



Anti-mitochondrial Antibody-Negative Primary Biliary Cholangitis Is Part of the Same Spectrum of Classical Primary Biliary Cholangitis

Guilherme Grossi Lopes Cançado^{1,2} · Michelle Harriz Braga³ · Maria Lucia Gomes Ferraz⁴ · Cristiane Alves Villela-Nogueira⁵ · Debora Raquel Benedita Terrabuio³ · Eduardo Luiz Rachid Cançado³ · Mateus Jorge Nardelli¹ · Luciana Costa Faria¹ · Nathalia Mota de Faria Gomes⁴ · Elze Maria Gomes Oliveira⁶ · Vivian Rotman⁵ · Maria Beatriz Oliveira⁷ · Simone Muniz Carvalho Fernandes da Cunha⁸ · Marlone Cunha-Silva⁹ · Liliana Sampaio Costa Mendes¹⁰ · Claudia Alexandra Pontes Ivantes¹¹ · Liana Codes^{8,12} · Valéria Ferreira de Almeida e Borges^{13,14} · Fabio Heleno de Lima Pace¹⁵ · Mario Guimarães Pessoa³ · Izabelle Venturini Signorelli¹⁶ · Gabriela Perdomo Coral¹⁷ · Paulo Lisboa Bittencourt^{12,18} · Cynthia Levy¹⁹ · Cláudia Alves Couto¹ · Members of the Brazilian Cholestasis Study Group Consortium

Received: 13 April 2021 / Accepted: 16 June 2021

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Background Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease in which anti-mitochondrial antibodies (AMA) are the diagnostic hallmark. Whether AMA-negative PBC patients represent a different phenotype of disease is highly debated.

Aims The purpose of our study was to compare AMA-positive and AMA-negative PBC patients in a large non-white admixed Brazilian cohort.

Methods The Brazilian Cholestasis Study Group multicentre database was reviewed to assess demographics, clinical features and treatment outcomes of Brazilian PBC patients, stratifying data according to AMA status.

Results A total of 464 subjects (95.4% females, mean age 56 ± 5 years) with PBC were included. Three hundred and eighty-four (83%) subjects were AMA-positive, whereas 80 (17%) had AMA-negative PBC. Subjects with AMA-negative PBC were significantly younger (52.2 ± 14 vs. 59.6 ± 11 years, $p = 0.001$) and had their first symptom at an earlier age (43.2 ± 13 vs. 49.5 ± 12 years, $p = 0.005$). Frequency of type 2 diabetes was significantly increased in subjects with AMA-negative PBC (22.5% vs. 12.2%, $p = 0.03$). Lower IgM (272.2 ± 183 vs. 383.2 ± 378 mg/dL, $p = 0.01$) and triglycerides (107.6 ± 59.8 vs. 129.3 ± 75.7 mg/dL, $p = 0.025$) and higher bilirubin (3.8 ± 13.5 vs. 1.8 ± 3.4 mg/dL, $p = 0.02$) levels were also observed in this subgroup. Response to ursodeoxycholic acid varied from 40.5 to 63.3% in AMA-positive and 34 to 62.3% in AMA-negative individuals, according to different response criteria. Outcomes such as development of liver-related complications, death and requirement for liver transplantation were similar in both groups.

Conclusions AMA-negative PBC patients are similar to their AMA-positive counterparts with subtle differences observed in clinical and laboratory features.

Keywords Primary biliary cholangitis · Anti-mitochondrial antibody · Ursodeoxycholic acid · Autoantibody · Disease phenotype

Abbreviations

PBC	Primary biliary cholangitis
AMA	Anti-mitochondrial antibodies
IIF	Indirect immunofluorescence
IB	Immunoblotting

AIH	Autoimmune hepatitis
ANA	Antinuclear antibodies
SMA	Anti-smooth muscle antibody
IgM	Immunoglobulin M
UDCA	Ursodeoxycholic acid
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	Gamma-glutamyltransferase

✉ Guilherme Grossi Lopes Cançado
guilhermegrossi@terra.com.br

Extended author information available on the last page of the article

SD	Standard deviation
IQR	Interquartile range
MAFLD	Metabolic dysfunction-associated fatty liver disease

Introduction

Primary biliary cholangitis (PBC) is an immune-mediated inflammatory liver disorder that affects interlobular bile ducts leading to bile duct injury, ductopenia and cirrhosis [1, 2]. It is much more common in Caucasian middle-aged women and is usually progressive without treatment toward end-stage liver disease requiring liver transplantation [1–3]. Anti-mitochondrial antibodies (AMA) are the serological hallmarks of PBC [4]. In subjects with cholestasis, their presence either by indirect immunofluorescence (IIF) or by other immunoassays such as ELISA or immunoblotting (IB) is regarded as sufficient for the diagnosis of PBC without requirement of further histological evaluation [3]. AMAs are found in 78–90% of patients when tested by IIF, and in 90–95% when more accurate immunoassays are used [4–8]. On the other hand, AMA can be detected in 0.1–0.5% of apparently healthy subjects [9–11] or in patients with other autoimmune liver diseases, mainly autoimmune hepatitis (AIH) [11, 12]. They are also considered as early markers of PBC even in the absence of cholestasis and predictors of disease development [13]. In fact, 10.2–16% of healthy AMA-positive patients have been shown to evolve to full-blown PBC during follow-up, while up to 83% of the individuals with baseline histological findings compatible with PBC developed clinical and biochemical features of PBC after the initial positive antibody test [14–17].

It is however well acknowledged that 5–15% of patients with PBC worldwide lack AMA [3, 8, 18]. This is challenging since immune-mediated damage to biliary epithelial cells in PBC is directed against the same E2 subunits of 2-oxo-acid dehydrogenase complex epitopes recognized by AMA [2]. It is also not entirely known whether the presence of AMA defines different subgroups of patients with AMA-positive and AMA-negative PBC, implying varying natural history [7, 19–28]. In the past, several authors have considered AMA-negative PBC as part of the spectrum of PBC and AIH overlap syndrome [29, 30]. Those authors coined the term autoimmune cholangitis to define AMA-negative PBC by the presence of high-titer antinuclear (ANA) and/or anti-smooth muscle (SMA) antibodies, prominent lobular and portal inflammation on liver biopsy and biochemical response to corticosteroids [31–33]. More recently, the term AMA-negative PBC has been used to define a subset of patients who lack AMA but have typical histological changes of PBC. More than half of these patients have detectable ANAs and 40–50% of these are PBC-specific

(multiple nuclear dots and rim-like membrane pattern), further supporting the diagnosis [34]. In spite of those findings, it is still unclear in the literature whether the presence of AMA could influence clinical expression and outcomes in subjects with PBC. In this respect, some [26, 27] but not all reports [19–25, 28] have described distinct clinical features in AMA-negative PBC patients including higher frequency of ANA and SMA and lower levels of serum immunoglobulin M (IgM) [20–23], reduced response to ursodeoxycholic acid (UDCA) and transplantation-free survival when compared to their AMA-positive counterparts [26, 27].

The purpose of this study was to compare clinical, laboratory and histological features of AMA-positive and AMA-negative PBC patients in a large non-white admixed Brazilian cohort.

Methods

Study Population

The study population included adult (≥ 18 years old) patients who were diagnosed with PBC between January 1st, 1992 and December 31st, 2019 in 28 different hepatology centers from all regions of the country. The diagnosis of PBC was considered if patient fulfilled at least two of the following diagnostic criteria as recommended by the American Association for the Study of Liver Disease guidelines: (i) positive serology for anti-mitochondrial antibodies (AMA); (ii) persistent increase in the serum alkaline phosphatase (ALP) levels; and (iii) liver histology compatible with PBC (3). Patients in whom the diagnosis could not be confirmed or who had another etiology of liver disease, including overlap syndrome with autoimmune hepatitis, were excluded.

Data Collection

Each investigator was asked to identify all PBC patients that have been followed in their Liver Center at the time of the survey, without any selection or exclusion whatsoever, and to fill-in a standardized database provided by the Brazilian Cholestasis Study Group to assess retrospectively demographics, real-life clinical, laboratory and histological features of PBC, as well as response to treatment with either UDCA and/or fibrates. Briefly, data obtained from medical records included sex; age at diagnosis; year of diagnosis; year of first symptoms or first biochemical changes; last date of follow-up; baseline clinical presentation, concurrent autoimmune diseases, dyslipidemia and type 2 diabetes; baseline liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT), bilirubin, albumin, IgM, immunoglobulin G, glucose, triglycerides

and cholesterol levels; autoantibody profile including ANA, SMA and AMA; liver histology staged according to the Ludwig system; presence of osteopenia or osteoporosis; development of liver-related complications; response to treatment with UDCA and/or fibrates; liver transplantation and death. The response to treatment either to UDCA or fibrates was analyzed according to international validated criteria including Barcelona, Paris I and II, Toronto, Rotterdam and POISE trial criteria [34–38]. The duration of follow-up was defined as the interval between the diagnosis and the last visit or the date of liver transplantation or death.

All demographics, clinical and laboratory data including response to treatment and outcomes were compared according to AMA status assessed by IIF in two groups of patients: AMA-positive and AMA-negative PBC. All AMA-positive patients had titers $\geq 1:40$. Liver histology specimens were available for all patients with AMA-negative and 256 AMA-positive PBC patients. Cirrhosis was diagnosed both histologically (when available) or clinically according to several parameters, such as (a) presence of esophagogastric varices on endoscopy; (b) suggestive imaging studies (abdominal ultrasound, computed tomography or magnetic resonance); (c) platelet count $< 150,000/\text{mm}^3$ without other possible explanations, (d) liver-related biochemical alterations, such as serum albumin < 3.5 g/dL and enlarged INR, (e) signs of liver failure on physical exam. This study was conducted in accordance with the ethical standards of the Helsinki Declaration and was approved by the Federal University of Minas Gerais Ethics Committee Board (CAAE 98627218.6.1001.5149).

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software (IBM, USA). Continuous variables distribution was assessed by Shapiro–Wilk test, and those with Gaussian distribution were expressed as mean and standard deviation (SD), or as median and interquartile range (IQR) if skewed distribution. Categorical variables were expressed as absolute number and percentage. Univariate analysis was performed using chi-square or Fisher exact test, as appropriate, for categorical variables. Continuous variables were analyzed by the Student t test or Mann–Whitney U test, according to the distribution. Pairwise deletion was applied to missing data. *P* values < 0.05 were considered statistically significant.

Results

Patient Characteristics

Four hundred sixty-four subjects (95.4% female, mean age 56 ± 5 years) with well-defined diagnosis of PBC were

included in this study. Three hundred eighty-four (83%) subjects were AMA-positive PBC patients, whereas 80 (17.2%) had AMA-negative PBC. Demographic, clinical and laboratory features are summarized in Table 1. Subjects with AMA-negative PBC were significantly younger (52.2 ± 14 vs. 59.6 ± 11 years in AMA-positive patients, $p = 0.001$) and had their first symptom at an earlier age (43.2 ± 13 vs. 49.5 ± 12 years in AMA-positive patients, $p = 0.005$) when compared to their counterparts with AMA-positive PBC. Age at diagnosis was also lower and time to diagnosis was longer in AMA-negative patients, but the difference was not statistically significant for either variable. With respect to AMA status, no other differences in demographics and baseline clinical features were observed, with the exception of the frequency of type 2 diabetes mellitus, that was significantly increased in those subjects with AMA-negative PBC (22.5% vs. 12.2% in patients with AMA-positive PBC, $p = 0.03$). Comparison of baseline laboratory features revealed that AMA-negative patients when compared to their AMA-positive counterparts have baseline lower IgM (272.2 ± 183 vs. 383.2 ± 378 mg/dL, $p = 0.01$) and triglycerides (107.6 ± 59.8 vs. 129.3 ± 75.7 mg/dL, $p = 0.025$) and higher bilirubin (3.8 ± 13.5 vs. 1.8 ± 3.4 mg/dL, $p = 0.02$) levels. No differences were observed in ANA prevalence (Table 1). Mean dose of UDCA was 12.86 ± 2.7 and 13.3 ± 2.2 mg/Kg in AMA-positive and negative groups, respectively ($p = 0.39$). Any patient was using fibrate at the baseline. Response to UDCA varied from 40.5 to 63.3% in AMA-positive and 34 to 62.3% in AMA-negative subjects, according to different response criteria. (Table 2). No difference was observed in the frequency of treatment response in those groups of patients using different available criteria. On the contrary, paired analysis of ALP and GGT levels over 5 years of UDCA treatment showed slower decline of both ALP and GGT in those AMA-negative patients when compared to their AMA-positive counterparts, but the difference was not statistically significant (Fig. 1), with the exception of 2 years follow-up time. Outcomes such as development of liver-related complications and death and requirement for liver transplantation were similar in both groups of patients.

Discussion

The present study analyzed 464 subjects with well-defined PBC. Eighty (17%) of them lacked AMA when tested by IIF in local reference laboratories in Brazil, one of the largest cohorts of AMA-negative patients with PBC in real-world setting published thus far. Our findings support the concept that AMA-negative PBC subjects have subtle differences in baseline clinical and laboratory features but similar outcomes when compared to their AMA-positive counterparts. AMA-negative PBC subjects were shown to be significantly

Table 1 Baseline Clinical and Laboratory Features in Patients with AMA-positive and AMA-negative Primary Biliary Cholangitis

Variables	AMA-negative (n=80)	AMA-positive (n=384)	p values
<i>Demographics</i>			
Age (yrs.)	52.2 ± 14.1	59.6 ± 11.3	0.001
Age at first symptoms (yrs.)	43.2 ± 13.3	49.5 ± 11.9	0.005
Mean time to diagnosis (yrs.)	2.7 ± 4.5	1.9 ± 4.7	0.076
Age at diagnosis (yrs.)	47.8 ± 13.5	51.7 ± 10.9	0.056
Female sex	92.50%	96.35%	0.132
<i>Clinical features</i>			
Pruritus	46.7%	49.5%	0.75
Fatigue	36.4%	38.3%	0.80
Jaundice	23.1%	20.8%	0.77
Splenomegaly	7.5%	4.95%	0.41
Hepatomegaly	14.1%	14.8%	1.0
Xanthoma	5.0%	4.2%	0.76
Xanthelasma	6.33%	7.0%	1.0
Type 2 Diabetes Mellitus	22.5%	12.2%	0.026
Dyslipidemia	19%	22%	0.39
<i>Concurrent autoimmune diseases</i>			
Hashimoto's thyroiditis	13.8%	19.8%	0.27
Sjogren syndrome	8.9%	7.8%	0.93
Rheumatoid arthritis	8.9%	3.7%	0.06
Scleroderma	2.5%	6.5%	0.28
<i>Laboratory features</i>			
ANA	56.6%	66.3%	0.1
SMA	4.4%	3.88%	0.74
IgG (mg/dL)	1553.9 ± 515	1483.7 ± 519	0.39
IgM (mg/dL)	272.2 ± 183	383.2 ± 378	0.01
AST (x ULN)	2.6 ± 1.95	2.5 ± 1.9	0.66
ALT (x ULN)	3.1 ± 2.5	2.7 ± 2.5	0.09
ALP (x ULN)	3.8 ± 2.9	3.70 ± 3.0	0.27
GGT (x ULN)	13.3 ± 13.7	11.4 ± 11.6	0.16
Bilirubin (mg/dL)	3.8 ± 13.5	1.8 ± 3.4	0.02
Albumin (g/dL)	3.9 ± 0.5	3.9 ± 0.5	1.0
Platelets (mm ³)	216,640 ± 97,296	221,158 ± 90,536	0.74
Triglycerides (mg/dL)	107.6 ± 59.8	129.3 ± 75.7	0.025
Total Cholesterol (mg/dL)	230.4 ± 75.7	232.5 ± 76	0.57
<i>Bone disease by densitometry</i>			
Absent (n=64)	29%	33%	0.46
Osteopenia (n=82)	51.6%	40%	
Osteoporosis (n=51)	19.4%	27%	
Cirrhosis at baseline	36.6%	32%	0.53

AMA anti-mitochondrial antibody; ANA antinuclear antibody; ALT alanine aminotransferase; ALP alkaline phosphatase; AST aspartate aminotransferase; GGT gammaglutamyl transferase; IgG immunoglobulin G; IgM immunoglobulin M; SMA anti-smooth muscle antibody; ULN upper limit of normality; Yrs. years

younger at disease onset, and to have a longer time from symptoms onset to diagnosis, probably due to requirement of histological evaluation for definite diagnosis. As reported by other authors [22, 23, 29, 30], IgM levels were lower in AMA-negative patients with PBC when compared to their AMA-positive counterparts, but in contrast to other reports [21, 23, 29, 30], no increase in the frequency of either ANA

or SMA was found in the former group of patients. Baseline higher bilirubin levels, usually associated with advanced disease [1–3], were more often encountered in subjects with AMA-negative PBC, indicating that those patients could have a more advanced liver disease at the time of diagnosis, possibly due to a delay in diagnosis. It is worth mentioning that a higher frequency of type 2 diabetes was also

Table 2 Outcomes and Response to Treatment in Patients with AMA-positive and AMA-negative PBC

Variables	AMA-negative (n = 80)	AMA-positive (n = 384)	p value
Mean follow-up time (years)	5.3 ± 4.8	6.4 ± 5.3	0.101
<i>Liver-related complications patients during follow-up</i>			
Variceal bleeding	5.6%	8.8%	0.36
Hepatic encephalopathy	5.7%	7.1%	1.0
Ascites	13.5%	15.2%	0.91
Spontaneous bacterial peritonitis	2.5%	4.0%	1.0
Hepatocellular carcinoma	0%	2.3%	0.6
<i>Response to UDCA at 12 months</i>			
Toronto criteria (n = 316)	151 (57.6)	28 (51.9)	0.435
Barcelona criteria (n = 312)	164 (63.3)	29 (54.7)	0.240
Paris-1 criteria (n = 315)	158 (60.3)	30 (56.6)	0.616
Paris-2 criteria (n = 315)	106 (40.5)	18 (34.0)	0.377
POISE trial criteria (n = 315)	179 (68.3)	33 (62.3)	0.391
Rotterdam criteria (n = 272)	150 (65.2)	23 (54.8)	0.195
Liver transplantation	5%	6.8%	0.74
Liver-related deaths	1.5%	3.6%	0.39

AMA anti-mitochondrial antibody; PBC primary biliary cholangitis; UDCA ursodeoxycholic acid

identified in those AMA-negative patients. Interestingly, Hindi et al. [40] have reported more advanced PBC in subjects with risk factors for metabolic syndrome and metabolic dysfunction-associated fatty liver disease (MAFLD) that is closely associated with type 2 diabetes mellitus. It is, thus, possible that those AMA-negative patients could have competing risks for advanced or progressive disease such as younger age at disease onset, higher bilirubin levels and associated MAFLD. On the other hand, lower levels of triglycerides were observed in this subgroup, a finding that might be linked to the use of hypoglycemic medications and/or insulin and reflect satisfactory glycemic control.

Differently from Sakauchi et al. [22], who reported a significantly higher prevalence of Sjogren's syndrome, rheumatoid arthritis, autoimmune thyroiditis, and scleroderma in AMA-negative patients, a similar distribution of concurrent autoimmune diseases was observed in AMA-positive and negative subjects in the present study. Furthermore, although ANA has been reported with extremely high proportions

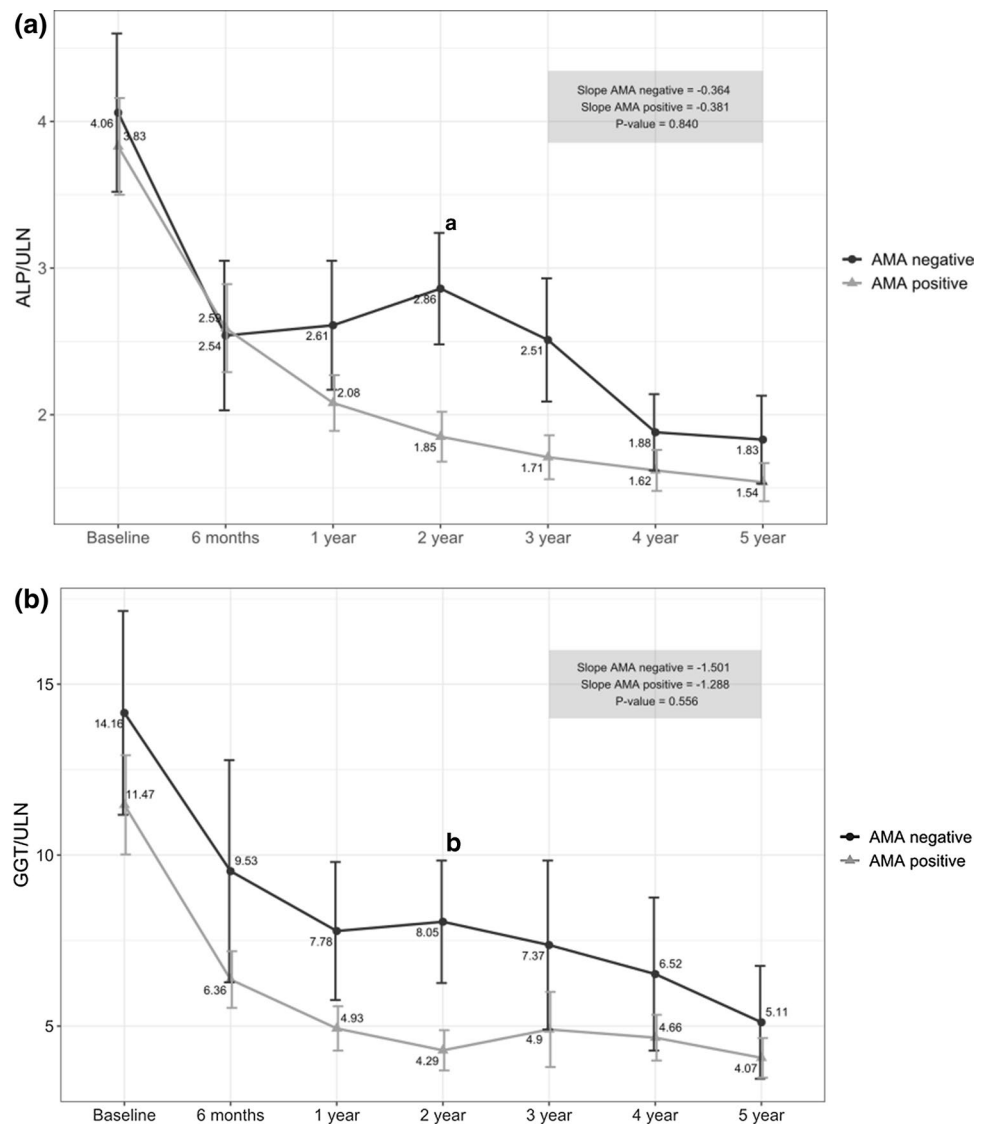
in AMA-negative PBC, in our study, we observed a relatively lower prevalence, but still very significant levels [21, 23]. This may be ascribed to differences in genetics and/or environmental factors related to each population or even to diverse methodology employed in each study [41, 42].

Several investigators have reported similar outcomes [21, 28] and treatment responses to UDCA [19, 24, 25] in patients with PBC irrespective of AMA status, whereas others reported conflicting results [26, 27]. Koulentani et al. [27] evaluated a very small cohort of patients with AMA-positive and AMA-negative PBC and suggested a lower effect of UDCA treatment in subjects with advanced disease and AMA-negative PBC. Juliusson et al. [26], on the other hand, reviewed 71 AMA-negative PBC matching them on year of diagnosis to the same number of AMA-positive counterparts. The authors reported reduced survival free of liver-related complications in the former group of patients. In the present study, the response to UDCA treatment was assessed using various internationally validated criteria with similar rates of response observed in AMA-negative PBC patients when compared to their AMA-positive counterparts. However, a slower decline in ALP and GGT levels was observed in AMA-negative PBC patients over 5-years, indicating that normalization or near normalization of ALP and GGT may take longer to achieve in AMA-negative patients. No difference in other outcomes such as liver-related complications, liver-related mortality or liver transplantation was noticed.

Our study has some limitations, including its retrospective design and lack of data regarding variable methods to test AMA (IB, beads and/or ELISA). It also important to highlight that our cohort presented a high prevalence of cirrhotic patients at baseline. This might reflect a referral bias to specialized hepatology centers or late diagnosis of PBC in Brazil. On the other hand, it has to be recognized that it reflects real-life practices of AMA detection that is currently based in IIF in large parts of the world.

In conclusion, our data show that AMA-negative PBC patients are remarkably similar to AMA-positive subjects in clinical and laboratory features, as well as in treatment responses and outcomes with very subtle differences. Even though treatment responses to UDCA are similar irrespective of AMA status, subjects with AMA-negative PBC may have a slower decline in ALP and GGT levels over time.

Fig. 1 ALP and GGT levels in subjects with PBC according to AMA status. Shown are the mean values of ALP and GGT at each time point of the follow-up. Bars indicate the standard deviation. ^a $p=0.02$; ^b $p=0.03$; ALP alkaline phosphatase; AMA anti-mitochondrial antibody; GGT gammaglutamyl transferase; PBC primary biliary cholangitis



Acknowledgments We acknowledge the support of the Brazilian Society of Hepatology.

Funding This work was supported by Brazilian Society of Hepatology and Instituto Brasileiro do Fígado—IBRAFIG.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.


References

- Lleo A, Wang G-Q, Gershwin ME, Hirschfield GM. Primary biliary cholangitis. *Lancet* (London, England) 2020; 396:1915–1926.
- Leung KK, Deeb M, Hirschfield GM. Review article: pathophysiology and management of primary biliary cholangitis. *Aliment Pharmacol. Ther.* 2020; 52:1150–1164.
- Lindor KD, Bowlus CL, Boyer J et al. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2019; 69:394–419.
- Sebode M, Weiler-Normann C, Liwinski T, Schramm C. Autoantibodies in Autoimmune Liver Disease—Clinical and Diagnostic Relevance. *Front Immunol.* 2018. <https://doi.org/10.3389/fimmu.2018.00609>.
- Hurlburt KJ, McMahon BJ, Deubner H et al. Prevalence of autoimmune liver disease in Alaska Natives. *Am J Gastroenterol.* 2002; 97:2402–7.
- Arbour L, Rupps R, Field L et al. Characteristics of primary biliary cirrhosis in British Columbia's First Nations population. *Can. J. Gastroenterol.* 2005; 19:305–10.
- Muratori P, Muratori L, Gershwin ME et al. “True” anti-mitochondrial antibody-negative primary biliary cirrhosis, low sensitivity of the routine assays, or both? *Clin Exp Immunol.* 2004; 135:154–8.

8. Selmi C, Zuin M, Bowlus CL, Gershwin ME. Anti-mitochondrial antibody-negative primary biliary cirrhosis. *Clin Liver Dis.* 2008; 12:173–85, ix.
9. Mattalia A, Quaranta S, Leung PS et al. Characterization of anti-mitochondrial antibodies in health adults. *Hepatology.* 1998; 27:656–61.
10. Cunha LM, Bittencourt PL, Abrantes-Lemos CP et al. Prevalence of non-organ-specific autoantibodies in a rural community from northeastern Brazil: a population-based study. *Hum Immunol.* 2012; 73:70–4.
11. Shibata M, Onozuka Y, Morizane T et al. Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. *J Gastroenterol.* 2004; 39:255–9.
12. Farias AQ, Goncalves LL, Bittencourt PL et al. Applicability of the IAIHG scoring system to the diagnosis of antimitochondrial/anti-M2 seropositive variant form of autoimmune hepatitis. *J Gastroenterol Hepatol.* 2006; 21:887–893.
13. Sun C, Xiao X, Yan L et al. Histologically proven AMA positive primary biliary cholangitis but normal serum alkaline phosphatase: Is alkaline phosphatase truly a surrogate marker? *J Autoimmun.* 2019; 99:33–38.
14. Metcalf J V., Mitchison HC, Palmer JM et al. Natural history of early primary biliary cirrhosis. *Lancet* (London, England) 1996; 348:1399–402.
15. Mitchison HC, Bassendine MF, Hendrick A et al. Positive anti-mitochondrial antibody but normal alkaline phosphatase: Is this primary biliary cirrhosis? *Hepatology.* 1986. <https://doi.org/10.1002/hep.1840060609>.
16. Dahlqvist G, Gaouar F, Carrat F et al. Large-scale characterization study of patients with antimitochondrial antibodies but non established primary biliary cholangitis. *Hepatology.* 2017; 65:152–163.
17. Zandanell S, Strasser M, Feldman A, Tevini J, Strebinger G, Niederseer D, Pohla-Gubo G, Huber-Schönauer U, Ruhaltinger S, Paulweber B, Datz C, Felder TK, Aigner E. Low rate of new-onset primary biliary cholangitis in a cohort of anti-mitochondrial antibody-positive subjects over six years of follow-up. *J Intern Med.* 2020 Apr;287:395–404.
18. Oertelt S, Rieger R, Selmi C et al. A sensitive bead assay for antimitochondrial antibodies: Chipping away at AMA-negative primary biliary cirrhosis. *Hepatology.* 2007; 45:659–65.
19. Liu B, Shi XH, Zhang FC et al. Antimitochondrial antibody-negative primary biliary cirrhosis: a subset of primary biliary cirrhosis. *Liver Int.* 2008; 28:233–9.
20. Mendes F, Lindor KD. Antimitochondrial antibody-negative primary biliary cirrhosis. *Gastroenterol Clin North Am.* 2008; 37:479–84, viii.
21. Invernizzi P, Crosignani A, Battezzati PM et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. *Hepatology.* 1997. <https://doi.org/10.1002/hep.510250507>.
22. Sakauchi F, Mori M, Zeniya M, Toda G. Antimitochondrial antibody negative primary biliary cirrhosis in Japan: utilization of clinical data when patients applied to receive public financial aid. *J Epidemiol.* 2006; 16:30–4.
23. Zhang F-K, Jia J-D, Wang B-E. Clinical evaluation of serum antimitochondrial antibody-negative primary biliary cirrhosis. *Hepatobiliary Pancreat Dis Int.* 2004; 3:288–291.
24. Kim WR, Poterucha JJ, Jorgensen RA et al. Does antimitochondrial antibody status affect response to treatment in patients with primary biliary cirrhosis? Outcomes of ursodeoxycholic acid therapy and liver transplantation. *Hepatology.* 1997; 26:22–6.
25. Joshi S, Cauch-Dudek K, Heathcote EJ et al. Antimitochondrial antibody profiles: are they valid prognostic indicators in primary biliary cirrhosis? *Am J Gastroenterol.* 2002; 97:999–1002.
26. Juliusson G, Imam M, Björnsson ES et al. Long-term outcomes in antimitochondrial antibody negative primary biliary cirrhosis. *Scand J Gastroenterol.* 2016; 51:745–52.
27. Koulentaki M, Moscardina J, Dimoulios P et al. Survival of anti-mitochondrial antibody-positive and -negative primary biliary cirrhosis patients on ursodeoxycholic acid treatment. *Dig Dis Sci.* 2004. <https://doi.org/10.1023/B:DDAS.0000037811.48575.da>.
28. Zandanell S, Strasser M, Feldman A et al. Similar clinical outcome of AMA immunoblot-M2-negative compared to immunoblot-positive subjects over six years of follow-up. *Postgrad Med.* 2021:1–8.
29. Michieletti P, Wanless IR, Katz A et al. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. *Gut.* 1994; 35:260–265.
30. Lacerda MA, Ludwig J, Dickson ER et al. Antimitochondrial antibody-negative primary biliary cirrhosis. *Am J Gastroenterol.* 1995; 90:247–249.
31. Kadokawa Y, Omagari K, Ohba K et al. Does the diagnosis of primary biliary cirrhosis of autoimmune cholangitis depend on the “phase” of the disease? *Liver Int.* 2005. <https://doi.org/10.1111/j.1478-3231.2005.01078.x>.
32. Sánchez-Pobre P, Castellano G, Colina F et al. Antimitochondrial antibody-negative chronic nonsuppurative destructive cholangitis. Atypical primary biliary cirrhosis or autoimmune cholangitis? *J. Clin. Gastroenterol.* 1996; 23:191–198.
33. Vierling JM. Primary biliary cirrhosis and autoimmune cholangiopathy. *Clin Liver Dis.* 2004. [https://doi.org/10.1016/S1089-3261\(03\)00132-6](https://doi.org/10.1016/S1089-3261(03)00132-6).
34. Levy C, Bowlus CL. Role of Antinuclear Antibodies in Primary Biliary Cholangitis. *Am J Gastroenterol.* 2020; 115:1604–1606.
35. Parés A, Caballería L, Rodés J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. *Gastroenterology.* 2006; 130:715–20.
36. Corpechot C, Abenavoli L, Rabahi N et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology.* 2008; 48:871–7.
37. Kumagi T, Guindi M, Fischer SE et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. *Am J Gastroenterol.* 2010; 105:2186–94.
38. Corpechot C, Chazouillres O, Poupon R. Early primary biliary cirrhosis: Biochemical response to treatment and prediction of long-term outcome. *J Hepatol.* 2011. <https://doi.org/10.1016/j.jhep.2011.02.031>.
39. Nevens F, Andreone P, Mazzella G et al. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *N Engl J Med.* 2016. <https://doi.org/10.1056/nejmoa1509840>.
40. Híndi M, Levy C, Couto CA et al. Primary biliary cirrhosis is more severe in overweight patients. *J Clin Gastroenterol.* 2013. <https://doi.org/10.1097/MCG.0b013e318261e659>.
41. Bittencourt PL, Palácios SA, Farias AQ et al. Analysis of major histocompatibility complex and CTLA-4 alleles in Brazilian patients with primary biliary cirrhosis. *J Gastroenterol Hepatol.* 2003; 18:1061–1066.
42. Tanaka A, Leung PSC, Gershwin ME. The genetics of primary biliary cholangitis. *Curr Opin Gastroenterol.* 2019. <https://doi.org/10.1097/MOG.0000000000000507>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Guilherme Grossi Lopes Cançado^{1,2}  · Michelle Harriz Braga³ · Maria Lucia Gomes Ferraz⁴ · Cristiane Alves Villela-Nogueira⁵ · Debora Raquel Benedita Terrabuio³ · Eduardo Luiz Rachid Cançado³ · Mateus Jorge Nardelli¹ · Luciana Costa Faria¹ · Nathalia Mota de Faria Gomes⁴ · Elze Maria Gomes Oliveira⁶ · Vivian Rotman⁵ · Maria Beatriz Oliveira⁷ · Simone Muniz Carvalho Fernandes da Cunha⁸ · Marlone Cunha-Silva⁹ · Liliana Sampaio Costa Mendes¹⁰ · Claudia Alexandra Pontes Ivantes¹¹ · Liana Codes^{8,12} · Valéria Ferreira de Almeida e Borges^{13,14} · Fabio Heleno de Lima Pace¹⁵ · Mario Guimarães Pessoa³ · Izabelle Venturini Signorelli¹⁶ · Gabriela Perdomo Coral¹⁷ · Paulo Lisboa Bittencourt^{12,18} · Cynthia Levy¹⁹ · Cláudia Alves Couto¹ · Members of the Brazilian Cholestasis Study Group Consortium

¹ Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Universidade Federal de Minas Gerais, Av. Professor Alfredo Balena 110, Belo Horizonte, Minas Gerais 30130-100, Brazil

² Hospital da Polícia Militar de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

³ Departamento de Gastroenterologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil

⁴ Disciplina de Gastroenterologia, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil

⁵ Hospital Universitário Clementino Fraga Filho e Departamento de Clínica Médica da Faculdade de Medicina, Universidade Federal Do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

⁶ Centro Universitário Lusíada—UNILUS, Santos, São Paulo, Brazil

⁷ Ambulatório Municipal de Hepatites Virais de São José Dos Campos, São José dos Campos, São Paulo, Brazil

⁸ Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil

⁹ Divisão de Gastroenterologia (Gastrocentro), Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

¹⁰ Hospital de Base do Distrito Federal, Brasília, Distrito Federal, Brazil

¹¹ Serviço de Gastroenterologia, Hepatologia e Transplante Hepático, Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil

¹² Hospital Português, Salvador, Bahia, Brazil

¹³ Instituto de Gastroenterologia, Endoscopia e Proctologia, Uberlândia, Minas Gerais, Brazil

¹⁴ Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil

¹⁵ Serviço de Gastroenterologia e Hepatologia, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil

¹⁶ Hospital Universitário Cassiano Antônio Moraes, Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil

¹⁷ Irmandade da Santa Casa de Misericórdia de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

¹⁸ Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil

¹⁹ Division of Digestive Health and Liver Diseases, University of Miami Miller School of Medicine, Miami, FL, USA