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Histological activity despite normal ALT and IgG serum levels in patients with autoimmune hepatitis and cirrhosis

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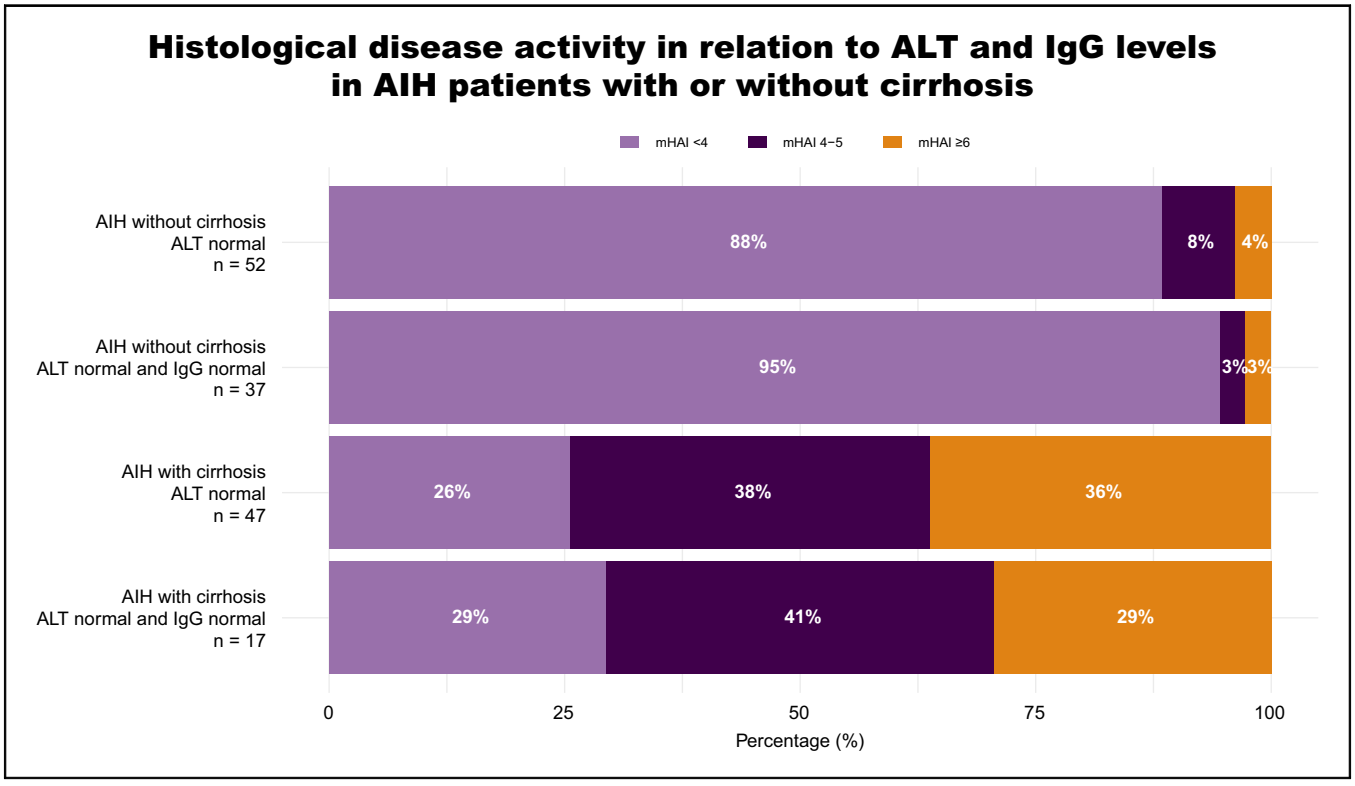
Graphical abstract



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Highlights

- A considerable proportion of patients with AIH cirrhosis show histological disease activity despite normal ALT levels.
- Addition of IgG only slightly improves the prediction of low histological activity in patients with AIH and cirrhosis.
- ALT and IgG do not correlate sufficiently with histological activity in AIH cirrhosis.
- New surrogate markers of histological activity are needed in patients with AIH cirrhosis.

Lay summary

Autoimmune hepatitis (AIH) is an inflammatory disease of the liver that usually responds to immunosuppressive therapy. Serum transaminases and IgG levels within the normal ranges define complete biochemical remission and are considered as surrogate markers for histological disease activity. Here, we show that those biochemical markers are not sufficient to indicate low disease activity in patients with AIH and already established cirrhosis. Consequently, until better biomarkers for disease activity are found, only liver biopsy can reliably indicate disease activity in the presence of cirrhosis. Additional investigations, such as measurements of liver stiffness, should be undertaken to monitor non-invasively for disease progression in patients with AIH and established cirrhosis.

Research article

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Histological activity despite normal ALT and IgG serum levels in patients with autoimmune hepatitis and cirrhosis

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Background & Aims: In autoimmune hepatitis (AIH), normal levels of transaminases and IgG define biochemical remission and are considered the best surrogate markers for histological remission. This study assessed whether this also applies to patients with AIH cirrhosis.

Methods: In this European multicentric study, we included 125 biopsies from 113 patients with AIH and histologically proven cirrhosis; 105 biopsies from 104 patients with AIH without cirrhosis served as controls. Biochemical parameters were available within 4 weeks of biopsy. AIH activity was graded according to the modified Hepatitis Activity Index (mHAI), with mHAI ≥4/18 considered to indicate risk of disease progression.

Results: In total, 47 out of 125 liver biopsies were obtained from patients with AIH cirrhosis and normal ALT levels at time of biopsy. Only 26% (12/47) of those livers showed histological remission (mHAI <4/18), whereas 36% (17/47) showed moderate to high histological activity (mHAI ≥6/18). In patients with noncirrhotic AIH, 88% (46/52 biopsies) of cases with normal ALT levels had histological remission and only 4% (2/52) had an mHAI ≥6/18 (p <0.001). The addition of IgG to define complete biochemical remission only slightly improved the association with histological remission in the limited number of patients with AIH cirrhosis available for analysis [29% (5/17) of biopsies with mHAI <4/18]. ALT correlated closely with mHAI in AIH without cirrhosis but poorly in AIH with cirrhosis.

Conclusions: In contrast to patients with noncirrhotic AIH, in patients with AIH cirrhosis, who are at risk of disease progression, normal ALT levels and potentially also complete biochemical remission are poor surrogate markers of histological remission. Thus, new biomarkers are needed to monitor disease activity and progression in patients with AIH cirrhosis.

Lay summary: Autoimmune hepatitis (AIH) is an inflammatory disease of the liver that usually responds to immunosuppressive therapy. Serum transaminases and IgG levels within the normal ranges define complete biochemical remission and are considered as surrogate markers for histological disease activity. Here, we show that those biochemical markers are not sufficient to indicate low disease activity in patients with AIH and already established cirrhosis. Consequently, until better biomarkers for disease activity are found, only liver biopsy can reliably indicate disease activity in the presence of cirrhosis. Additional investigations, such as measurements of liver stiffness, should be undertaken to monitor non-invasively for disease progression in patients with AIH and established cirrhosis.

Keywords: Autoimmune hepatitis; Biochemical remission; Histological activity; Liver biopsy; Cirrhosis; mHAI.

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Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease that usually responds to immunosuppression.[1] Insufficient treatment response with continued histological activity harbours the risk of disease progression, cirrhosis development, and liver failure.[2–5] Approximately one-third of patients already present with cirrhosis at the time of AIH diagnosis, which adds to

the risk of experiencing liver-related death or requiring liver transplantation.[1,4–6] Given that a histological scoring system specific for AIH is lacking, the modified Hepatitis Activity Index (mHAI) is frequently used for the grading of histological inflammatory activity in AIH.[5] Current guidelines recommend treatment of AIH in patients with an mHAI ≥4/18, whereas an mHAI ≥6/18 represents moderate to high histological activity.[1,7] Normal levels of transaminases and IgG (complete biochemical remission) are considered the best available biochemical surrogate markers for histological remission.[1,5,8] Nevertheless, some patients with AIH have residual histological activity on liver histology despite normal biochemical markers, which is an independent risk factor for a reduced long-term transplant-free survival.[5,9,10] Of note, in a long-term observational study, even moderate histological activity in patients with normal transaminases and normal globulins was implicated in AIH disease progression.[9] In clinical practice, and before the publication of the European Association for the Study of the Liver (EASL) Practice Guidelines on AIH, transaminases were often used to indicate remission and IgG was measured only infrequently. We hypothesized, based on our personal experience, that patients with AIH cirrhosis might harbour histological activity that warrants treatment escalation despite normal transaminases. To test this hypothesis, we investigated in a multicentre European study whether transaminases and IgG levels suffice to indicate histological remission in AIH and whether the presence of cirrhosis reduces the value of transaminases and IgG as surrogate markers for histological remission in AIH.

Methods

Patient population

In our retrospective European multicentre study, 125 biopsies from 113 patients with AIH cirrhosis from five European high-volume centres were included: Hamburg (HH), n = 69; Larissa (LA), n = 26; Hannover (HN), n = 19; Nijmegen (NIJ), n = 10; and London (LON), n = 1. As a sex- and age-matched control group, we identified 104 patients with AIH without cirrhosis on histology, resulting in 105 biopsies (HH, n = 71; HN, n = 25; LA, n = 10). The diagnosis of AIH was based on clinical, biochemical, serological and histopathological findings in accordance with current EASL Guidelines.[1] Patients with additional signs of primary biliary cholangitis or primary sclerosing cholangitis were excluded. Patients were also excluded if the time between biopsy and blood samples was more than 4 weeks and in cases of changed dosages of immunosuppression within these 4 weeks. Biopsies were either taken at first diagnosis or as follow-up biopsies because of clinical and imaging signs of disease progression or decompensation, exclusion of differential diagnoses during treatment, absence of biochemical remission, before starting a second- or third-line therapy, increases in liver stiffness, or before an attempt at treatment. Biopsies were taken under immunosuppressive treatment in 34% (42/125) of patients with AIH cirrhosis and in 81% (85/105) of AIH patients without cirrhosis. The cohorts of patients with AIH with and without cirrhosis were divided in groups based on normal and elevated transaminases and/or IgG. For patients with AIH cirrhosis and normal ALT and IgG levels at time of biopsy, the presence of repeatedly normal values in the months before biopsy was recorded. Fig. S1 details a flow chart of all patients with AIH included in this study is displayed. As a non-AIH control group, we included 17 biopsies of patients from HH with either nonalcoholic fatty liver disease (NAFLD, n = 6) or alcoholic liver disease (ALD, n = 11) and already established cirrhosis. Diagnosis was based on clinical, histopathological, and serological findings.

Clinical and biochemical parameters

We assessed data on biochemical parameters, immunosuppressive drug intake, and concomitant autoimmune diseases within 4 weeks of biopsy. The following biochemical markers were evaluated: immunoglobulin G (IgG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (gamma-GT), haemoglobin, albumin, bilirubin, creatinine, platelet count, and international normalised ratio (INR). Parameters are displayed with the upper or lower limit of normal (ULN and LLN, respectively). In accordance with the current EASL guideline,[1] we defined normal levels of transaminases (ALT) and IgG as biochemical remission regardless of the presence or absence of therapy.

Liver histology

Histopathological evaluation included the mHAI, which uses up to 18 points to grade inflammatory activity and necrosis.[11] Most liver specimens from HH and HN (AIH with cirrhosis, n = 78/88; AIH without cirrhosis, n = 71/96; NAFLD/ALD, n = 17/17) were independently and blindly evaluated by S.W. and T.K. and mean values of mHAI scores were included in the analysis. Inter-rater reliability between S.W. and T.K. was tested by calculating the intraclass correlation coefficient (ICC), showing a good to very good strength of agreement (ICC1 = 0.78; 95% CI, 0.72–0.83; p <0.001) with a mean difference of 1.19 points (95% CI, 0.87–1.48; p <0.001). All liver specimens of NIJ (n = 10) were evaluated by S.W., whereas histopathological evaluation in LA was undertaken by one local expert pathologist for all specimens (n = 36). Given that the remaining specimens (n = 35, 14.5%) were not available for centralised re-evaluation, existing mHAI scores evaluated by local expert pathologists at the corresponding centres were used for analysis. Cirrhosis was diagnosed on histology as either F4 fibrosis[12] or F6 fibrosis[11] according to local standards. For the control group without cirrhosis, only patients with early liver fibrosis (F0–F2 according to Desmet *et al.*[12] or Ishak *et al.*[11]) were included to avoid erroneous classifications of stage-3 patients because of sampling error. Liver biopsies were taken between 1989 and 2019, with one biopsy was taken in 1979. The study was approved by the local ethics committees and informed consent was obtained as needed by local regulations. Examples of liver histologies are shown with magnifications of 50× and 200 × and a Zeiss Axiolab 5 microscope was used. Staining was performed with haematoxylin and eosin.

Statistical analysis

Percentages and counts are given for categorical data. Mean and median values with the corresponding SD or range were calculated for continuous data. To test for differences between groups, parametric and nonparametric tests, including the Student's t test or the Wilcoxon signed rank test, were performed. A comparison of categorical data between groups was performed using the chi-square test or Fisher's exact test as appropriate. Correlation between mHAI scores and biochemical parameters within patients with cirrhotic and noncirrhotic AIH was performed using Pearson's correlation analysis. To calculate the percentage of explained variance of histological activity score by biochemical parameters, a variance analysis was performed. A linear

Table 1. Biochemical and clinical features at time of liver biopsy of all patients with AIH.

Feature	AIH cirrhosis (n = 125)	AIH non-cirrhosis (n = 105)	*p* value*
Sex, female	71%	74%	0.657
Age (years ± SD)	54 ± 17 (56)	51 ± 15 (52)	0.204
ALT/ULN (range)	1.6 (0.2–118.4)	1.1 (0.3–57.8)	0.042
AST/ULN (range)	1.5 (0.4–103.6)	1 (0.3–49.8)	<0.001
IgG/ULN (range)	1.2 (0.5–3.2)	0.8 (0.4–2.9)	<0.001
mHAI (0–18)†	6 (0–13.5)	2.5 (0–11)	<0.001
Immunosuppressive treatment	34%	81%	<0.001
Child-Pugh Score (A, B, C)	72%, 23%, 5%	–	–
Concomitant autoimmune disease‡	18%	24%	0.237

Mean values are presented with median in brackets.
AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; mHAI, modified Hepatitis Activity Index; ULN, upper limit of normal.
* Continuous variables were compared using Wilcoxon signed rank test. Fisher's exact test was used for comparing percentages.
† According to mHAI.[19]
‡ Rheumatoid arthritis, type 1 diabetes mellitus, Sicca syndrome, coeliac disease, scleroderma, thyroid disease, multiple sclerosis, vitiligo, inflammatory bowel disease.

regression model was used to evaluate the predictive value of the combination of biochemical parameters for the mHAI and the interaction effect of cirrhosis and noncirrhosis. If not normally distributed, logarithmised parameters were used. All *p* values were two-tailed; $p < 0.05$ was considered statistically significant. Figure design and statistical testing were carried out using R Version 3.6.0 and R Studio Version 1.2.1335.

Results

Patient characteristics at time of liver biopsy

A total of 125 biopsies from 113 patients with AIH and cirrhosis were included. As a control group, we analysed 105 biopsies from 104 patients with AIH without cirrhosis. Biochemical and histological features of all patients are detailed in Table 1. Distribution of age and sex were similar between the two groups. A greater proportion of patients with AIH cirrhosis was biopsied without immunosuppression and had higher levels of ALT and IgG compared with patients with noncirrhotic AIH [median ALT/ULN = 1.6 (0.2–118.4) *vs.* 1.1 (0.3–57.8), $p = 0.042$; median IgG/ULN = 1.2 (0.5–3.2) *vs.* 0.8 (0.4–2.9), $p < 0.001$]. Of note, inclusion into the study was weighted toward patients with normal levels of transaminases and IgG to assess the association between biochemical and histological remission, which might not reflect the natural distribution of biochemical parameters in patients with cirrhosis. Histological activity graded by mHAI was higher in the group of patients with AIH cirrhosis compared with patients with AIH without cirrhosis [median mHAI = 6 (0-13.5) *vs.* 2.5 (0–11), $p < 0.001$]. In the group of patients with AIH cirrhosis, biopsies were mainly taken at first diagnosis, whereas lack of remission before starting a second-line therapy or the decision to withdraw the treatment indicated the sampling of liver biopsies in most patients without cirrhosis. Indications for biopsies of patients with AIH with or without cirrhosis depending on ALT levels are detailed in Table S1.

Patients with AIH and cirrhosis show histological disease activity despite normal ALT levels

In a first step, we investigated the association of normal transaminases with the presence of histological remission in patients with AIH without cirrhosis: 49% (52/105) of liver biopsies were taken with ALT levels within the normal range. Of those, 88% (46/52) were compatible with histological remission (mHAI <4/18), 8% (4/52) had residual (mHAI 4–5/18), and only 5% (2/52) moderate to high histological activity (mHAI ≥6/18), confirming previous reports.[8–10,13] Within the group of patients with AIH cirrhosis, 38% (47/125) of liver biopsies were taken in patients with ALT levels within the normal range. Of those, only 26% (12/47) had evidence of histological remission (mHAI <4/18), whereas 38% (18/47) had residual (mHAI 4–5/18) and 36% (17/47) moderate to high inflammatory activity (mHAI ≥6/18) ($p < 0.001$). Histological disease activity in relation to ALT levels and illustrative examples of liver specimens are detailed in Figs. 1 and 2. In patients with AIH and normal ALT levels, the mHAI was significantly higher in those with cirrhosis than in patients without cirrhosis [median mHAI = 5 (1–10) *vs.* 2 (0–6), respectively; $p < 0.001$]. Biochemical and histological features of patients with AIH and without cirrhosis depending on ALT levels are detailed in Table 2 and histological disease activity in relation to IgG in Fig. S2. Using AST levels as a surrogate marker did not improve the association of transaminases with histological remission: within the group of patients with AIH cirrhosis, 28% (35/125) of liver biopsies were taken in patients with AST levels within the normal range. Of those, only 20% (7/35) had evidence of histological remission (mHAI <4/18), whereas 51% (18/35) had residual (mHAI 4–5/18) and 29% (10/35) moderate to high inflammatory activity (mHAI ≥6/18). Histological disease activity in relation to AST levels in patients with AIH or without cirrhosis is detailed in Fig. S3.

To investigate whether cirrhosis *per se* implies a basic inflammatory activity, we identified a comparison group of patients with cirrhosis resulting from NAFLD (n = 6) or ALD (n = 11) from HH. Of the liver biopsies, 53% (9/17) were taken with ALT levels within the normal range and showed a lower histological activity compared with those from patients with AIH cirrhosis and normal ALT levels at time of biopsy. Of those 9 patients, 78% (7/9) showed low hepatitis activity (mHAI <4/18) and 22% (2/9) had mHAI 4–5/18, whereas none showed moderate to high histological activity (mHAI ≥6/18). Histological activity in relation to ALT is detailed in Fig. S4.

Histological disease activity despite normal ALT and IgG levels in AIH cirrhosis

Given that ALT serum levels alone turned out to be insufficient markers of histological remission in patients with AIH cirrhosis, we investigated whether the addition of IgG serum levels improved this association. IgG levels at time of biopsy were available in 87% (109/125) of the group with AIH cirrhosis, and 17/109 biopsies were taken with ALT and IgG levels within the normal ranges. The addition of normal IgG levels only slightly improved the association with histological remission and could not exclude the presence of relevant histological activity: only 29% (5/17) of biopsies taken in patients with AIH cirrhosis in complete biochemical remission were compatible with

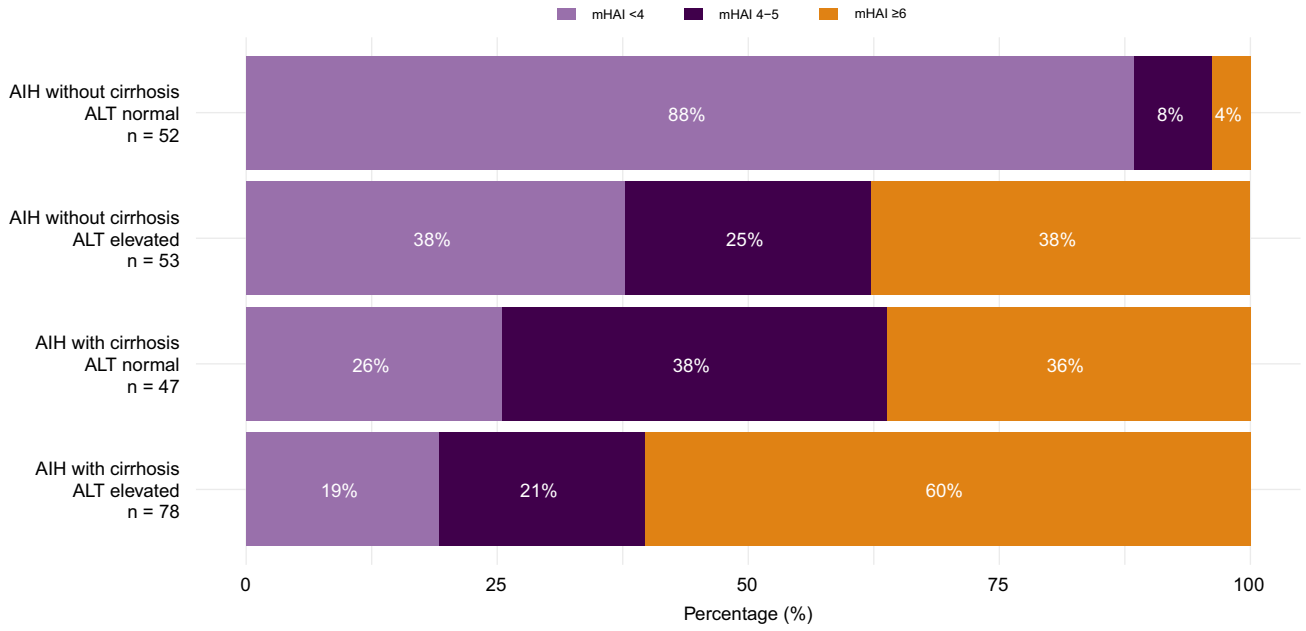

Fig. 1. Histological activity of patients in patients with AIH with and without cirrhosis depending on ALT levels. Histological activity scores subdivided into histological remission (mHAI <4), mild residual disease activity (mHAI 4–5), or moderate–high inflammatory activity (mHAI ≥6). Patients were subdivided into ALT levels within or above the normal ranges. AIH, autoimmune hepatitis; ALT, alanine aminotransferase; mHAI, modified Hepatitis Activity Index.

histological remission (mHAI <4/18) *vs.* 26% in patients with normal ALT levels only. Of this subgroup, 41% (7/17) still presented with residual inflammatory activity (mHAI 4–5) and another 29% (5/17) with moderate to high histological inflammation (mHAI ≥6).

To exclude that the poor association of biochemical remission in AIH cirrhosis resulted from variations in liver enzyme levels and, thus, did not reflect stabile biochemical remission, we additionally investigated time points before liver biopsy in the 17 patients with AIH cirrhosis and normal ALT and IgG levels at time of biopsy. In 14/17 of those patients, ALT levels before biopsy were available and 11/14 showed ALT levels repeatedly within the normal range. Accordingly, IgG levels were available in 12/17 patients, and 11/12 showed repeatedly normal IgG levels before biopsy. The time courses of those biochemical parameters and the corresponding indications for biopsy in those patients are detailed in Figs S5 and S6.

To evaluate the clinical relevance of the observed residual, moderate, or high inflammatory activity (mHAI ≥4/18) in those 12 patients with AIH cirrhosis and normal ALT and IgG levels at time of biopsy, we investigated the clinical course of those patients: In 6 out of 12 patients, immunosuppressive therapy was initiated or intensified after liver biopsy. Of those, 5/6 patients showed a stable clinical course over 1–10 years, as indicated by liver stiffness measurement or follow-up biopsy. In only 1 case was an increased liver stiffness documented, 3 years after biopsy.

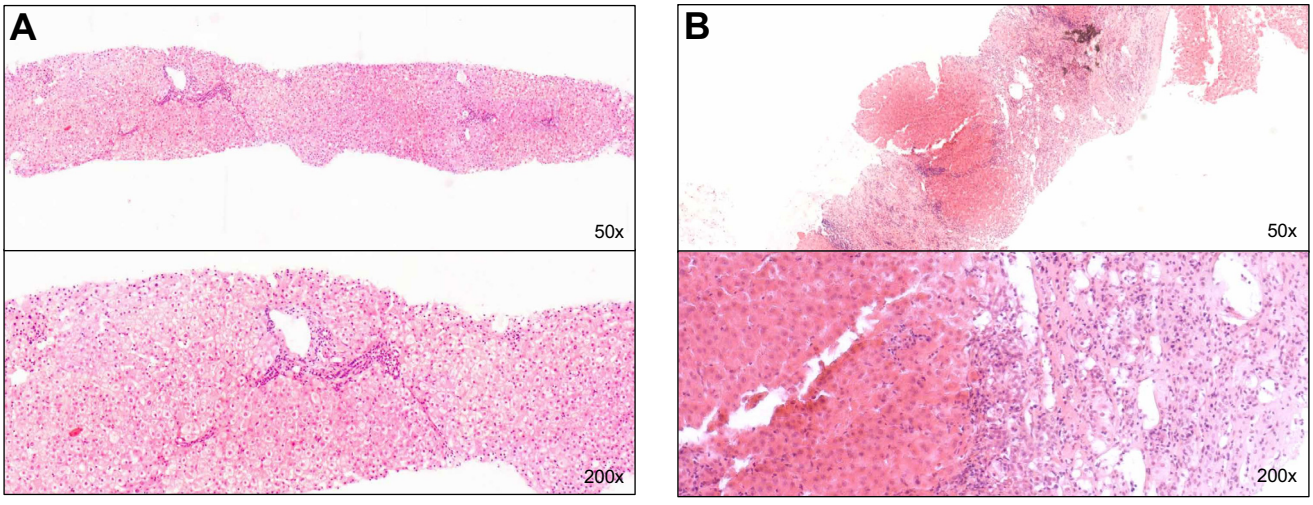

Fig. 2. Examples of liver histologies of patients with AIH with and without cirrhosis with normal ALT serum levels. (A) Specimen of a patient with AIH without cirrhosis and normal ALT levels at time of biopsy; mHAI 1/18. (B) Specimen of a patient with AIH cirrhosis and normal ALT levels at time of biopsy; mHAI 8/18. Staining was performed with haematoxylin and eosin. AIH, autoimmune hepatitis; ALT, alanine aminotransferase; mHAI, modified Hepatitis Activity Index.

Table 2. Biochemical, histological, and clinical features at time of liver biopsy from patients with AIH with and without cirrhosis.

Feature	AIH cirrhosis with elevated ALT levels (n = 78)	AIH cirrhosis with normal ALT levels (n = 47)	AIH non-cirrhosis with elevated ALT levels (n = 53)	AIH non-cirrhosis with normal ALT levels (n = 52)	*p* value*	
					AIH cirrhosis: ALT elevated *vs.* ALT normal	ALT normal: AIH cirrhosis *vs.* non-cirrhosis
Haemoglobin/LLN	1 ± 0.2 (1)	1 ± 0.2 (1)	1.1 ± 0.1 (1.1)	1.1 ± 0.1 (1.1)	0.098	0.029
Platelets/LLN	1 ± 0.5 (0.9)	1.1 ± 0.6 (1.1)	1.7 ± 0.5 (1.7)	1.6 ± 0.5 (1.6)	0.229	<0.001
Albumin/LLN	1 ± 0.3 (1)	1 ± 0.1 (1.1)	1.1 ± 0.2 (1.1)	1.2 ± 0.1 (1.2)	0.092	<0.001
ALP/ULN	1.5 ± 0.8 (1.2)	0.9 ± 0.5 (0.8)	1.2 ± 0.9 (0.9)	0.6 ± 0.3 (0.6)	<0.001	0.001
Bilirubin/ULN	1.1 (0.3-45.2)	0.8 (0.3-3.7)	0.7 (0.2-15.9)	0.4 (0.2–1.2)	0.002	0.001
Creatinine/ULN	0.8 ± 0.2 (0.8)	0.8 ± 0.2 (0.7)	0.9 ± 0.2 (0.8)	0.8 ± 0.2 (0.8)	0.892	0.771
INR	1.3 ± 0.2 (1.3)	1.2 ± 0.2 (1.1)	1.1 ± 0.2 (1)	1 ± 0.1 (1)	0.001	<0.001
IgG/ULN	1.5 ± 0.7 (1.5)	1.2 ± 0.4 (1.1)	1.1 ± 0.5 (0.9)	0.8 ± 0.2 (0.8)	0.026	<0.001
mHAI (0–18)	6.8 (0–13.5)	5 (1–10)	4 (0–11)	2 (0–6)	<0.001	<0.001
mHAI <4, 4–5, ≥6	19%, 21%, 60%	26%, 38%, 36%	38%, 24%, 38%	88%, 8%, 4%	0.024	<0.001
Immunosuppressive treatment	26%	47%	72%	92%	0.019	<0.001
Child-Pugh score (A, B, C)	68%, 24%, 8%	79%, 21%, 0%	–	–	0.153	–
MELD	10 (6–22)	8 (6–13)	–	–	0.01	–

Mean values are presented with median in brackets.
AIH, autoimmune hepatitis; ALT, alanine aminotransferase; LLN, lower limit of normal; MELD, model for end-stage liver disease; mHAI, modified Hepatitis Activity Index; ULN, upper limit of normal.
* Continuous variables were compared using Wilcoxon signed rank test. A comparison of categorical data between groups was performed using the chi-square test or Fisher's exact test as appropriate.

By contrast, immunosuppressive therapy was not initiated or intensified in 3/12 patients. All 3 patients had hepatic decompensation and variceal bleeding with consecutive liver-related death, or were in need of transjugular intrahepatic portosystemic shunt (TIPS) implantation because of recurring hydropic decompensation. In 3/12 patients, no clinical long-term follow-up data were available. Clinical courses are detailed in Table S2.

In the group of patients with AIH without cirrhosis, IgG levels at time of biopsy were available in 86% (90/105) and 37 of those were taken with ALT and IgG levels within the normal ranges. In contrast to the group of patients with AIH cirrhosis, 95% (35/37) of biopsies taken in patients with AIH without cirrhosis in complete biochemical remission showed histological remission (mHAI <4/18), whereas only 3% (1/37) had residual activity (mHAI 4–5/18) and another 3% (2/37) moderate to high inflammatory activity (mHAI ≥6/18) (p <0.001). Histological disease activity depending on normal ALT and IgG levels compared with normal ALT levels is detailed in Figs. 3 and 4.

Elevated ALT and IgG levels in patients with AIH with or without cirrhosis indicated histological activity that warrants intensified immunosuppressive therapy in most patients: 92% of patients with cirrhosis and 76% of patients without cirrhosis

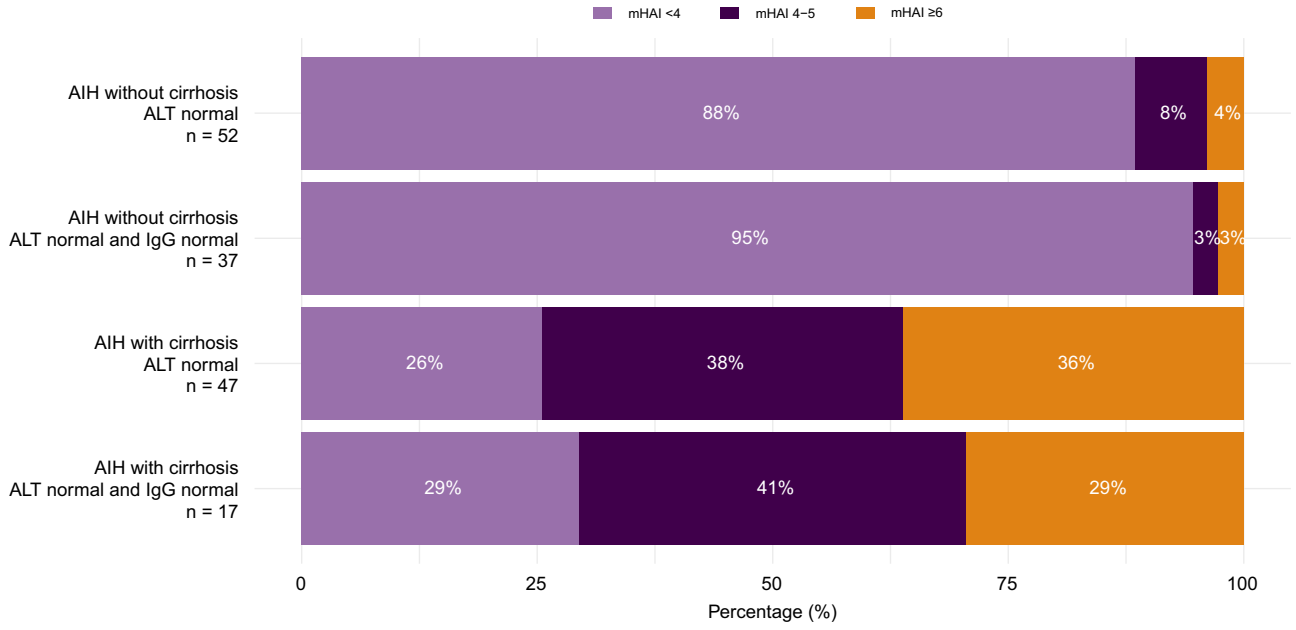

Fig. 3. Histological activity of patients with AIH with and without cirrhosis depending on normal ALT and IgG levels. Histological activity scores subdivided into histological remission (mHAI <4), mild residual disease activity (mHAI 4–5), or moderate–high inflammatory activity (mHAI ≥6). AIH, autoimmune hepatitis; ALT, alanine aminotransferase; mHAI, modified Hepatitis Activity Index.

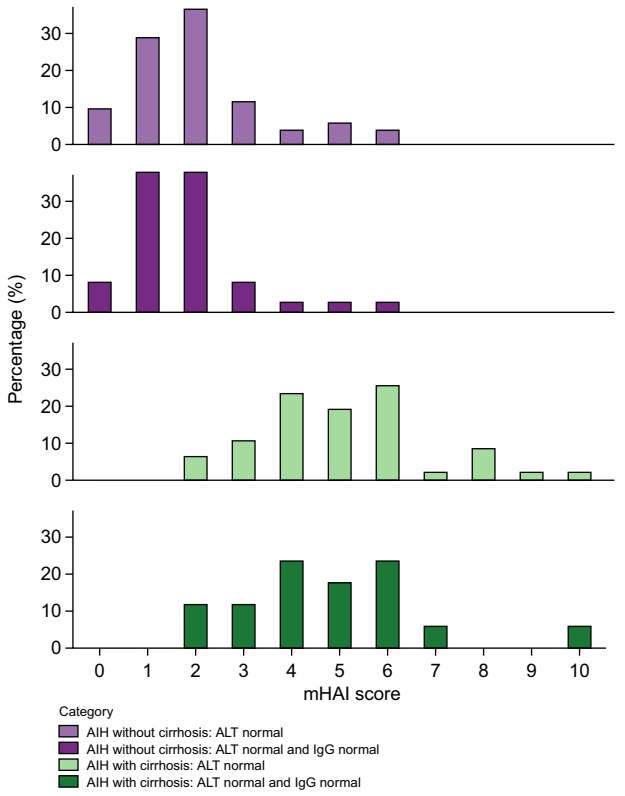

Fig. 4. Distribution of histological activity scores of patients with AIH with and without cirrhosis depending on normal ALT and IgG levels. AIH, autoimmune hepatitis; ALT, alanine aminotransferase; mHAI, modified Hepatitis Activity Index.

with elevated ALT and IgG levels showed an mHAI ≥4/18 (Fig. S7).

Low correlation of biochemical markers with histological activity in AIH cirrhosis

Given that normal levels of ALT and IgG could not reliably exclude the presence of histological disease activity in patients with AIH cirrhosis, we compared the correlation of ALT and IgG with histological grading between patients with and without cirrhosis. In patients with AIH cirrhosis, biochemical parameters correlated poorly with mHAI scores [AIH cirrhosis: r = 0.38 with log(ALT/ULN, 95% CI, 0.22–0,52; p <0.001; r = 0.51 with IgG/ULN, 95% CI, 0.36–0.64; p <0.001). By contrast, in patients with AIH without cirrhosis, we found a high correlation of ALT levels with histological activity, whereas IgG levels showed a poor correlation, comparable with that of patients with AIH cirrhosis [AIH noncirrhosis: r = 0.77 with log(ALT/ULN), 95% CI, 0.68–0.84, p <0.001; r = 0.54 with IgG/ULN, 95% CI, 0.38–0.67, p <0.001).

To evaluate the spread of histological activity by means of biochemical parameters (ALT and IgG), we performed a variance analysis. It showed that, in patients with AIH cirrhosis, the combination of biochemical parameters (ALT and IgG) explained less variance of histological activity compared with patients with AIH without cirrhosis [AIH cirrhosis: adj-R^2, 0.34 of log(ALT/ULN) + IgG/ULN for mHAI; p <0.001; AIH noncirrhosis: adj-R^2, 0.63 of log(ALT/ULN) + IgG/ULN for mHAI; p <0.001).

Finally, we performed a linear regression model to evaluate the combination of biochemical parameters with the interaction effect of the presence of cirrhosis in predicting histological activity. The transformed data used in the linear model are detailed in Fig. 5. Samples of patients with AIH without cirrhosis separated more clearly based on mHAI levels than did samples of

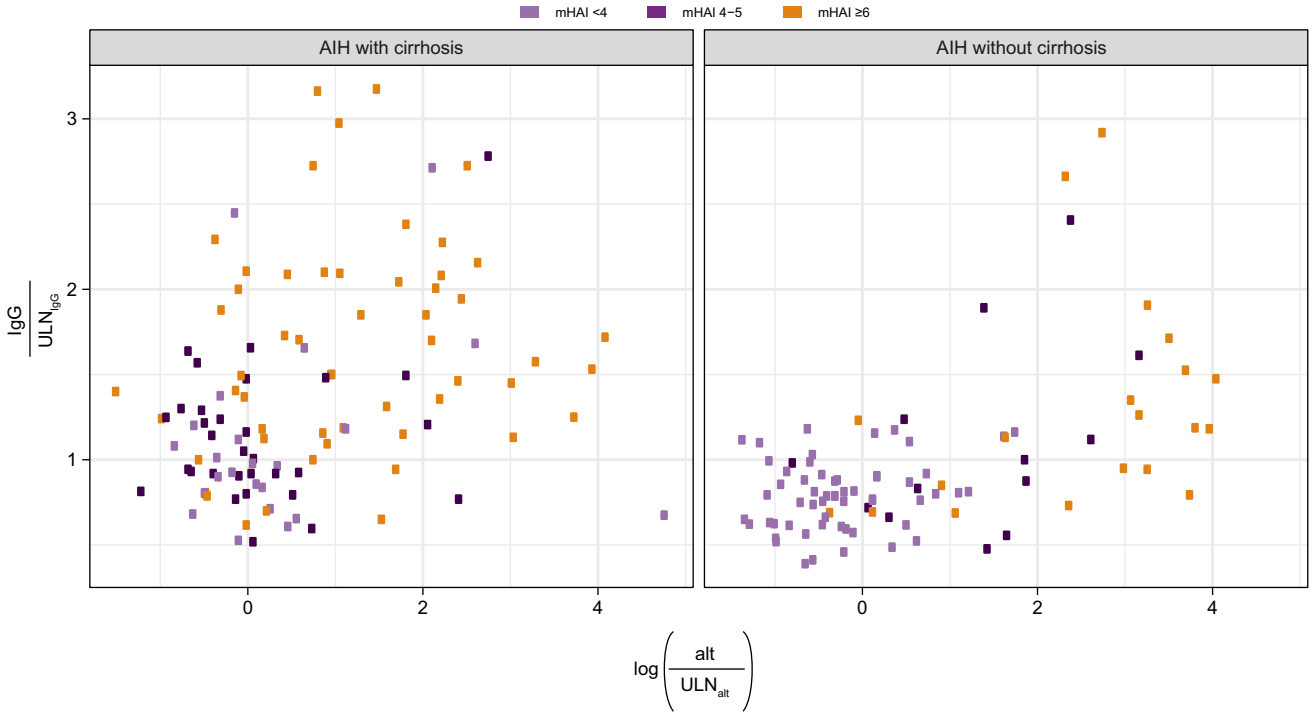

Fig. 5. The mHAI activity in correlation of the transformed ALT and IgG data of patients with AIH with and without cirrhosis. AIH, autoimmune hepatitis; ALT, alanine aminotransferase; mHAI, modified Hepatitis Activity Index.

Table 3. Linear regression analysis for mHAI with ALT and IgG in patients with AIH with and without cirrhosis.

	Regression coefficient	2.5% CI	97.5% CI
Intercept	3.22***	2.33	4.11
log(ALT/ULN)	0.74***	0.41	1.07
IgG/ULN	1.69***	1.1	2.29
Category (AIH non-cirrhosis)	-2.10***	-2.81	-1.39
log(ALT/ULN)*category (AIH non-cirrhosis)	0.56*	0.13	1

AIH, autoimmune hepatitis; ALT, alanine aminotransferase; mHAI, modified Hepatitis Activity Index; ULN, upper limit of normal. Regression coefficients and confidence intervals are given.
*p <0.05, **p <0.01, ***p <0.001.

patients with AIH cirrhosis, supporting the finding that biochemical parameters are more useful to predict histological activity in patients with AIH without cirrhosis than in those with cirrhosis. This was further corroborated by the linear regression model: on a first iteration, we considered the interaction between IgG/ULN with the presence of cirrhosis. That term was removed because of a lack of significance. The linear model showed that the effect of ALT for the prediction of mHAI in patients with AIH cirrhosis was significantly smaller (0.73) than in patients with AIH without cirrhosis (0.73 + 0.56 = 1.29) [log(ALT/ULN): *B*, 0.73; 95% CI, 0.41–1.07; p <0.001; IgG/ULN: *B*, 1.7; 95% CI, 1.1–2.3; p <0.001; category (AIH noncirrhosis): *B*, -2.1, 95% CI, -2.81,-1.39; p <0.001, log(ALT/ULN)*category (AIH noncirrhosis): *B*, 0.56; 95% CI, 0.13–1; p <0.05). Regression coefficients and confidence intervals for the linear regression model are detailed in Table 3. Moreover, in agreement with the results displayed in Fig. 5, using ALT in combination with IgG to predict histological activity was less reliable in patients with AIH cirrhosis compared with patients with AIH without cirrhosis (AIH cirrhosis: root mean square error, 2.3; AIH noncirrhosis: root mean square error, 1.81).

Discussion

In patients with AIH, biochemical remission is considered the best surrogate marker available to indicate histological remission, defined as mHAI less than 4/18 points. Patients with AIH and established cirrhosis are at risk of disease progression.[14] Our study demonstrates that normal ALT levels are not sufficiently associated with histological remission in patients with AIH cirrhosis: only 26% of liver biopsies taken in patients with AIH cirrhosis and normal ALT levels had histological remission, whereas this was the case in 88% of liver biopsies taken in patients with AIH without cirrhosis and normal ALT levels. The addition of normal IgG levels only slightly improved the diagnostic performance, with 29% (5/17) of patients with AIH cirrhosis and normal ALT and IgG levels showing histological remission. Given that insufficient treatment response with continued histological inflammatory activity and presence of cirrhosis harbour the highest risk of disease progression and decompensation in patients with AIH,[2–5] our results are of particular clinical relevance: Whereas initiating or intensifying immunosuppressive treatment in patients with AIH cirrhosis and relevant histological activity (mHAI ≥4/18) despite normal ALT and IgG levels at time of biopsy led to a stable clinical course in 5 out of 6 patients, all 3 patients that did not receive intensified treatment developed signs of hepatic decompensation.

Previous studies point at histological activity despite biochemical remission and immunosuppressive treatment in patients with AIH, but without focusing on the difference between patients with AIH with or without cirrhosis[8–10,13]: Dhaliwal *et al.* showed that nearly 50% of treated patients with AIH (27/120 with cirrhosis at presentation) had persisting histological activity despite normal ALT and gamma-globulin levels, which was shown to be an independent risk factor for reduced long-term transplant-free survival.[9] This might relate, in part, to the potential difference between individual 'normal' transaminase levels and ULN defined for the general population, a problem not sufficiently addressed in several liver diseases. Patients with persisting histological disease activity despite biochemical remission were reported to have higher serum transaminases within the normal range compared with those who achieved histological remission.[9] Similar findings were reported by another study on the presence of histological remission in follow-up biopsies,[13] suggesting that patients have their own target ULN, which should be aimed for during therapy.

Most patients with AIH without cirrhosis and in biochemical remission were shown to have low to moderate histological activity (94% below mHAI; 6/18).[8] In addition, it has been previously reported that, in follow-up biopsies of patients with AIH (81% without cirrhosis), 82% of patients with biochemical remission also had histological remission.[13] In the present multicentric study, we confirmed these data to support the notion that in patients with AIH without cirrhosis, biochemical remission is an adequate surrogate for histological remission. This study is the first to investigate in detail whether this also holds true for patients with AIH and established cirrhosis.

Activated stellate cells are the cell population driving the development of fibrosis and cirrhosis. Independent of their direct involvement in fibrosis formation, they also act as antigen-presenting cells and stimulate immune responses by the secretion of cytokines, such as IL-1 and IL-4, which might contribute to a baseline inflammatory activity in cirrhotic livers.[15–17] Nevertheless, biopsies of patients with NAFLD/ALD with cirrhosis and normal ALT levels showed a lower histological activity compared with biopsies of patients with AIH cirrhosis (mHAI <4 in biopsies of patients with cirrhosis and normal ALT levels: 78% in NALFD/ALD *vs*. 26% in AIH). This finding indicates a relevant disease activity induced by AIH in patients with cirrhosis regardless of baseline inflammation in cirrhosis, and underlines the need for an AIH-specific histological grading and staging system.

Our study has obvious strengths and limitations: it represents a large cohort of patients with AIH without and with histological proven cirrhosis collected from several expert centres, thereby reflecting real-life practice. A high proportion of IgG serum levels could be analysed, which, in many centres, had not been standard over previous years. As limitations, despite the large cohorts of patients included, the subgroup analyses were restricted to small group sizes: from patients with AIH cirrhosis, we were only able to include 17 patients with normal serum ALT and normal IgG levels. Aside from that, our study was hampered by

the limitations of being retrospective, including several liver pathologists, and the lack of a specific AIH disease score. Furthermore, we aimed to include as many patients as possible with surrogate markers within the normal ranges, which led to the relatively high percentage of patients with cirrhosis and normal ALT and IgG levels.

In conclusion, our results underline the need for new biomarkers of disease activity in patients with AIH cirrhosis, a patient group at highest risk of disease progression. Follow-up biopsies have already been discussed as an adequate tool for disease monitoring.[6,9,13] However, biopsies might harbour increased risk in patients with established cirrhosis. Furthermore, our study calls for a clearly defined threshold of histological activity in patients with AIH cirrhosis to define those at low *vs*. high risk of hepatic decompensation (indication for liver transplantation *vs*. fatal outcome because of liver failure). Given that transient elastography (TE) has already been proved as a non-invasive tool for monitoring fibrosis development,[13,18] more data are needed to assess the value of TE for monitoring disease activity and progression in patients with AIH cirrhosis. Until better biomarkers of histological remission are established, we propose to monitor patients with AIH cirrhosis by using non-invasive tools of fibrosis stage to not miss disease progression in patients with ongoing histological activity.

Abbreviations

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EASL, European Association for the Study of the Liver; gamma-GT, gamma glutamyl transferase; ICC, intraclass correlation coefficient; INR, international normalised ratio; LLN, lower limit of normal; MELD, model for end-stage liver disease; mHAI, modified Hepatitis Activity Index; NAFLD, non-alcoholic fatty liver disease; TE, transient elastography; TIPS, transjugular intrahepatic portosystemic shunt; ULN, upper limit of normal.

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Conflict of interest

The authors have declared no conflicts of interest.

Authors' contributions

A.L: substantial contribution to the conception and design, data acquisition and analysis, interpretation of data, and drafting of the article. SW and TK: histopathological examination and critical revision. Z.K., V.L., S.P., M.A.T., F.D., E.J., F.P., J.P.H.D., G.D., M.H., and R.T.: data acquisition and critical revision. S.O-M., G.S., and A-M.L.: statistical analysis and interpretation, and critical revision. A.W.L.: substantial contribution to the design, and critical revision for important intellectual content. C.S.: substantial contribution to the design, interpretation of data, drafting of the article, and critical revision for important intellectual content.

Data availability statement

The data that support the findings of this study are available from the corresponding authors, C.S. and A.L., upon reasonable request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100321.

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