Stool Color Card

To the Editor:

With great interest, we read the report by Lien et al.[1] on their experience with using a stool color card (SCC) for the early identification of babies with biliary atresia. So far, Taiwan is the only country with regular, nationwide screening for this devastating disease. With this screening, the age for Kasai hepatoportoenterostomy has significantly dropped, and this has meant a significant increase in jaundice-free survival with the native liver at the age of 3 years. The same Taiwanese group has shown that the sensitivity and specificity of the SCC for the detection of biliary atresia are 97.1% and 99.9%, respectively.[2]

Biliary atresia is one of the most progressive fibrogenic liver diseases. The more advanced the liver fibrosis is at the time of the Kasai operation, the worse the chances are for the child to live with his or her own liver; increased age at the time of surgery has a progressive and sustained deleterious effect on the results of the Kasai operation until adolescence.[3-5]

The criteria for a condition to be considered appropriate for newborn screening are as follows: (1) the condition is an important health problem; (2) there is a recognizable latent period or an early symptomatic period during which intervention may be beneficial; (3) there are suitable screening tests or examinations that are acceptable, reliable, easy to apply, and available; and (4) there are accepted treatments that are available and beneficial when they are applied early.[6,7] The implementation of screening for biliary atresia is thus justified. Moreover, the reported results convincingly demonstrate that in Taiwan, the SCC is a simple, noninvasive, efficient, low-cost, and applicable mass screening method for the early diagnosis and management of biliary atresia.

These considerations led to the design of a Swiss national biliary atresia screening pilot program, which was started in 2009. The Swiss SCC is available in German, French, Italian, and English (Fig. 1). It is explained and handed out to the parents after their child's birth by the attending pediatrician or midwife. An instructive Web site for parents and health care personal has been established (http://www.basca.ch). The SCC and the baby's stool color are checked during the first visit with the treating physician, usually at the age of 4 weeks. During the current pilot period for the screening, SCC data are immediately transmitted to the coordination center in Geneva, Switzerland, to evaluate the feasibility and acceptance of the screening program: Switzerland obviously has a culture and mentality different from those of Taiwan. If an abnormal stool color is discovered, a further evaluation of the baby is immediately performed. By signing the SCC, the parents give their informed, written consent to the physician to communicate the data to the coordination center.

The current program is open to all interested physicians, and SCCs can be ordered for free via the Web site (http://www.basca.ch). The screening is voluntary, and money is not received by the patient, the participating physician, or the coordination center.

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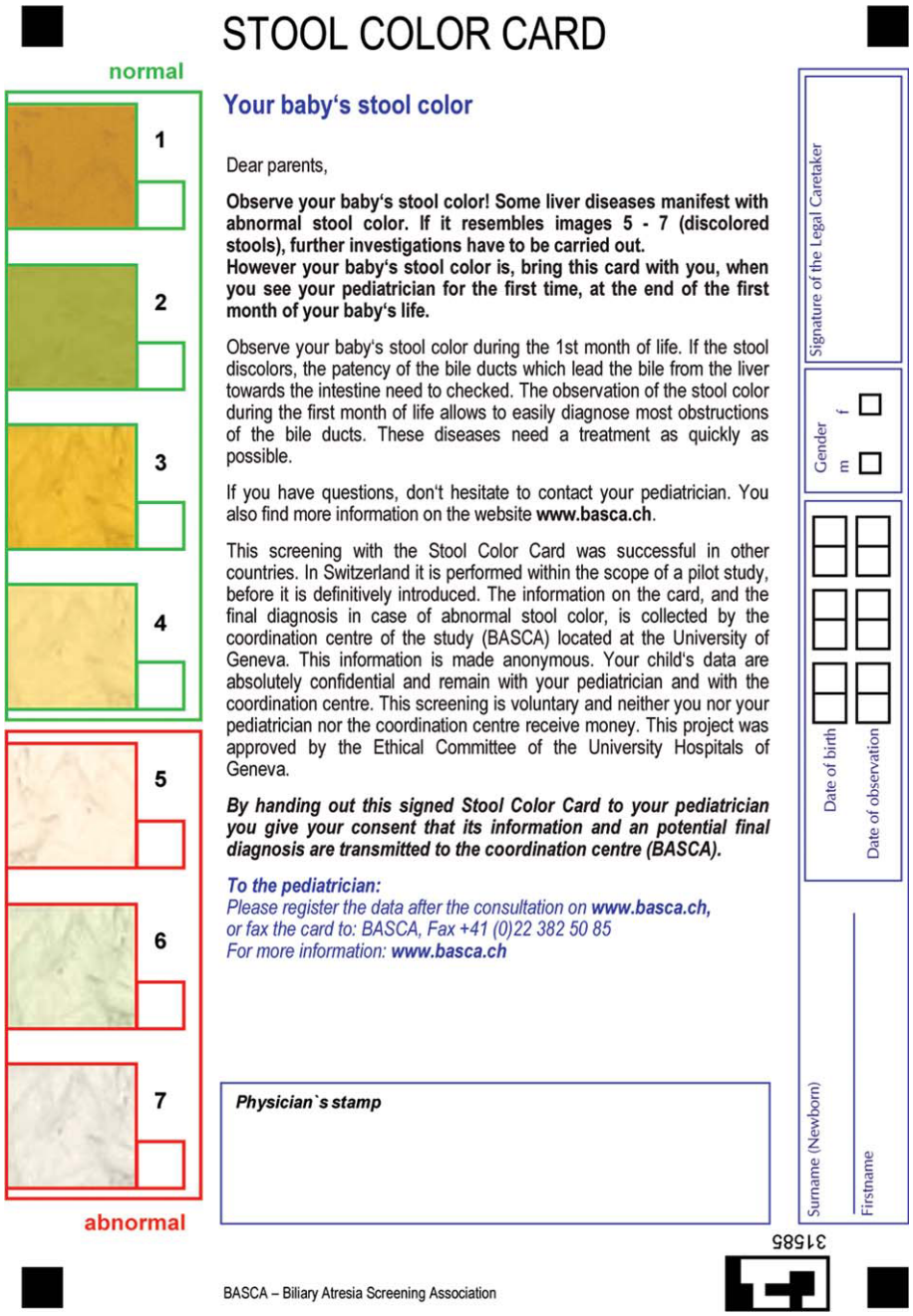

Fig. 1. English version of the Swiss SCC for biliary atresia screening. Published with the permission of the program director.

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Reply:

We appreciate the comments from Dr. Wildhaber. Biliary atresia (BA) is the most common cause of liver death in children. Through the stool color card screening program, the prognosis of BA can be improved remarkably by early detection and timely surgery. The stool color card screening program is indeed a noninvasive, low-cost, and simple screening tool that is suitable for mass screening.[1,2] We are pleased to learn of the launch of the Swiss national biliary atresia screening program. This is a positive support to our stool card screening program in Taiwan. We hope to see more countries begin the implementation of the universal stool color card screening program for BA in children. As we understand, experts from quite a number of countries, including Canada, Malaysia, Australia, and the Philippines, among others, have started or are planning a pilot study for the stool color card screening program for BA.

Our stool color card is available in other languages for new immigrants to Taiwan, including versions in English, Vietnamese, Thai, Indonesian, and Khmer (Kampuchea). In addition, the stool color card has been integrated into a child health booklet. Educational lectures were given to health and medical personnel, and periodically to day care workers.. Posters were also put up in local clinics and hospitals to propagate the related knowledge. Many previous studies had shown the importance of earlier detection for BA.[3–5] Our experience provides evidence that the stool color card is a good screening tool for BA. Continuous efforts to actively promote early detection is mandatory to improve the long-term outcome of BA. We believe that the stool color card is a blessing for children's health.

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Misuse of Scoring Systems

To the Editor:

We wish to comment on the article entitled "Pathologic Criteria for Nonalcoholic Steatohepatitis: Interprotocol Agreement and Ability to Predict Liver-Related Mortality" by Younossi et al.,[1] which was recently published in Hepatology. The stated goals of the study were 2-fold: (1) to compare the results of biopsy interpretations made according to three previously published histological scoring systems[2-4] and a previously unpublished system[1] for the histopathological diagnosis of nonalcoholic steatohepatitis (NASH) and (2) to compare the use of these systems for the prediction of long-term mortality. The study cohort consisted of patients with biopsy-proven nonalcoholic fatty liver disease (NAFLD) who had at least 5 years of follow-up.

Details of the utilization of the previously described histological scoring systems provide insight into our concerns. Matteoni et al.[2] described a system for the categorization of the spectrum of diseases in NAFLD and classified cases into four types: (1) steatosis, (2) steatosis plus inflammation, (3) steatosis plus ballooning, and (4) steatosis plus Mallory-Denk bodies or fibrosis. This classification system was not used to identify the presence or absence of NASH in the original study according to Younossi et al. The degree of inflammation and its location (portal or lobular) were not specified in the system, nor was the degree of fibrosis or its zonality (perisinusoidal or portal). On the other hand, the Brunt proposal[3] for grading and staging NASH was just that: a proposal, published in the same year as the Matteoni classification system, that followed the same paradigm used in chronic hepatitis for separating grading (activity) and staging (fibrosis). This proposal was, however, not intended for establishing a diagnosis of NASH. This system was applied to liver biopsy samples only after NASH had been diagnosed and not to cases with steatosis only or steatosis and inflammation. Thus, the use of "fat plus lobular inflammation" for a "Brunt" diagnosis of "NASH" by Younossi et al. is the result of a misunderstanding, and this misapplication of the proposal has led to their conclusion that the system results in the overdiagnosis of NASH.

The National Institutes of Health–sponsored NASH Clinical Research Network system, which is called the Kleiner scoring system,[4] also separates the activity [nonalcoholic fatty liver disease activity score (NAS)] and the stage (fibrosis). The NAS comprises steatosis, inflammation and ballooning only, and no fibrosis, as implied by Younossi et al.[1] Importantly, the NAS scoring system was not intended to be used as a surrogate for a diagnostic determination of NASH versus NAFLD without NASH. Although, as noted by Younossi et al., other authors have used the NAS as a surrogate for establishing a diagnosis of NASH, neither the NASH Clinical Research Network nor we as the participating pathologists have ever supported the use of the system for diagnosis in writing or presentations. Furthermore, as we have recently demonstrated, although higher NAS scores correlate with a diagnosis of NASH statistically, they have separate and distinct clinical meanings, and the NAS cannot replace the histological diagnosis.[5]

Unfortunately, Younossi et al.[1] also assessed both the Brunt and Kleiner scoring systems for another purpose for which they were not designed: the prediction of liver-related mortality. Interestingly, they tested these systems against the Matteoni system, which was developed specifically for this purpose. Several statistical analyses

were performed and led to a final conclusion confirming what has been shown in many studies of NASH over the past decades: hepatic fibrosis is a predictor of long-term morbidity/mortality.

In conclusion, the comparison of histological grading and staging systems and the validation of their elements against clinical outcomes are important goals in clinical investigation. Of utmost importance in these types of studies, however, is careful attention to the details of the methods that are used. The "Brunt" and "NAS" systems, as applied in this article,[1] have not been appropriately used in this context, and we emphasize this to the editor and the readers of this article to dispel any potential misunderstandings about the usefulness of these grading systems when they are applied in the appropriate way.

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Reply:

We thank Dr. Brunt and colleagues for their interest in our article. We agree that validation against clinical outcome is important for any grading and staging system. Our study was designed to examine the relationship of histopathologic features to liver-related mortality in nonalcoholic fatty liver disease (NAFLD), and so among other assessments, we included two widely used scoring systems.[1,2]

Regarding NAFLD activity score (NAS), we agree that the original description and subsequent confirmation discouraged its use for diagnosis of nonalcoholic steatohepatitis (NASH),[2,3] and as noted in our article, our results confirmed this.

Regarding the score presented by Brunt et al., we strictly adhered to the definitions in its original description.[1] It is true that the score was intended for grading biopsies diagnosed with NASH. However, the article's description of mild NASH is quite clear and precise: "Steatosis (predominantly macrovesicular) involving up to 66% of biopsy; *may* see occasional ballooned zone 3 hepatocytes; scattered rare intra-acinar polymorphonucleocytes and/or intra-acinar lymphocytes; no or mild portal chronic inflammation." Steatosis and mild lobular inflammation are mandatory parts of this definition of mild NASH, but ballooning is optional. Elsewhere, the description of mild NASH includes "Ballooning and disarray are minimally present, *if at all*." It also noted "these biopsies meet the minimum criteria for steatohepatitis." By contrast, the description of moderate NASH says "Ballooning and disarray are *always* present." Therefore, this means that any biopsy with steatosis and mild lobular inflammation can be interpreted as mild NASH according to the *original* criteria described in Brunt et al.

It is important to remember that regardless of intended use, all pathologic grading and staging systems are based on the premise that what looks bad to the pathologist will be worse for patients. If the goal of a clinical trial is to improve histologic characteristics, it is with the presumption that histologic improvement will decrease an adverse long-term outcome. A true evidence-based test of that hypothesis requires a study with long-term outcome as the endpoint. If under the rubric of NASH we exclude Brunt's grade 1 or mild NASH, its performance in predicting liver-related mortality improves significantly. In contrast, NAS does not directly translate to a diagnosis of NASH and is more useful for assessing short-term histologic changes in clinical trials, as was its intent.

Finally, we must disagree with the statement that "many" studies have shown that hepatic fibrosis is a predictor of long-term morbidity and/or mortality in NASH. We know of only three other nonalcoholic fatty liver disease studies (combined patients = 302) that assessed histology and long-term outcome. It seems likely that further advances in histologic predictors of natural history will require a much larger number of patients with long-term follow-up.

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Efforts at Making the Diagnosis of Acute-Onset Autoimmune Hepatitis

To the Editor:

We read with great interest the article by Stravitz et al.,[1] who report that patients with indeterminate acute liver failure (ALF) often have features of autoimmune hepatitis (AIH) according to histological, serological, and clinical analyses. Fifty-eight percent of their patients with indeterminate ALF were diagnosed with probable AIH-ALF on the basis of pathological features, and they had higher serum globulin levels and higher levels of anti-nuclear antibodies, anti–smooth muscle antibodies, or both in comparison with patients without histological findings suggestive of probable AIH-ALF.

As hepatologists struggling against intractable liver diseases in Japan, we applaud their efforts at making the diagnosis of acute-onset AIH.

In past Japanese surveys of ALF, a specific etiology could not be identified in 30% to 40% of adult patients.[2] Since the establishment of the criteria of the International Autoimmune Hepatitis Group[3] and the recognition of acute-onset AIH, patients with autoimmune ALF have begun to be diagnosed.[4] However, in the early stages of their illness, they often demonstrate a histological pattern atypical for AIH that consists of centrilobular necrosis with or without portal changes.[5-7]

Recently, we have also reported that AIH is not a rare cause of ALF in our unit, and the number of patients with unknown causes could decrease according to the precise diagnosis of AIH, which is based on a combination of the aforementioned pathological features and the original revised criteria.[8] In our unit, AIH has been involved in 29% of ALF cases, and unknown causes have been involved in 12%; this means that in comparison with the results of a national survey, approximately half of our patients with unknown causes have been diagnosed with AIH-ALF.

In our recent studies,[7-10] the severity of acute-onset AIH was not high at its onset in most patients, but some of them advanced to severe diseases without a precise diagnosis or treatment. For an early diagnosis, it is most important to exclude other causes systematically, to remember acute-onset AIH in the differential diagnosis, and then to apply the scoring system; comprehensive evaluations of clinical, biochemical, radiological, and histological features are necessary. In particular, a precise pathological evaluation plays an important role in the differential diagnosis, as the authors describe. However, this is complicated by the fact that there is still no gold standard for making the diagnosis of acute-onset AIH, as the authors repeatedly note.

We believe that one of the pathological characteristics of acute-onset AIH is its histological heterogeneity, especially in severe and fulminant AIH. Histological heterogeneity leads to radiological heterogeneity. Unenhanced computed tomography often shows hypoattenuated and hyperattenuated areas, with the former reflecting massive hepatic necrosis and the latter reflecting regenerative islands. Ultrasound shows similar heterogeneity. Histological heterogeneity also leads to clinical heterogeneity. The time from onset to admission to our unit did not differ with the clinical severity (nonsevere, severe, or fulminant), and the time from onset to histological examination did not differ with the histological features (chronic hepatitis, severe acute hepatitis, or massive/submassive necrosis; Fig. 1).

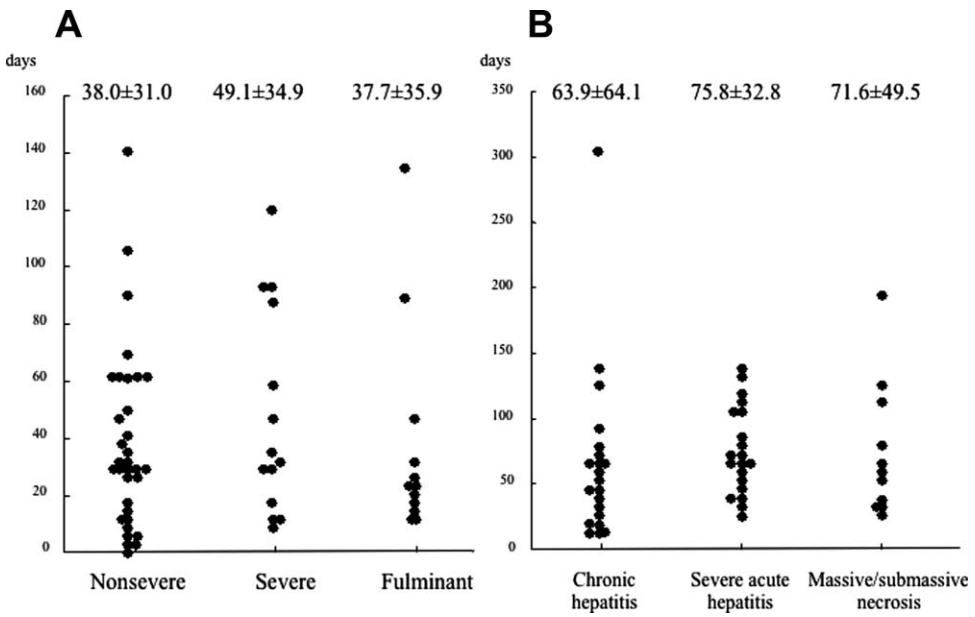

Fig. 1. Associations between (A) the clinical severity and the time from onset to admission and (B) the histological features and the time from onset to histological examination.

Characteristic morphological patterns of liver necrosis and regeneration should exist in patients with acute-onset AIH, and a better understanding of these patterns would be helpful in making the diagnosis.

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Potential conflict of interest: Nothing to report.

Autoimmune Acute Liver Failure

To the Editor:

We read with great interest the article entitled "Autoimmune Acute Liver Failure: Proposed Clinical and Histological Criteria" by Stravitz et al.[1]

Our reading of this article has given rise to several comments. It is necessary to be very cautious when one is ranking histological features first in the diagnosis and management of severe forms of autoimmune disease; during their study, Stravitz et al.[1] examined liver biopsy samples from 72 of 204 patients (i.e., 35% of the total cohort). However, the use of different liver biopsy techniques, such as transjugular liver biopsy, native liver biopsy, and postmortem biopsy, may have induced variations in the histological patterns. Centrilobular necrosis (CN), which corresponds to massive hepatic necrosis type 1 in this study, is an important but infrequent histopathological pattern of autoimmune hepatitis; centrilobular necrosis with sparing of the portal tracts was present in 3.5% of the cases reported by Hofer et al.[2] This particular pattern is of crucial importance because it may be indicative of an early stage of the disease. For the series described by Stravitz et al., it would be interesting to have a description of the phenotype and, more specifically, the prognosis of the patients with isolated centrilobular necrosis. The fact that the centrilobular zone is damaged during an early stage by the immune process is intriguing and suggests that specific autoantigens in this area could be presented to the immune system early during the course of liver disease. Clearly, the identification of these potential targets during an initial phase of the disease would be of considerable interest. In addition, it is unfortunate that the identification of a pattern typical of severe autoimmune hepatitis (AIH) is based only on this experience; in several reports, researchers have attempted to describe this entity, and experiences besides those of the US Acute Liver Failure Study Group should be cited.[3-6] In particular, the characteristics of the patients may differ between the studies. In our cohort, 8 of 16 patients (50%) suffered from grade 3/4 encephalopathy,[3] whereas 26 of 72 patients (39%) in Stravitz et al.'s study did.

The most important and problematic issue in the management of severe autoimmune liver disease is corticosteroid therapy. Of course, if a response to corticosteroid therapy is an important argument in favor of an autoimmune process, it is important that any decision to administer this therapy be balanced against the high potential risk of sepsis; infections occurred in 5 of 12 patients (42%) during steroid therapy in our study.[3] If treatment failure seems to be predicted by changes in the Model for End-Stage Liver Disease–Sodium score and the UK Model for End-Stage Liver Disease score on day 7,[7] specific scores on entry must be defined for making decisions about the administration of steroid therapy.

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Reply:

We appreciate the comments of Drs. Fujiwara and Duclos-Vallée regarding our article, "Autoimmune acute liver failure: proposed clinical and histological criteria."[1] Dr. Fujiwara reports that approximately 50% of their patient population with acute liver failure (ALF) of unclear etiology was ultimately diagnosed with autoimmune ALF (AI-ALF) on the basis of liver histology and clinical parameters, similar to findings in our series (58%). The investigators advise caution in interpreting our work regarding the heterogeneity of histological findings in liver specimens from patients with ALF. Dr. Fujiwara presents data that demonstrate no difference between the time of onset of hepatitis to time of admission or liver biopsy between patients with autoimmune hepatitis presenting as acute-onset hepatitis, chronic hepatitis, or ALF, with a large degree of overlap, supporting the clinical heterogeneity of the disease.

Dr. Duclos-Vallée also raises concern about the fact that two-thirds of our liver specimens were sections from explants, whereas the remainder were transjugular needle biopsies. The lack of uniform biopsy technique, they suggest, may have influenced our results by increasing the probability of finding specific features of autoimmunity in the larger specimens obtained from an explant. We did not, however, find statistically significant differences in the prevalence of the four features of autoimmunity between specimens obtained from explanted livers and those obtained by transjugular biopsy.

Dr. Duclos-Vallée also requested further correlation between patients with centrilobular necrosis and their clinical phenotype and outcomes. Patients with central perivenulitis were characterized by a longer jaundice-to-encephalopathy interval, and a poorer prognosis (higher incidence of liver transplantation). These attributes can be ascribed to a more subacute liver injury in patients with AI-ALF compared to those with other indeterminate etiologies. Perhaps due to small numbers (13 of 72 patients; 18%), those patients with exclusive centrilobular necrosis were not clinically different than those without this feature.

Finally, Dr. Duclos-Vallée and colleagues raise concern about administering corticosteroids to patients with suspected AI-ALF, a major rationale for performing our study. We strongly agree with their contention that corticosteroid administration may introduce a significant risk of infection, and that no convincing evidence of efficacy exists.[2] It should be noted, however, that no randomized, placebo-controlled studies have explored the efficacy of corticosteroids in patients with rigorously defined AI-ALF. In the absence of such information, we would reserve the administration of corticosteroids to patients with recent-onset AI-ALF and low-grade hepatic encephalopathy. Certainly, corticosteroids are unlikely to improve the transplant-free survival of a patient with AI-ALF late in the clinical course of the syndrome or with clinical attributes of poor outcome.

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Occult Hepatitis B Virus Infection and Hepatocellular Carcinoma Development in Patients with Chronic Hepatitis C

To the Editor:

We read with interest the article by Lok et al. that assessed occult hepatitis B virus (HBV) infection in patients who are negative for hepatitis B surface antigen and who have advanced chronic hepatitis, from the Hepatitis Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial, who did or did not develop hepatocellular carcinoma (HCC).[1] They conclude by affirming that occult HBV infection has no role in HCC development in U.S. patients with chronic hepatitis C.

After a detailed evaluation, we have several concerns regarding this conclusion. The authors themselves admit that their study has at least four main limitations. First, a limited number of patients with HCC were evaluated, and the diagnosis of cancer was simply presumed in some cases. In the HALT-C trial, the patients were randomly assigned to maintenance pegylated interferon or to no further treatment, and it would be relevant to know how the occult-positive patients were distributed according to treatment received and to definite or presumed HCC diagnosis. The second and third stated limitations concern the long storage duration and the very limited size of

biopsy specimens examined: 2-3 mm of tissue obtained by percutaneous needle biopsy cannot provide reliable results. Theoretically, such a small piece of tissue may not actually be liver or could be fibrotic tissue. The fourth stated limitation concerns the lower number of HBV genomic regions tested as compared to previous studies that provided different results. It is also highly surprising that the X genomic region (the viral gene mainly involved in the direct oncogenic role of HBV) was not searched in a study evaluating the association between HBV persistence and HCC development. Moreover, there is generic information about patient origins and, consequently, about the presumable infecting genotypes. Thus, the possibility that sensitivity and specificity of both amplification primers and probes was inadequate in a number of cases cannot be ruled out.

This study confirms previously reported (and not denied) data concerning the association between occult HBV and severe chronic hepatitis C in the United States.[2] Considering the very low prevalence of HBV infection in the United States, this observation is interesting,[3] and it would be very important to know the prevalence of occult HBV in U.S. patients infected with hepatitis C virus, with minimal liver damage.

Altogether, we feel that there is still ample room for a role of occult HBV infection in the development of HCC in patients with chronic hepatitis, and that its categorical exclusion in the U.S. population is not sufficiently proven in the study by Lok et al.

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Potential conflict of interest: Nothing to report.

Reply:

We appreciate the interest of Dr. Raimondo and colleagues in our article.[1] Of the 91 patients with hepatocellular carcinoma (HCC) who were tested for hepatitis B core antibody (anti-HBc) in serum, 39 of 53 (74%) patients negative for anti-HBc and 32 of 38 (84%) patients positive for anti-HBc met criteria for definite HCC. Among the 28 patients with HCC who were tested for hepatitis B virus (HBV) DNA in the liver, 18 of 25 (72%) HBV DNA–negative and two of three (67%) HBV DNA–positive patients met criteria for definite HCC. We would like to point out that the diagnostic criteria for presumed HCC in the HALT-C Trial protocol—a new mass on ultrasound in conjunction with two other imaging studies showing a lesion with arterial enhancement, with or without washout or evidence of progression on follow-up—are very stringent and most, if not all, patients with presumed HCC would have met the current guidelines for diagnosis of HCC. Of the 25 patients with HCC who had undetectable HBV DNA in the liver, 10 were randomized to maintenance peginterferon and 14 to no treatment, whereas one developed HCC during the lead-in phase and was not randomized. All three patients with HCC who had detectable HBV DNA in the liver were in the control group. We acknowledge the limitations of the data based on testing of HBV DNA in the liver; however, serological data showed clearly that despite evidence of a high prevalence of prior HBV infection, there was absolutely no association with HCC. We note that failure to find an association of prior or occult HBV infection and HCC among patients with HCV-related cirrhosis is not confined to the United States. Even in Italy, one large study found no association between anti-HBc in serum and HCC or liver-related deaths.[2]

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Association Between Human Leukocyte Antigen DP Gene Polymorphisms and Chronic Hepatitis B in a Chinese Population

To the Editor:

We read with great interest the article by Guo et al.,[1] which suggested that 11 polymorphisms of human leukocyte antigen DP genes were significantly associated with chronic hepatitis B in a Chinese population. These significant associations were first reported by a genome-wide association study in Japanese and Thai populations.[2] Since then, besides the study by Guo et al, [1] two studies further confirmed the significant associations.[3,4] These studies are very important, because the findings would help us more deeply understand the genetic mechanism of chronic hepatitis B. However, after carefully inspecting the article by Guo et al.,[1] we noted four issues that should be considered.

First, the authors stated that the Bonferroni correction was the current *P* value times 4. In my opinion, it is not true. On the basis of data in Table 2, the *P* value should be multiplied by 8 [(2 haplotype blocks + 2 variants (rs3135021+rs11752643)) × 2 group comparisons]; in Table 3, the multiplication should be by 12 [((2 haplotype blocks + 1 variant (rs3135021)) × 2 genotype comparisons for each variant × 2 group comparisons)]. Therefore, the *P* value for statistical significance in Table 2 should be 0.05/8 = 0.00625 and that in Table 3 should be 0.05/12 = 0.00417. On the basis of the new *P* values, the association between rs2395309 and chronic hepatitis B is not statistically significant after Bonferroni correction (Table 3).

Second, the authors did not provide the statistical powers of their studied sample for each variant. Therefore, I am not certain whether the statistically significant results are the true ones or are due to chance. It is always better to present the statistical powers.

Third, it is more proper to move the notes of Armitage's trend test in Table 2 to the notes in Table 3, because the three genotypes for each variant in Table 3, rather than those in Table 2, showed the trends.

Fourth, the order in which the three genotypes are listed under each SNP variant, namely rs9277341, rs9277535, rs3117222, and rs9380343, is not uniform in Table 2 and Table 3, and this would confuse the readers. Therefore, we suggest that the authors should keep odds ratios with 95% confidence intervals in the same direction in order to present their results more clearly.

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Reply:

We do appreciate that there are legitimate differences of opinion with respect to correction for multiple tests as the letter by Dr. Liu aptly illustrates. Our study[1] was designed to replicate a previously published genome-wide association study.[2] One opinion is, in this case, that a correction for multiple comparisons is unnecessary. In our study, however, we still address it. We state that a Bonferroni test may be too conservative given the nonindependence of adjacent single-nucleotide polymorphisms (SNPs) strongly associated by linkage disequilibrium[3] (see Johnson et al.[3] for our detailed discussion of linkage disequilibrium statistics on genome-wide association studies). Nonetheless, even if we concede to the more stringent statistical correction offered by Dr. Liu in his comment (0.05/8 = 0.00625 for table 2 and 0.05/12 = 0.00417 for table 3), nearly all the associations for SNP alleles (table 2) or genotypes (table 3) still achieve statistical significance after correction. Only rs3135021 fails significance. We feel that our data are robust and statistically strong in support of the associations of the SNP alleles, genotypes, and haplotypes illustrated in table 2 to table 4 and figure 1.

The statistical power of our study for the additive model is 89%-100%, based on case number, control number, disease prevalence (0.08), significance level (0.05), disease allele frequency (0.334–0.797), and genotype relative risk (1.5). Also, the data reported in table 2 were actually from Armitage's trend test (computed with SAS Genetics software), which is most useful when there is an additive allele effect on the disease susceptibility.

As for the direction (i.e., the polarity of odds ratios [ORs]), this format is also a matter of taste. We chose to base our tables on the risk allele (protective allele as a reference) with OR < 1.0, which we state. For example, for rs2395309, the OR should be 1/0.71 = 1.41, and 95% confidence interval should be 1.16–1.69 (formula is 1/0.86 = 1.16, 1/0.59 = 1.69) for risk allele G. We realize that use of the term "risk allele" might be confusing, because some alleles protect whereas others are susceptible, as indicated by the OR. For this confusion, we stand corrected. We apologize for the confusion, but when one examines the OR, it seemed clearly stated to us.

For table 3, we list the reference group (OR = 1.0), and the most common genotype was used as a reference for each SNP.

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Neutralization of Lipopolysaccharide Effects in Liver Diseases, the Quest for the Holy Grail

To the Editor:

We read with great interest the article in Hepatology by Nolan on the role of lipopolysaccharide (LPS) in liver injury.[1] This review, written by a pioneer of this approach, details the main studies that progressively established gut-derived LPS as a key cofactor in acute and chronic liver disease in the last half-century and more recent studies that tried to prevent LPS-induced damages by reducing or by neutralizing plasma LPS or proinflammatory cytokines. However, we think some important recent studies should have been discussed in this review.

Among the studies exploring possibilities to neutralize LPS, those which assessed the effects high-density lipoprotein (HDL) have not been mentioned.[2-4] HDL particles are multifunctional lipoprotein complexes that transport lipids and have several anti-inflammatory properties. In cirrhosis, HDL plasma concentrations are decreased and could impair the host's ability to neutralize LPS.[3] In cirrhotic rats with ascites, two recent studies have shown that HDL administration reduced the effects of LPS on tumor necrosis factor-α production[2,4] and systemic hemodynamics, restoring liver endothelial nitric oxide synthase activity and decreasing portal pressure.[2] These studies suggest that the excessive proinflammatory response to LPS in cirrhosis may be attributable, at least in part, to reduced LPS neutralization by HDL. Incubation of whole blood with reconstituted HDL prevents LPS-induced tumor necrosis factor-α and interleukin-6 overproduction by monocytes of patients with cirrhosis.[3] Therefore, we believe that restoring HDL content in patients with cirrhosis may be a research avenue to follow in the future.

We are conscious that mentioning all the studies related to this crucial subject is very difficult, but we think that the studies we report here are worth being cited in such a review.

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Potential conflict of interest: Nothing to report.

Alpha-Fetoprotein in Hepatocellular Carcinoma Surveillance: Wake Not the Dead

To the Editor:

We read with interest the letter by Marrero and El-Serag that calls for the inclusion of alpha-fetoprotein (AFP) in the American Association for the Study of Liver Diseases (AASLD) updated guidelines for the management of hepatocellular carcinoma (HCC).[1,2] However, we disagree with their conclusions and feel that the AASLD recommendation to perform HCC surveillance with ultrasonography (US) alone is supported by solid evidence.[1,2]

The evidence supporting surveillance programs for HCC with liver US with or without AFP testing stems from the results of a randomized controlled trial and from cohort studies showing that surveillance improves both detection rate of early HCCs and patient survival.[3-5] However, it is clear that the authors of the AASLD

guidelines took into account the numerous limitations of AFP testing, and therefore it is no surprise that they did not include this serological marker in their HCC surveillance recommendations.[2] In fact, although we may agree with Marrero and El-Serag that the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial is a suboptimal setting to assess the role of AFP for the early detection of HCC, this study had the precious gifts of providing prospectively collected data and to include a large population of patients who were mainly at risk of developing HCC.[6] Furthermore, data were available both at HCC diagnosis and 1 year before, thus being as close as possible to everyday clinical practice and therefore providing the best evidence currently available.[2,6] In this study, the sensitivity of AFP at a cutoff of 20 ng/mL was low (i.e., 61%) at the time of HCC diagnosis, yet at 22% it was unacceptably low 12 months before, when HCC was likely present in the majority of patients.[6] These results are strikingly similar to those obtained in a case-control study carried out in a completely different population, where a 20 ng/mL AFP cutoff showed 60% sensitivity for the diagnosis of HCC,[7] although sensitivity was unacceptably low (i.e., approximately 50%) for single nodules and/or lesions smaller than 3 cm.[8]

We feel that the extensors of the updated AASLD guidelines did not ignore the "highest level of evidence for the efficacy of US combined with AFP in research studies"[2] as affirmed by Marrero and El-Serag,[1] but evaluated both efficacy and cost-effectiveness. Indeed, the combination of AFP and US leads to a mere 6%-8% increase in sensitivity for the detection of early HCC as compared to US alone, with a doubling in the rate of false-positives and at an unbearable increase (by 84%) in surveillance-related costs.[9,10] Therefore, AFP provides no additional benefit to US, as recently concluded even in the meta-analysis by the Marrero group,[10] with a significant worsening of the cost-effectiveness of surveillance.[9] To conclude, we feel that the use of AFP as a surveillance test for HCC should be regarded as a memory, and any effort to increase the awareness and application of the currently proposed surveillance guidelines among physicians in clinical practice should be embraced.

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Treatment Implication from the Potential Association Between Nonalcoholic Fatty Liver Disease and Cardiovascular Disease

To the Editor:

In recent years, the potential association between nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) has attracted much interest,[1] generating discussion that NAFLD patients should perhaps be treated not only for their liver disorder but also owing to their associated cardiovascular risk factors. However, it seems that at present we cannot draw a definitive conclusion on the association between NAFLD and CVD. I read with great interest the article by Ghouri et al.,[2] who reviewed data from recent prospective studies and concluded that a diagnosis of NAFLD is insufficient to consider patients as being at high risk for CVD. Nevertheless, it is interesting to note that Targher et al.[3] recently reviewed the rapidly growing body of clinical evidence supporting a strong link between NAFLD and the CVD risk. Therefore, additional effort is encouraged to shed light on the link between the two disorders.

I do not intend to judge the association between NAFLD and CVD; however, I sincerely hope the current debatable status will not hamper the research community to optimize the treatment strategy. Before drawing a definitive conclusion on the association between the two disorders, I submit that the NAFLD treatment strategy, which also possesses the potential to prevent/reduce the associated risk of CVD, may serve as a good approach for NAFLD patients. The advantage of this strategy is that if no or only a weak association between NAFLD and CVD is found, the approach will not bring additional burden to the patients. There have been numerous clinical trials of various treatment modalities for NAFLD as comprehensively reviewed by Lam and Younossi,[4] including weight loss agents, insulin-sensitizing agents, lipid-

lowering agents, antioxidants, probiotics, and other novel compounds. In addition, Satapathy and Sanyal[5] recently discussed the status of current and emerging treatment strategies for nonalcoholic steatohepatitis patients and highlighted the great potential of antioxidant therapy. Because the treatment strategies for NAFLD and CVD are similar, it is promising to consider that one of the abovementioned treatment modalities for NAFLD could also be applied for associated risk of CVD.

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Are There More Factors Other than Hepatitis B Virus DNA Level and A1762T/G1764A Mutation in Liver Tissue that Also Independently Predict Postoperative Survival in Hepatocellular Carcinoma?

To the Editor:

We read with great interest the study by Yeh et al.[1] on hepatitis B virus (HBV)-DNA level and basal core promoter A1762T/G1764A mutation in liver tissue independently predicting postoperative survival in hepatocellular carcinoma (HCC). According to the article, the amount of HBV-DNA in liver tissue and the presence of the basal core promoter mutation were two independent predictors for postoperative survival in HCC. The authors also found that a short-stretch pre-S deletion located between codons 107 and 141 was associated with a poorer postoperative prognosis. This study can be hailed as an original contribution in terms of predicting postoperative survival in HCC; however, we have some concerns about it.

First, as is well known, evidence suggests that HBV genetic mutations contribute to the risk of HCC. Recent studies have shown that HBV genotype- or subgenotype-specific mutations, including C1653T in the EnhII region, T1753V, and the double mutant A1762T/G1764A in the BCP region, are independent risk factors for HCC.[2] Another study has indicated that pre-S deletions, I68T in surface gene, T1762/A1764, and A1899 are independent risk factors for HCC.[3] A recent meta-analysis revealed that the HBV pre-S mutations C1653T, T1753V, and A1762T/G1764A are associated with an increased risk of HCC. These mutations alone and in combination may be predictive of hepatocarcinogenesis.[4] We wonder whether the other gene mutations correlated with HCC in HBV other than the basal core promoter A1762T/G1764A mutation (e.g., C1653T, T1753V, A1899) can independently predict postoperative survival in HCC.

Second, it has not been determined whether hepatitis B e antigen (HBeAg) is associated with postresection survival in HCC. According to Sun et al.,[5] HBeAg was associated with a higher risk of early recurrence and poorer survival in patients after curative resection of small HCC. However, Chen et al.[3] indicated that HBeAg positivity is not a negative factor for resection in HCC patients and has no significant influence on postresection survival. Therefore, we wonder whether HBeAg was associated with postresection survival in HCC in the study by Yeh et al. Given that the serum samples used in the study had been stored in the serum bank, we think that the detection of HBeAg in both groups is convenient and has great significance.

Finally, a study by Tomimaru et al.[6] showed that histological assessment of the degree of fibrosis in noncancerous tissue is a unique prognostic factor for primary HCC without hepatitis B or C viral infection. If this is the case, then is histological assessment of the degree of fibrosis in noncancerous tissue one of the prognostic factors for HBV-related HCC as well?

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Risk Factors for Infection in Chronic Hepatitis C: A High Prevalence of Sexual Exposure Among Human Immunodeficiency Virus–Coinfected Women

To the Editor:

We have read with great interest Tohme and Holmberg's review[1] in HEPATOLOGY on the sexual transmission of hepatitis C virus (HCV). They distinguished between heterosexual and homosexual contacts and also between monoinfected and human immunodeficiency virus (HIV)–coinfected patients, and they affirmed the recently reported increasing incidence of HCV infection among HIV-positive men who have sex with men.

We analyzed the risk factors for infection in a series of 886 consecutive patients [median age = 40 years, interquartile range = 33-53 years, 521 males (58.8%)] with chronic hepatitis C who were followed up in our liver unit; 198 of these patients (22.3%) were HIV-coinfected. A risk-factor questionnaire was prospectively collected by the members of the unit (i.e., us). We considered the risk factor for HCV infection to be sexual exposure (SEXEXP) only in patients who fulfilled the following criteria: (1) a negative history for intravenous drug use (IDU), inhalatory drug use (INHDU), or blood transfusions (BTs) before 1994 and (b) a sexual partner who was recognized to be anti-HCV–positive.

The main risk factors in the whole group of patients were IDU (32.5%), BTs (19.4%), INHDU (8.9%), and SEXEXP (8.6%). In 20.2% of the patients, no risk factors were identified. However, we found significant differences in the risk factors between males and females [the main ones were IDU (47.4%) and BTs (30.5%), respectively; SEXEXP was considered to be the probable risk factor in only 1.7% of men but in 18.3% of women ($P = 0.0000$)]. There were also significant differences between monoinfected HCV patients (n = 687, age = 46 ± 14 years) and HIV-coinfected patients (n = 198, age = 35 ± 6 years). In the first group, 24.4% had a history of BTs, 23.5% had a history of IDU, and 9.1% had a history of INHDU; in the second group, a history of IDU was predominant (62.1%), and it was followed by SEXEXP (20.5%).

In our opinion, the more interesting finding is the relationship between females (n = 365) and SEXEXP as the probable route of HCV transmission. The definition of SEXEXP was fulfilled by 10% of monoinfected women (n = 292, age = 51 ± 15 years), whereas in the group of HIV-coinfected women (n = 73, age = 35 ± 7 years), the percentage was more impressive: 49%. Although this subgroup of coinfected women is small, it seems to us that this finding is worthy of being reported. The sexual partners of these women are also our patients; most have the same HCV genotype as their wives, and they usually have a history of IDU. Thus, we have to rely on clinical histories to exclude this background in women. In conclusion, we have found SEXEXP to be a very prevalent risk factor for HCV infection in HIV-coinfected women. The transmission of HCV might be secondary to high viremia levels in their partners in the period before antiretroviral treatment. This result should be further addressed in a larger population.

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