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Histologically proven AMA positive primary biliary cholangitis but normal serum alkaline phosphatase: Is alkaline phosphatase truly a surrogate marker?

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ABSTRACT

Background and aims: The most highly directed and specific autoantibody in human immunopathology is the serologic hallmark of primary biliary cholangitis (PBC), antimitochondrial antibodies (AMAs). However the clinical significance of finding a positive AMA, with normal alkaline phosphatase (ALP) remains enigmatic.
Methods: We took advantage of 169 consecutive outpatients who were identified as having a positive AMA, but normal ALP levels between January 2012 and January 2018. A liver biopsy was performed on 67/169 of these AMA positive normal ALP patients.
Results: In all 169 patients we reconfirmed the AMA and also performed anti-gp210 and anti-sp100, liver stiffness (LSM) assessed by vibration-controlled transient elastography (VCTE), an abdominal computed tomography (CT) scan, and either a magnetic resonance imaging (MRI) or ultrasound. The liver biopsies were reviewed by two unbiased observers. 87.6% of the 169 patients were females with a mean age of 46; the median AMA titer 1:320; an elevated serum IgM was found in 53.3%. Importantly, in patients with a liver biopsy, 55 (82.1%) out of 67 had varying degrees of cholangitis activity, diagnostic of PBC.
Conclusion: In patients who were AMA-positive but had normal ALP levels, more than 80% were associated with

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; ANA, antinuclear antibody; AST, aspartate aminotransferase; CA, chronic cholangitis activity; CI, confidence interval; CK7, cytokeratin 7; CK19, cytokeratin 19; CoH, canals of Hering; GGT, gamma-glutamyl transpeptidase; HA, hepatic activity; IgM, immunoglobulin M; LSM, liver stiffness; OR, odds ratio; PBC, primary biliary cholangitis; ROC, receiver operating characteristic; SLE, systemic lupus erythematosus; TB, total bilirubin; UDCA, ursodeoxycholic acid; ULN, upper limit of normal; VCTE, vibration-controlled transient elastography

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histological classic PBC. These data emphasize the importance of a positive AMA, even with a normal ALP and also question the role of ALP as a sole surrogate marker of cholangitis.

1. Introduction

The classic serologic hallmark of PBC are the presence of AMA, observed in greater than 90–95% of patients with a specificity that exceeds 95% [1,2]. Indeed, the presence of AMA with elevations of ALP are sufficient to diagnose PBC [3]. However, it is not unusual for a clinician to order an ANA and have the results reported as a negative ANA, but positive AMA. In patients who have a positive AMA but normal ALP, the diagnosis and prognosis are not well defined. The new EASL clinical practice guidelines [4] recommend following such patients for annual biochemical assessment. A new grading and staging system for PBC has recently been proposed, which takes into account the degree of both chronic cholangitis activity (CA) and hepatic activity (HA) and that of fibrosis, bile duct loss, and chronic cholestasis for staging [5,6]. This grading system is suggested to more accurately reflect clinical outcome [7,8]. We have taken advantage of a unique large cohort of consecutive patients identified in our center as having a positive AMA and normal levels of ALP. We report herein that in patients who are AMA positive but have normal ALP levels, more than 80% are associated with histologically proven PBC. Moreover, our data question the reliability of relying on ALP as the solo biochemical surrogate marker of cholangitis.

2. Patients and methods

2.1. Patient population

During the period between January 2012 and January 2018, a total of 169 consecutive patients were identified to have a positive AMA but normal ALP levels; all 169 of these patients were referred to the Department of Gastroenterology and Hepatology of Shanghai Renji Hospital for further evaluation. None of these patients were suspected of having PBC and the elevation of AMA was an incidental finding as part of routine testing for ANA in patients with arthralgias and/or undiagnosed fatigue, irrespective of suspicion of PBC. The AMA was reconfirmed in our laboratory both by immunofluorescence and immunoblotting. All 169 patients were offered a liver biopsy and 67/169 provided informed consent to biopsy.

2.2. Study design

The patients' medical records were reviewed, including their symptomatology, biochemical, serological, radiological, and possible presence of either autoimmune disease or other chronic illness, including hepatitis B and C. All laboratory values were expressed as multiples of the upper limit of normal (ULN), whereas albumin and platelet levels were expressed as the multiples of the lower limit of normal. In addition to AMA, anti-gp210 and anti-sp100 were tested. Patients underwent a LSM, assessed by VCTE. Patients also underwent a physical examination as well as an abdominal CT scan, MRI or ultrasound. All liver biopsies were analyzed using the new EASL criteria, including CA, HA and stages [5,6,9]. The histopathology was interpreted by two “blinded” pathologists. All patients provided informed consent according to a protocol approved by the Ethics Committee of Renji Hospital, Shanghai Jiao Tong University.

2.3. Laboratory assessments

Serum IgM and IgG levels were assessed by immunonephelometry. AMA were assessed via indirect immune fluorescence (IIF) on HEp-2 cells (EUROIMMUN, Germany) with known positive and negative standards in duplicate, and also by immunoblotting using the EUROASSAY test kit (EUROIMMUN, Germany), again with known positive and negative controls. Anti-gp210, and anti-sp100 were assessed by immunoblotting using the EUROASSAY test kit, using known positive and negative controls.

Table 1
Characteristics of patients with AMA-positive results and normal ALP levels.

	Whole group N = 169	With liver biopsy N = 67	Without liver biopsy N = 102	P
Age at accession (mean)	46, (16–68)	44, (20–67)	47, (16–68)	P = 0.064
Female sex (%)	148 (87.6%)	55 (82.1%)	93 (91.2%)	P = 0.080
Fatigue + (n, %)	18 (10.7%)	7 (10.4%)	11 (10.8%)	P = 0.945
Pruritus + (n, %)	0 (0%)	0 (0%)	0 (0%)	–
AMA[a] titer	1:320, (0–1:1000)	1:320, (0–1:1000)	1:320, (0–1:1000)	P = 0.382
Gp210 + (n, %)	38 (22.5%)	12 (17.9%)	26 (25.5%)	P = 0.248
Sp100 + (n, %)	24 (14.2%)	9 (13.4%)	15 (14.7%)	P = 0.817
IgM × UNL	1.19, (0.26–5.78)	1.24, (0.35–3.84)	1.16, (0.26–5.78)	P = 0.667
IgG × UNL	0.98, (0.50–1.69)	1.01, (0.65–1.69)	0.97, (0.50–1.68)	P = 0.297
ALT × UNL	1.40, (0.13–8.30)	1.23, (0.15–6.30)	1.51, (0.13–8.30)	P = 0.112
AST × UNL	1.13, (0.23–5.95)	0.94, (0.30–3.93)	1.26, (0.23–5.95)	P = 0.056
ALP × UNL	0.59, (0.21–0.99)	0.57, (0.22–0.99)	0.60, (0.21–0.99)	P = 0.187
GGT × UNL	1.69, (0.14–10.08)	1.69, (0.14–10.08)	1.69, (0.18–6.90)	P = 0.163
TB × UNL	0.65, (0.23–0.99)	0.64, (0.23–0.99)	0.67, (0.28–0.99)	P = 0.535
Albumin (g/L)	1.27, (1.00–1.57)	1.27, (1.00–1.57)	1.27, (1.03–1.47)	P = 0.687
Platelet (1×10^9/L) [b]	1.59, (0.45–2.84)	1.61, (0.82–2.84)	1.57, (0.45–2.67)	P = 0.575
Splenomegaly +(n,%)	10 (5.9%)	4 (6.2%)	6 (5.9%)	P = 0.943

Normal value: ALP 40–150 U/I, ALT 10–40 U/I, AST 10–40 U/I, GGT 10–50 U/I, TB 3.4–17.1 μmol/L, albumin 34–54 g/L, PLT 101–320 × 109/L, IgG 7–16 g/L, IgM 0.4–2.3 g/L. The titration interval of AMA was provided by the dilution factor of 3.162 (square root of 10). Thus, every second step represented in its denominator an integral power of 10 with the recommended lowest dilution of 1:100 (1:100, 1:320, 1:1,000, 1:3,200, etc.)
P values refer to comparisons between patients with a liver biopsy and the whole patients with AMA-positive results and normal ALP levels.
ALT alanine aminotransferase, UNL upper normal limit, AST aspartate aminotransferase, ALP alkaline phosphatase, GGT gamma-glutamyl transpeptidase, TB total bilirubin, IgG immunoglobulin G, IgM immunoglobulin M, AMA antimitochondrial antibody.
[a] AMA titer are expressed as median (range).
[b] Performed in 157 patients.

2.4. Histological analyses

Liver samples, performed using a 1.6-gauge needle, were fixed in 10% neutral buffered formalin, embedded in paraffin, and 4 mm thick sections were cut from each paraffin block. Slides were stained with hematoxylin-eosin (H&E), Masson and Cytokeratin 7(CK7), Cytokeratin 19(CK19) immunohistochemistry (Shanghai Long Island Biotec. Co. Ltd). More than six portal tracts were identifiable in all of the liver biopsies. All specimens were independently reviewed by two experienced "blinded" liver pathologists at our institute. In the event of disparate interpretation by the two pathologists (approximately 10% of cases), the biopsies were reviewed together until agreement. An advantage of the Nakanuma scoring system is the objective criteria and reproducibility. Hepatic activity (HA), cholangitis activity (CA), bile duct loss, fibrosis were measured according to the system proposed by Nakanuma Y [5,6]. The canals of Hering (CoH) were assessment as fibrosis 0 and 1 in the Nakanuma Y score. The CoH were counted and calculated according to Khan et al. [10]. A single CK19-positive cell or cell cluster or a single CK19-positive linear string was counted as one unit. The CoH to portal tract (C/P) ratio was calculated for each PBC specimen. Ductular reaction immunostaining for CK7 was assessed semiquantitatively: 0, absent; 1, occupying < 10% portal tract circumferences; 2, 11%–25%; 3, 26%–50%; 4, 51%–100%.

2.5. Statistical analyses

Continuous statistics were presented as means (range or ± SD), and categorical data were expressed as the number of subjects (or percentage). Mann–Whitney tests were used for the comparisons of continuous statistics, and the Chi-square test, or the Fisher's exact test, when appropriate, was used for categorical variables. Receiver operating characteristic (ROC) analysis was performed to determine the cutoffs of continuous data and to estimate the sensitivity and specificity associated with the cut-offs. Univariate and multivariate regression analyses were performed using a Logistic regression model. Odds ratio (OR) was calculated by a logistic regression model in both univariate and

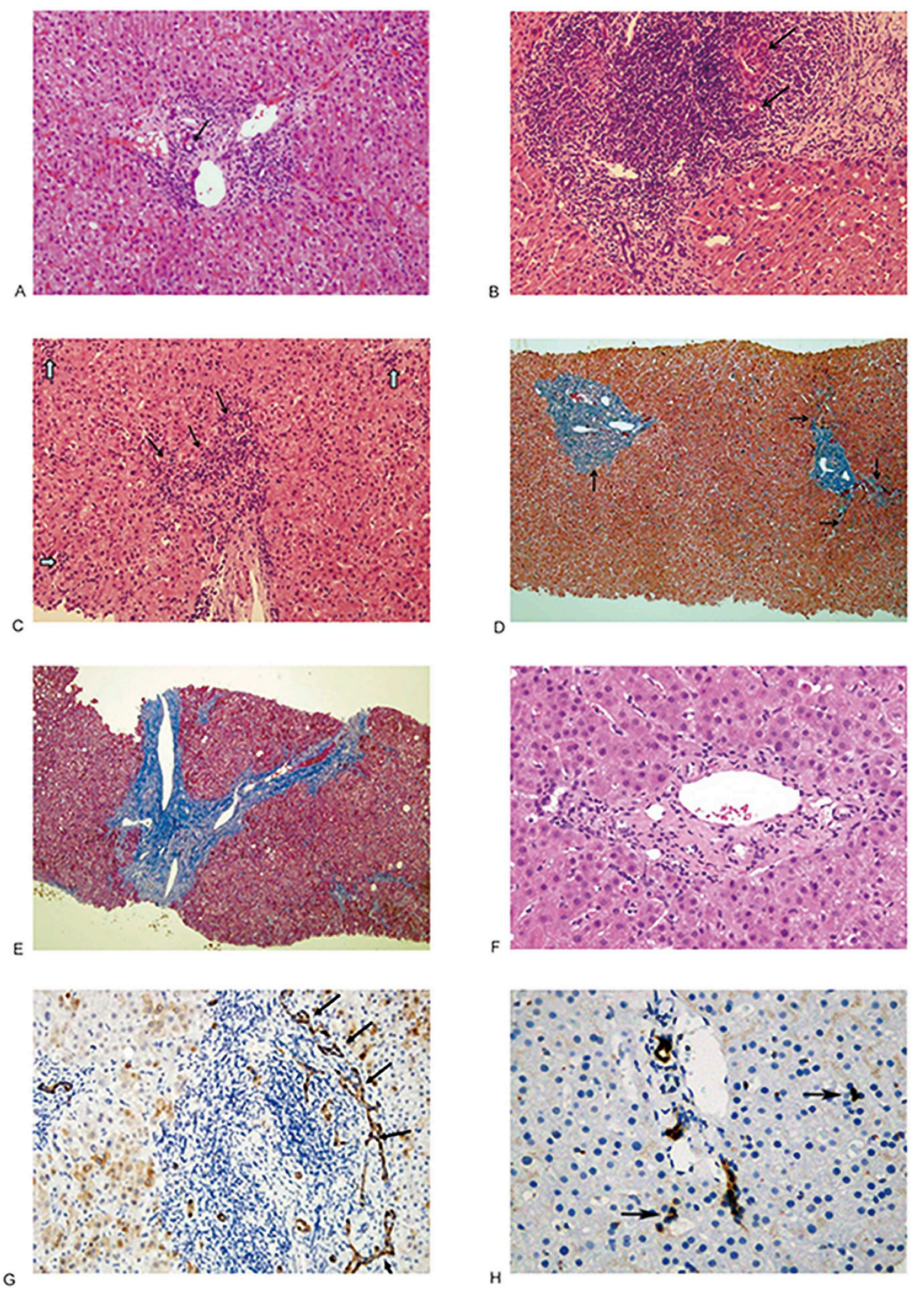

Fig. 1. Representative histology findings "AMA-positive ALP-normal" PBC. A) 1 bile duct with evident chronic cholangitis (arrow). Chronic cholangitis activity 1 (CA1, H&E staining, ×200); B) Chronic nonsuppurative destructive cholangitis (arrow). Chronic cholangitis activity 3 (CA3, H&E staining, ×200); C) Interface hepatitis affecting approximately 10 hepatocytes at the interface in 1 portal tract (arrow) and mild to moderate lobular hepatitis (blue arrow). Hepatic activity 1 (HA1, H&E staining, ×100); D) Portal tract showing fibrous enlargement and periportal fibrosis (arrow) (score 1 of fibrosis, Masson staining, ×100); E) Bridging fibrosis with variable lobular disarray (score 2 of fibrosis, Masson staining, ×100); F) Bile duct loss (H&E staining, ×200); G) Ductular reaction (arrow, CK7 immunostaining, ×200); H) Canals of Hering (arrow, CK19 immunostaining ×400). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

multivariate analyses. All analyses were performed using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL, USA); p values were two-sided and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of patients with AMA-positive results and normal ALP levels

The clinical characteristics of the 169 patients are noted in Table 1. We should emphasize the uniqueness of our Center, allowing us to capture all such patients for more detailed analysis. Although only 67 of the 169 patients provided informed consent for the biopsy, we note that the clinical features of those patients who underwent a biopsy are similar to the group who declined a biopsy. Patients were primarily women (87.6%) with a mean age of 46. None of the patients complained of pruritus and there were no patients with cirrhosis, but there were 10/169 (5.9%) with splenomegaly, 25/169 (14.8%) with Sjögren's syndrome, 6/169 (3.6%) with Hashimoto's thyroiditis' and 2/169 (1.2%) with systemic lupus erythematosus (SLE). In addition, there were 4/169 (2.4%) patients with alcoholic liver disease, 3/169 (1.8%) with chronic hepatitis B and 2/169 (1.2%) with chronic hepatitis C. The serum levels of total bilirubin (TB, except for one SLE patient with autoimmune hemolysis) were normal. As noted, all ALP were all in the normal range. The data in the group without a liver biopsy had similar and comparable data.

3.2. Histological features of patients with AMA-positive results and normal ALP levels

A liver biopsy were performed in sixty-seven cases with AMA-positive results and normal ALP levels. Representative findings on grading and staging are shown in Fig. 1. The distribution of CA, HA and stages by the new system of these 67 cases are shown in Fig. 2. The percentage of cases categorized as CA0/1/2/3 was 12/14/15/26 (17.9%/20.9%/22.4%/38.8%), respectively. Nearly 40% of the cases had marked cholangitis activity. The distribution of HA0/1/2/3 was 42/18/6/1 (62.7%/26.9%/9.0%/1.5%), respectively. The percentage of cases categorized as stages 1/2/3/4 was 17/45/5/0 (25.4%/67.2%/7.5%/0%). Five patients in stage 3 demonstrated bridging fibrosis with variable lobular disarray (score 2), and bile duct loss in < 1/3 of portal tracts (score 1). Among the five stage 3 patients, 2/5 patients had chronic hepatitis B, and 1/5 had an estimated daily alcohol consumption of over 10 gm per day for ten years. Twelve of the biopsied 67 patients were histologically normal and coined herein “AMA-positive only” patients. The remaining 55/67 patients were coined “AMA-positive ALP-normal” PBC. All of the 12/67 “AMA-positive only” patients were CA0, HA0 and stage 1.

The degree of ductular reaction (CK7 immunostaining, 0/1/2/3/4) of the “AMA-positive ALP-normal” PBC group was 2/3/2/14/17 (5.3%/7.9%/5.3%/36.8%/44.7%, n = 38), and that of the “AMA-positive only” cases was 3/1/5/0/0 (33.3%/11.1%/55.6%/0%/0%, n = 9).

Table 2
Characteristics of “AMA-positive ALP-normal” PBC Patients and “AMA-positive only” Patients.

	“AMA-positive AKP-normal” PBC N = 55	“AMA-positive only” patients N = 12	P
Age at accession (mean)	45, (20–66)	42, (23–67)	P = 0.291
Female sex (%)	45 (81.8%)	10 (83.3%)	P = 0.901
Fatigue +(n,%)	6 (10.9%)	1 (8.3%)	P = 0.792
Pruritus +(n,%)	0 (0.0%)	0 (0.0%)	–
AMA titer	1:320, (0–1:1000)	1:100, (0–1:320)	P = 0.010
Gp210 +(n,%)	11 (20.0%)	1 (8.3%)	P = 0.340
Sp100 +(n,%)	9 (16.4%)	0 (0%)	P = 0.132
IgM × UNL	1.40, (0.35–8.84)	0.94, (0.41–2.27)	P = 0.295
IgG × UNL	1.03, (0.65–1.69)	0.91, (0.66–1.16)	P = 0.118
ALT × UNL	1.25, (0.15–6.30)	1.15, (0.18–4.13)	P = 0.418
AST × UNL	0.95, (0.33–2.90)	0.90, (0.30–3.93)	P = 0.104
ALP × UNL	0.61, (0.23–0.99)	0.41, (0.22–0.87)	P = 0.001
GGT × UNL	1.75, (0.16–10.08)	1.39, (0.14–7.98)	P = 0.202
TB × UNL	0.63, (0.23–0.99)	0.68, (0.39–0.89)	P = 0.486
Albumin (g/L)	1.26, (1.00–1.47)	1.30, (1.21–1.57)	P = 0.545
Platelet (1×10^9/L)	1.60, (0.82–2.84)	1.68, (1.18–2.04)	P = 0.273
Elastography (LSM kpa)[a]	4.92, (2.3–9.8)	5.34, (3.0–6.8)	P = 0.286
Splenomegaly +(n,%)	3[b] (6.0%)	1[c], (9.1%)	P = 0.657

[a] Performed in 47 patients.
[b] One patient was splenomegaly due to autoimmune hemolysis, so date was not calculated.
[c] One patient accepted splenectomy due to trauma.

There was a significant difference between the “AMA-positive ALP-normal” PBC group and the “AMA-positive only” group ($p < 0.001$). The “AMA-positive ALP-normal” PBC group had mean 0.58 ± 0.48 CoH per portal tract (n = 23, range: 0 to 2.17; median: 0.50) compared to the “AMA-positive only” group mean 0.61 ± 0.53 CoH per portal tract (n = 8, range: 0.17 to 1.75; median: 0.37; P = 0.947).

3.3. The clinical circumstance in need of a liver biopsy

The clinical characteristics of patients with and without histological evidences of PBC were compared (Table 2). ALP levels were normal in all patients. However, there were variations within the normal range with higher levels in patients who were histologically diagnostic for PBC (0.61 × ULN vs. 0.41 × ULN) (Fig. 3A). In addition, the titer of AMA (1:320 vs. 1:100) was significantly different between patients with and without histological diagnosis of PBC (Fig. 3B). In further analysis, the ALP level (0.475 × ULN) was studied by ROC analysis (Fig. 3C). In univariate logistic regression analysis, ALP > 0.475 × ULN (OR 26.81, CI 3.19–225.22, p = 0.002) and AMA ≥1:320 (OR 6.95; CI 1.39–34.80; p = 0.018) were associated with the histological diagnosis of PBC. Multivariate regression analysis, including ALP and AMA, revealed evidence for an association between higher levels of ALP (OR 21.04; CI = 2.43–181.86; p = 0.006) with “AMA-positive ALP-normal” PBC.

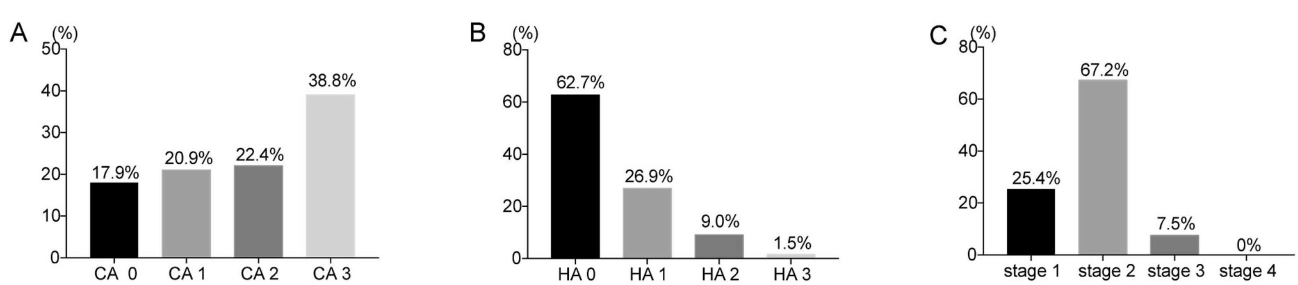

Fig. 2. The distribution of Hepatic Activity (HA), Cholangitis Activity (CA) and stages of the 67 patients with AMA-positive results and normal ALP. A) Chronic cholangitis activity; B) Hepatitis; C) Stages.

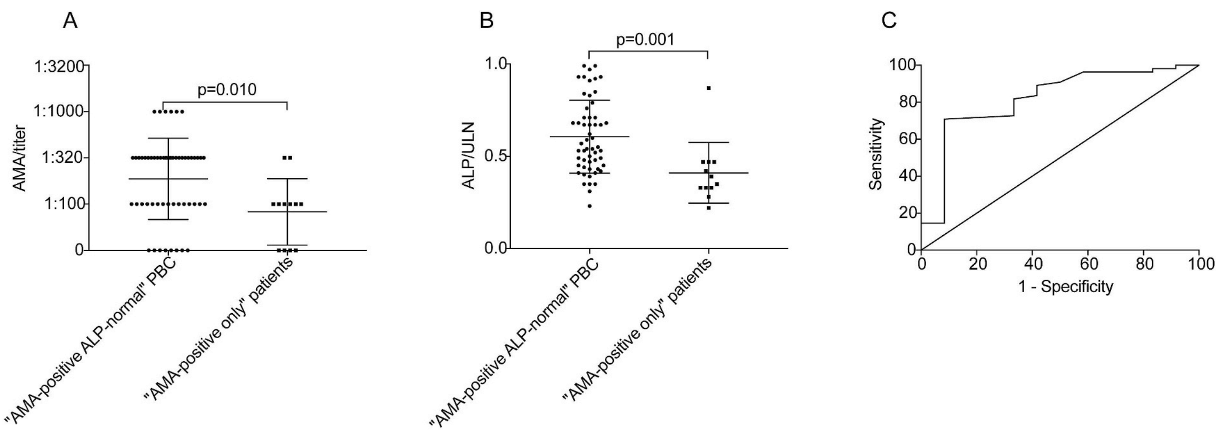

Fig. 3. The different clinical characteristics of "AMA-positive ALP-normal" PBC and "AMA-positive only" patients. A) The ALP level of "AMA-positive ALP-normal" PBC were higher than that of "AMA-positive only" patients; B) The titer of AMA in "AMA-positive ALP-normal" PBC was higher than that in "AMA-positive only" patients. C) The ROC curves presenting the predicted level of ALP in the clinical circumstance in need of a liver biopsy. Among patients with AMA-positive results and normal ALP levels (n = 67), ALP (xULN) had an area under the ROC curve of 0.82 (95%CI: 0.678 to 0.961) to identify "AMA-positive ALP-normal" PBC.

However, the AMA titer was not significantly associated with the "AMA-positive ALP-normal" PBC in multivariate analysis (OR 4.50; CI = 0.78–25.78; p = 0.092). All the patients with both ALP > 0.475 × ULN and AMA ≥1:320 were in the "AMA-positive ALP-normal" PBC group, and 50.0% of the patients who were not ALP > 0.475 × ULN nor AMA ≥1:320 were "AMA-positive ALP-normal" PBC. This indicates that high levels of ALP within the normal range and a high titer of AMA could potentially provide additional information on the liver histology.

4. Discussion

Our study population is unique because we have the ability to identify and follow thereafter each and every patient who presented to our medical center. This is in striking contrast to earlier studies that followed AMA positive patients either in the U.K. or in France. Our study is also unique in our ability to collect histologic data. We should emphasize that in this study, we demonstrate that 82.1% of patients who were AMA positive and who underwent a liver biopsy with normal ALP levels were found to have a histological diagnosis of PBC. Our data is comparable to that in earlier studies [11–13] but different from that of a recent French study. In one early UK cohort, 24/29 AMA-positive patients without signs of liver disease were noted to have histological findings compatible or consistent with the diagnosis of PBC [11]. During a follow-up (median 17.8 years), 22/29 patients developed symptoms typically attributable to PBC, and 24/29 patients had persistently abnormal cholestatic liver enzymes after a median time of only 5.6 years [12]. A more recent study reported that liver biopsy on 4/6 AMA positive patients with cholestatic liver enzymes had bile duct lesions of early stage PBC whereas 2/6 did not have any specific histological findings [13]. Clearly histological lesions precede the abnormal liver biochemistry. We are currently serially following our patient cohort. We should emphasize that the 67 patients with a liver biopsy were nearly identical to the patients in our study who did not undergo a biopsy.

There were several studies that reported that AMA positivity in healthy subjects is higher than the prevalence of PBC [14–17]. However these later studies rarely had the intense clinical and biochemical work-up noted herein. A multicenter study in France followed 229 AMA positive subjects without established diagnosis of PBC for up to seven years after AMA detection. ALP data was available in 119 subjects. 74% of them had normal ALP, only 1 in 6 (16%) with a positive AMA and normal ALP developed PBC within 5 years. The cumulative incidence rate of PBC was 2%, 7% and 16% at 1, 3 and 5 years respectively [14]. However, this study came from multiple centers, the AMA assay was rarely recapitulated, and thus there remains the potential issue of selection bias. Further, only 28 subjects had a liver biopsy at AMA detection and none of these patients displayed histological lesions suggestive of PBC. Our study has the advantage of being a single center study and we were able to "capture" each and every patient with a positive AMA. In our study we diagnosed PBC by histological evidence, while in the French study the diagnosis was based on physician opinions. Furthermore, our patients were younger than those in the French study, which is consistent with data that histological lesions precede abnormal biochemical liver tests. Our study does have the disadvantage that Chinese patients may be referred later in the disease course and were symptomatic with arthralgias and fatigue. This group may thus be different from Western patients who may be detected earlier in their disease course as part of a routine physical examination for employment. Nonetheless, our ability to capture a large consecutive cohort minimizes selection bias and emphasizes the clinical significance of the AMA as well as the confounding finding of normal ALP levels. Our first patient entered the study in 2012 and the most recent patient in 2018. The patient herein are now enrolled in a detailed long-term follow-up study.

To explore the need of a liver biopsy for diagnostic purpose, we analyzed a variety of factors. The AMA titers and the ALP levels were associated with histological diagnosis of PBC and the need for a liver biopsy. Our study reflects that high AMA titers were associated with diagnosis of PBC, which is consistent with several published studies [13,18], while inconsistent with that of Cancado et al. [19]. The elevation of serum ALP level is a traditional marker of cholestatic conditions and one of the diagnostic criteria for PBC in the EASL clinical practice guidelines [4]. In cholestasis, an elevated serum ALP level is mainly due to increased hepatic synthesis and the subsequent release of ALP in the sinusoidal blood flow [20]. Indeed, changes in ALP levels is a laboratory index in evaluating treatment responses in PBC [21,22], with the concept of the lower serum ALP level, the better the outcome. However, ours and other studies have shown that patients with AMA-positive results and normal ALP levels also have the possibility of suffering from PBC [11–13], and our study also pointed out that AMA-positive patients should be carefully followed when ALP > 0.475 ULN. We acknowledge the limitations of our study, like the small sample size. The future studies in other centers were needed to validated the ALP ROC analyses.

The diagnostic values of anti-sp100 and anti-gp210 in AMA negative PBC patients have been validated in many studies [23,24]. Anti-sp100 was negative in all patients without PBC histological evidence, and positive in 9 (16.4%) of "AMA-positive ALP-normal" PBC patients. Anti-gp210 antibodies were positive in 11 (20.0%) "AMA-positive ALP-normal" PBC patients, and in 1 (8.3%) patient of the "AMA-positive only" group. Hence, we suggest that it is necessary for patients with anti-gp210 or anti-sp100 positive results and normal ALP levels to undergo a liver biopsy. LSM, which has been shown as one of the best surrogate markers for the detection of cirrhosis or severe fibrosis in patients with PBC [25,26], did not provide any information on the diagnosis of "AMA-positive ALP-normal"

PBC. GGT levels were not significantly different between the two groups. In clinical practice, isolated serum GGT elevation has little specificity for cholestasis, and may also result from enzyme induction in response to alcohol or drug intake [27].

There were no significant differences of CoH loss between the "AMA-positive ALP-normal" PBC patients and "AMA-positive only" group. The CK19-positive CoH in the "AMA-positive only" group was 0.61 ± 0.53C/P, which was significantly lower than in previous studies 9.2 ± 6.0 C/P (n = 6) [10] or 9.19 ± 6.04 C/P (n = 6) [28], but similar to the "minimal change PBC" 0.41 ± 0.57 C/P (n = 9) [10]. Perhaps the "AMA-positive only" group in this study was not normal in histology, but was at the very early stage of PBC with the loss of CoH. This hypothesis is similar to the concept that autoantibodies to mitochondria can be detected long before the typical pathological changes of PBC [29–32].

In conclusion, in patients with normal ALP levels and positive AMA results, approximately 80% were associated with histological PBC. Clinicians should be aware that ALP may not be entirely reflective of histology and also that ALP levels may not always be reflective of cholestasis; this may be particularly important when ALP is used as the sole surrogate marker in clinical trials.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.01.005.

Conflicts of interest

The authors disclose no conflicts.

Author contributions

XM, QM and MEG designed and supervised the project. XM, JF, RT, QW, QM and XX obtained funding. CS contributed to acquisition of data, analysis of data and drafting of the manuscript. LY and XX performed interpretation of data and drafting of the manuscript. CS, JZ, YW,YL, ML and BL and collected samples. XX and LS performed clinical diagnosis. QM and YP provided technical support. XM, QM, MEG and PL revised the manuscript for important intellectual content.

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References

[1] P. Invernizzi, A. Lleo, M. Podda, Interpreting serological tests in diagnosing autoimmune liver diseases, Semin. Liver Dis. 27 (2007) 161–172.
[2] S. Oertelt, R. Rieger, C. Selmi, P. Invernizzi, A.A. Ansari, R.L. Coppel, M. Podda, et al., A sensitive bead assay for antimitochondrial antibodies: chipping away at AMA-negative primary biliary cirrhosis, Hepatology 45 (2007) 659–665.
[3] C.L. Bowlus, M.E. Gershwin, The diagnosis of primary biliary cirrhosis, Autoimmun. Rev. 13 (2014) 441–444.
[4] EASL clinical practice guidelines: the diagnosis and management of patients with primary biliary cholangitis, J. Hepatol. 67 (2017) 145–172.
[5] Y. Nakanuma, Y. Zen, K. Harada, M. Sasaki, A. Nonomura, T. Uehara, K. Sano, et al., Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: interobserver agreement, Pathol. Int. 60 (2010) 167–174.
[6] K. Harada, M. Hsu, H. Ikeda, M. Zeniya, Y. Nakanuma, Application and validation of a new histologic staging and grading system for primary biliary cirrhosis, J. Clin. Gastroenterol. 47 (2013) 174–181.
[7] A.W. Chan, R.C. Chan, G.L. Wong, V.W. Wong, P.C. Choi, H.L. Chan, K.F. To, Evaluation of histological staging systems for primary biliary cirrhosis: correlation with clinical and biochemical factors and significance of pathological parameters in prognostication, Histopathology 65 (2014) 174–186.
[8] Y. Kakuda, K. Harada, S. Sawada-Kitamura, H. Ikeda, Y. Sato, M. Sasaki, H. Okafuji, et al., Evaluation of a new histologic staging and grading system for primary biliary cirrhosis in comparison with classical systems, Hum. Pathol. 44 (2013) 1107–1117.
[9] O. Chazouilleres, D. Wendum, L. Serfaty, S. Montembault, O. Rosmorduc, R. Poupon, Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy, Hepatology 28 (1998) 296–301.
[10] F.M. Khan, A.R. Komarla, P.G. Mendoza, H.C. Bodenheimer Jr., N.D. Theise, Keratin 19 demonstration of canal of Hering loss in primary biliary cirrhosis: "minimal change PBC"? Hepatology 57 (2013) 700–707.
[11] H.C. Mitchison, M.F. Bassendine, A. Hendrick, M.K. Bennett, G. Bird, A.J. Watson, O.F. James, Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis? Hepatology 6 (1986) 1279–1284.
[12] J.V. Metcalf, H.C. Mitchison, J.M. Palmer, D.E. Jones, M.F. Bassendine, O.F. James, Natural history of early primary biliary cirrhosis, Lancet 348 (1996) 1399–1402.
[13] T. Berdichevski, O. Cohen-Ezra, O. Pappo, Z. Ben-Ari, Positive antimitochondrial antibody but normal serum alkaline phosphatase levels: could it be primary biliary cholangitis? Hepatol. Res. 47 (2017) 742–746.
[14] G. Dahlqvist, F. Gaouar, F. Carrat, S. Meurisse, O. Chazouilleres, R. Poupon, C. Johanet, et al., Large-scale characterization study of patients with anti-mitochondrial antibodies but nonestablished primary biliary cholangitis, Hepatology 65 (2017) 152–163.
[15] A. Mattalia, S. Quaranta, P.S. Leung, M. Bauducci, J. Van de Water, P.L. Calvo, F. Danielle, et al., Characterization of antimitochondrial antibodies in health adults, Hepatology 27 (1998) 656–661.
[16] K. Ohba, K. Omagari, H. Kinoshita, H. Soda, J. Masuda, H. Hazama, M. Tagawa, et al., Primary biliary cirrhosis among atomic bomb survivors in Nagasaki, Japan, J. Clin. Epidemiol. 54 (2001) 845–850.
[17] B.H. Chen, Q.Q. Wang, W. Zhang, L.Y. Zhao, G.Q. Wang, Screening of anti-mitochondrial antibody subtype M2 in residents at least 18 years of age in an urban district of Shanghai, China, Eur. Rev. Med. Pharmacol. Sci. 20 (2016) 2052–2060.
[18] A. Dellavance, E.L. Cancado, C.P. Abrantes-Lemos, M. Harriz, V. Marvulle, L.E. Andrade, Humoral autoimmune response heterogeneity in the spectrum of primary biliary cirrhosis, Hepatol. Int. 7 (2013) 775–784.
[19] E.L. Cancado, M. Harriz, The importance of autoantibody detection in primary biliary cirrhosis, Front. Immunol. 6 (2015) 309.
[20] M.M. Kaplan, A. Righetti, Induction of rat liver alkaline phosphatase: the mechanism of the serum elevation in bile duct obstruction, J. Clin. Invest. 49 (1970) 508–516.
[21] G.M. Hirschfield, J.K. Dyson, G.J.M. Alexander, M.H. Chapman, J. Collier, S. Hubscher, I. Patanwala, et al., The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines, Gut 67 (2018) 1568–1594.
[22] P.J. Trivedi, C. Corpechot, A. Pares, G.M. Hirschfield, Risk stratification in autoimmune cholestatic liver diseases: opportunities for clinicians and trialists, Hepatology 63 (2016) 644–659.
[23] P. Muratori, L. Muratori, R. Ferrari, F. Cassani, G. Bianchi, M. Lenzi, L. Rodrigo, et al., Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis, Am. J. Gastroenterol. 98 (2003) 431–437.
[24] O. Bandin, J.C. Courvalin, R. Poupon, L. Dubel, J.C. Homberg, C. Johanet, Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis, Hepatology 23 (1996) 1020–1024.
[25] C. Corpechot, F. Carrat, A. Poujol-Robert, F. Gaouar, D. Wendum, O. Chazouilleres, R. Poupon, Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis, Hepatology 56 (2012) 198–208.
[26] A. Floreani, N. Cazzagon, D. Martines, L. Cavalletto, V. Baldo, L. Chemello, Performance and utility of transient elastography and noninvasive markers of liver fibrosis in primary biliary cirrhosis, Dig. Liver Dis. 43 (2011) 887–892.
[27] EASL Clinical Practice Guidelines: management of cholestatic liver diseases, J. Hepatol. 51 (2009) 237–267.
[28] R. Saxena, P. Hytiroglou, S.N. Thung, N.D. Theise, Destruction of canals of Hering in primary biliary cirrhosis, Hum. Pathol. 33 (2002) 983–988.
[29] K.E. Kisand, K. Metskula, K.V. Kisand, T. Kivik, M.E. Gershwin, R. Uibo, The follow-up of asymptomatic persons with antibodies to pyruvate dehydrogenase in adult population samples, J. Gastroenterol. 36 (2001) 248–254.
[30] R. Uibo, K. Kisand, C.Y. Yang, M.E. Gershwin, Primary biliary cirrhosis: a multi-faced interactive disease involving genetics, environment and the immune response, Apmis 120 (2012) 857–871.
[31] W. Huang, K. Kachapati, D. Adams, Y. Wu, P.S. Leung, G.X. Yang, W. Zhang, et al., Murine autoimmune cholangitis requires two hits: cytotoxic KLRG1(+) CD8 effector cells and defective T regulatory cells, J. Autoimmun. 50 (2014) 123–134.
[32] M.J. Pollheimer, P. Fickert, Animal models in primary biliary cirrhosis and primary sclerosing cholangitis, Clin. Rev. Allergy Immunol. 48 (2015) 207–217.