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ORIGINAL ARTICLE

Biliary features in liver histology of children with autoimmune liver disease

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Abstract

Objectives and study Various degrees of biliary changes are considered to be part of the histological picture of children with pediatrics autoimmune liver disease (AILD), but the literature is scarce and confusing. We aimed to describe the characteristics of children with AILD (autoimmune hepatitis, AIH, and autoimmune sclerosing cholangitis, ASC) focusing on the prevalence and type of biliary abnormalities on initial biopsy to see whether ASC was predictable on histological ground.

Methods The files of children diagnosed with AILD were reviewed. The Ishak score was used to grade inflammation and fibrosis on biopsy; a biliary score was built to grade bile duct injury. Demographic, laboratory and histological features at diagnosis were reported and compared between the two groups (AIH vs ASC).

Results Forty-one patients were diagnosed with AIH ($n=24$), ASC ($n=13$) and PSC ($n=4$) between 2009 and 2018. Twenty-nine patients [$F=76\%$, AIH = 20, ASC = 9, median age at diagnosis 11.7 (range 2.2–17.8)] were included in the study; 12 (4 with PSC) were excluded. Prevalence of inflammatory bowel disease was higher in ASC group (56% vs 10% in AIH, $p<0.05$). On histology 17% had cirrhosis. The grade of biliopathy with AILD was moderate in 72% and severe in 31%, and overall more prominent in ASC ($p=0.031$). The inflammation of the bile ducts was classified as "multifocal" or "diffuse" mainly in ASC patients (89% vs 45% in AIH, $p=0.043$). Periductular fibrosis was reported in 52% of AILD patients, with a higher mean score in ASC group ($p<0.05$). However, ductular reaction, biliary metaplasia and granulomatous cholangitis were equally reported in AIH and ASC, providing no clear-cut for the distinction of the two entities in the global histological evaluation.

Conclusions Majority of patients with pediatrics AILD have "moderate" or "severe" features of biliopathy; AIH and ASC are not easily distinguishable on histological ground at diagnosis, and therefore, the cholangiogram remains the only effective tool to differentiate patients with AIH from those with ASC. Further prospective studies are needed to better define histological biliary features in AILD, assess if the biliopathy responds to immunosuppressive treatment and evaluate its impact on long-term outcome.

Keywords Autoimmune hepatitis · Autoimmune sclerosing cholangitis · Overlap syndrome · Children

Abbreviations

AILD	Autoimmune liver disease
AIH	Autoimmune hepatitis
ASC	Autoimmune sclerosing cholangitis
PSC	Primary sclerosing cholangitis
PBC	Primary biliary cirrhosis
SC	Sclerosing cholangitis
ANA	Anti-nuclear antibodies
SMA	Anti-smooth muscle antibodies
LKM-1	Anti-liver/kidney microsomes antibodies
LC1	Anti-liver cytosol antibodies
MRCP	Magnetic resonance cholangiopancreatography

Introduction

Autoimmune hepatitis (AIH) is a chronic and progressive liver disease affecting both children and adults [1]. In children, unlike in adults, the clinical and histological features of AIH are commonly associated with abnormalities of the biliary tree that can be demonstrated at cholangiography, making a picture overlapping with sclerosing cholangitis

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(SC), defined autoimmune sclerosing cholangitis (ASC) [2]. For this reason the European Association for the Study of the Liver (EASL), the American Association for the Study of Liver Disease (AASLD), and more recently, the European Society for Pediatrics Gastroenterology Hepatology and Nutrition (ESPGHAN) recommend to include the magnetic resonance cholangiopancreatography (MRCP) in the baseline work-up of children with a diagnosis of autoimmune liver disease (AILD) to distinguish those with AIH (normal cholangiogram) from those with ASC (abnormal cholangiogram) [3–5].

Nevertheless, the small bile ducts involvement, common in children with AILD and generally not picked up by MRCP, remains a challenging matter, leading to confusion and misleading diagnosis as far as, for instance, the diagnosis of overlap syndrome or small duct PSC [6]. Verdonk and co-authors showed that bile duct injury is common in adults with newly diagnosed AIH, suggesting that biliary features in this setting do not necessarily point towards an overlap syndrome [7]. Besides, guidelines by the International Autoimmune Hepatitis Group (IAIHG) on overlap syndromes and AASLD reported that patients who present with a liver biopsy compatible with primary sclerosing cholangitis (PSC), but with a normal cholangiogram, are classified as "small duct PSC" [6, 8, 9]. This concept has been transferred from adults to children and adopted in many centres, but no criteria to diagnose small duct PSC has been defined in pediatrics so far, suggesting a probable overestimation or underestimation of this diagnosis [1, 5–7].

In pediatrics there are no studies on histological features of bile duct injury in AILD patients, and therefore, children with AIH might be wrongly classified, in presence of some biliary changes, as overlap syndrome. This is relevant for the understanding of pediatrics AILD and its progression over time. In this study, we aimed to report demographic, laboratory and histological features of children diagnosed as AILD, describe the prevalence and type of biliary abnormalities on initial biopsy, and comparing AIH to ASC, to see whether, at the time of diagnosis, these conditions are distinguishable on histological ground.

Methods

Data collection

We reviewed retrospectively the medical records of children with AILD (AIH or ASC) diagnosed at Hospital Papa Giovanni XXIII, Bergamo, Italy, which is the largest referral centre for pediatrics hepatology and transplantation in Italy, in the last 10 years. This period was chosen because in the last 10 years all patients underwent strict criteria for the differential diagnosis between AIH and ASC including magnetic resonance cholangiopancreatography (MRCP) performed at diagnosis.

The following categories of patients were excluded from this study:

(a) Patients with a diagnosis of primary sclerosing cholangitis (PSC) or secondary SC.
(b) Patients in whom the initial biopsy was not available for review.

We prepared a database recording the following features: age, sex, liver function tests (aspartate aminotransferase, AST, alanine aminotransferase, ALT, gamma glutamyltranspeptidase, GGT, total and direct bilirubin, alkaline phosphatase, ALP, international normalised ratio, INR), serum, autoantibodies (antinuclear, ANA, anti-smooth muscle, SMA, anti-liver kidney microsomes, LKM-1, anti-liver cytosol 1, LC1, perinuclear anti-neutrophil cytoplasmic, pANCA, anti-soluble liver antigen, SLA). IgG levels were corrected according to the normal values for age [10].

Diagnosis of autoimmune liver disease

The diagnosis was based on elevated transaminases and IgG levels, positive autoantibodies, compatible liver histology, and exclusion of other liver diseases [11]. A lower threshold for autoantibody positivity was applied to children compared to adults, i.e. titre $\geq$ 1:20 for ANA and SMA and $\geq$ 1:10 for anti-LKM-1 were used, as indicated by the International Autoimmune Hepatitis Group (IAIHG) consensus statement on liver autoimmune serology [12]. All children underwent MRCP at or soon after presentation to investigate possible cholangiopathy consistent with sclerosing cholangitis (SC) [2, 8].

Patients without cholangiopathy on MRCP were diagnosed as AIH type 1 (AIH-1) in case of seropositivity for SMA and/or ANA antibody, or type 2 (AIH-2) if they were positive for LKM-1 and/or LC1 [1]. Patients with cholangiopathy were diagnosed as ASC [1, 2].

Histological re-evaluation

The histology slides were reviewed blindly and independently by two expert liver pathologists (AS and LL). Inflammation and fibrosis (stained with haematoxylin/eosin and Masson Trichrome) were evaluated and graded according to the Ishak score [13]. The inflammatory infiltrate was typified by lymphocytes immunophenotyping. In the absence of a standardized scoring system for classifying the biliary abnormalities on histology of children with AILD, we built a detailed score expanding a previously reported grading system adopted for PSC in adults [14]. The evaluation of

biliary abnormalities was performed on cytokeratin-7 (CK-7) stained specimens.

Five biliary abnormalities were considered as "biliary features suggestive for biliopathy" (Table 1).

1. Inflammatory injury of the bile duct, referring to epithelial lymphocyte infiltration of the bile duct.
2. Ductular reaction, in the presence of multiple small bile ductular structures that are seen at the edge of portal tracts. These include ductular proliferation, cholangiocyte proliferation and ductular hyperplasia.
3. Periductular fibrosis, in case of fibrosis around the bile duct in the portal area.
4. Granulomatous cholangitis, represented by epithelial granulocytes infiltration with ruptured basal membrane.
5. Biliary metaplasia, the expression of biliary type CK-7 in hepatocyte cytoplasm [15].

To assess the grade of biliopathy each parameter was scored from 0 (absent) to +3 (diffuse); cholangitis was scored as 0 (absent) or +1 (present) (Table 1). Final mean score was calculated in all patients and compared between the two groups (AIH vs ASC).

Table 1 Biliary features score

Biliary features	Score
Inflammatory injury of the bile duct (epithelial lymphocyte infiltration)	
Absent	0
Focal (up to 10% of the interlobular bile ducts)	+1
Multifocal (10–50% of interlobular bile ducts)	+2
Diffuse (> 50% of interlobular bile ducts)	+3
Ductular reaction	
Absent	0
Focal (up to 10% of the portal tracts)	+1
Multifocal (10–50% of the portals tracts)	+2
Diffuse (> 50% of the portals tracts)	+3
Periductular fibrosis	
Absent	0
Focal (up to 10% of the interlobular bile ducts)	+1
Multifocal (10–50% of interlobular bile ducts)	+2
Diffuse (> 50% of interlobular bile ducts)	+3
Biliary metaplasia	
Absent	0
Focal (up to 10% of hepatocytes)	+1
Multifocal (10–50% of hepatocytes)	+2
Diffuse (> 50% of hepatocytes)	+3
Cholangitis (epithelial granulocytes infiltration with ruptured basal membrane)	
Absent	0
Present	+1

The prevalence of biliopathy on histology was classified as follow:

(a) Absent: no biliary feature;
(b) Mild: 1 biliary feature;
(c) Moderate: ≥ 2–4 biliary features;
(d) Severe: presence of all 5 biliary features.

Treatment protocol

The patients were treated according to a previously described protocol [16]. Briefly, the immunosuppressive (IS) treatment consisted of first line use of prednisone at a dose of 2 mg/kg/day (up to a maximum of 60 mg/day) for 14 days followed by 4–6 weeks tapering schedule reaching a total maintenance dose of 5 or 2.5 mg/day. If the response to treatment was not satisfactory, azathioprine was added at the dose of 1.5–2 mg/kg/day. Patients with ASC were also administered ursodeoxycholic acid (UDCA) at the dose of 15–20 mg/kg/day. Discontinuation of IST was attempted in patients with normal value of transaminases and IgG, negative or low titer positive autoantibodies at least 3 years after starting the IS treatment, and no inflammation on histology. Nonetheless, this study considers only the features at diagnosis, therefore, this is provided for protocol description completeness, and no evaluations on treatment response and follow-up are presented here.

Statistical analysis

Data are reported as medians and ranges or means and standard deviation (DS), and specified in the text. Comparison between categorical values was performed using X^2 or Fisher's exact tests as appropriate. The Student t test for independent samples was used to determine if two sets of data were significantly different from each other. A p value of 0.05 or less was assigned significance.

The Cohen's kappa test was adopted to evaluate the agreement between the two observing pathologists. Kappa is constructed to be zero when the agreement obtained can be entirely attributed to chance, and it attains a maximal value of 1 in the case of complete agreement. Values greater than 0.40 are considered in keeping with good interobserver agreement. The analysis was performed with IBM-SPSS 13.0 for Windows. Retrospective studies are approved by ethics committee provided patient anonymisation.

Results

We collected data on 41 pediatrics patients (AIH = 24, ASC = 13 and PSC = 4) between 2009 and 2018. Twelve children were excluded from the study: 4 patients (male = 3)

because of the diagnosis of PSC, 8 patients (4 with AIH, 4 with ASC) had the diagnosis of AILD at local Hospital (3 patients came to our centre from different Countries: Egypt, England and Israel), and therefore, the liver tissue from the initial biopsy was not available to perform the histological review.

Demographic and laboratory features

Twenty-nine patients fulfilled the evaluation criteria for the diagnosis of AILD and were included in the study; 22 (76%) were female and the median age at diagnosis was 11.7 years (range 2.2–17.8). All patients underwent liver biopsy and MRCP. Twenty patients ($F=16$, 80%) were diagnosed with AIH (15 with AIH-1; 5 with AIH-2), and 9 ($F=6$, 66%), having abnormal cholangiogram, with ASC.

All patients were positive for at least one of the tested autoantibodies (Table 2). Median values of transaminases were higher in AIH group compared to ASC ($p<0.05$); GGT and ALP/AST ratio were found higher in ASC patients ($p<0.05$). Seven patients (25%) had an associated IBD [ulcerative colitis, UC, in 6 (2 with AIH and 4 with ASC] and Crohn's disease (CD) in 1 with ASC]. The prevalence of IBD was higher in ASC group (56% vs 10% in AIH, $p<0.05$). ANCA was positive in 71% of patients with IBD and in 27% without IBD ($p=0.07$). No patient with ASC was LKM-1 positive (Table 2).

When we retrospectively applied a recent scoring system for pediatrics AILD proposed by the ESPGHAN society all patients met the criteria for the diagnosis of AIH and ASC [5].

Histological features

The interobserver agreement between the two pathologists who reviewed blindly all the available specimens was very good (Cohen's kappa = 0.6).

Biliary features (Table 3)

Inflammation injury of bile duct was reported in 100% of patients. It presented as "focal" form mainly in AIH patients (50% vs 11% in ASC, $p<0.05$) and "multifocal" or "diffuse" in ASC patients (89% vs 45% in AIH, $p<0.05$); the mean score was higher among ASC patients [2.0 ($\pm$0.58) vs 1.4 ($\pm$0.51) in AIH, $p<0.05$]. Periductular fibrosis was reported in 52% of patients ($n=15$) as "mild" form in 11 patients and as "multifocal" form in 4 patients (14%, all with ASC, $p<0.05$). No patients showed a "diffuse form" of periductular fibrosis. The mean score of periductular fibrosis was significantly higher among ASC patients compared to AIH group [1.11 ($\pm$92) vs 0.45 ($\pm$0.51), $p<0.05$]. Ductular reaction, biliary metaplasia and granulomatous cholangitis were reported in 100, 72 and 65% of patients without

Table 2 Baseline features on 29 children with pediatrics autoimmune liver disease

	All pts ($n=29$)	AIH ($n=20$)	ASC ($n=9$)	p value
Age (years)	11.7 (2.2–17.8)	11.7 (2.2–17.8)	14.3 (4.3–16.1)	0.370
Female prevalence	22 (76%)	16 (80%)	6 (67%)	0.642
AST (nv: < 50 IU/l)	330 (66–2037)	452 (66–2037)	155 (85–875)	**0.049**
ALT (nv: < 50 IU/l)	285 (71–2183)	671 (71–2183)	156 (116–890)	**0.049**
GGT (nv: < 40 IU/l)	158 (10–655)	110 (10–594)	287 (48–655)	**0.028**
Total bilirubin (nv: < 1 mg/dl)	2.3 (0.3–20.8)	3.0 (0.3–20.8)	1.2 (0.8–6.5)	0.205
Conjugated bilirubin (mg/dl)	1.6 (0.1–16.2)	1.9 (0.1–16.2)	0.4 (0.1–4.3)	0.155
ALP (nv: < 350 IU/l)	268 (107–1209)	265 (107–1209)	285 (173–817)	0.719
ALP/AST ratio	1.2 (0.1–9,3)	0.4 (0.1–5)	2.0 (0.3–9.3)	**0.047**
INR (nv: 0.8–1.2)	1.3 (0.9–2.9)	1.3 (1–2.9)	1.1 (0.9–1.4)	0.071
IgG (g/dl)	1.8 (0.6–4.0)	1.9 (0.6–4.0)	1.7 (1.3–3.4)	0.754
IgG > ULN, *n* (%)	19 (65%)	13 (65%)	6 (66%)	1.00
ANA ≥ 1:20	24 (83%)	15 (75%)	9 (100%)	0.152
SMA ≥ 1:20	21 (72%)	14 (70%)	7 (78%)	1.00
LKM-1 ≥ 1:10 (or LC1 positive)	5 (17%)	5 (25%)	0	0.152
pANCA positive	11 (38%)	6 (30%)	5 (56%)	0.231
Associated IBD	7 (25%)	2 (10%)	5 (56%)	**0.016**

Bold values indicate that p value is statistically significant

AIH autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl-transpeptidase, *INR* international normalised ratio, *ALP* alkaline phosphatase, *ANA* anti-nuclear antibodies, *SMA* anti-smooth muscle antibodies, *LKM-1* anti-liver/kidney microsomes antibodies, *pANCA* perinuclear anti-neutrophil cytoplasmic, *IBD* inflammatory bowel disease

Table 3 Biliary abnormalities on histology of children with autoimmune liver disease and comparison between AIH and ASC patients

	All pts ($n=29$)	AIH ($n=20$)	ASC ($n=9$)	p value
1. Inflammation injury of bile duct, *n* (%)	29 (100%)	20(100%)	9 (100%)	ns
Focal	12	11	1	
Multifocal	16	9	7	
Diffuse	1	0	1	
Mean score of inflammatory injury (±SD)	1.62 (±0.56)	1.4 (±0.51)	2.0 (±0.58)	**0.011**
2. Ductular reaction, *n* (%)	29 (100%)	20 (100%)	9 (100%)	ns
Focal	7	6	1	
Multifocal	8	5	3	
Diffuse	14	9	5	
Mean score of ductular reaction (mean ± SD)	2.24 (±0.83)	2.1 (±0,87)	2.4 (±0,72)	ns
3. Periductular fibrosis, *n* (%)	15 (52%)	9 (45%)	6 (67%)	ns
Focal	11	9	2	
Multifocal	4	–	4	
Diffuse	–	–	–	
Mean score of periductular fibrosis (±SD)	0.62 (±0.72)	0.45 (±0.51)	1.11 (±0.92)	**0.019**
4. Biliary metaplasia, *n* (%)	21 (72%)	14 (70%)	7 (78%)	ns
Focal	16	10	6	
Multifocal	3	3	0	
Diffuse	2	1	1	
Mean score of biliary metaplasia (±SD)	0.96 (±0.82)	0.95 (±0.83)	1.1 (±0.86)	ns
5. Granulomatous cholangitis, *n* (%)	19 (65%)	12 (60%)	7(78%)	ns
Mean score of cholangitis	0.75(±0.57)	0,6 (±0,50)	1.0 (±0,50)	0.057
Grade of biliopathy, *n* (%)				
Mild (1 biliary feature)	29 (100%)	20 (100%)	9 (100%)	ns
Moderate (≥2 to 4 biliary features)	21	14	7	ns
Severe (all 5 biliary features)	9	6	3	ns
Final score of biliopathy (±SD)[a]	6.20 (±2.30)	5.60 (±2.28)	7.56 (±1.81)	**0.031**

Bold values indicate that p value is statistically significant

ns not statistically significant, *SD* standard deviation

[a]It includes the mean score from all 5 biliary features

difference between the 2 groups ($p>0.05$). The mean score of the granulomatous cholangitis tended to be significantly higher in ASC compared to AIH patients [1.0 (±0.50) vs 0.6 (±0.50), $p=0.057$].

The overall prevalence of biliopathy was moderate (≥2 to 4 biliary features) in 72% of patients and severe (all 5 biliary features) in 31% without differences between the two groups ($p>0.05$). However, the mean score of biliopathy was slightly higher among ASC patients [7.56 (±1.81) vs 5.60 (±2.28), $p<0.05$)] (Table 3).

Inflammation, fibrosis and cirrhosis

Stage of inflammation and necrosis was reported in a high percentage of cases without differences between AIH and ASC patients ($p>0.05$) (Table 4). Fibrosis was reported in 24 patients (82%) mainly as "moderate" form (15 patients, 63%). Cirrhosis was documented in 5 patients (17%) (Table 5).

Lymphocytes immunophenotyping

In the entire cohort of patients CD3 (marker of T lymphocytes), CD20 (marker of B lymphocytes) and CD79a (marker of plasma cells) were expressed in 70%, 9%, 21% of the portal tract infiltrate and 80%, 4%, 16% of the lobular infiltrate, respectively. The comparison of the two groups showed that patients with ASC had a significantly higher expression of CD20 in the portal tract than patients with AIH.

Outcome

All patients responded well to IS treatment. At the last follow-up (median of 4.2 years, range 2.6–9.9) all patients survived. Among AIH patients (follow up 4.5 years, range 2.6–9.9), all patients (100%) had normal transaminases, 18 (90%) had normal GGT, 10/13 (77%) normalized serum IgG, 12 (7 with AIH-1 and 5 with AIH-2, 60%) still had

Table 4 Stage of inflammation and necrosis in children with autoimmune liver disease according to Ishak scores

	All pts ($n=29$)	AIH ($n=20$)	ASC ($n=9$)	p value
1. Interface hepatitis, n (%)	28 (96%)	20 (100%)	8 (89%)	ns
Mild score (score 1)	10	8	2	
Moderate (score 2–3)	17	11	6	
Severe (score 4)	1	1	0	
Mean score ± SD	1.86 (±0.91)	1.85 (±0.87)	1.89 (±1.05)	ns
2. Confluent necrosis, n (%)	21 (72%)	14 (70%)	7 (78%)	ns
Focal (score 1)	15	11	4	
Multiple areas (score 2–5)	6	3	3	
Multiacinar necrosis (score 6)	–	–	–	
Mean score ± SD	0.96 (±0.77)	0.90 (±0.79)	1.11 (±0.78)	ns
3. Focal lytic necrosis/apoptosis, n (%)	28 (96%)	19 (95%)	9 (100%)	ns
One focus (score 1)	5	2	3	
More than one foci (score 2–3)	23	17	6	
More than 10 foci (score 4)	–	–	–	
Mean score ± SD	2.03 (±0.77)	1.95 (±0.68)	2.22 (±0.97)	ns
4. Portal inflammation, n (%)	29 (100%)	20 (100%)	9 (100%)	ns
Mild (score 1)	1	–	1	
Moderate (score 2–3)	22	17	5	
Severe (score 4)	6	3	3	
Mean score ± SD	2.72 (±0.84)	2.65 (±0.74)	2.89 (±1.05)	ns
Final mean score ± SD[a]	7.3 (±2.9)	7.3 (±2.6)	7.4 (±3.7)	ns

ns not statistically significant

[a]It includes the scores from all 4 necroinflammatory parameters

Table 5 Stage of fibrosis and cirrhosis according to Ishak scores

	All pts ($n=29$)	AIH ($n=20$)	ASC ($n=9$)	p value
Fibrosis	24 (82%)	16 (80%)	8 (89%)	1.00
Mild (score 1)	7	6	1	0.381
Moderate (score 2–3)	15	9	6	0.427
Severe (score 4)	2	1	1	1.00
Cirrhosis	5 (17%)	4 (20%)	1 (11%)	1.00
Incomplete (score 5)	3	2	1	1.00
Complete (score 6)	2	2	–	NA
Final mean score ± SD*	2.72 (±1.52)	2.80 (±1.67)	2.56 (±1.23)	0.698

*It includes the scores from the stage of fibrosis and cirrhosis

positive autoantibodies. Among ASC patients (median follow-up of 3.8 years, range 3.0–6.2), 7 patients (78%) had normal transaminase levels, 5 (55%) normal GGT, 3/6 normalised serum IgG, and 5 (55%) still had positive autoantibodies.

Fifteen patients (AIH = 10, ASC = 5, 52%) were on prednisone plus azathioprine, 7 (AIH = 5, ASC = 2, 24%) on prednisone alone, 3 (AIH = 1, ASC = 2, 10%) on prednisone plus MMF and 1 (with AIH) on prednisone plus tacrolimus. Medications were discontinued in 4 patients (3 with AIH-1 and in 1 with AIH-2) after liver biopsy which confirmed the absence of inflammation 3.8, 4.2, 4.8 and 6.5 years from the diagnosis, respectively. At the last follow-up, all three patients with AIH-1 are still on remission after 6, 11 and 52 months from stop treatment. Conversely, the patient with AIH-2 relapsed 1 year later and IS treatment was re-started.

Discussion

In this study, we report data on 29 children diagnosed with AILD according to IAIHG simplified criteria but with a lower threshold for autoantibody positivity, as recommended by IAIHG consensus in 2004 [12], and with the use of cholangiogram to distinguish patients with ASC from those with AIH. Indeed, when we retrospectively

applied to our cohort of patients a novel scoring system recently proposed by ESPGHAN society, which includes both a lower threshold for autoantibody positivity and the cholangiogram, 100% of patients fulfilled the diagnosis of pediatrics AILD [5].

Similarly, to previous studies, one-third of patients with a serological and histological pattern consistent with AIH-1 had an abnormal cholangiogram at diagnosis, and was classified as ASC [2]. Only a small proportion (17%) had the diagnosis of AIH-2 confirming that this phenotype of AIH represents a minority of all cases of pediatrics AILD [17–19]. Higher values of transaminases were reported among AIH patients and higher values of GGT and ALP/AST ratio in ASC group, suggesting a more severe hepatocellular cytolysis and a biochemical pattern of biliopathy in the two groups, respectively. No patient with ASC was LKM-1 positive, confirming the previously reported rare association between LKM-1 positivity and ASC [19–21]. Similarly, to other studies a higher prevalence of IBD was documented among ASC patients, ulcerative colitis being more common [19–21].

With regard to biliary features on biopsy at diagnosis, this is the first study providing details on the prevalence, the type and the grade of biliopathy on histology of children with AILD. At diagnosis, on histology all children had a various degree of biliopathy classified as moderate in the majority of patients and severe in one-third of cases, more prominent in ASC, but without clear-cut differences between AIH and ASC patients.

Similarly, Gregorio and co-authors reported histological biliary changes in 65% of ASC and 31% of AIH patients, but no details on type and grade of biliary abnormalities were reported in this study [2].

In our studied group, the inflammatory injury of the bile ducts was more severe among ASC patients compared to AIH and this may probably favor the cholangiocyte necrosis, and consequently, the higher values of GGT and ALP/AST ratio found in ASC patients compared to AIH group. Granulomatous cholangitis of the interlobular bile ducts constitutes the hallmark of PBC, but it is also found in livers with other longstanding biliary diseases, in particular PSC [22]. In this study it was found in 65% of patients suggesting its important role in pediatrics AILD as well. Periductal fibrosis is the hallmark for the diagnosis of PSC [22, 23]. We found a mild form of periductular fibrosis in some 50% of patients with AILD (both AIH and ASC) supporting the intriguing hypothesis that children with AILD might eventually switch from a parenchymal to a biliary disease over time. However, no patients had at the time of diagnosis the classical features of periductal concentric ("onion-skin") fibrosis, nor other histological features of PSC, confirming that a better understanding of the evolution from mild to severe form of periductal fibrosis may be achieved by prospective studies which include follow-up biopsies [22, 23].

Interestingly, prevalence and type of biliopathy on histology were similar between AIH and ASC patients. This is a crucial point since in pediatrics there are published studies in which the cholangiogram is not routinely included in the diagnostic work-up of AILD, and the diagnosis of ASC is based on liver histology alone [24, 25]. This may well lead to overdiagnosing ASC. Our results showed that AIH and ASC are not clearly distinguishable on histological ground, and strongly support the need to perform the cholangiogram to differentiate patients with ASC from those with AIH.

In previous reports, the relevant presence of biliary features on histology of these patients and the lack of standardised diagnostic criteria, has caused confusion and ambiguity, as far as, for instance, its significance for the diagnosis of "small duct PSC" or of "overlap syndrome" [4, 6–8].

In this study, the majority of patients (75%) had histological features usually detected in patients with PBC, PSC or other cholestatic disorders, and therefore, they may be classified as having an overlap syndrome. This high percentage mainly depends on the high rate of patients with ductular reaction which is a histological feature of PSC, as well as of other liver disorders [4–8]. In adults, several overlap syndromes are recognised, but, in the absence of international criteria, the IAIHG discouraged to consider overlap syndromes as separate entities [6]. To support this recommendation stands the common finding of bile duct injury associated with AIH described previously, showing its prevalence 30–50% in chronic active hepatitis [26–28].

Interestingly, Verdonk and co-authors recently reported on bile duct injury and ductular reaction in biopsies of 35 adult patients with AIH. The authors showed that, using cytokeratin-7 stain, bile duct injury and ductular reaction were present in 83% and 94% of cases, respectively, similar to our study [7]. The authors concluded that these findings do not necessarily support the diagnosis of an overlap syndrome [7]. Previous studies reported a lower rate of biliary features in patients with AIH likely because of the different preparation of the histology slides for review. In fact, in both Verdonk's and our study, biliary abnormalities were scored on cytokeratin-7 stained slices, whereas the previous reports were evaluated on haematoxylin and eosin stain alone [27]. Cytokeratin-7 staining allows to evaluate some biliary features which are not assessable on hematoxylin–eosin alone, including mild ductular reaction as well as biliary metaplasia; thus it should always be included in the histological work-up of children with AILD.

Our findings, along with those described in adults by Verdonk, suggest that biliary features, rather than being atypical, are part of the histology pattern most commonly seen in AIH.

In pediatrics this represents a novelty in the field, and suggests the need of prospective studies to better stage the biliopathy at diagnosis, assess whether biliary changes may response to IS treatment, and analyse the impact of biliopathy on long-term outcome of children with AILD.

In recent studies some authors proposed a new histological scoring system for the diagnosis of AIH in adult patients. Similar to our results, in Gurung et al study the authors reported features of bile duct injury in the majority of cases (60%) [29]; conversely, in Balitzer et al study, CK7 staining was reported as positive in a small proportion of patients (20%) [30]. These differences suggest the need to use a standardized scoring system to assess the prevalence and type of biliopathy in the adult population as well.

The involvement of the biliary tract may represent a collateral damage to the cholangiocytes caused by an inflammatory process that in AIH is mainly targeted to the hepatocytes [31]. Some recent evidence points at ductular reaction as a marker of progenitor cell derived liver regeneration that follows hepatic injury of any sort. Therefore, it seems that biliary features in AIH may well be the result of a regenerative response driven by the necroinflammatory activity [32, 33].

Children with AIH and ASC did not significantly differ in terms of stage of inflammation, fibrosis, and necrosis although patients with ASC tended to have a higher percentage of $CD20^+$ cells in the portal tract. Remarkably this finding may be in agreement with a study reporting a good response to B-cell depletion in a murine model of autoimmune hepatitis, suggesting that B cells play an active role in the pathogenesis of the disease, despite its classification as a T cell-mediated autoimmune process [34].

In children with AILD a histological diagnosis of cirrhosis has been reported between 11% and 68% of cases [2, 20]. Our prevalence of 17% is in keeping with other pediatrics series and suggests a late diagnosis in a significant proportion of cases [2, 35].

Overall, the outcome in our cohort is excellent, with 100% of patients alive at last follow up. Medications were discontinued in a small proportion of cases (4 patients, 14%) although 1 girl with AIH-2 relapsed 1 year after stop treatment, confirming that this condition is usually steroid-dependent and regarded to require treatment for life [16].

In conclusion, this is the first pediatrics study reporting prevalence, type and grade of biliopathy in children with AILD. Our study shows that children with AIH and ASC present biliary features on histology in a high percentage of cases. Despite minor differences, both conditions are not clearly distinguishable on histological ground, making the cholangiogram the only tool to differentiate AIH and ASC in children. Cytokeratin-7 stain should be always included in the histological work-up of children with AILD. Further studies on larger groups of patients and with longer follow-up are required to better understand the determinants and the progression of biliary involvement in pediatrics AILD.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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