Re: Indeterminate 1-2 Cm Nodules Found on Hepatocellular Carcinoma Surveillance: Biopsy for All, Some, or None?

To the Editor:

We read with interest the article by Khalili et al.[1] addressing the management of small liver nodules detected in patients with cirrhosis under surveillance with abdominal ultrasound (US) that gave indeterminate results by contrast imaging. To optimize American Association for the Study of the Liver Disease (AASLD) guidelines,[2] the authors suggest performing a fine-needle biopsy examination of nodules showing either arterial hypervascularity on computed tomography (CT) / magnetic resonance imaging (MRI) or accompanied by a synchronous hepatocellular carcinoma (HCC) only, since these were the only independent variables associated with malignancy in their retrospective study. According to this algorithm, approximately 20% of additional tumors will be identified, with a sensitivity of 62%, a specificity of 79%, and a 73% save of liver biopsies.

When we applied this algorithm to our patients with a *de novo* liver nodule prospectively detected during surveillance[3] (Fig. 1), the corresponding figures were 44% for sensitivity and 55% for specificity, with a positive predictive value of 44% and a negative predictive value of 55%, respectively. Overall, among 36 1-2 cm indeterminate nodules the modified algorithm would have diagnosed 7 (44%) of tumors only of the 16 identified by histology, including 15 HCC and 1 intrahepatic cholangiocarcinoma (ICC). At the same time, the diagnosis of HCC would have been significantly delayed in nine (56%) patients compared with none if treated according to AASLD guidelines. The fact that the majority (75%) of delayed diagnoses were in patients with a very early HCC, i.e., the ideal candidates for radical treatment with local ablation,[4] attenuates the appeal of the modified algorithm, which in addition would have also led to a misdiagnosis of ICC in one nodule devoid of contrast uptake during the arterial phase of CT/MRI. Due to the high incidence of HCC in patients with compensated cirrhosis and the low risk of liver biopsy complications, we strongly endorse unmodified AASLD guidelines for the management of patients with cirrhosis with 1-2 cm liver nodules with undefined radiological diagnosis.

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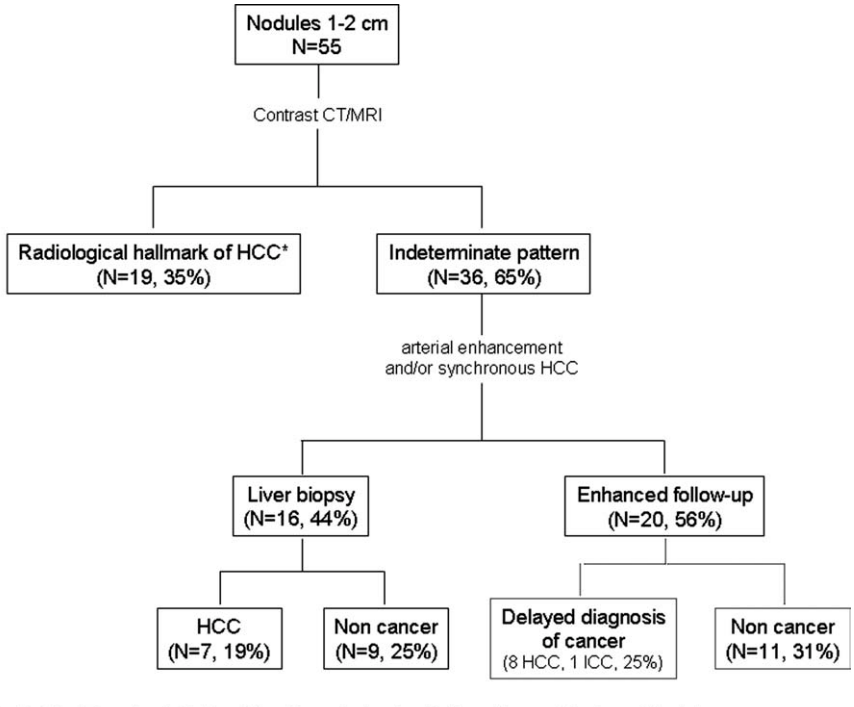

Fig. 1. The diagnostic yield of revised AASLD algorithm in our patients undergoing surveillance. Liver histology was the gold standard for the diagnosis of 1-2 cm HCC.

*contrast uptake in the early arterial phase followed by contrast wash-out in the portal venous/late phase of the study.
HCC= hepatocellular carcinoma, ICC=intrahepatic cholangiocarcinoma

Reply:

We thank Drs. Iavarone and Sangiovanni for the interest in our study,[1] but caution the authors with regard to several points in their letter. First, their conclusions are based on a small sample size of 36 indeterminate nodules. While they calculate sensitivity and specificity of 44% and 55%, respectively, using our proposed criteria, the 95% confidence interval was not reported. We calculate their confidence interval to be 21%-69% for sensitivity and 32%-76% for specificity. Small sample sizes lead to real uncertainty.

Second, the authors report that 16/35(46%) of their malignant nodules did not demonstrate a typical enhancement pattern on imaging. This rate is well above those for <2 cm nodules reported by Forner et al. (15%),[2] Leoni et al. (7%),[3] or us (25%).[1] The substantial lower sensitivity reported by Iavarone and Sangiovanni may be a result of their small sample size, but should lead to reexamination of their methodology. It is unclear whether the authors used the critical delayed phase in assessment of washout.[4] They also used fixed imaging times after contrast injection for all magnetic resonance imaging (MRI) and an indeterminate number of computed tomography (CT) scans, compromising phase timing.

Third, we disagree with fine-needle biopsy (FNB) as the reference standard. The substantial false-negative rate of biopsy is ignored by the authors in both their study and their letter. FNB, as opposed to core biopsy, further compromises the diagnosis of very early hepatocellular carcinomas (HCCs) due to its inability to detect architectural changes such as sinusoidal invasion.[5] Biopsy relies on the judgment of a pathologist to predict future behavior of a nodule, whereas close imaging follow-up demonstrates *actual* behavior: growth. For the purposes of a study, long-term stability represents a stronger reference standard than biopsy.

Finally, Iavarone and Sangiovanni worry that our proposed criteria may lead to "significantly delayed" diagnosis in a proportion of patients. Our role is detection and treatment of only malignancies that cause morbidity or shorten life. If the term "significant" is to be used outside its statistical definition, it should be within such a framework. In the setting of a competing potentially fatal disease (cirrhosis), the treatment of "very early HCCs" has yet to be justified.

We invite the authors and others to perform a new prospective trial to independently evaluate our proposals.

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Diagnostic Algorithms for Liver Fibrosis in Hepatitis C: Are They Ready to Avoid Liver Biopsy?

To the Editor:

We appreciated the article by Boursier et al.[1] about the comparison of diagnostic algorithms for liver fibrosis in hepatitis C. The purpose of combining unrelated noninvasive methods is to increase the performance of each individual method and to minimize the number of liver biopsies needed. The authors found an impressive 0% rate in liver biopsies needed with a synchronous combination of FibroScan and FibroMeter. We believe that this article deserves several comments.

Boursier et al. refer to SAFE biopsy as intended for binary diagnosis. The authors state that their synchronous algorithm guarantees a more precise classification of liver fibrosis because it provides six diagnostic classes. We wish to underline that SAFE biopsy algorithms have been modeled to address the main clinical endpoints for decision-making: significant fibrosis (≥F2 by METAVIR) and cirrhosis, as defined by international guidelines.[2,3] Importantly, some of the classes (F2 ± 1 and F3 ± 1) included in the classification of Boursier et al. imply a delta of up to two stages of fibrosis in the same class. This may make it difficult to distinguish between stages that have a different management in clinical practice, such as F1 versus F2 or F3 versus F4.

An advantage of SAFE biopsy in clinical practice is that it uses APRI as an initial screening test, which has virtually no cost and global availability. A recent meta-analysis concluded that APRI should still be regarded as a first-line screening test for liver fibrosis in hepatitis C in countries with limited health care resources.[4]

Another important issue is that SAFE biopsy algorithms adopt widely available and validated tests. When compared with APRI and FibroTest, FibroMeter has been less evaluated independently. Moreover, FibroMeter is not licensed in as many countries as FibroTest.[5]

Finally, even though liver biopsy is an imperfect standard, it is still regarded as the standard of reference by international guidelines.[2]

We conclude that combination algorithms are excellent tools to screen liver fibrosis in hepatitis C in clinical practice. The choice of the algorithm could be based on local resources, the clinical setting, and clinician preference. Whether combination algorithms could completely avoid liver biopsy deserve further independent investigation.

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Reply:

We read with interest the comments by Sebastiani and Alberti on our new FibroMeter plus Fibroscan combination in chronic hepatitis C (CHC).

We acknowledge that SAFE was a significant improvement in the noninvasive diagnosis of liver fibrosis by increasing the diagnostic accuracy and reducing the rate of liver biopsy. SAFE uses the aspartate aminotransferase-to-platelet ratio index (APRI) as first-line test because it is easily calculated at bedside with virtually no cost. Moreover, Sebastiani and Alberti argue that APRI can be used as a first-line screening test.[1] However, as stated in the pivotal study,[2] SAFE for significant fibrosis (Metavir F$\geq$2) requires liver biopsy in low APRI values due to an insufficient negative predictive value and does not require liver biopsy in high APRI values. Finally, SAFE does not use APRI as a screening test and has is inconvenient to select low-risk patients for an invasive procedure like liver biopsy. In fact, fibrosis evaluation in CHC does not correspond to a screening but to a diagnostic procedure for patient management and must use the most accurate tests. In this setting, FibroMeter and Fibroscan have been independently validated as accurate fibrosis tests in large series,[3,4] and their combination is the most accurate among six noninvasive fibrosis tests.[5]

We agree that significant fibrosis and cirrhosis are important diagnostic targets for, respectively, antiviral therapy and screening procedures. However, in clinical practice physicians have to successively use SAFE for F$\geq$2 and then, in case of noninvasive "F$\geq$2" diagnosis, SAFE for F4 to secondarily discriminate F4 from F2/3 patients. Our results clearly show that this successive use of algorithms devoted for a binary diagnosis of fibrosis significantly decreases the diagnostic accuracy and increases the rate of liver biopsy. Our new FM+FS classification circumvents this limitation by giving a precise evaluation (six diagnostic classes) of liver fibrosis in a one-step procedure.

We agree that F2$\pm$1 and F3$\pm$1 diagnoses in our classification might induce difficult decisions in clinical practice. A classical solution is to perform liver biopsy in these cases. FM+FS classification would thus require 36.8% biopsies, which is still significantly lower than with Successive SAFE (63.8%, $P < 0.001$) or Successive Bordeaux Algorithms (49.8%, $P < 0.001$). A modern solution is to evaluate the prognostic value of FM+FS classification for clinical events. Recent works have shown that noninvasive fibrosis tests had a prognostic value equal or superior to histological fibrosis staging[6,7] and that the FM+FS combination might increase this prognostic value.[8] This clearly supports a possible patient management without liver biopsy.

In conclusion, a combination of noninvasive fibrosis tests is an excellent diagnostic tool for liver fibrosis in CHC patients. The fibrosis tests must provide the most accurate combination. Combining a blood test with a physical test such as Fibroscan seems the better clinically available choice. In this setting, the synchronous combination of FibroMeter with Fibroscan allows for a precise and accurate diagnosis of liver fibrosis, with a minimum or, even more, without any liver biopsies according to the physician choice.

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Is M65 Really Better Than M30 as a Biomarker of Hepatic Fibrosis?

To the Editor:

We read with great interest the article by Joka et al.,[1] who demonstrated that, compared with the widely used apoptosis marker M30, the M65 assay had a better diagnostic performance and even differentiated between lower fibrosis stages as well as between healthy individuals and patients with simple steatosis. However, there are some issues in terms of data analysis and interpretation that merit consideration.

First, the authors claimed that, unlike the M30 assay, only serum levels of total M65 significantly discriminated between patients with nonalcoholic fatty liver disease (NAFLD) and healthy controls.[1] However, this finding is not surprising given the very small number of patients with simple steatosis (n = 10) and nonalcoholic steatohepatitis (n = 12) enrolled in this study. Actually, the results concerning M30 may be just a false-negative finding due to the fact that the study was underpowered for such a comparison. Indeed, we have shown that among patients with NAFLD, M30 and M65 distinguished between advanced fibrosis and early stage fibrosis with a similar sensitivity and specificity.[2] Second, the authors used Ishak fibrosis stage in all patients with chronic liver disease, regardless of the underlying etiology.[1] One may argue whether the application of a disease-specific score for fibrosis (such as the METAVIR score[3] for HCV fibrosis or the Kleiner et al.[4] criteria for NAFLD fibrosis) would yield different results. Finally, the authors pooled together all patients with chronic liver diseases for the purpose of comparing the diagnostic value of M30 and M65 assays for fibrosis.

We believe that this approach is not methodologically robust and can yield unreliable results. In our own experience, patients with NAFLD and mild fibrosis may display greater levels of M30 compared with those with a diagnosis of Wilson disease and severe fibrosis. It is thus likely that M30 levels are driven chiefly by apoptosis rather than hepatic fibrosis.[5,6] In light of these caveats, a word of caution is needed to avoid overinterpreting the diagnostic utility of M65 assays in the noninvasive assessment of liver fibrosis in chronic liver disease.

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Reply:

We thank Drs. Yilmaz and Kurt for their comment on our article by Joka et al.[1] In that study we evaluated different CK-18 cell death biomarkers for their diagnostic value to differentiate between various fibrosis stages in sera of patients with chronic liver disease (n = 121).

We applied the internationally accepted Ishak score for staging of fibrosis.[2] Compared to the Metavir score, the Ishak score allows for a more differentiated histopathological assessment, which enables its application not only for chronic viral hepatitis but also for other disease entities. It was not our intention to selectively evaluate the biomarkers in hepatitis C virus (HCV) patients but rather in a broad range of chronic inflammatory liver diseases. As the definition of disease stages, however, does not differ substantially between scores, we would not expect any relevant differences by applying alternative fibrosis scoring systems.

Dr. Yilmaz and colleagues found higher levels of M30 in nonalcoholic fatty liver disease (NAFLD) patients with minimal fibrosis compared to those with Wilson's disease and severe fibrosis. In our NAFLD cohort, patients showed no or only minimal fibrosis, which did not influence the diagnostic value of the M30 or M65 assay for prediction of nonalcoholic steatohepatitis (NASH). However, we found that, in contrast to the M65 assays, detection of NASH by the M30 enzyme-linked immunosorbent assay (ELISA) depends on alanine aminotransferase (ALT) levels. Thus, inflammatory disease activity might contribute to higher M30 levels in NAFLD patients despite lower fibrosis stages. In this context, it is also worth mentioning that differences of M30 antigen stability between sera and plasma might contribute to discrepant observations, since concerns about M30 antigen stability in plasma samples during storing have been reported.[3] Using serum samples, we did not observe stability issues of the M30 antigen during storing.

We certainly agree that NAFLD patients represent a relatively small subgroup in our analysis. However, despite the limited number of NAFL (nonalcoholic fatty liver; n = 10) and NASH (n = 12) patients included, we could demonstrate that, unlike the M30 ELISA, the M65 assays are able to significantly ($P < 0.01$) distinguish between healthy individuals and patients with NAFL or NASH. This observation is in line with a previous study of NAFLD patients that failed to distinguish between patients with simple steatosis and healthy controls by the M30 assay but demonstrated a higher predictive value of the M65 compared to the M30 assay to identify NASH patients.[4] Furthermore, we disagree that the study was substantially underpowered. Boxplot analysis

demonstrated a wide overlap for the M30 assay with regard to NAFL and NASH or healthy controls. To be acceptable as a marker for clinical decision making, the discriminatory power of an assay must yield significant results if subgroups of more than 10 patients are studied. In our opinion, one major factor contributing to the lower diagnostic accuracy of the M30 ELISA might be the lower values obtained by the M30 compared to the M65 assays, especially in patients with mild liver diseases.

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Rifaximin Reduces Endotoxemia and Improves Liver Function and Disease Severity in Patients With Decompensated Cirrhosis

To the Editor:

We read with great interest the article by Nolan regarding the pathogenetic role of intestinal endotoxin in the development of liver injury.[1] Increased circulating levels of gut-derived endotoxin[2] and specific sensitivity to endotoxin have been shown in cirrhosis,[3] which could worsen further hepatic impairment. However, the effects of changing gut flora on liver function in patients with

Table 1. Plasma Endotoxin Levels and Measures of Liver Function and Disease Severity Evaluated Before and After Rifaximin Treatment in Patients With Decompensated Cirrhosis

	Baseline	End of Observational Period (Week 8)	End of Rifaximin Treatment (Week 16)
Plasma endotoxin levels (EU/mL)	3.14 ± 1.23	3.32 ± 1.05	1.62 ± 0.85*,†
Ascites (suppressed by medication/refractory)	8/1	8/1	8/1
Encephalopathy (none/suppressed by medication)	4/5	4/5	4/5
Serum albumin (g/dL)	3.21 ± 0.08	3.14 ± 0.06	3.45 ± 0.06*,†
Serum albumin improved (yes/no)		1/8	7/2*,†
INR	1.66 ± 0.09	1.7 ± 0.07	1.51 ± 0.07*,†
INR improved (yes/no)		1/8	7/2*,†
Serum total bilirubin (mg/dL)	2.17 ± 0.16	2.24 ± 0.1	1.72 ± 0.13‡,§
Serum total bilirubin improved (yes/no)		2/7	8/1*,†
Serum alanine aminotransferase (U/L)	56 ± 4	57 ± 4	52 ± 6
Serum aspartate aminotransferase (U/L)	48 ± 5	48 ± 6	46 ± 4
Serum creatinine (mg/dL)	1.18 ± 0.09	1.17 ± 0.05	1.13 ± 0.07
Child-Pugh score	8.7 ± 0.4	9 ± 0.5	8.1 ± 0.5*,†
Child-Pugh score improved (yes/no)		0/9	7/2‡,§
MELD score	16 ± 1.2	16.2 ± 1.1	13.7 ± 1‡,§
MELD score improved (yes/no)		0/9	9/0‡,§

Data are expressed as means ± standard error.

Encephalopathy before entry was controlled with lactulose/lactitole. The MELD score was calculated according to the formula of the United Network for Organ Sharing (UNOS) available at www.unos.org. For the estimation of Child-Pugh score in the present series: INR substituted prothrombin time prolongation (<1.7, 1 point; 1.71-2.2, 2 points); encephalopathy was scored 1 point when never having occurred and 2 points when suppressed with medication; ascites was scored with 2 when suppressed with diuretics and 3 when refractory.

Abbreviations: INR, international normalized ratio; MELD, model for end-stage liver disease.

*$P < 0.01$ versus Baseline.

†$P < 0.01$ versus observational period.

‡$P < 0.001$ versus Baseline.

§$P < 0.001$ versus observational period.

cirrhosis remain unclear. Treatment with synbiotics improved the Child-Pugh class as a result of significant improvements in serum bilirubin and albumin levels and in prothrombin activity.[4] Additionally, probiotics reduced endotoxemia and improved the Child-Pugh score, although not significantly, in patients with compensated cirrhosis.[5] Finally, treatment with paromomycin and neomycin for 3-6 months significantly improved serum albumin levels[6] and the Child-Pugh score, mainly because of a decreased incidence of ascites and encephalopathy,[7] respectively.

Herein, we present preliminary data on the effects of rifaximin, a virtually unabsorbable antibiotic with broad-spectrum antimicrobial activity and an excellent safety profile[8] on endotoxemia and liver function and disease severity in 9 liver transplant candidates with alcoholic cirrhosis (male, n = 7; mean age = 56 ± 6 years; Child-Pugh class B/C: 5/4). All patients abstained from alcohol for at least 1 year before inclusion. Plasma endotoxin levels were detected by the Limulus amebocyte lysate chromogenic endpoint assay (Hycult Biotech, Uden, The Netherlands). Clinical infection, upper gastrointestinal bleeding, and use of antibiotics or prebiotics 6 weeks before or during the study were exclusion criteria. Patients were evaluated after an 8-week observational period and after an 8-week course of rifaximin (1,200 mg/day).

All measures remained unchanged during the observational period. Rifaximin significantly reduced plasma endotoxin levels, together with a significant increase in serum albumin levels and significant decreases in serum total bilirubin levels and international normalized ratio. Child-Pugh and model for end-stage liver disease scores decreased significantly after treatment (Table 1).

In conclusion, intestinal decontamination by rifaximin could be a feasible, safe approach to prevent endotoxin-induced liver injury and improve liver function and disease severity in patients with decompenstaed cirrhosis. The present findings should be confirmed in a placebo-controlled trial.

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Hepatitis B Virus Mutants Associated with Hepatitis B Surface Antigen Loss: Chicken or Egg?

To the Editor:

We read with great interest the article entitled "Emergence of Hepatitis B Virus S Gene Mutants in Patients Experiencing Hepatitis B Surface Antigen Seroconversion After Peginterferon Therapy" by Hsu and Yeh in the July 2011 issue of Hepatology.[1] Peginterferon is one of the preferred agents for the treatment of chronic hepatitis B, with a higher incidence of hepatitis B surface antigen (HBsAg) loss than nucleos(t)ide analogues, which is closest to the cure of hepatitis B virus (HBV) infection.[2] Hsu and Yeh found that two patients achieved HBsAg loss after receiving peginterferon therapy but retained high serum HBV DNA levels nevertheless.[1] They identified two new HBV variants, sT125A and sW74*, from the serum samples at HBsAg-negative phase, and these mutant HBsAg proteins could not be detected in *in vitro* studies. They therefore concluded that these S gene mutations were responsible for the failure of detecting HBsAg.

Although Hsu and Yeh's findings are interesting, several issues need to be addressed further. First, the variant of sT125A was shown to be a minor strain of the total viral population (14.3%) in patient 1 according to the cloning results. If HBsAg loss is caused by viral mutation, this HBsAg loss–related viral strain is supposedly the major strain; otherwise, we cannot explain why patients achieving HBsAg loss still harbor more than 50% of viral strains, which are competent for producing detectable HBsAg. In other words, proving the *in vitro* phenotype of a minor viral strain does not explain the loss of circulating HBsAg in these patients. Second, the variant sW74* was shown to represent 83.1%-100% of viral strains in a patient with HBsAg loss. It is noteworthy that the S ORF is overlapped with polymerase ORF in the HBV genome.[3] Assuming the deletion of sW74* from nucleotide 1284-1744 (W64 to the end of S ORF), this deletion mutant also destroys amino acids 429-581 of polymerase, an important part of the reverse-transcriptase domain. This viral strain is therefore supposed to not replicate by itself. Virologically, other viral strains with competent replication must become the major strain. However, the results derived from the cloning and pyrosequencing failed to confirm this inference. In other words, if the pyrosequencing results are correct, the viral strain is supposed to not replicate profoundly, and this patient is unlikely to have such a high viral load. On the contrary, if the pyrosequencing results are not valid, the authors need to document the existence of other competent viral strains and prove that the HBsAg produced by this viral strain could not be detected by common HBsAg antibody.

Taken together, these observations suggest that the peginterferon-related HBsAg loss reported by Hsu and Yeh may not be attributed to these viral mutants, but may instead be caused by certain epigenetic or genetic modifications in hepatocytes that are driven by host immunity. These mutant strains are merely the products selected by host immune pressure. In conclusion, HBsAg

loss after peginterferon therapy cannot be convincingly explained by these viral mutants. Further studies are required to examine the underlying mechanisms involved in peginterferon-induced HBsAg loss.

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Histological Discrimination Between Autoimmune Hepatitis and Drug-Induced Liver Injury

To the Editor:

We read with interest the article by Suzuki et al.[1] We were surprised that only 1 (4%) of the autoimmune hepatitis (AIH) cases whose diagnoses were made by experienced hepatologists in Mayo Clinic showed "typical" histology, and that complete agreement on histological diagnosis among four experienced hepatopathologists was less than 50%, if biopsy slides were evaluated blinded to the clinical information. We realized that we should renew our awareness of evaluating liver histology.

In daily clinical practice, it is often very difficult in distinguishing drug-induced liver injury (DILI) from AIH with acute presentation of the disease (i.e., acute AIH) as a cause of acute hepatitis. As the investigators described, there is no pathognomonic feature for AIH or DILI, so the evaluation of liver histology in determining AIH versus DILI is important.

The diagnosis of AIH is challenging and that of acute onset AIH is even more challenging and difficult, because patients show acute presentation, such as acute hepatitis, and may not have typical clinicopathological features of AIH, and because there is no gold standard for it. Some acute AIH cases are at risk of losing the timing of starting immunosuppressive therapy, develop into severe or fulminant form, and are sometimes resistant to immunosuppressive therapy and have a poor prognosis. It is most important to exclude other causes systematically and apply the International AIH Group original revised scoring system,[2] rather than simplified the scoring system.[3,4] Especially, precise pathological evaluation plays an important role in the differential diagnosis.[5]

As the investigators commented in the Discussion, the sample size was too small and there was a possibility that some of the observed histological features may have been influenced by clinical presentation of AIH (i.e., acute versus chronic presentation). Therefore, it is important to show how many patients of the examined 28 AIH cases were clinically and histologically "acute AIH" who usually present atypical clinicopathological features and may have influenced the histological findings of their study.

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