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Original article

Prevalence and significance of antimitochondrial antibodies in autoimmune hepatitis (AIH): Results from a large multicentre study of the International AIH Group

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ABSTRACT

Background & Aims: Antimitochondrial antibodies (AMA) are specific markers for the diagnosis of primary biliary cholangitis (PBC) but can also be found occasionally in patients with autoimmune hepatitis (AIH). The present large multicentre cohort study assessed the prevalence and significance of AMA in AIH-patients.
Methods: 123 AMA-positive AIH-patients were investigated and compared with 711 age-matched AMA-negative AIH-patients and 69 patients with AIH/PBC variant.

Abbreviations: AIH, Autoimmune hepatitis; AMA, Antimitochondrial antibodies; PBC, Primary biliary cholangitis; Primary sclerosing cholangitis, (PSC); UDCA, Ursodeoxycholic acid; IAIHG, International autoimmune hepatitis Group; IQR, (Interquartile range); AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; γ-GT, γ-glutamyl transpeptidase; ALP, Alkaline phosphatase; INR, International normalized ratio; IgG, Immunoglobulin class G; IgM, Immunoglobulin class M; ULN, Upper limit of normal; LLN, Lower limit of normal; IIF, Indirect immunofluorescence; WB, Western immunoblotting; SMA, Smooth muscle antibodies; anti-LKM, Anti-liver/kidney microsome antibodies; anti-LC1, Anti-liver cytosol type-1 antibodies; anti-SLA/LP, Antibodies against soluble liver antigen/liver pancreas; ANA, Antinuclear antibodies; CBR, Complete biochemical response; NR, No response; OR, Odds ratio; CI, Confidence interval; HR, Hazard ratio; RLM, Rim like membranous; MND, Multiple nuclear dots.

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Antimitochondrial antibodies
Outcome

Results: AMA prevalence in AIH-patients was 5.1% (range: 1.2%-11.8%). AMA-positivity was associated with female sex ($p = 0.031$) in AMA-positive AIH-patients but not with liver biochemistry, bile duct injury on liver biopsy, disease severity at baseline and response to treatment compared to AMA-negative AIH-patients. Comparing AMA-positive AIH-patients to those with AIH/PBC variant, there was no difference in disease severity. Regarding liver histology, AIH/PBC variant patients were characterized by the presence of at least one feature of bile duct damage ($p<0.001$). Response to immunosuppressive treatment was similar among groups. From AMA-positive AIH patients only those with evidence of non-specific bile duct injury had higher risk to progress to cirrhosis (HR=4.314, 95%CI: 2.348–7.928; $p<0.001$). During follow-up, AMA-positive AIH-patients had higher risk to develop histological bile duct injury (HR 4.654, 95%CI 1.829–11.840; $p = 0.001$).
Conclusions: AMA presence is relatively common among AIH-patients, but their clinical significance seems important only when they co-exist with non-specific bile duct injury at the histological level. Therefore, a careful evaluation of liver biopsy seems of utmost importance in these patients.

1. Introduction

Autoimmune hepatitis (AIH) is a chronic progressive liver disease leading to end-stage liver disease and/or death, if left untreated [1–3]. Within the spectrum of AIH, some patients exhibiting characteristics of primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) are classified as variant forms of AIH [1–4]. The prevalence of AIH/PBC variant is generally considered to be about 10% of adult patients with AIH or PBC [4,5]. The ``Paris criteria'' are the most widely used diagnostic tools for the definition of AIH/PBC variant [6]. Once PBC is diagnosed, this variant should always be considered, if particularly there is poor response to ursodeoxycholic acid (UDCA) monotherapy [1–5]. The presence of both diseases' characteristics at the same time is the most common presentation, but it should be emphasized that the onset of AIH and PBC might be temporally separated, with PBC frequently appearing earlier [4–6].

Antimitochondrial antibodies (AMA) constitute the serological hallmark of PBC which are detected in 90–95% of PBC patients when sensitive diagnostic assays are used [4,7]. AMA are so strongly associated with PBC diagnosis, that in the revised score for AIH diagnosis published by the International Autoimmune Hepatitis Group (IAIHG), four points are subtracted towards the final score if AMA are present [8]. However, AMA have been occasionally found in patients with other liver diseases than PBC, although the relevance of this finding is currently unknown. In this context, AMA detection has been reported in about 5–10% of AIH-patients (up to 35% in some Japanese studies), depending mainly on the method employed in each study, with a higher prevalence seen when more sensitive methods like indirect immunofluorescence (IIF) or immunoblotting were used [9–13]. Regarding their significance in AIH, most of the studies indicate that AMA simply represent a bystander phenomenon without any obvious clinical significance. However, the limitations of short follow-up, infrequent sequential histologic examination and the small numbers of patients included may have led to the abovementioned inconclusive results [9–13].

In this retrospective multicentre study, we aimed to investigate the AMA prevalence in a large cohort of AIH-patients at presentation and during long-term follow-up as well as to assess their significance in terms of baseline clinical, biochemical, and histological characteristics, treatment response and long-term outcome as similar data in adequate number of AIH-patients with long-term follow-up is missing. Furthermore, we compared AMA-positive AIH-patients to a well-defined group of patients with AIH/PBC variant syndrome.

2. Patients and methods

This is a large retrospective multicentre study of prospectively collected data in all AMA-positive AIH-patients followed in 12 centres of the IAIHG. All patients had an established diagnosis of AIH according to the simplified criteria of the IAIHG [14]. As a result, other causes of liver diseases such as viral, metabolic, genetic, and toxic, including alcohol abuse, were excluded in all patients by appropriate investigation. Particularly according to guidelines, all acute AIH cases tested negative apart from hepatitis B and C viral markers, also for IgM antibodies against hepatitis A and E viruses. AIH patients with concomitant viral, PSC, drug-induced liver injury, metabolic fatty liver disease or alcoholic liver disease were excluded from the analysis. The records of patients were reviewed and the clinical, biochemical, serological, and histological characteristics at diagnosis and during follow-up were collected. In total, 123 AIH-patients positive for AMA at diagnosis were evaluated. None of these patients fulfilled the ``Paris criteria'' [6] for a definite diagnosis of AIH/PBC variant syndrome. Seven hundred and eleven age-matched AIH-patients negative for AMA at baseline were used as the disease comparator group of the study (approximately 6:1 ratio to the AMA-positive AIH group). Additionally, the AMA-positive AIH-patients ($n = 123$) were compared to a well-defined population of patients with AIH/PBC variant syndrome ($n = 69$) according to the ``Paris criteria'' [6]. The median (interquartile range, IQR) of follow-up in the total group of patients ($n = 903$) was 6.4 (9.1) years [6.4 (8.8) in AMA-positive, 6.6 (9.3) in AMA-negative and 5.3 (5.9) years in AIH/PBC variant; $p = 0.525$].

2.1. Clinical assessments

Follow-up was performed by periodic evaluation every 3–6 months, according to the clinical practice of each center. Each patient underwent a comprehensive clinical review and complete physical examination at presentation and at every follow-up visit. During follow up, the severity of liver disease (presence of cirrhosis, hepatic decompensation, and development of hepatocellular carcinoma) was assessed by clinical examination and/or imaging studies and/or liver histology, according to the clinical practice of each expertise center. According to our previous publications [15–17], the diagnosis of cirrhosis in patients without available liver biopsy was based on ultrasonography (coarse echo pattern of the liver parenchyma along with irregular hepatic margins, splenomegaly, portal vein diameter>16 mm), endoscopic evidence of cirrhosis (varices, portal gastropathy), and/or clinical findings of decompensation (ascites, variceal bleeding, encephalopathy).

2.2. Biochemical data and immunoglobulins

Conventional laboratory tests were collected from medical records. At the time of initial presentation, the laboratory data that were analyzed were aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), bilirubin, albumin, international normalized ratio (INR), platelets, immunoglobulin G (IgG) and immunoglobulin M (IgM). Laboratory values were expressed as times the upper limit of normal (ULN), except for albumin which was expressed as times the lower limit of normal (LLN).

2.3. Autoantibodies serology

AMA were determined according to practice guidelines by standard methods such as IIF on rodent liver-kidney-stomach substrate and/or specific ELISAs against the known target-autoantigens of AMA or Western immunoblotting (WB) [4,7,18]. The same standard assays were used for the detection of smooth muscle antibodies (SMA), anti-liver/kidney microsome antibodies (anti-LKM) and anti-liver cytosol type-1 antibodies (anti-LC1) [1-3,18]. Antibodies against soluble liver antigen/liver pancreas (anti-SLA/LP) were also detected by ELISAs or WB as described [18–20]. Antinuclear antibodies (ANA) were detected by IIF on HEp2 or rodent liver-kidney-stomach substrates. PBC-specific ANA, such as anti-gp210 and anti-sp100 antibodies were also determined by ELISAs or WB as described [4,7,18,21,22]. Patients positive with at least one method for each autoantibody were considered positive for the respective autoantibody.

2.4. Histological evaluation

Available liver biopsies at baseline (720/834, 86.3%) were evaluated locally at each expertise center by experienced liver pathologists. According to previous publications [23–25], the presence of no more than one or two of the following lesions, such as florid duct injury, ductopenia, ductular reaction/proliferation, cholestasis or granuloma was considered as incidental features of non-specific bile duct injury. In addition, sequential liver biopsies were available during follow-up in 217 AIH-patients.

2.5. Treatment regimens

Patients were treated according to current guidelines with corticosteroids alone or in combination with azathioprine or mycophenolate mofetil [1-3,17,26,27].

Treatment endpoints were defined according to the EASL guidelines, and the recent new definitions of response criteria and endpoints proposed by the IAIHG (Supplementary Table 1) [1,28]. Treatment response was considered as complete biochemical response (CBR) when transaminases and IgG have normalized in less than 6-months after treatment initiation. However, because of the retrospective kind of the present study and to homogenize this endpoint among centres, CBR was assessed only at last follow-up. Insufficient response was defined as lack of CBR. No response (NR) was defined as the absence of less than 50% decrease of serum transaminases from baseline values after 4 weeks of immunosuppression despite intensive immunosuppression and confirmation of adherence to therapy (Supplementary Table 1). Treatment was withdrawn in accordance with EASL recommendations when immunosuppression had been administered for at least 3 years and at least 2 years following CBR [1]. Apart from immunosuppressive treatment, 43/123 (35%) AMA-positive AIH and 39/69 (56.5%) AIH/PBC patients received treatment with UDCA for at least 12 months at a dose of 13–15 mg/kg/day at the discretion of each center.

The study was conducted in accordance with the protocol and principles of the 1975 Declaration of Helsinki. The protocol was approved by the Institutional Research Board of the coordinating center, and at each participating center, in accordance with local regulations. All patients agreed to the use of their data after anonymous analysis by written consent at the time of initial evaluation.

2.6. Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. Quantitative values are expressed as median (IQR). Data were analyzed by x^2 test (two by two with Yate's correction), Fisher's exact test, Mann-Whitney *U test*, Kruskal-Wallis test (with Dunn's multiple comparisons test), multivariate logistic regression, and Cox-regression analyses where applicable. Two-sided *p*-values <0.05 were considered statistically significant.

3. Results

3.1. AMA prevalence in AIH-patients

To evaluate the prevalence of AMA in AIH-patients, each center provided the total number of AIH-patients that have been followed at each center at the time of data collection. All patients were tested locally in their respective expertise center for the presence of AMA using at least one well-established method [4,7]. A total of 2436 AIH-patients were followed across all 12 centres of the study, with 123 of them being AMA-positive at baseline (mean prevalence: 5.1%; range: 1.2% to 11.8%; Supplementary Table 2). Serial samples for sequential determinations of AMA were available in 104/123 AMA-positive AIH patients [89/104 (85.6%) remained consistently AMA-positive during follow up].

3.2. Baseline characteristics of AMA-positive AIH-patients

Baseline characteristics of patients are shown in Table 1. Comparison between AMA-positive ($n = 123$) and AMA-negative AIH-patients ($n = 711$) showed that female sex ($p = 0.031$) was more frequent in AMA-positive AIH-patients (Table 1). AMA-positive patients were characterized by increased levels of IgM ($p<0.001$) and higher rates of ANA with multiple nuclear dots pattern ($p = 0.028$), anti-sp100 ($p = 0.002$) and anti-gp210 ($p<0.001$), whereas AMA-negative patients had higher rates of SMA positivity ($p<0.001$; Table 1). In terms of liver histology, no statistically significant difference was found between AMA-positive and AMA-negative AIH-patients regarding the presence of non-specific bile duct injury. Finally, there was no difference in disease severity (i.e. presence of cirrhosis, decompensation, or development of hepatocellular carcinoma) between AMA-positive and AMA-negative AIH-patients at the time of initial presentation (Table 1).

Comparison between AMA-positive AIH-patients ($n = 123$) and AIH/PBC variant patients ($n = 69$) showed no difference regarding the liver autoimmune serology and disease severity. Patients with AIH/PBC variant had higher levels of AST ($p = 0.045$), ALT ($p = 0.037$), γ-GT ($p<0.001$), ALP ($p<0.001$), and IgG ($p<0.001$) per the definition of the Paris criteria.

3.3. Response to treatment

Data on response to immunosuppression after at least 6 months of treatment was available in 697 patients (91 AMA-positive AIH, 552 AMA-negative AIH, and 54 AIH/PBC variant).

There was no difference in response to treatment for the management of AIH, in terms of the on treatment CBR, rates of corticosteroids withdrawal, the rapidity of corticosteroids cessation and the maintenance of CBR after treatment withdrawal between AMA-positive and AMA-negative AIH-patients (Table 2).

Moreover, there was no effect of the presence of AMA on treatment response (CBR vs. insufficient response and NR) [odds ratio (OR)= 0.724, 95% confidence interval (CI): 0.415–1.264; $P = 0.256$], withdrawal of corticosteroids (OR=0.734, 95%CI: 0.448–1.204; $P = 0.221$) and maintenance of CBR after immunosuppressive treatment cessation (OR=0.407, 95%CI: 0.156–1.059; $P = 0.065$), after adjustment for the presence of non-specific bile duct injury and female sex (Supplementary Table 3).

In addition, no difference was found between AMA-positive AIH-patients and patients with AIH/PBC variant in terms of response to immunosuppressive treatment, although the latter group was characterized by earlier discontinuation of corticosteroids (Table 2).

Among the 697 patients with available data on treatment response after at least 6 months of immunosuppression, 34/91 (37.4%) AMA-positive and 31/54 (57.4%) AIH/PBC patients also received UDCA at

Table 1
Baseline characteristics of AIH-patients according to AMA positivity and AIH/PBC variant.

	N[†]	AMA-positive AIH ($n = 123$)	AMA-negative AIH ($n = 711$)	AIH/PBC variant ($n = 69$)	*P*-value[*]	*P*-value[**]
Females	903	110 (89.4%)	575 (80.9%)	59 (85.5%)	**0.031**	0.567
Age (years)	903	48 (20)	50 (29)	50 (17)	0.719	0.368
Follow-up	874	6.4 (8.7)	6.6 (9.3)	5.3 (5.8)	0.878	0.377
Baseline laboratory parameters						
AST (x ULN)	826	5.8 (15.9)	7 (17)	11.8 (17.3)	0.520	**0.047**
ALT (x ULN)	839	5.5 (16.6)	8.1 (15.3)	9.8 (15.6)	0.147	**0.037**
γ-GT (x ULN)	767	2.5 (2.8)	2.5 (4.3)	6.3 (5.7)	0.116	**<0.001**
ALP (x ULN)	814	1.3 (0.8)	1.2 (0.9)	2 (2.2)	0.685	**<0.001**
Bilirubin (x ULN)	817	1.3 (4.7)	1.4 (4.4)	1.6 (4.2)	0.724	0.467
Albumin (x LLN)	763	1.07 (0.33)	1.03 (0.29)	1.06 (0.31)	0.753	0.938
INR (x ULN)	760	0.97 (0.25)	0.94 (0.27)	0.96 (0.23)	0.859	0.937
Platelets (x LLN)	803	1.29 (1.04)	1.35 (0.76)	1.26 (0.64)	0.836	0.787
IgG (x ULN)	799	1.27 (0.51)	1.32 (0.80)	1.59 (0.78)	0.342	**<0.001**
IgM (x ULN)	721	0.9 (0.84)	0.69 (0.6)	1.04 (1.29)	**<0.001**	0.085
Baseline autoantibodies profile						
ANA positivity with any method	890	88 (72.1%)	535 (76.2%)	44 (66.7%)	0.393	0.539
ANA with RLM pattern	385	6 (12%)	33 (10.4%)	1 (5.9%)	0.921	0.699
ANA with MND pattern	385	14 (28%)	46 (14.5%)	5 (29.4%)	**0.028**	1.000
anti-sp100 positivity	271	8 (20.5%)	10 (4.6%)	1 (6.3%)	**0.002**	0.258
anti-gp210 positivity	263	7 (18.9%)	3 (1.4%)	1 (6.7%)	**<0.001**	0.412
SMA positivity	775	57 (52.8%)	447 (73.8%)	51 (83.6%)	**<0.001**	<0.001
anti-LKM positivity	609	1 (1.2%)	25 (5.1%)	0 (0%)	0.155	1.000
anti-SLA/LP positivity	470	5 (6.7%)	49 (13.2%)	2 (8.7%)	0.167	0.665
anti-LC1 positivity	414	0 (0%)	7 (2.2%)	0 (0%)	0.358	NA
Family history of autoimmune diseases	681	2/78 (2.6%)	15/567 (2.6%)	3/36 (8.3%)	1.000	0.323
Bile duct injury	785	39/105 (37.1%)	168/615 (27.3%)	47/65 (72.3%)	0.052	<0.001
Severity of liver disease						
Cirrhosis	903	33 (26.8%)	179 (25.2%)	17 (24.6%)	0.782	0.872
Decompensated cirrhosis	229	10 (30.3%)	64 (35.8%)	3 (17.6%)	0.686	0.499
Hepatocellular carcinoma	903	0 (0%)	1 (0.1%)	0 (0%)	1.000	NA
UDCA treatment more than 12 months	903	43 (35%)	NA	39 (56.5%)	NA	0.006

AMA, antimitochondrial antibodies; PBC, primary biliary cholangitis; AST, aspartate aminotransferase; ULN: upper limit of normal; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LLN, lower limit of normal; INR, international normalized ratio; IgG, immunoglobulin class G; IgM, immunoglobulin class M; ANA, antinuclear antibodies; RLM, rim like membranous; MND, multiple nuclear dots; SMA, smooth muscle antibodies; anti-LKM, anti-liver/kidney microsome, anti-SLA/LP, anti-sol`uble liver antigen/liver pancreas; anti-LC1, anti-liver cytosol type-1. NA, non-applicable.

[†] Patients with available data.

[*] *P* value is derived from the comparison of AMA-positive to AMA-negative AIH-patients.

[**] *P* value is derived from the comparison of AMA-positive AIH-patients to AIH/PBC variant patients. Numerical values are present as median (interquartile range).

Table 2
Response to treatment according to AMA presence.

	N[*]	AMA-positive AIH	AMA-negative AIH	AIH/PBC variant	*P*-value[*]	*P*-value[**]
On treatment response						
Complete biochemical response (CBR)	697	71/91 (78%)	445/552 (80.6%)	44/54 (81.5%)	0.847	0.835
Insufficient response	697	17/91 (18.7%)	91/552 (16.5%)	8/54 (14.8%)		
No-response	697	3 /91 (3.3%)	16/552 (2.9%)	2/54 (3.7%)		
Withdrawal of corticosteroids	666	31/91 (34.1%)	220/523 (42.1%)	24/52 (46.2%)	0.188	0.211
Time to corticosteroids withdrawal (years)	272	3.4 (5.9)	2.9 (6.1)	1.4 (3.3)	0.598	**0.014**
Maintenance of CBR after stopping treatment	175	11/23 (47.8%)	92/144 (63.9%)	6/8 (75%)	0.215	0.240

AMA, antimitochondrial antibodies; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; CBR, complete biochemical response. [†]Patients with available data and treatment duration more than 6 months.

[*] *P* value is derived from the comparison of AMA-positive to AMA-negative AIH-patients.

[**] *P* value is derived from the comparison of AMA-positive AIH-patients to AIH/PBC variant patients.

Table 3
Impact of UDCA on response to immunosuppression treatment in AMA-positive AIH and AIH/PBC patients.

	N*	AMA-positive AIH		*P*-value	AIH/PBC variant		*P*-value
		UDCA	no-UDCA		UDCA	no-UDCA	
On treatment response							
Complete biochemical response (CBR)	145	23/34 (67.6%)	48/57 (84.2%)	0.128	25/31 (80.6%)	19/23 (82.6%)	0.934
Insufficient response	145	10/34 (29.4%)	7/57 (12.3%)		5/31 (16.1%)	3/23 (13%)	
No-response	145	1/34 (2.9%)	2/57 (3.5%)		1/31 (3.2%)	1/23 (4.3%)	
Withdrawal of corticosteroids	143	13/34 (38.2%)	18/57 (31.6%)	0.675	14/29 (48.3%)	10/23 (43.5%)	0.948
Time to corticosteroids withdrawal (years)	54	3.2 (4.6)	4.1 (7.4)	0.621	2 (3.5)	0.8 (2.4)	0.371
Maintenance of CBR after stopping treatment	31	3/8 (37.5%)	8/15 (53.3%)	0.667	5/5 (100%)	1/3 (33.3%)	0.107

UDCA, ursodeoxycholic acid; AMA, antimitochondrial antibodies; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; CBR, complete biochemical response.

a dose 13–15 mg/kg/day for at least 12 months. The median (IQR) duration of UDCA was 84.5 (126) months for AMA-positive and 58 (59) months for AIH/PBC patients. Separate sub-analysis in each group revealed that UDCA treatment was not associated with the on-treatment response to immunosuppression both in AMA-positive AIH ($P = 0.128$) and in AIH/PBC variant patents ($P = 0.934$) (Table 3). Similarly, UDCA did not affect withdrawal of corticosteroids ($P = 0.675$ and $P = 0.948$), time to corticosteroids withdrawal ($P = 0.621$ and $P = 0.371$) and maintenance of CBR after stopping treatment ($P = 0.667$ and $P = 0.107$) in both groups respectively (Table 3).

3.4. Outcome of patients

From 834 AIH-patients, 212 (25.4%) had cirrhosis at baseline. Ninety-two of the remaining 622 patients (14.8%) developed cirrhosis during follow-up. Multivariate Cox-regression analysis revealed that progression to cirrhosis was associated with AMA positivity ($p = 0.02$) and the presence of non-specific bile duct injury ($p<0.001$) (Table 4). From the 304 cirrhotic patients (212 at baseline and 92 who developed cirrhosis during follow-up), 100 (32.9%) developed at least one episode of decompensation during follow-up, although no association was found with the AMA status and the presence of bile duct injury at baseline liver biopsy. During follow-up, 95 out of 834 patients (11.4%) died due to liver-related cause or had undergone liver transplantation. There was no effect of the presence of AMA or non-specific bile duct injury at the histological level on patients' survival (Table 4). AMA positivity and presence of non-specific bile duct injury retained their negative impact regarding the progression to cirrhosis even after adjustment according to decade of diagnosis ($p = 0.018$ and $p<0.001$, respectively). A separate multivariate sub-analysis in AMA-positive AIH patients revealed that progression to cirrhosis was associated only with the presence of bile duct injury only in AMA-positive patients (HR=2.98, 95%CI: 1.09–8.1; $p = 0.033$) and was independent of UDCA administration (HR=2, 95% CI: 0.74–5.41; $P = 0.173$) (Supplementary Table 4).

Comparison of the 3 groups (AMA-positive AIH-patients, AMA-negative AIH-patients and AIH/PBC variant patients) after adjustment for sex revealed that AMA-positive AIH-patients had a higher risk of progression to cirrhosis compared to AMA-negative AIH patients [hazard ratio (HR)=2.074, 95%CI: 1.272–3.383; $p = 0.003$; Fig. 1a], while no difference was identified between them and patient with AIH/PBC variant (HR=1.134, 95%CI: 0.521–2.468; $P = 0.752$; Fig. 1a). Sub-analysis of AMA-positive patients with available liver biopsy revealed that only those with non-specific bile duct injury on histology were more prone to progress to cirrhosis (HR=4.314, 95%CI: 2.348–7.928; $p<0.001$), while no difference was found for AMA-positive patients without histological evidence of bile duct damage (HR=1.101, 95%CI: 0.0.504–2.405; $p = 0.809$) when compared to AMA-negative AIH-

Table 4
Outcome of patients according to AMA positivity and the presence of non-specific bile duct injury.

	HR	95% CI	*P*-value
Progression to cirrhosis during follow-up			
AMA positivity	1.83	1.10–3.05	**0.020**
Presence of bile duct injury	2.41	1.56–3.74	**<0.001**
Progression to decompensation			
AMA positivity	1.26	0.72–2.19	0.415
Presence of bile duct injury	1.44	0.91–2.28	0.124
Liver transplantation or Liver Related Death			
AMA positivity	1.57	0.90–2.75	0.110
Presence of bile duct injury	1.29	0.80–2.07	0.296

AMA, antimitochondrial antibodies; HR, hazard ratio; CI: confidence interval. AMA positivity and presence of non-specific bile duct injury retained their negative impact regarding the progression to cirrhosis even after adjustment according to decade of diagnosis (HR=1.85, 95%CI: 1.11–3.08; $P = 0.018$ and HR=2.41, 95%CI: 1.56–3.74; $P<0.001$, respectively).

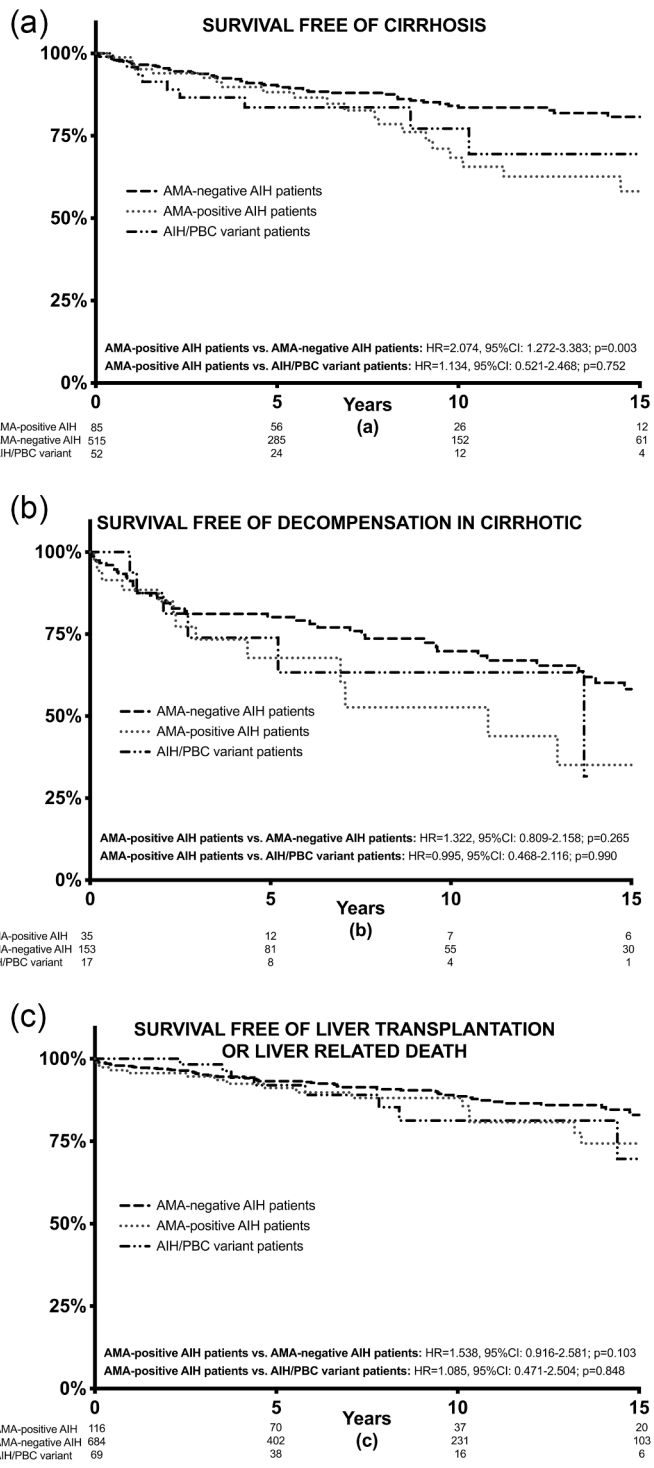

Fig. 1. Outcome of AMA-positive AIH, AMA-negative AIH and AIH/PBC variant patients. (a) AMA-positive had a higher risk of progression to cirrhosis compared to AMA-negative AIH-patients. (b) No difference was found among the 3 groups, regarding the development of decompensation of cirrhosis and (c) concerning the survival free of liver-related death or liver transplantation. Hazard ratios (HR) were determined after adjustment for sex. AIH, autoimmune hepatitis; AMA, antimitochondrial antibodies; PBC, primary biliary cholangitis.

patients.

In addition, there was no difference among the 3 groups, regarding the development of decompensation of liver disease (Fig. 1b) and survival free of liver-related death or liver transplantation (Fig. 1c).

3.5. Histological characteristics of bile duct injury in patients with available serial liver biopsies

Sequential liver biopsies were available in 217/834 AIH-patients (AMA-positive, $n = 36$; AMA-negative, $n = 181$; presence or absence of non-specific bile duct injury at baseline biopsy, $n = 69$ and $n = 148$, respectively). The median (IQR) duration between the baseline and the last liver biopsy was 5.8 (7.7) years.

Twenty-four out of these 217 (11.1%) patients developed new lesions of bile duct damage, 46/217 (21.2%) resolved their lesions, 23/217 (10.6%) had persistent lesions, while 124/217 (57.1%) patients were consistently free of histological features of bile duct damage. AMA-positive patients were more likely to develop bile duct injury throughout follow-up (HR=4.654, 95%CI: 1.829–11.840; $P = 0.001$, Fig. 2a), whereas the resolution of bile duct injury at baseline was not associated with the presence of AMA (HR=0.708, 95%CI: 0.323–1.554, $P = 0.389$, Fig. 2b).

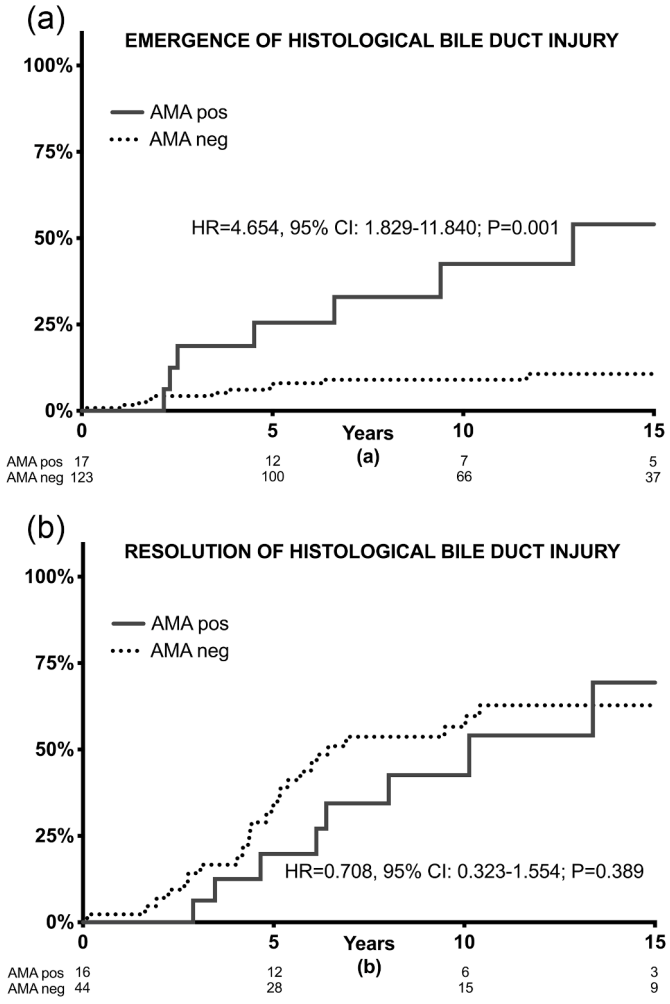

Fig. 2. Alterations of histological characteristics of PBC in AIH patients with available sequential liver biopsies. (a) AMA-positive patients were more likely to develop histological characteristics of PBC. (b) Resolution of histological characteristics of PBC was not associated with the presence of AMA. PBC, primary biliary cholangitis; AMA, antimitochondrial antibodies; HR, hazard ratio; CI: confidence interval.

4. Discussion

To the best of our knowledge, this is the largest study ever conducted on the clinical importance of AMA positivity in patients with well-established AIH, from 12 centres across 8 different countries. We showed that AMA prevalence is relatively common among AIH-patients, but their clinical significance seems important only when they co-exist at baseline biopsy with some unspecific lesions of the bile ducts. Previous studies in relatively small number of AIH-patients with short follow-up have reported AMA detection as a common event [9–13]. Czaja et al. found a prevalence up to 8% by using ELISAs for the detection of autoantibodies [29]. When more sensitive techniques were used, AMA positivity increased up to 12% via IIF [10] and up to 34% in Japan using immunoblotting with recombinant mitochondrial autoantigens [9]. Our multicentre study confirmed these previous findings, revealing an average AMA prevalence of 5% (range: 1.2–11.8%) across different centres. This wide range suggests probably that there are differences in the procedures utilized by different centres.

Regarding the clinical significance of AMA positivity in AIH-patients at baseline characteristics, our findings showed no association with disease severity and biochemical markers of cholestasis. In line with our findings, other studies have found no significant variations in many parameters, such as liver enzymes and autoantibody profiles between AMA-positive and AMA-negative AIH-patients, concluding that the clinical characteristics are unrelated to the AMA status of the patients [9,11,12].

At the histological level, several studies have shown so far that biliary changes can occur frequently even in typical AIH cases, mainly as a coincidental finding associated with classic AIH or an incomplete manifestation of a variant syndrome [23–25]. Similarly, we showed that almost one-third of our patients with AIH had at least one feature of non-specific bile duct injury at baseline liver biopsy, irrespectively of the presence of AMA.

Of note, the presence of AMA did not affect the response to immunosuppressive treatment after adjustment for the presence of non-specific bile duct injury and sex in terms of on treatment CBR, the achievement of permanent withdrawal of corticosteroids, the rapidity of corticosteroids cessation, and the maintenance of CBR after complete treatment withdrawal. These findings support previous research that found a comparable response to immunosuppression in AIH-patients irrespective of AMA positivity [6,9,11,30] or the presence of concurrent bile duct changes [23,24,30]. However, AMA-positive patients seem to progress to more severe disease compared to AMA-negative AIH-patients even though, this was mainly observed only among those with evidence of non-specific bile duct lesions at baseline liver biopsy.

AMA are very specific for PBC diagnosis and often predict the onset of liver damage by several years, even in subjects who are asymptomatic and have no other signs or symptoms of chronic liver disease [31–33]. Indeed, Dahlqvist et al. showed that about 16% of AMA positive individuals with non-established PBC at the time of AMA detection developed PBC during a course of 5-years follow-up period [33]. This observation along with a recent Chinese study [31], is expanded to the AIH-patients in our study. Specifically, in patients with available serial liver biopsies during follow-up, we found that a small proportion of AIH-patients (11.1%) eventually developed histological features of bile duct injury, with AMA-positive having a fourfold risk compared to AMA-negative AIH-patients. In a published clinic experience of long-term follow-up evaluation of 15 AMA-positive AIH-patients, O'Brien et al. reported that these patients had no clinical or histological evidence of PBC in up to 27 years of follow-up [10]. However, Dinani et al. reported one patient with ongoing review from this series along with two similar cases with strong histologic and serologic evidence of the development of PBC after longer follow-up [13].

The inclusion of the largest cohort of 123 AMA-positive AIH-patients ever reported in comparison to 711 age-matched AMA-negative AIH-patients from various geographic areas is a major strength of our

study. There are, however, some inherent limitations in our study because of its large-scale design. These limitations can be summarised as a limited set of available laboratory data, different methods for determining AMA (IIF, ELISA, WB) among centres and different time periods, different treatment and follow-up strategies among centres, retrospective design resulting in not assessment of CBR on month 6 after initiation of induction treatment as defined recently by the IAIHG [28], the absence of sex matching, and the evaluation of liver biopsies locally and not centrally.

Nevertheless, we believe that the reliability and generalizability of our findings are enhanced by the long-term follow-up and management of patients from expertise and experienced tertiary centres with proven expertise in the diagnosis and management of autoimmune liver diseases and liver autoimmune serology investigation. The strategy of prospective data collection, and the collection of many clinical events are additional strengths of the study, while response to treatment and outcome was not affected by gender, era of diagnosis and UDCA usage. Although, the presence of cirrhosis was not evaluated by a liver biopsy in all patients, the vast majority of them (86.3%) had an available liver biopsy at baseline, while a considerable number (26%) had also available sequential liver biopsies. Furthermore, our patients were followed on a regular (at least biannually) basis by specialized centers, allowing for the early detection of cirrhosis and decompensating events by using several non-invasive methods. Besides, multivariate models and subanalyses were performed only in patients who had available liver biopsies. Future prospective studies are required to validate our findings, using standardised treatment strategies, a central expertise laboratory for liver autoimmune serology and histopathology. Furthermore, by including a group of patients with AIH/PBC variant in our analyses, we were able to draw some interesting conclusions. The comparison of patients with AIH/PBC variant with AMA-positive AIH-patients did not reveal differences regarding disease severity at baseline and outcome. Response to treatment was almost identical between the two groups, though the former group achieved a faster withdrawal of corticosteroids.

In conclusion, patients with AIH could also be positive for AMA either at diagnosis or later during the disease course. AIH-patients who are merely AMA-positive appear to respond well to immunosuppressive treatment with no effect on liver-related mortality. However, AMA positivity seems of considerable importance in the subgroup of AMA-positive AIH-patients who have concurrently incidental features of bile duct injury on baseline liver histology.

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Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2023.06.001.

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