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Original article

Can liver biopsy be spared for the diagnosis of autoimmune hepatitis in selected children? A multicenter retrospective study

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ABSTRACT

Introduction and Objectives: Autoimmune hepatitis (AIH) is diagnosed based on clinical, biochemical, immunological, and histological parameters, and on the exclusion of other liver diseases. Multiple scoring systems are available for AIH diagnosis, all of which require liver biopsy (LB). With the aim of reducing invasive procedures to minimize patient's risks, this study evaluated whether LB may be spared for AIH diagnosis in some children, similar to primary biliary cholangitis.
Materials and Methods: Children with histologically confirmed autoimmune liver disease (AILD) were evaluated from 5 Pediatric Units. We retrospectively collected clinical, laboratory, imaging and histological data to assess AIH diagnostic scores (International Autoimmune Hepatitis Group [IAIHG] criteria, juvenile AIH score [JAIH], and simplified criteria [s-IAIHG]) in each patient pre- and post-LB. The diagnosis of autoimmune sclerosing cholangitis (ASC) was based on magnetic resonance cholangiopancreatography and liver histology.
Results: Ninety-one patients (55 females) were evaluated (36 with AIH type I, 24 with AIH type II, 8 with seronegative AIH, and 23 with ASC). The mean age at diagnosis and duration of follow-up were 8.9 ± 4.8 and 9.6 ± 7.8 years, respectively. Based on IAIHG, JAIH and s-IAIHG scores, pre-LB scores were *"definite"* for 15.3%, 49.5%, and 0% of patients, respectively. Post-LB, the diagnosis was confirmed in all these patients. We found no associations between liver histological findings or diagnostic scores and relapses or treatment withdrawal.
Conclusions: In patients with a *"definite"* AIH score pre-LB, LB is not necessary for diagnosis. Histological findings and scoring systems do not predict relapses or treatment withdrawal.
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1. Introduction

Autoimmune hepatitis (AIH) is a progressive inflammatory disease that affects both children and adults, with a predominance in women [1]. It is characterized by complex interactions among genetic, immunological, and environmental factors that cause the loss of tolerance to hepatic antigens in genetically susceptible individuals [2]. The diagnosis of AIH is based on a combination of clinical, biochemical, immunological and histological features and on the exclusion of other known causes of liver disease that may present with the same clinical and laboratory profile [3]. Patients with AIH usually have high serum aminotransferase activity, elevated immunoglobulin G (IgG) levels, positive circulating autoantibodies, typical histopathological features, [4] and respond to immunosuppressive treatment [5].

AIH, such as autoimmune sclerosing cholangitis (ASC) [6] and *de novo* AIH after liver transplantation, is an autoimmune liver disease

Abbreviations: AIH, autoimmune hepatitis; AILD, autoimmune liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibodies; ASC, autoimmune sclerosing cholangitis; AST, aspartate aminotransferase; IAIHG, International Autoimmune Hepatitis Group; LB, liver biopsy; MRCP, magnetic resonance cholangiopancreatography; PBC, primary biliary cholangitis; SMA, anti-smooth muscle antibodies; anti-LC1, anti–liver cytosol type 1 antibodies; anti-LKM-1, anti–liver-kidney microsome type 1 antibodies

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(AILD) [7]. The diagnosis of AIH can be challenging because it can present at any age and with a broad spectrum of clinical manifestations, including increased aminotransferase serum levels and cirrhosis and its complications. In some cases, AIH onset may present with acute liver failure. Therefore, AIH must be considered in the differential diagnosis of several types of liver involvement patterns [8]. The International Autoimmune Hepatitis Group (IAIHG) proposed a diagnostic score for AIH that has also been used in children [9]. The IAIHG subsequently revised the criteria and published a simplified version, the simplified IAIHG score (s-IAIHG). More recently, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published the juvenile AIH score (JAIH), which is specific for children and is designed to differentiate AIH from ASC [10]. All these scores include histological features; in fact, liver biopsy (LB) is currently considered mandatory for all patients with suspected AIH, unless there are significant contraindications [10].

The pivotal role of LB in the scoring system for AIH [9,11] diagnosis is documented by the value they attribute to histological lesions. For example, according to the simplified criteria for the diagnosis of AIH, a *"definite"* AIH diagnosis necessarily requires histological examination. The histological hallmark of AIH is interface hepatitis (i.e., a dense inflammatory infiltrate composed of mononuclear and plasma cells), which crosses the limiting plate and disrupts the surrounding parenchyma [12]. Histological findings allow us to classify the severity of inflammatory activity, evaluate the stage of fibrosis, and exclude alternative diagnoses [13,14]. However, in AIH patients, classic histological features, such as dense portal lymphoplasmacytic infiltrates with moderate/severe interface hepatitis, are not always observed. In fact, interface hepatitis is absent in a considerable percentage of patients with this condition, particularly if they were previously exposed to immunosuppressive treatment for associated conditions [6]. Furthermore, such histological findings are not pathognomonic of AIH, as they can also be found in other liver diseases, although they are generally more severe in AIH [6]. Therefore, the diagnosis of AIH requires additional tests.

Typically, AIH patients respond well to immunosuppressive treatment, which should be started as soon as possible and carried out for several years [10]. First-line treatment includes prednisone alone or in combination with azathioprine [15]. Notably, at present, the choice of immunosuppressive drugs in children is not influenced by histological findings [16,17].

Over the last two decades, physicians have made efforts to minimize invasive procedures to reduce patient risk as well as healthcare costs. In certain chronic diseases, such as primary biliary cholangitis (PBC), celiac disease, and primary immune thrombocytopenia, mandatory histological or cytological evaluation via biopsy or fine-needle aspiration has been partially or completely abandoned in selected cases [18–22].

Hepatologists are also adopting less invasive approaches to manage chronic diseases. For example, vibration-controlled transient elastography has become a routine alternative to LB for assessing liver stiffness, and its use has been validated in several chronic liver diseases, including hepatitis C [23,24].

Moreover, there is currently considerable debate regarding the need for LB before the withdrawal of immunosuppressive treatment in AIH patients. Maggiore et al. reported that 45 % of children discontinued immunosuppressive treatment without relapse, on the basis of only normal serum transaminase activity without histological evaluation [25]. Thus, in selected cases LB could be omitted.

Since LB is an invasive procedure that is not free from side effects, the aim of this study was to investigate whether LB for AIH diagnosis can be spared in selected children.

2. Materials and Methods

This multicenter, observational and retrospective study was conducted at the Pediatric Gastroenterology and Liver Unit of the University of Naples Federico II (Naples, Italy), Pediatric Gastroenterology Unit of Regina Margherita Pediatric Hospital (Turin, Italy), Pediatric Unit of Pediatric Hospital Giovanni XXIII (Bari, Italy), Pediatric Gastroenterology and Liver Unit, Hospital Umberto I (Rome, Italy) and Pediatric Gastroenterology Unit of Maggiore Hospital (Bologna, Italy). The study was undertaken between 01 January 2023 and 30 November 2024.

All children with suspected AILD were considered for the study and the scores available for diagnosis were assessed pre- and post-LB [9–11]. All patients with a histologically documented diagnosis of AIH were evaluated and compared with those found not to have AIH after LB. Patients with *de novo* AIH after liver transplantation were excluded. The IAIHG revised diagnostic criteria, JAIH score and simplified criteria for the diagnosis of AIH (s-IAIHG) were calculated pre- and post-LB [9–11]. For the IAIHG revised diagnostic criteria, only the pretreatment scores were included in the analysis.

The group with suspected AILD was defined by the presence of: persistent elevated serum aminotransferase levels, serum IgG levels over the upper limit of normal with or without positivity of circulating autoantibodies, exclusion of the main alternative causes of pediatric liver disease (drug-induced liver injury (DILI), genetic metabolic disorders, metabolic dysfunction-associated steatotic liver disease, infections, alcohol consumption). As recently suggested, suspected AILD was also considered in children with acute elevation of liver enzymes associated with hypergammaglobulinemia with or without positivity of circulating autoantibodies and with complete exclusion of the other causes of acute liver disease.

We reviewed the medical records of each patient. Age, sex, exclusion of other causes of liver disease (viral hepatitis, alcohol consumption history, medication history, genetic metabolic disorders, metabolic dysfunction-associated steatotic liver disease), family history of autoimmune diseases and presence of extrahepatic autoimmune disorders at the time of diagnosis were recorded. Data from the following laboratory tests were collected (i.e., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total and conjugated bilirubin levels, serum bile acids, serum IgG levels, and the presence of autoantibodies (antinuclear antibody [ANA], anti-smooth muscle antibody [SMA], anti-liver kidney microsome [LKM] type 1 antibody, anti-liver cytosol type 1 antibodies [anti-LC1], anti-soluble liver antigen [SLA], perinuclear anti-neutrophil nuclear antibody [p-ANCA]).

Patients were classified based on serum antibodies as affected by AIH type I (in the presence of ANA and/or SMA), AIH type II (in the presence of LKM-1 and/or LC-1) [26] or seronegative AIH (in the absence of autoantibodies) [27].

Autoantibody testing was performed by indirect immunofluorescence, and titers were categorized as low or high according to internationally criteria for AIH [10].

We analyzed the abdominal ultrasound findings, with a focus on the liver, biliary tree, and spleen. ASC diagnosis was based on magnetic resonance cholangiopancreatography (MRCP) findings, in combination with immunological and histological data [28]. Data on immunosuppressive treatment, relapses or treatment withdrawal were collected for each patient, and the response to therapy was assessed according to guidelines [8,10].

2.1. Histology

We recorded the histological features of the hepatic samples obtained with LB and calculated the related histological scores. The presence of interface hepatitis, predominantly lymphoplasmacytic infiltration, rosetting of liver cells, biliary changes, and other changes, or none of the above, were evaluated for the IAIHG revised diagnostic criteria, and the corresponding scores were assigned [9–11]. Following the s-IAIHG criteria and the JAIH score, histological findings were

classified as *"typical"* or *"compatible"* with AIH. "*Typical*" AIH features include the presence of interface hepatitis, lymphocytic/lymphoplasmacytic infiltrates in portal tracts, emperipolesis and rosette formation, whereas "*compatible*" features include the presence of only lymphocytic/lymphoplasmacytic infiltrates in portal tracts without other *"typical"* features [11].

2.2. Scoring system

According to the IAIHG revised diagnostic criteria, [9] AIH diagnosis was classified as *"definite"* or *"probable"*. Based on this score, histological parameters provide a maximum of 5 points, 3 of which are given in the presence of moderate/severe interface hepatitis, whereas negative points are assigned in the absence of *typical* features or in the case of biliary and/or other changes suggestive of an alternative etiology.

According to the JAIH score, [10] a diagnosis of *"probable"* AIH/ASC is made with a score ≥7, whereas a diagnosis of *"definite"* AIH/ASC is made if the score is ≥8. Negative points are not foreseen, and only *"typical"* or *"compatible"* histology is considered for scoring. The JAIH is the only score that distinguishes between the diagnosis of AIH and ASC.

According to the s-IAIHG criteria, [11] the diagnosis is *"definite"* with a score of 7 or more, and *"probable"* with a score ≥6. Liver histology accounts for 2 out of 8 points; 2 points are assigned in the presence of the *typical* histological picture, whereas 1 point is given if *compatible* features are present. This score requires LB to obtain a *"definite"* diagnosis.

To assess the impact of histological evaluation, we compared pre- and post-LB scores of patients diagnosed with AIH, classified as *"definite", "probable" or "not probable"*. In addition, we examined potential differences among the AILD groups (i.e., AIH I, II, seronegative and ASC) in terms of baseline laboratory and histological findings, responses to therapy, relapses, and permanent treatment withdrawal.

2.3. Ethics statement

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Ethical consent for data collection was waived, given the retrospective nature of the study and the anonymous treatment of data.

2.4. Statistical analysis

The data were managed and analyzed via SPSS software v.29. Descriptive data are reported as the means and standard deviations for quantitative variables, or as absolute numbers and relative frequencies for qualitative variables. Univariate analysis was performed using the unpaired Student's t-test or ANOVA. We used the two-tailed Fisher's exact test to assess associations between variables. A *P value* <0.05 indicated statistical significance.

3. Results

No patient with a *"definite"* or *"probable"* diagnosis of AILD prior to LB was found not to have AILD after LB. Ninety-one patients (55 females, 60.4 %) had a confirmed AIH diagnosis. The mean age at AILD diagnosis was 8.9 ± 4.8 years, and the mean duration of follow-up was 9.7 ± 7.8 years.

All patients had elevated serum levels of aminotransferases (ALT and AST) at initial presentation (mean ALT levels 926.2 ± 832.9 IU/L). GGT values were abnormal in 61 patients (67 %; mean 141.9 ± 183.8 IU/L), and ALP levels were altered in 13 patients (14.3 %; mean 347.9 ± 207.2 IU/L). Serum total bilirubin was elevated in 35 patients (38.4 %; mean 2.7 ± 3.7 mg/dL). The ALP/ALT ratio was >1.5 in 57/71 patients (62.6 %). IgG levels were elevated in 72 patients (79.1 %; mean value 23.5 ± 10.2 g/L). No patients presented with acute liver failure at AIH onset.

Thirty-six patients (39.5 %) were diagnosed with AIH type I, 24 (26.4 %) with AIH type II, 8 (8.8 %) with seronegative AIH, and 23 (25.3 %) with ASC. The clinical and biochemical laboratory data based on the type of AIH are summarized in Table 1.

All patients underwent an abdominal ultrasound at the time of diagnosis. Forty-eight patients (52.7 %) presented with abnormalities (36 with hepatomegaly, 26 with splenomegaly, and 14 with both hepatomegaly and splenomegaly). No patients had biliary tree abnormalities. Patients with hepatomegaly and/or splenomegaly had higher levels of liver enzymes at onset (p = 0.037) and, more frequently, hypothyroidism ($p < 0.001$).

At LB, 60 patients (65.9 %) displayed histological findings *"typical"* of AIH (24 [40 %] with AIH type I, 16 [26.7 %] with type II, 6 [10 %] with seronegative AIH, and 14 [23.3 %] with ASC). Thirty-one patients (34.1 %) displayed a histological picture *"compatible"* with AIH, of whom 14 (45.2 %) were type I, 7 (22.6 %) were type II, 1 (3.2 %) was seronegative and 9 had ASC (29 %). No patient with normal MRCP

Table 1
Clinical and laboratory features of 91 children with different types of autoimmune liver disease.

	Type I AIH (n = 36)	Type II AIH (n = 24)	Seronegative AIH (n = 8)	ASC (n = 23)	Overall Population (n = 91)	p value
Female gender, n (%)	19 (53 %)	20 (83.3 %)	6 (75 %)	10 (43.5 %)	55 (60.4 %)	0.004
Age, years, mean ± sd	9.1 ± 4.4	6.7 ± 4.8	8.3 ± 4.2	11 ± 4.8	8.9 ± 4.8	0.018
Follow-up, years, mean ± sd	9.3 ± 7.4	13.1 ± 9.0	11.0 ± 9.2	6.5 ± 5.4	9.6 ± 7.8	0.035
ALT, IU/L (mean ± sd)	863.3 ± 838.7	1073.3 ± 919.1	1453.1 ± 806.4	722.5 ± 686.8	926.1 ± 832.9	0.164
ALP, IU/L (mean ± sd)	370.4 ± 191.1	299.4 ± 170.8	274.3 ± 110.7	379.8 ± 261.2	347.8 ± 207.1	0.498
ALP/ALT ratio <1.5, n (%)	16 (44.4 %)	20 (83.3 %)	4 (50 %)	17 (74 %)	57 (62.6 %)	0.113
GGT, IU/L, mean ± sd	178.4 ± 253.1	73.5 ± 70.8	134.1 ± 142.6	148.6 ± 98.3	141.8 ± 183.7	0.222
Total bilirubin, mg/dL, mean ± sd	2.4 ± 3.6	3.2 ± 4.8	3.9 ± 3.5	2.4 ± 2.6	2.65 ± 3.5	0.728
Conjugated bilirubin, mg/dL, mean ± sd	1.1 ± 2.1	2.5 ± 4.0	2.7 ± 2.7	1.4 ± 1.8	1.76 ± 2.9	0.254
Serum Bile Acids, μmol/L, mean ± sd	20.7 ± 11	101.5 ± 172.5	27.5 ± 17.7	121.2 ± 107.8	77.1 ± 103.8	0.163
Immunoglobulin G, g/L, mean ± sd	25.7 ± 10.5	19.4 ± 10.6	17.6 ± 6.5	26.1 ± 8.8	23.4 ± 10.2	0.025
High-titer ANA, n (%)	22 (61.1 %)	0	0	10 (43.4 %)	35 (38.4 %)	
High-titer LKM-1, n (%)	0	18 (75 %)	0	0	19 (20.8 %)	
High-titer SMA, n (%)	11 (30.5 %)	0	0	11 (47.8 %)	23 (25.2 %)	
Anti-LC-1 positivity, n (%)	0	4 (16.6 %)	0	0	4 (4.4 %)	
Other extrahepatic autoimmune conditions, n (%)	14 (38.9 %)	7 (29.2 %)	2 (25 %)	6 (26.1 %)	29 (31.8 %)	0.839
Relapsers, n (%)	10 (27.7 %)	12 (50 %)	3 (37.5 %)	11 (47.8 %)	36 (39.5 %)	0.374
Treatment withdrawal, n (%)	6 (16.6 %)	1 (4.2 %)	0 (0 %)	1 (4.3 %)	8 (8.7 %)	0.248

AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ASC, autoimmune sclerosing cholangitis; GGT, gamma-glutamyl transferase.

results had signs of small duct cholangitis. We found no differences between histological findings or types of AIH.

Patients with ASC had a greater mean age at diagnosis than did those with normal MRCP (11 ± 4.8 vs 8 ± 4.6 years, $p = 0.011$). No other differences were recorded between AIH and the ASC group. The *"typical"* and *"compatible"* histological findings were similar among AIH and ASC patients (61.4 % vs 60.9 % and 38.6 % vs 39.1 %, respectively).

3.1. Primary response and permanent treatment withdrawal

All patients showed a complete initial clinical and biochemical response to immunosuppressive treatment. Thirty-six patients (39.6 %) had one or more episodes of relapse during the follow-up. In 8 patients (8.8 %), immunosuppressive therapy was successfully discontinued. At diagnosis, relapsers had higher ALT levels ($p = 0.003$). Relapses or permanent treatment withdrawal did not significantly differ between AIH patients and ASC patients, or among the different AIH types. In addition, patients who successfully discontinued treatment were younger (5.6 ± 3.6 vs 9.2 ± 4.8, $p = 0.039$) and had lower levels of total and conjugated bilirubin at diagnosis (0.5 ± 0.2 vs 3 ± 3.8, $p < 0.001$, and 0.2 ± 0.1 vs 1.9 ± 2.8, $p < 0.001$, respectively).

3.2. IAIHG revised diagnostic criteria

Using the IAIHG revised diagnostic criteria, the pre-LB scores categorized 14 patients (15.3 %) as *"definite"* AIH, 76 patients (83.5 %) as *"probable"* AIH, and 1 patient (1 %) as *"not probable"* AIH. Post LB, the results remained unchanged in all patients with a *"definite"* AILD diagnosis. Among the 76 patients with *"probable"* pre-LB scores, 48 (63.1 %) were reclassified as *"definite"* post LB, whereas the diagnosis remained unchanged in the other 28 patients (36.8 %). In the only patient with a *"not probable"* pre-LB score, the result changed to *"probable"* post LB (Fig. 1).

Histological features *typical* of AIH were observed in 10 patients with a *"definite"* score and 51 patients with a *"probable"* score, according to the IAIHG score. However, histological findings *compatible* with AIH were found in 4 patients with a *"definite"* score and in 28 patients with a *"probable"* score.

No difference in terms of relapse during the follow-up was observed in the group of patients with *"probable"* and *"definite"* scores, both pre- and post-LB.

Considering only the ASC group, 2 (8.7 %) patients had *"definite"* and 21 (91.3 %) had *"probable"* pre-LB scores. Post LB, the score remained unchanged in the 2 patients with a *"definite"* score, whereas it changed to *"definite"* post LB in 11/21 patients with a *"probable"* pre-LB score. The score remained unchanged in the remaining 10 patients.

3.3. JAIH score

According to the JAIH score, 45 (49.5 %) patients had *"definite"*, 21 (23.1 %) had *"probable"* and 25 (27.4 %) had *"not probable"* pre-LB scores. Post LB, the score remained unchanged in all the patients with a *"definite"* score. Among patients with a *"probable"* pre-LB score, the score was reclassified as *"definite"* in 21 patients post LB. In the group with a *"not probable"* pre-LB score, the score remained unchanged in 2 patients (8 %), whereas the others were reclassified post LB as *"definite"* and *"probable"* (18 patients (72 %) and 5 patients (20 %), respectively) (Fig. 2).

Histological features *typical* of AIH were observed in 26 patients (28.6 %) with a *"definite"* score and in 14 patients (15.4 %) classified as *"probable"* according to the JAIH score. Histological findings *compatible* with AIH were found in 20 patients with a *"definite"* score and in 6 patients with a *"probable"* score. No difference in terms of relapse during the follow-up was found in the group of patients with different scores both pre- and post-LB.

In the ASC group, 15 (65.3 %) patients had a *"definite"*, 5 a *"probable"* (21.7 %) and 3 (13 %) a *"not probable"* pre-LB diagnosis. Post LB, all 23 patients obtained a *"definite"* score.

3.4. s-IAIHG score

Using the *s-IAIHG* score, 55 (60.5 %) patients had a *"probable"* score and 36 (39.5 %) had a *"not probable"* pre-LB score. Post LB, the score was confirmed in all patients with a *"probable"* (60.5 %) pre-LB score. In the group of patients with *"not probable"* pre-LB scores, the post-LB score became *"definite"* in 8 patients (22.3 %), whereas it changed to *"probable"* in 19 patients (52.7 %) and it remained *"not probable"* in 9 patients (25 %) (Fig. 3).

Sixty-seven percent (37/55) of patients with a *"probable"* AIH diagnosis, according to the s-IAIHG, presented histological features *typical* of AIH, whereas 32 % (18/55) presented histological features *compatible* with AIH.

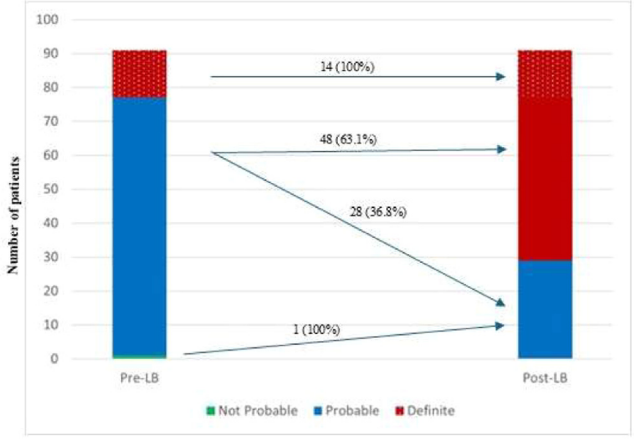


Fig. 1. Distribution of patients before and after liver biopsy (LB) in the three diagnostic AIH categories "not probable" (green), "probable" (blue), "definite" (red).

The arrows indicate the transition of patients from the pre-LB to the post-LB category. Patients with a "definite" AIH diagnosis before LB are indicated by a dotted red line. LB, liver biopsy.

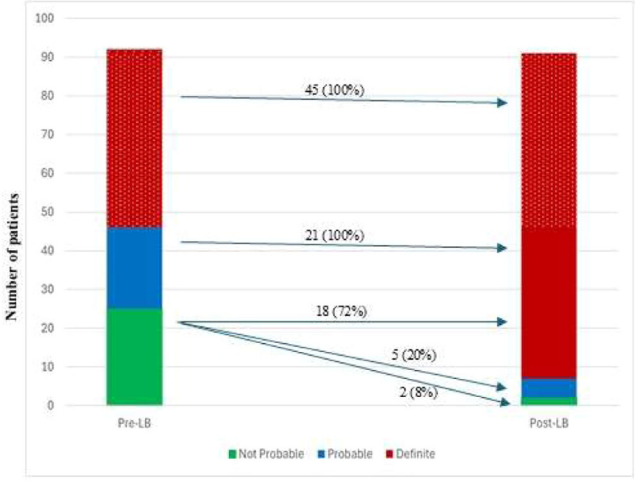


Fig. 2. Distribution of patients before and after liver biopsy (LB) in the three diagnostic AIH categories "not probable" (green), "probable" (blue), "definite" (red).

The arrows indicate the transition of patients from the pre-LB to the post-LB category. Patients with an AIH "definite" diagnosis before LB are indicated by dotted red line. LB, liver biopsy.

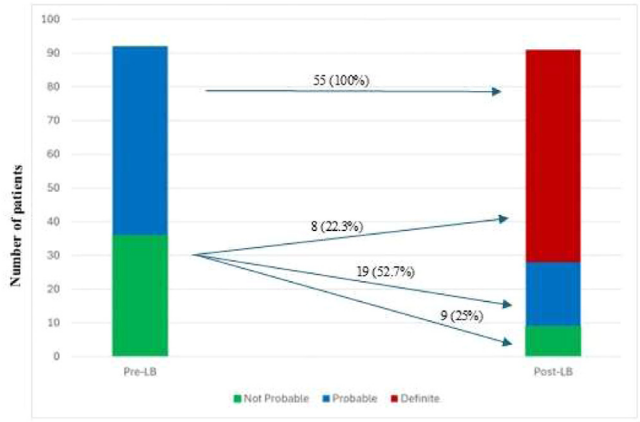


Fig. 3. Distribution of patients before and after liver biopsy (LB) in the three diagnostic AIH categories "not probable" (green), "probable" (blue), "definite" (red).

The arrows indicate the transition of patients from the pre-LB to the post-LB category. LB, liver biopsy.

In the ASC group, 19 patients (82.6 %) had a *"probable"* pre-LB score, and 4 patients (17.4 %) had a *"not probable"* pre-LB score. Post LB, it became *"definite"* in 20 patients (87 %) and remained *"probable"* in 3 (13 %).

During the study period, no patient with a *"definite"* or *"probable"* pre-LB score was found with histological finding not suggestive of AIH.

4. Discussion

Current recommendations require LB to obtain a definite diagnosis of AIH [10]. However, there is a growing trend toward less invasive diagnostic procedures to minimize risks to patient health and reduce associated costs. This shift is supported by the increasing use of elastography and fibroscan as alternatives to LB for evaluating liver fibrosis [23,24]. Additionally, in the case of PBC—a typical adult liver disease—diagnosis can often be made without LB in patients with significantly high ALP levels and elevated titers of anti-mitochondrial antibodies [18]. This multicenter study was conducted to evaluate whether LB could be spared for AIH diagnosis in some patients.

Our results indicate that all patients with a *"definite"* pre-LB score according to the IAIHG and JAIH systems had their diagnosis confirmed post-LB. This consideration could not be applied to the s-IAIHG score, which by definition requires LB to obtain a *"definite"* diagnosis of AIH. Notably, in the group with a *"definite"* pre-LB diagnosis, the histological evaluation revealed findings *typical* of AIH in most patients, thereby confirming the reliability of the pre-LB score. These observations support the idea that LB could be spared in patients classified with a *"definite"* diagnosis based on pre-LB scores using IAIHG score and JAIH score. In contrast, our findings confirm the need for LB in patients with a *"probable"* or *"not probable"* pre-LB score. In our cohort, no significant correlation was found between histological findings and the likelihood of either response to immunosuppressive therapy or relapse rates or the ability to achieve permanent treatment discontinuation. Based on these data, omitting LB in patients with a *"definite"* pre-LB diagnosis does not seem to be associated with a higher risk of relapse or other unfavorable events during follow-up. Furthermore, the choice of immunosuppressive therapy in children — typically prednisone, with or without azathioprine — is made independently from liver histology results [10]. On the other hand, histology may modify therapeutic decisions in adult patients, as budesonide is a first-line option for those with AIH without advanced fibrosis but less suitable for cirrhotic patients because of impaired hepatic metabolism and increased systemic bioavailability [29]. Currently, there are insufficient data on the use of budesonide in children with AIH. Therefore, based on these considerations, the results of our study cannot be extended to adults without further research.

One of the primary arguments for routinely performing LB in AIH patients is the need for a highly certain diagnosis, as this condition requires long-term therapy. Importantly, the histological features used to diagnose AIH are not pathognomonic. Indeed, findings such as interface hepatitis, hepatocellular rosettes, and emperipolesis, while suggestive of AIH, can also occur in other conditions including viral hepatitis, PBC, and drug-induced liver injury [30].

Another relevant aspect in the diagnosis of AIH is the differential diagnosis of sclerosing cholangitis. Biliary alterations indicative of sclerosing cholangitis are preferably detected with MRCP thus LB is limited to patients in which small duct cholangitis is suspected. In our study, we did not find cases of small duct cholangitis, and no significant hepatic histological differences were identified between patients with AIH and those with ASC, thereby confirming the key diagnostic role of MRCP in identifying patients with ASC [15].

Moreover, assessment of atypical p-ANCA may be helpful as an adjunctive tool in the differential diagnosis between AIH and ASC [31]. Concerning the reliability of the currently available diagnostic scores, the JAIH score is the only score designed for children and the only one suitable for differential diagnosis between AIH and ASC, [10] since the other two scores do not foresee cholangiography. In our study, we observed that the percentage of *"definite"* pre-LB diagnoses was higher in the ASC group (65 %) than in the AIH group. In addition, after LB, the score became *"definite"* in all patients, suggesting that this score may have high sensitivity in patients with ASC. Moreover, the lack of differences in histological findings, rates of relapses and treatment withdrawal between patients with ASC and AIH also suggests that LB is not indispensable for the diagnosis of AIH in patients with *"definite"* pre-LB scores. This finding could have significant benefits in clinical practice by reducing psychological stress for patients and their families, as well as lowering the costs associated with the biopsy procedure. However, avoiding LB should not be a reason to formulate AIH diagnoses with less accuracy. This implies that other causes of liver disease (e.g., DILI, genetic metabolic disorders, metabolic dysfunction-associated steatotic liver disease, infections, alcohol) should be excluded and that supporting factors (e.g., serum immunoglobulin levels, and the presence of autoantibodies) should be identified with great accuracy. Indeed, AIH can often be misdiagnosed with other causes of liver disease such as Wilson disease, due to the aspecific presence of autoantibodies such as ANA and SMA [32,33]. DILI may also mimic AIH [34]. Furthermore, the indirect immunofluorescence technique (IIF) is the recommended method for autoantibody assessment and its interpretation must account for the fact that cutoff values are different between children and adults. Similarly, serum IgG levels, which should be considered indicative of AIH in children, have different cutoff values in children than in adults. Additionally, variability in the quality of substrates for autoantibody detection may influence test results. Therefore, it is recommended that autoantibodies be tested in a reference center where standardized protocols and high-quality substrates are employed [35].

Recently, increasing attention has been directed toward anti–F-actin smooth muscle antibodies with anti-microfilament actin (MF-SMA), as a strong and reliable marker for the diagnosis of AIH. Incorporating the MF-SMA test into diagnostic algorithms may improve patient selection and, in appropriately selected cases and in specialized settings, may reduce the need for liver biopsy without compromising diagnostic accuracy [36]. Long-term follow-up with the assessment of the clinical course over time contributed to confirm AIH diagnosis in this study, and represents a necessary key point for AIH monitoring.

5. Conclusions

Although LB remains a fundamental tool in the diagnostic process of AIH, especially in patients with a *"not probable"* AIH score and in patients with seronegative AIH, this study is a preliminary step toward the development of a less invasive path for the diagnosis of AIH, at least in the subgroup of children in which the diagnosis can be obtained based on other diagnostic elements. Prospective multicenter studies are needed to better establish the specificity and sensitivity of available diagnostic scores and to promote a noninvasive and reliable diagnosis of AIH/ASC.

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Declaration of generative AI and AI-assisted technologies in the writing process

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Authors contribution

RI, VDC and FDD conceptualized and designed the study and contributed to the discussion. RI, VDC and FDD analyzed data, interpreted results, and drafted the manuscript. RI, VDC, FDD, PLC, MP, MA, FZ, RF, ER, FS and PA enrolled patients, collected data, and critically revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of interests

None.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.aohep.2026.102198.

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