Autoantibodies in Primary Biliary Cholangitis

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KEYWORDS

• Antimitochondrial antibodies • Autoantibodies • Diagnosis • Cholangitis
• Biliary liver disease

KEY POINTS

- Antimitochondrial antibodies (AMAs) have a significant role in the diagnosis of primary biliary cholangitis (PBC) and are highly specific and sensitive in the context of cholestasis.
- Patients with cholestasis who are AMA negative should have serology for PBC-specific antinuclear antibodies sent.
- Patterns of serology in patients with PBC are not only helpful diagnostically but can also provide prognostic and biologic insights into disease course.

Abbreviations	
AMA	antimitochondrial antibody
PBC	primary biliary cholangitis

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic immune-mediated liver disease with an estimated global prevalence of 14.6 per 100,000 population (range from 1.91 to 40.2).[1,2] Geographic variation is noted with North American prevalence at 21.8 per 100,000, Europe at 14.6 per 100,000 and the Asian-Pacific region at 9.8 per 100,000.[2] Most patients (85%) identify as women.[2,3] PBC is characterized by an immune-mediated destruction of small bile duct biliary epithelial cells, with a characteristic nonsuppurative destructive cholangitis and ductopenia. There is subsequent cholestasis, progressive liver fibrosis, and complications arise from end-stage liver disease.[4]

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The diagnosis of PBC is generally based on 2 of 3 criteria being met: (1) an elevated alkaline phosphatase (ALP) as a marker of elevated cholestasis parameters, (2) positive autoantibodies for antimitochondrial antibody (AMA) with a minimum titer of 1:40, or specific antinuclear antibodies (ANAs) relating to PBC, and/or (3) liver histopathology consistent with PBC.[5] First-line treatment of PBC involves daily ursodeoxycholic acid (UDCA), a hydrophilic bile acid that improves cholestasis parameters and delays histologic and clinical progression to end-stage liver disease and its complications.[6] Second-line treatment options (obeticholic acid, fibrates, and clinical trial therapies) are available for those who have persistently elevated ALP and/or elevated conjugated bilirubin despite UDCA treatment.[7]

This review focuses on the role of autoantibodies in the diagnosis of PBC, as well as the relationship between autoantibodies with pathophysiology and prognostication, along with a discussion regarding novel and other related disease autoantibodies.

The Role of Antimitochondrial Antibodies in Primary Biliary Cholangitis

Mitochondria and their components are known to be recognized as damage-associated molecular patterns that activate the innate immune system and are implicated in signaling with both the innate and active immune response in many diseases.[8,9] Although this has yet to be directly demonstrated in PBC, AMAs have a clearly significant role in the diagnosis of PBC. Unlike most autoantibodies that are found in multiple diseases, AMA is unique in that it is both a highly specific and sensitive marker for PBC: more than 90% of patients with PBC have a positive AMA, whereas 0.5% of healthy individuals without PBC are AMA positive, and AMA can be found in up to 1% of individuals presenting with extrahepatic disorders.[10–13] AMA was discovered in initial investigations searching for autoantibodies associated with PBC. Walker and colleagues performed indirect immunofluorescence testing (IFT) on PBC-sera-stained human gastric mucosa and thyrotoxic human thyroid, and noted a granular cytoplasmic fluorescence pattern in these tissues that were especially rich in mitochondria.[14] The group then confirmed antimitochondrial reactivity of the sera to subcellular fractions of rat mitochondria and not other subcellular fractions.[14]

The target of AMA in PBC is a family of proteins called the 2-oxo-acid dehydrogenase complexes that participate in oxidative phosphorylation and decarboxylation of keto acid substrates along the inner mitochondrial matrix.[15] This includes the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), the branched-chain 2-oxo-acid dehydrogenase complex, the ketoglutaric acid dehydrogenase complex, and the anchoring dihydrolipoamide dehydrogenase-binding protein (E3) for PDC-E2.[15] The E2 subunits exhibit lipoyl domain homology, and several studies have demonstrated that the dominant epitope recognized by AMA is located within the lipoyl domain. Most patients with AMA reactivity react against PDC-E2, along with reactivity against the branched-chain 2-oxo acid dehydrogenase E2 complex, the ketoglutaric acid dehydrogenase E2 complex, or both.[15] AMA binding to epitopes within the lipoic acid binding domain disrupts the domain and inhibits enzymatic function.[16]

The role of molecular mimicry between pathogenic molecules and mitochondrial antigens is of particular interest in PBC because human E2 PDC subunits share similar epitope regions with those of *Escherichia coli* bacteria[17]. Notably, recurrent urinary tract infections in women are associated with PBC,[18] of which *E. coli* is a commonly detected pathogen. Thus, one concept (among many) involves *E. coli* PDC-E2 exposure leading to the development and production of AMA.[19,20] Although this may not be the only mechanism in the breakdown in immunologic tolerance in PBC (as other chemicals, infectious and drug triggers have been speculated about), AMA specificity to PDC-E2

hints at a potential contributory role of AMA and PDC-E2 in the immune-mediated pathophysiology of PBC. Notably, the destruction of biliary epithelial cells in PBC is mediated by infiltrative autoreactive T cells, of which some have found to be specific for PDC-E2.[21] Moreover, patients with PBC have abnormal expression of either PDC-E2 or cross-reacting molecules in the apical region of biliary epithelium.[22] However, the titer of AMA does not correlate with symptom duration, jaundice, or serum levels of ALP or immunoglobulins; the titer may fluctuate and fall with treatment, although without overt significance in terms of clinical outcomes.[23–26] Patients positive for immunoglobulin G (IgG) AMA may have significantly more severe disease (as defined by worse histology and elevated biochemical markers), whereas higher IgG and IgA AMA titers were associated with higher Mayo risk score; however, none of the isotypes or titer level was able to predict disease outcome.[26] Furthermore, mitochondria are present in all nucleated cells, including leukocytes and hepatocytes; yet the immune-mediated damage seen in PBC is focused on bile duct epithelial cells. Future research to further delineate the autoantigen and antibody interaction in bile duct epithelial ductal cells will be relevant to understanding the pathophysiology underpinning PBC.

Due to the specificity and sensitivity of AMA in PBC, AMA is an important criterion incorporated into diagnosing PBC. Thus, all patients being evaluated for unexplained chronic cholestasis or suspected PBC should have autoantibody testing for AMA (**Fig. 1**). Preferably, testing for AMA should be done using IFT on rodent kidney/liver tissue and confirmation of fluorescence pattern using human larynx epithelial cancer cell line (Hep-2) cells and solid-phase test systems such as enzyme-linked immunosorbent assays (ELISAs) using bovine or porcine heart mitochondrial fractions.[7] Confirmatory testing after IFT is usually required because other cytoplasmic antibodies (such as cardiolipin antibodies and anti-liver kidney muscle antibodies) can be misinterpreted for AMA.[27]

AMA may also be present for many years before the emergence of biochemical cholestasis and/or other PBC-associated symptoms. In the study by Dahlqvist and colleagues who followed AMA-positive patients without cholestasis or clinical evidence of liver disease, 9 of 92 patients developed clinical features of PBC (with a 5-year incidence rate of PBC of 16%).[28] Longitudinal follow-up of PBC patients with isolated AMA positivity demonstrate these patients often have less advanced histology compared with symptomatic patients at diagnosis, yet remain at risk for progression.[29] Notably, with increased availability of assays to evaluate for AMA, PBC-specific ANA, and other autoantibodies in the modern era, PBC is now often diagnosed at much earlier stages. Most recently, in the Swiss PBC Cohort Study, 24 of 30 patients (80%) with isolated positive PBC serology (with AMA and/or PBC-specific ANA) and normal ALP had histologic features of mild PBC, with 2 patients with Nakanuma stage 3 of 4 disease.[30] Notably, other liver enzymes were frequently mildly elevated. Initiation of UDCA treatment in early stage PBC may be beneficial, as evidenced by normalization of survival in early PBC patients to rates similar to the general population (including those with normal baseline ALP) with treatment.[31] Furthermore, a delay in starting UDCA treatment once PBC is diagnosed is associated with a lower probability of UDCA response, and thus, an increased risk for progression in disease.[32] These findings support the notion that patients who do not exhibit typical clinical cholestasis but have AMA positivity should be evaluated by a hepatologist with consideration for ultrasound and elastography (e.g., Fibroscan), as well as be evaluated for other cholestatic liver diseases (see **Fig. 1**). Thus, in patients with AMA positivity with normal liver enzymes, these patients usually remain with a mild PBC phenotype, but are at risk of PBC-associated complications and require risk stratification, ongoing follow-up with annual liver bloodwork, and monitoring.[29,33]

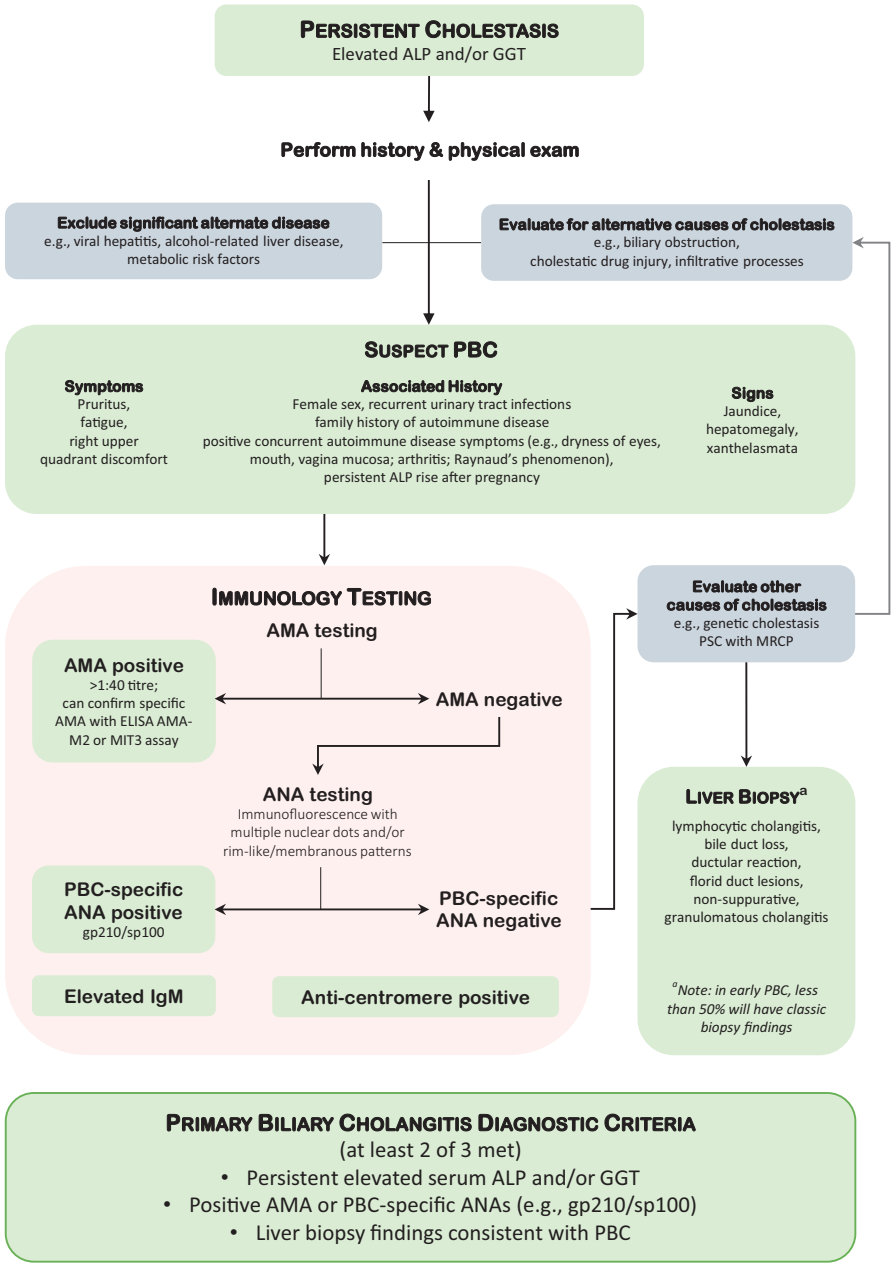

Fig. 1. Approach to persistent cholestasis and diagnosis of primary biliary cholangitis. ALP, alkaline phosphatase; AMA, antimitochondrial antibody; ANA, antinuclear antibody; GGT, gammaglutamyltransferase; IgM, immunoglobulin M; MRCP, magnetic resonance cholangiopancreatography; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

Although both sensitive and specific for PBC, AMA can be seen in other hepatic and nonhepatic conditions. AMA positivity has been reported in patients with acute liver failure of any cause,[13] as well as other liver conditions including autoimmune hepatitis (AIH), hepatitis C, and alcohol-related liver disease.[28,34] In one series examining sera in patients with acute liver failure from multiple causes, AMAs were detected in 33% of patients, with reactivity found against the same major antigens also seen in PBC.[13] The authors concluded that this finding provides support for the hypothesis that oxidative stress-induced liver injury can lead to the induction of AMAs. Interestingly, AMA positivity waned with time from the initial onset of injury with only one patient retaining AMA positivity at 24 months.[13] AMAs have also been found in other autoimmune conditions such as systemic lupus erythematosus (SLE), Sjogren syndrome, and chronic graft versus host disease after allogeneic stem cell transplantation.[28,35] It is unclear whether AMA serologies persisted in these patients due to sparse longitudinal data. Additionally for such studies, it must be recognized that different AMA assays are usually used making generalization difficult.

Antimitochondrial Antibody-Negative Primary Biliary Cholangitis and Primary Biliary Cholangitis-Specific Antinuclear Antibodies

Depending on the laboratory assays used, 5% to 17% of patients with PBC do not have AMA reactivity. Previously, several studies have demonstrated that this could be partially overcome by using recombinant proteins for 2-oxo-acid dehydrogenase complexes in ELISA or using IgG and IgA specific isotopes of AMA in an M2-enhanced performance ELISA (MIT3), which incorporates the 3 immunodominant epitopes recognized by AMA.[26,36–38] It is thought that AMA-negative PBC is a similar disease process to AMA-positive PBC involving abnormal expression of PDC-E2 and/or molecular mimicry of PDC-E2. Tsuneyama and colleagues demonstrated that sera from AMA-negative patients react similarly to sera from AMA-positive patients, with intense staining of the apical region of the bile duct epithelial cells for PDC-E2, suggesting similar disease processes.[39] It has been previously proposed that a worse prognosis exists for patients diagnosed with AMA-negative PBC compared with AMA-positive PBC, with reduced transplant-free survival[40] and worse bile duct damage around portal areas on histopathology with increased levels of B-cell infiltrates in early phases of bile duct damage.[41] It is unclear whether this reflects diagnostic delay or true pathogenic differences; as such, contemporary research is required to evaluate this further. In clinical practice, it is important to manage patients with PBC the same regardless of AMA serology.

Table 1
Prevalence, sensitivity, and specificity of primary biliary cholangitis autoantibodies

			PBC Diagnosis	
Autoantibody	**PBC Prevalence (%)**		**Sensitivity (%)**	**Specificity (%)**
AMA	95		73–100[10]	76–100[10]
ANA	50–56[42,105]		-	-
	AMA positive	*AMA negative*		
Anti-gp210	16–18[105]	15–45[42,105]	6–55[42,106]	62–100[42,50,106]
Anti-sp100	24–31[59,105]	13–54[42,105]	8–42[42,106]	64–100[42,50,106]
Anti-hexokinase-1	39–56[66,67]	12–40[66,67]	45[67]	95[67]
Anti-Kelch	19–26[66,67]	10–29[66,67]	25[67]	95[67]

In the search for identifying other autoantibodies associated with PBC, researchers have also documented ANA serology in PBC patients, with 50% to 56% of all PBC having ANA positivity, and up to 85% of AMA-negative PBC patients having ANA positivity.[42,43] Approximately half of AMA-negative PBC patients will have at least 1 of 3 PBC-specific ANA: namely sp100 (a transcription stimulating factor), promyelocytic leukemia (a transcription coactivator), or gp210 (a nuclear pore glycoprotein).[38,44,45] These antibodies carry high specificity for PBC (**Table 1**)[46] and are particularly useful in diagnosing suspected AMA-negative PBC because these patients may experience a delay in diagnosis, and thus, a delay in appropriate care and treatment (see **Fig. 1**). PBC-specific ANA can be detected by IFT on rodent tissue, followed by confirmation with Hep-2 cell staining in various staining patterns depending on the nuclear antigen target. Nuclear body antigens (eg, sp100) stain in a "multiple nuclear dots pattern", whereas nuclear envelope antigens (eg, gp210) stain with a "dotted or discontinuous rim-like/membranous pattern."[47–49] Previously, multiple nuclear dots and/or rim-like/membranous patterns were found in 31 of 101 (31%) AMA-positive patients and 17 of 22 (77%) AMA-negative patients.[50] Hep-2 cells are usually avoided as an initial test due to the presence of low-titer ANA antibodies in healthy subjects.[27] ELISA can also be used to confirm results of IFT.

The mechanisms behind development and production of ANA in PBC remains unknown but is thought to be related to molecular mimicry with environmental or pathogenic antigens similar to the development of AMA,[51] or to mimicry between the nuclear proteins gp210 and sp100 to the E2 subunit of the PDC.[52] Studies have demonstrated human leukocyte antigen (HLA) alleles (including DRB1*rs9277535, DRB1*03:01, DRB1*15:01, DRB1*01, and DPB1*03:01) with the presence of sp100 antibodies, suggesting a significant genetic predisposition with the sp100 autoantibody.[53] Moreover, a study in Japanese patients reported association between the HLA alleles DRB1*04:04 and DRB1*08:03 with the presence of anti-gp210 and anticentromere antibodies, respectively.[54] These specific ANA in PBC carry clinical significance. An sp100 autoantibody level may have prognostic utility with respect to the development of fibrosis on liver biopsy,[25] and positivity is associated with worse disease severity and worse prognosis in European studies[55,56]; however, this has not been consistently seen in other populations, particularly in Japan.[57] Of note, with treatment with UDCA, anti-sp100 has been shown to decrease in some patients (similar to AMA titer on UDCA treatment), suggesting modulation by UDCA in the response to the sp100 antigen; however, the clinical significance of this decrease in titer with treatment is not yet known.[58] In patients with high gp210 antibodies, these patients have worse cholestasis and impaired liver function[59] and are associated with more severe interface hepatitis lobular inflammation.[57,60] In a study evaluating serial anti-gp210 levels at diagnosis and with UDCA therapy, patients who had sustained anti-gp210 levels despite treatment were at increased risk of progressing to liver failure.[61] In another study evaluating newly diagnosed PBC patients, those with reactivity to gp210 and/or p62 (a nucleoporin) exhibited an unfavorable disease course characterized by decreased time to death, transplantation, and complication-free survival.[62] More recently, a study evaluating clinical utility of specific ANA in PBC found that anti-gp210 autoantibodies were significantly associated with elevated serum aminotransferase activity, bilirubin, and liver stiffness at presentation, as well as being independently associated with death or liver transplantation, even when accounting for other baseline determinants and UDCA treatment response.[42]

Besides the ANA described, other specific ANA targets in PBC include other nuclear body proteins such as sp140 and small ubiquitin-like modifiers, and nuclear envelope antigens such as the nucleoporin autophagy receptor p62.[48,59,63–65] Of note, patients

with PBC-specific ANA to nuclear body proteins often react to 2 to 3 proteins in the family, suggesting clustering of autoantigens. Their exact contribution to PBC pathophysiology remains to be elucidated in future studies.

Other Novel Autoantibodies in Primary Biliary Cholangitis

Although advances in IFT solid phase immunoassays can detect most patients with PBC, a small proportion of patients remain both AMA and ANA negative. More recently, anti-kelch-like 12 protein (anti-Kelch) and anti-hexokinase-1 antibodies have been identified as potential new biomarkers for PBC, with a pooled prevalence of 24.9% positive for anti-Kelch and 45.7% positive for anti-hexokinase-1 in AMA-positive PBC patients, and 19.2% positive for anti-Kelch and 24.7% positive for hexokinase-1 in AMA-negative PBC patients.[66,67] The Kelch protein family participate in numerous cellular processes, including cytoskeletal organization, ion channel gating, transcription suppression, and targeting of proteins for ubiquitination.[68] The higher prevalence of hexokinase-1 in PBC may be related to the fact that this enzyme is found on the outer membrane of mitochondria and is responsible for phosphorylating glucose, as well as being involved in the adaptive coupling of mitochondrial metabolism to cell survival and sensitivity to apoptosis.[69] Anti-hexokinase-1 status was associated with lower transplant-free survival and time to liver decompensation.[56] Other autoantibodies of interest include anti-p97/valosin containing protein antibodies that have been found in approximately 13% of PBC patients.; The presence of these autoantibodies seem to suggest a slower progressive disease course and decreased mortality; however, further studies are required as the data is very preliminary.[70]

Secondary Hepatitis in Patients with Primary Biliary Cholangitis (Overlap/Primary Biliary Cholangitis with Features of Autoimmune Hepatitis)

A common clinical scenario is one where a patient presents with features of both PBC and AIH, including elevated transaminase activity and cholestatic liver chemistry, who is found to have AMA positivity along with serology or histology with features of AIH. In a recent study by Haldar and colleagues, 32 of 499 PBC patients (6.4%) had features of PBC with clinically classified overlapping AIH.[42] Such patients have been reported to have suboptimal responses to UDCA therapy, higher rates of decompensated liver disease complications (including bleeding and ascites), and lower transplant-free survival.[71–73] Whether patients with features of both PBC and AIH represent a high-risk PBC phenotype versus the coexistence of 2 disease entities is debated, as some studies show these patients benefit from initiation of UDCA alone, whereas other studies show augmented results with the addition of immunosuppression.[74] It is key to recognize that bile acid metabolism can affect immune responses, raising the concept that hepatitis activity in cholestatic liver disease could be secondary to the underlying biliary process. Two relevant articles recently demonstrated how bile acids can impact the balance of Th17 and Treg cells through the production of mitochondrial reactive oxygen species and modulating expression of key transcription factors as well as function.[75,76] Future studies are required to further understand and characterize these patients in objective ways.

Currently hepatology societal guidance suggests concurrent AIH with PBC can be diagnosed if 2 of 3 criteria are present: (1) elevation of ALT levels greater than 5 times upper limit the normal (ULN), (2) elevation of serum IgG levels greater than 2 times ULN or positive anti-smooth muscle antibody (SMA), and (3) moderate-to-severe interface hepatitis on histology.[7,77] Of note, the presence of anti-double stranded DNA (dsDNA) or anti-p53 (an important tumor-suppressor protein) has been associated with PBC-AIH overlap. In particular, double positivity for AMA and anti-dsDNA are reported to

be present in 38% to 50% of AIH-PBC overlap patients, compared with 4% to 10% of PBC only patients and 26% of AIH only patients,[78–80] whereas autoantibodies to p53 were found in 50% of AIH-PBC overlap patients compared with 2% of PBC only patients.[79] Neither of these autoantibodies are specific to PBC, as anti-dsDNA are characteristically seen in SLE,[81] and anti-p53 are seen in variety of solid organ cancers[82] as well as SLE, rheumatoid arthritis, dermatomyositis, autoimmune thyroiditis, and type 1 diabetes.[79] Evaluation of other PBC-specific autoantibodies including anti-Kelch and anti-hexokinase-1 are not significantly associated with PBC-AIH overlap.[80] Further investigation and larger scale studies are required to evaluate the role of autoantibodies and other serum biomarkers in so-called PBC-AIH overlap.

Other Nonspecific Autoantibodies in Primary Biliary Cholangitis and Disease Associations

Patients with PBC will frequently test positive for other autoantibodies that associate with other rheumatological conditions, some of which may occasionally be concurrently present with PBC.[83] PBC is commonly associated with autoimmune thyroid disease; however, the presence of thyroid disease does not influence the natural history or progression of PBC.[83,84] Patients with PBC often have concurrent reactivity to thyroid disease antibodies, with increased anti-thyroglobulin antibody in 55% of patients and anti-thyroid peroxidase antibody in 46% of PBC patients without any known thyroid disease seen in one study.[85]

PBC is also associated with celiac disease, with variable prevalence of celiac disease diagnosis among PBC patients ranging from 1% to 12%.[86–88] However, the question as to whether to screen PBC patients for celiac disease is debated.[89] Some studies have demonstrated that not all PBC patients who screen positive for autoantibodies for celiac disease (such as anti-endomysium antibodies and transglutaminase antibodies) with have truly elevated titers of celiac autoantibodies, nor histologic patterns suggestive of celiac disease.[90,91] Bizzaro and colleagues concluded that a true association was present in only 2% of patients, and in most cases, false positives were due to substrate variability in the assay.

Commonly, patients with PBC also have autoantibodies for non-PBC-specific ANA and thrombophilia-associated autoantibodies.[92] Among the ANA family of autoantibodies, patients with PBC may exhibit positivity for anti-centromere, anti-nuclear envelope, anti-Sjögren's-syndrome-related antigen A (SSA) (both ro-52 and ro-60), anti-Sjögren's-syndrome-related antigen B (SSB) (La), anti-double stranded DNA (dsDNA), anti-single strand DNA (ssDNA), anti-histone, anti-topoisomerase I (aka. scl-70), anti-Smith, anti-Jo-1, and anti-U1RNP antibodies.[85,92,93] Anti-nuclear envelope antibodies target proteins of the nuclear lamina, the innermost layer of the nuclear envelope. These anti-lamin and anti-lamin receptor antibodies present with a smooth membrane fluorescence pattern on IFT in 6% to 9% of PBC patients[94]; however, their role is unclear, and anti-lamin antibodies are often also found in other autoimmune conditions such as SLE and chronic fatigue syndrome.[95] Anti-centromere antibodies present with a discrete speckled centromere pattern with IFT staining and are seen targeting the centromere–kinetochore macrocomplex in up to 30% of patients with PBC, while also being seen in a third of patients with systemic sclerosis (SSc) and 10% of those with Sjogren's syndrome.[47,57,96] In patients with both PBC and SSc, 9% to 30% have anti-centromere antibodies.[97–99] The importance of anti-centromere antibodies in PBC disease course is debated, with some data suggesting no difference, whereas others point to a higher risk for ductular reaction, progression to portal hypertension and cirrhosis.[57,100,101] It is also seen in practice that sometimes patients with AMA-

negative PBC are anti-centromere positive with consistent PBC histology, and in this scenario, anti-centromere reactivity helps reach the PBC diagnosis.

Thrombophilia-associated autoantibodies (such as those reacting to cardiolipin, beta2-glycoprotein 1, phosphatidylserine, and prothrombin) also have notable presence in sera of PBC patients, with a range of 2% to 70% positivity reported depending on the specific antigen target.[102–104] The presence of these thrombophilia-associated autoantibodies have been reported (but not validated) with later PBC stage, with anti-prothrombin IgM associated with worse prognosis.The underlying mechanisms have yet to be elucidated[92], and additional studies are required to validate these findings and evaluate this association further.

SUMMARY

Testing for autoantibodies is essential to the diagnosis of PBC, as well as evaluation of related disease processes and potential prognostic factors. Future efforts to further characterize autoantibody profiles in PBC will be important in advancing our understanding of the pathophysiological mechanisms while providing new ways to risk stratify patients and possibly insights into therapeutic targets.

CLINICS CARE POINTS

- PBC is an archetypal autoimmune disease. Patients can be diagnosed accurately by appropriate use of immune serology. In particular upto 90-95% of patients are anti-mitochondrial antibody positive.
- Where patients are AMA negative, other serologic profiles based on anti-nuclear antibody patterns can be diagnostic. Some serologic findings, in particular gp210 reactivity, are associated with worse prognosis for patients.

DISCLOSURE

No relevant disclosures for all authors.

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