Primary Biliary Cirrhosis (PBC), PBC Autoantibodies, and Hepatic Parameter Abnormalities in a Large Population of Systemic Sclerosis Patients

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ABSTRACT. ***Objective.*** To investigate the diagnostic accuracy of antimitochondrial antibodies (AMA), sp100, and gp210 antibodies for primary biliary cirrhosis (PBC) in a large population of patients with systemic sclerosis (SSc); to examine concordance of these antibodies with subsets of SSc. Further, to assess the association of SSc-related antibodies with hepatic parameter abnormalities.

Methods. We obtained medical records to verify the diagnoses of SSc and PBC. Sera from all participants were examined for the presence of SSc- and PBC-related antibodies, as well as for abnormalities in hepatic parameters.

Results. We examined 817 patients with SSc, of whom 16 (2%) had confirmed PBC. The sensitivity and specificity of AMA by a MIT3 ELISA for PBC were 81.3% and 94.6%, respectively. Sp100 had a sensitivity and specificity of 31.3% and 97.4%, respectively, while gp210 had an even lower sensitivity. We were able to detect all PBC cases using AMA(MIT3) and sp100 as a combined marker, resulting in a significantly improved sensitivity of 100% ($p = 0.042$) with an incremental decrease in specificity to 92.6%. Independent of AMA or sp100 status, there was an association of anticentromere B (CENP-B) and anti-topoisomerase antibodies (ATA) with higher alkaline phosphatase levels ($p = 0.051$ and $p = 0.003$, respectively) while anti-RNA polymerase III (anti-RNAP) was associated with lower alkaline phosphatase levels ($p = 0.019$) among the patients with SSc.

Conclusion. Utilization of AMA(MIT3) and sp100 antibodies as a combined diagnostic marker leads to an improved detection of PBC in patients with SSc. CENP-B and ATA are associated with alkaline phosphatase elevation. (First Release Sept 1 2009; J Rheumatol 2009;36:2250–6; doi:10.3899/jrheum.090340)

Key Indexing Terms:
LIVER CIRRHOSIS BILIARY SCLERODERMA SYSTEMIC SCLEROSIS

Scleroderma or systemic sclerosis (SSc) is an acquired, multisystem autoimmune disease characterized by organ fibrosis that can involve the skin, lungs, gastrointestinal tract, heart, or the musculoskeletal system; an obliterative vasculopathy primarily involving small blood vessels; and immune activation with production of disease-specific autoantibodies. SSc is classified into limited and diffuse forms based primarily on the extent of skin involvement[1]. Further, the 3 relatively specific anticentromere, anti-topoisomerase (ATA), and anti-RNA polymerase III (anti-RNAP) antibodies are biomarkers for distinct clinical manifestations and prognostic subsets of SSc[2]. The overall incidence of SSc in the United States adult population is approximately 19.3 per million new cases per year with a prevalence estimate of approximately 276 per million[3].

Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic disease characterized by destruction of intrahepatic bile ducts causing fibrosis and eventually cirrhosis of the liver. The cause of PBC is not known, but it is thought to be of an autoimmune origin. The incidence of PBC is estimated at 27 per million per year and prevalence is approximately 402 per million[4]. PBC often occurs in association with a variety of other autoimmune conditions[5-7]. Since its first description in 1970[7-9], a number of studies have found a high frequency of SSc in patients with PBC, estimating its

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prevalence at 2% to 18.2%[5-7,9-14]. These studies also indicated that PBC was most highly correlated with limited SSc and more specifically with anticentromere[12,13,15]. The only study investigating the prevalence of PBC in patients with SSc reported a frequency of 2.3% in 230 patients with SSc[12]. Another study comparing clinical and immunological characteristics of patients with SSc/PBC overlap and SSc alone indicated that the patients with overlap syndrome are more likely to have anticentromere, calcinosis, and telangiectasia[16]. There is no published evidence that SSc-related autoantibodies are associated with liver function abnormalities independent of the coexistence of PBC and antimitochondrial antibodies (AMA).

AMA are reported to be present in 80% to 96.5% of patients with PBC[6,7,14,17]. Previous studies indicated a higher frequency of other autoimmune diseases in patients with AMA-negative PBC in comparison to AMA-positive PBC cases[7,18]. Eighteen percent to 44% of patients with PBC have autoantibodies against sp100[19,20], a nuclear protein identified as a "multiple nuclear dots" pattern when examined by indirect immunofluorescence (IIF) microscopy. According to published reports, these antibodies are highly specific for PBC and have been detected in very few subjects without the disease[19-22]. In addition, gp210 autoantibodies, which are directed against a component of the nuclear pore complex, have been reported to have a sensitivity of 9.4% to 29% and a specificity of more than 99% for PBC[23-25]. Antibodies to gp210 are associated with a poor prognosis and clinical outcome[26]. The diagnostic accuracy of gp210 and sp100 antibodies for PBC has not been investigated in patients with SSc.

The purpose of our study was to investigate the diagnostic accuracy of AMA, sp100, and gp210 antibodies for PBC in patients enrolled in the Scleroderma Family Registry and DNA Repository, and to examine the concordance of these antibodies with clinical subsets of SSc, as well as SSc-related antibodies (anticentromere, ATA, and anti-RNAP). We also assessed the association of these SSc-related antibodies with abnormalities in hepatic parameters.

MATERIALS AND METHODS

Study population. All study subjects were enrolled in the Scleroderma Family Registry and DNA Repository. All patients either met the 1980 American College of Rheumatology preliminary criteria for the classification of SSc[27] or had at least 3 of the 5 CREST syndrome features (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias)[1]. The participants were recruited from across the US by referral from other physicians, clinical practices of the investigators, and appeals to the Scleroderma Foundation patient support groups. We obtained medical records on all participants to verify their diagnosis and to characterize the disease. The registry databases also had clinical disease characteristics, including information on the extent of organ system involvement, and date of disease onset. The repository contained DNA, serum, and plasma samples. All study subjects provided written informed consent and the study was approved by the institutional review board of the University of Texas Health Science Center at Houston. The funding sources had no role in the study design, conduct, analysis, or reporting of the results.

We also screened all available medical records for chart diagnosis of PBC; additional documents for confirmation of this diagnosis were requested if necessary. Further, all patients who had AMA and an alkaline phosphatase elevation on our laboratory testing were asked to undergo a gastroenterological evaluation, and the results of this assessment were followed up. The diagnosis of PBC was made if 2 or more of the following criteria were present: positive AMA, increased hepatic enzymes suggesting cholestatic pattern for more than 6 months (mainly alkaline phosphatase), and a compatible or diagnostic liver biopsy[28].

The patients were classified as having limited or diffuse cutaneous SSc according to published criteria[1]. SSc-associated pulmonary fibrosis was defined as presence of typical findings on chest high-resolution computerized tomography (CT), regular chest CT or radiograph, or restrictive pattern on pulmonary function test. Pulmonary hypertension was defined as estimated peak right ventricular systolic pressure ≥ 40 mm Hg on echocardiography or pulmonary arterial systolic pressure ≥ 40 mm Hg by right-heart catheterization. Myositis was defined as inflammatory myositis referenced in patient's chart or objective muscle weakness and elevated creatine kinase levels. Scleroderma renal crisis was characterized by presence of new-onset accelerated systemic hypertension with evidence of renal impairment.

Autoantibody determination. Autoantibodies were detected with commercially available kits. Antinuclear antibody (ANA) testing was performed by IIF using HEp-2 cell substrates (Antibodies Inc., Davis, CA, USA). The CENP-B and ATA were determined utilizing a line immunoassay (recomLINE Scleroderma, Mikrogen GmbH, Neuried, Germany); the anti-RNAP results were obtained by ELISA (Quanta Lite ELISA, Inova Diagnostics, San Diego, CA, USA). AMA was detected by IIF on HEp-2 cell substrate and by ELISA using an M2 enhanced (MIT3) assay (Inova Diagnostics) based on the triple hybrid fusion MIT3 antigen, which contains the 3 immunodominant epitopes recognized by AMA[29]. Further, the gp210 and sp100 antibodies were ascertained by ELISA (Quanta Lite ELISA, Inova Diagnostics). The cutoff for MIT3, sp100, and gp210 assays was 25 units (negative ≤ 20, equivocal 20.1–24.9; and positive ≥ 25 units). In addition, levels of bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase were determined in all subjects using standard protocol at Clinical Pathology Laboratories, Inc., Austin, TX. Laboratory personnel were not aware of the subjects' primary diagnoses.

Statistical analysis. The analysis of categorical values was conducted by chi-squared test or by Fisher's exact test if the values were < 5. Continuous variables were analyzed by t-test. If the assumption of equal variance was not met we used the Aspin-Welch unequal variance test. The concordance between measurements was investigated utilizing the McNemar test. Linear regression analysis was used to adjust for the effect of multiple independent variables; an extended model that included an interaction term did not show any interaction between the investigated variables. Two-sided p values < 0.05 were considered significant. The analyses were performed utilizing the NCSS 2007 statistical program (NCSS, Kaysville, UT, USA).

RESULTS

The serum samples were collected from May 2001 to January 2007. At the time of analysis, 817 patients with SSc were enrolled in our study. PBC was present in 16 cases (2%). In this group of patients, a gastroenterologist evaluated 15 (93.8%) subjects for confirmation of PBC diagnosis. Moreover, 10 (62.5%) had undergone a liver biopsy and had biopsy results compatible with or diagnostic of PBC; 5 (31.3%) patients were given the diagnosis of PBC by their gastroenterologist based on the presence of AMA and persistent alkaline phosphatase elevation. One patient with PBC was deceased at the time of our laboratory testing but had AMA and persistent alkaline phosphatase and gamma-

glutamyl transferase elevation that could not be explained otherwise. Table 1 shows the clinical and serological features of the patients with PBC.

All SSc and PBC cases fulfilled the requirements for entry into our study. Demographic features of patients with SSc alone and with SSc/PBC overlap are shown in Table 2. The SSc/PBC overlap cases were significantly older (mean age 62.7 vs 53.77 yrs; p = 0.007) and had a longer disease duration at the study entry (mean 16.21 vs 9.02 yrs; p = 0.002). All SSc/PBC overlap cases were women, whereas the female to male ratio in the SSc-alone group was 8 to 1. Further, 94% of overlap cases were Caucasian while 79% of patients in the SSc-alone group were Caucasian. In both patient groups the majority of subjects had limited SSc, but the frequency of limited cutaneous skin involvement was higher in the SSc/PBC group (81% vs 59%).

The majority of patients in the overlap and SSc-alone groups were ANA-positive (88% and 90%, respectively). In the overall population, 56 (7%) patients had AMA(MIT3) antibodies. Only 3 patients had gp210 antibodies (1 in SSc/PBC, 2 in SSc-alone group) whereas 26 patients had sp100 antibodies (5 in SSc/PBC, 21 in SSc-alone group). In the SSc/PBC group, all patients had anticentromere B antibodies (CENP-B); 1 patient also had ATA. The CENP-B positivity was strongly associated with PBC (p < 0.001, OR 81.82, 95% CI 4.89–1369.45), whereas ATA and anti-RNAP were not associated with PBC (p = 0.224 and p = 0.09, respectively). The association of PBC with limited SSc did not reach statistical significance (p = 0.12, OR 2.63, 95% CI 0.78–13.14). Table 3 shows frequency of investigated antibodies in the SSc-alone and SSc/PBC overlap groups.

AMA as detected by IIF had a sensitivity of only 43.8% but a high specificity of 98.9%, whereas the sensitivity and specificity of AMA by (MIT3) ELISA for PBC was 81.3%

Table 1. Clinical and serological features of patients with primary biliary cirrhosis (PBC).

Patient	SSc antibodies	Alkaline Phosphatase	AMA(MIT3)*	sp100	gp210	PBC by Liver Biopsy
1	CENP-B**	Abnormal	–	+	–	Yes
2	CENP-B	Abnormal	+	–	–	ND
3	CENP-B	Abnormal	+	–	–	Yes
4	CENP-B, ATA†	Abnormal	+	–	–	ND
5	CENP-B	Abnormal	+	–	+	Yes
6	CENP-B	Abnormal	+	–	–	Yes
7	CENP-B	Abnormal	+	+	–	Yes
8	CENP-B	Abnormal	+	–	–	ND
9	CENP-B	Abnormal	+	+	–	ND
10	CENP-B	Abnormal	+	–	–	Yes
11	CENP-B	Abnormal	+	–	–	Yes
12	CENP-B	Abnormal	–	+	–	Yes
13	CENP-B	Normal††	+	–	–	Yes
14	CENP-B	Abnormal	–	+	–	Yes
15	CENP-B	Abnormal	+	–	–	ND
16	CENP-B	Abnormal	+	–	–	ND

* Antimitochondrial antibodies by MIT3 assay. ** Anticentromere B antibodies. † Antitopoisomerase I. †† Patient was treated with ursodiol. SSc: systemic sclerosis. ND: not done.

Table 2. Demographic features of the study subjects.

Characteristic	SSc/PBC Overlap, n = 16	SSc Alone, n = 801	All Subjects, n = 817
Mean age at enrollment (SD), yrs	62.7 (7.81)	53.77 (13.12)	53.95 (13.1)*
Mean disease duration (SD), yrs	16.21 (9.49)	9.02 (7.85)	9.16 (7.94)†
N female/N male	16/0	709/92	725/92
Race (%)			
Caucasian	15 (94)	632 (79)	647 (79)
African American	0	75 (9)	75 (9)
Hispanic	1 (6)	67 (8)	68 (8)
Other	0	27 (3)	27 (3)
Limited SSc (%)	13 (81)	474 (59)	487 (60)
Diffuse SSc (%)	3 (19)	323 (40)	326 (40)

* p = 0.007. † p = 0.002. SSc: systemic sclerosis; PBC: primary biliary cirrhosis.

Table 3. Frequency of autoantibodies.

	SSc/PBC Overlap, n = 16 (%)	SSc Alone, n = 801 (%)	All Subjects, n = 817 (%)
AMA by IIF	7 (44)	9 (1)	16 (2)
AMA by MIT3	13 (81)	43 (5)	56 (7)
ELISA			
sp100	5 (31)	21 (3)	26 (3)
gp210	1 (6)	2 (0.3)	3 (0.4)
ANA	14 (88)	721 (90)	735 (90)
CENP-B	16 (100)	230 (29)	246 (30)
ATA	1 (6)	155 (19)	156 (19)
Anti-RNAP	0	145 (18)	145 (18)

AMA: antimitochondrial antibodies; IIF: indirect immunofluorescence; CENP-B: anticentromere B antibodies; ATA: antitopoisomerase I antibodies; Anti-RNAP: antiRNA-polymerase III antibodies; ANA: antinuclear antibodies.

and 94.6%, respectively. These 2 tests were highly concordant (p = 0.001). Further, 3 out of 16 (18.7%) SSc patients with PBC did not have AMA(MIT3) antibodies. The sensitivity and specificity of gp210 for PBC was 6.3% and 99.8%, respectively, while sp100 had a sensitivity of 31.3% and specificity of 97.4% for PBC. Using gp210 and AMA(MIT3) as a combined marker did not increase the diagnostic accuracy for PBC. Utilization of a similar combination of AMA(MIT3) and sp100 detected all PBC cases, resulting in a sensitivity of 100%, which was significantly higher than the sensitivity of AMA(MIT3) alone (p = 0.042). This combined diagnostic tool had only a slightly lower specificity than AMA(MIT3) alone (92.6% vs 94.6%). The diagnostic accuracy of PBC-related antibodies for PBC in our population is shown in Table 4.

In a subgroup analysis, we calculated the diagnostic accuracy of AMA, sp100, and gp210 for only biopsy-confirmed PBC cases (n = 10). AMA as detected by IIF had a sensitivity of 50% with specificity of 98.6%, whereas the sensitivity and specificity of AMA by (MIT3) ELISA for PBC was 70% and 93.9%, respectively. The sensitivity and specificity of gp210 for PBC was 10% and 99.8%, respectively, while sp100 had a sensitivity of 40% and specificity of 97.3% for PBC. Moreover, AMA(MIT3) and sp100 as a combined diagnostic tool detected all PBC cases, with sensitivity and specificity of 100% and 91.9%, respectively.

Table 4. Diagnostic accuracy of PBC-related antibodies in SSc.

	Sensitivity, %	Specificity, %	LR+	LR–
AMA by IIF	43.8	98.9	39.9	0.6
AMA by MIT3 ELISA	81.3	94.6	15.1	0.2
sp100	31.3	97.4	11.9	0.7
gp210	6.3	99.8	25	0.9
AMA by MIT3 ELISA or sp100	100	92.6	13.6	0

AMA: antimitochondrial antibodies; IIF: indirect immunofluorescence; LR: likelihood ratio.

CENP-B was strongly associated with AMA(MIT3) positivity (p = 0.001, OR 3.41, 95% CI 1.97–5.89), while ATA and anti-RNAP were not associated with this antibody (p = 0.05, OR 0.43, 95% CI 0.12–1.01 and p = 0.15, OR 0.54, 95% CI 0.23–1.25). Upon further examination, sp100 positivity was associated with CENP-B (p = 0.001, OR 4.64, 95% CI 2.08–10.34) but not with the other SSc-related autoantibodies. We did not find any association between gp210 and any of the 3 SSc-related autoantibodies.

Because all SSc/PBC cases were CENP-B-positive, we compared the clinical features of 16 CENP-B-positive overlap cases with those of CENP-B-positive patients who did not have PBC. The frequency of interstitial lung disease, pulmonary arterial hypertension, cutaneous calcinosis, telangiectasia, thyroid disease, myositis, Raynaud's phenomenon, gastroesophageal reflux disease, fatigue, Sjögren's syndrome, and digital ulcers, pits or amputation did not differ significantly between these 2 groups (data not shown).

In the second part of our study, we investigated the relationship of hepatic parameters at enrollment with SSc-related antibodies among all patients with SSc. An elevated alkaline phosphatase was observed in 39 patients. Our univariate analysis of relationship between SSc-related antibodies and alkaline phosphatase abnormality revealed that CENP-B and ATA were associated with higher levels of alkaline phosphatase (p = 0.008 and p = 0.031, respectively), whereas anti-RNAP was associated with lower alkaline phosphatase levels (p = 0.001). Even after adjusting for the combined marker of AMA(MIT3) or sp100 positivity, there was still an association of CENP-B and ATA with higher alkaline phosphatase levels (p = 0.051 and p = 0.003), while anti-RNAP was associated with lower alkaline phosphatase values (p = 0.019). The relationship between alkaline phosphatase and SSc-related antibodies is shown in Table 5. In our study, 45 patients had an elevated AST; only 8 of these subjects had simultaneously an elevation of alkaline phosphatase. Further, 4 of these 8 subjects (50%) had PBC. Only 11 patients had an elevated ALT, of whom 4 also had an alkaline phosphatase elevation and 1 had PBC. The SSc-related antibodies did not show any relationship to either AST or ALT elevation (data not shown). Only 1 patient had a borderline elevation of total bilirubin level.

DISCUSSION

This is the first study that has specifically investigated PBC, PBC-related autoantibodies, and hepatic parameter abnormalities in a large population of patients with SSc. The prevalence of PBC in our study was 2%, similar to the only other reported prevalence of 2.3% in a population of 230 patients with SSc[12]. Considering that the prevalence of PBC in the general population is 0.04%[4], this finding confirms

Table 5. Alkaline phosphatase in relationship to SSc-related autoantibodies.

	Mean Difference, IU/l*	95% CI	p	Adjusted Mean Difference, IU/l*	Adjusted 95% CI†	Adjusted p†
CENP-B	9.50	2.52 to 16.47	0.008	5.50	–0.03 to 11.03	0.051
ATA	7.23	0.63 to 13.84	0.031	9.56	3.19 to 15.93	0.003
Anti-RNAP	–9.33	–14.01 to 4.66	0.001	–7.87	–14.4 to –1.34	0.019

* Mean difference in alkaline phosphatase levels between antibody-positive and negative patients. † Adjusted in a bivariate model for the combined marker of MIT3 or sp100. CENP-B: anticentromere B antibodies; ATA: anti-topoisomerase I antibodies; Anti-RNAP: anti-RNA-polymerase III antibodies.

the relatively high prevalence of PBC in patients with SSc. The association of PBC with anticentromere antibodies confirms similar findings[12,13,15], while the association of PBC with limited cutaneous involvement did not reach statistical significance in our population. This finding is supported by observations that certain SSc-related autoantibodies, which tend to be mutually exclusive, are strongly associated with various clinical subsets of scleroderma[30]. Further, the gene association and familial aggregation studies suggest that genetic factors exert their influence primarily on autoantibody expression in SSc[31-34]. While multiple studies have reported an association between anticentromere and PBC[12,13,15], Whyte, *et al* reported that there was no evidence of shared antigenic targets between AMA and anticentromere C antibodies (ACA), concluding that AMA and ACA represent discrete autoantibody populations that may coexist but do not show any cross-reactivity[35]. Moreover, we observed an association of sp100 antibodies with CENP-B; this finding has not been previously reported.

Despite utilizing an enhanced performance AMA(MIT3) ELISA for determination of AMA, which has a higher sensitivity for AMA detection than immunofluorescence-based methods[36], AMA(MIT3) antibodies in our study population had a sensitivity for PBC of 81.3%, which falls in the lower range of previously reported sensitivity of 80% to 96.5%[6,7,14,17]. In a study comparing the clinical features of SSc/PBC overlap cases to patients with PBC alone, AMA had a sensitivity of 93% in the overlap group compared to 94% in the PBC-alone group. This study was performed in a tertiary referral center for PBC; in addition, the cases and controls were matched for the serum bilirubin concentration at the initial visit, which might have masked differences in the baseline characteristics between the 2 comparison groups[15]. A study comparing the clinical features of AMA-negative to AMA-positive PBC in 5805 Japanese patients found that AMA-negative cases had a higher frequency of other associated autoimmune diseases in addition to lower serum levels of alkaline phophatase, gamma-glutamyl transferase, and IgM[18]. A population-based study conducted in England also reported a higher frequency of other associated autoimmune diseases in AMA-negative PBC cases[7].

Nakamura, *et al* investigated the predictive role of PBC-associated autoantibodies in PBC[26]. In this study, gp210 and anticentromere antibodies were associated with 2 different PBC progression types: anticentromere antibodies were predictive for development of portal hypertension, whereas gp210 antibodies were associated with progression to hepatic failure; sp100 antibodies were not predictive of any investigated clinical outcomes. That study did not report the frequency of coexistence of these antibodies. Further, multiple groups have reported that gp210 antibodies in PBC are associated with more severe disease and a higher rate of progression to endstage hepatic failure[37-40]. Rigamonti, *et al*, comparing the clinical features of SSc/PBC overlap with PBC alone, reported that the SSc/PBC group had a slower rate of bilirubin increase and a lower rate of liver transplantation or liver-related deaths than the PBC-alone group[15]. That study did not determine the frequency of gp210 antibodies in these 2 groups. The sensitivity of gp210 for PBC was lower in our population than in previous reports; we found a sensitivity of only 6.3%, whereas previous studies report a sensitivity of 9.4% to 26%. This finding might be explained by the fact that all our SSc/PBC overlap cases were CENP-B-positive. The lower frequency of gp210 antibodies in SSc/PBC patients might partially explain the milder course of PBC in this group of patients.

The sensitivity of sp100 for PBC in the investigated population was similar to published studies. In our study population, a combined diagnostic marker of AMA(MIT3) and sp100 was able to detect all PBC cases, resulting in a significantly improved sensitivity in comparison to AMA(MIT3) alone (100% vs 81.3%). This finding suggests that determination of sp100 in AMA-negative SSc patients will lead to an improved detection of PBC. Although sp100 had a high specificity of 97.4%, an indiscriminate use of this antibody in patients with SSc will lead to a high number of false-positive results, because PBC has a relatively low prevalence of 2% in this population. Therefore we recommend that sp100 should be ordered only in AMA-negative patients that have a clinical picture consistent with PBC, such as unexplained and persistent cholestasis.

There were no differences in the other clinical manifestations, including the sicca symptoms and Sjögren's syndrome, among CENP-B-positive patients in the 2 study groups. Our study might have been underpowered to detect

more minor clinical differences between the SSc/PBC overlap and SSc-alone groups.

We could not investigate whether the SSc patients with PBC-related antibodies but no clinical evidence of PBC will develop PBC over time; the specificity of PBC-related antibodies will increase further if some of these patients develop PBC in the future. Moreover, we relied on outside pathologists' evaluation of liver biopsies because the logistical limitations of our registry precluded an independent confirmation of liver biopsy results by a pathologist at our site. This shortcoming was offset by the fact that almost all our patients had been evaluated by a gastroenterologist. In our study, not all PBC cases were confirmed by a liver biopsy, but a subgroup analysis with biopsy-confirmed PBC cases showed very similar results for the diagnostic accuracy of the PBC-related antibodies. Considering the low prevalence of SSc in the general population and the fact that only 2% of SSc cases have PBC, the conduct of a large-scale SSc study where all PBC cases are confirmed in a uniform fashion regardless of their gastroenterologists' discretion is very difficult.

To our knowledge, the prevalence of hepatic parameter abnormalities in SSc and their association with SSc-related autoantibodies have not been reported in any other study. Our data suggest that anticentromere and ATA are associated with an alkaline phosphatase elevation, while anti-RNAP are linked to lower alkaline phosphatase levels among patients with SSc. This finding remained valid even after adjustment for the effect of AMA(MIT3) or sp100 positivity. The SSc-related antibodies were not associated with an AST or ALT elevation, suggesting that these associations are mainly caused by a hepatic cholestasis. This finding might be related to differences in the pathophysiology, comorbidities, or medication regimens of SSc serological subtypes. The clinical significance of these associations is unclear at this point.

Our findings confirm the relatively high prevalence of PBC in SSc, and suggest that the utilization of AMA(MIT3) and sp100 antibodies as a combined diagnostic tool improves the relatively low sensitivity of AMA(MIT3) antibodies for detection of PBC in this population. Further, our data indicate that anticentromere and ATA are associated with alkaline phosphatase elevation.

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REFERENCES

1. Leroy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202-5.
2. Silver RM, Medsger TA Jr. Systemic sclerosis and scleroderma variants: Clinical aspects. In: Koopman WJ, Moreland LW, editors. Arthritis and allied conditions. Philadephia: Lippincott Williams & Wilkins; 2005:1633-80.
3. Mayes MD, Lacey JV Jr, Beebe-Dimmer J, Gillespie BW, Cooper B, Laing TJ, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum 2003;48:2246-55.
4. Feld JJ, Heathcote EJ. Epidemiology of autoimmune liver disease. J Gastroenterol Hepatol 2003;18:1118-28.
5. Gershwin ME, Selmi C, Worman HJ, Gold EB, Watnik M, Utts J, et al. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. Hepatology 2005;42:1194-202.
6. Marasini B, Gagetta M, Rossi V, Ferrari P. Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. Ann Rheum Dis 2001;60:1046-9.
7. Watt FE, James OF, Jones DE. Patterns of autoimmunity in primary biliary cirrhosis patients and their families: a population-based cohort study. QJM 2004;97:397-406.
8. Murray-Lyon IM, Thompson RP, Ansell ID, Williams R. Scleroderma and primary biliary cirrhosis. Br Med J 1970;3:258-9.
9. Reynolds TB, Denison EK, Frankl HD, Lieberman FL, Peters RL. Primary biliary cirrhosis with scleroderma, Raynaud's phenomenon and telangiectasia. New syndrome. Am J Med 1971;50:302-12.
10. Bernstein RM, Callender ME, Neuberger JM, Hughes GR, Williams R. Anticentromere antibody in primary biliary cirrhosis. Ann Rheum Dis 1982;41:612-4.
11. Clarke AK, Galbraith RM, Hamilton EB, Williams R. Rheumatic disorders in primary biliary cirrhosis. Ann Rheum Dis 1978;37:42-7.
12. Jacobsen S, Halberg P, Ullman S, Van Venrooij WJ, Hoier-Madsen M, Wiik A, et al. Clinical features and serum antinuclear antibodies in 230 Danish patients with systemic sclerosis. Br J Rheumatol 1998;37:39-45.
13. Powell FC, Schroeter AL, Dickson ER. Primary biliary cirrhosis and the CREST syndrome: a report of 22 cases. Q J Med 1987;62:75-82.
14. Sakauchi F, Mori M, Zeniya M, Toda G. A cross-sectional study of primary biliary cirrhosis in Japan: utilization of clinical data when patients applied to receive public financial aid. J Epidemiol 2005;15:24-8.
15. Rigamonti C, Shand LM, Feudjo M, Bunn CC, Black CM, Denton CP, et al. Clinical features and prognosis of primary biliary cirrhosis associated with systemic sclerosis. Gut 2006;55:388-94.
16. Akimoto S, Ishikawa O, Muro Y, Takagi H, Tamura T, Miyachi Y. Clinical and immunological characterization of patients with systemic sclerosis overlapping primary biliary cirrhosis: a comparison with patients with systemic sclerosis alone. J Dermatol 1999;26:18-22.
17. van de Water J, Cooper A, Surh CD, Coppel R, Danner D, Ansari A, et al. Detection of autoantibodies to recombinant mitochondrial proteins in patients with primary biliary cirrhosis. N Engl J Med 1989;320:1377-80.
18. 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.
19. Evans J, Reuben A, Craft J. PBC 95k, a 95-kilodalton nuclear autoantigen in primary biliary cirrhosis. Arthritis Rheum 1991;34:731-6.
20. Zuchner D, Sternsdorf T, Szostecki C, Heathcote EJ, Cauch-Dudek K, Will H. Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology 1997;26:1123-30.
21. Guldner HH, Szostecki C, Grotzinger T, Will H. IFN enhance expression of Sp100, an autoantigen in primary biliary cirrhosis. J Immunol 1992;149:4067-73.

22. Szostecki C, Guldner HH, Netter HJ, Will H. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J Immunol 1990;145:4338-47.
23. Bandin O, Courvalin JC, Poupon R, Dubel L, Homberg JC, Johanet C. 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 1996;23:1020-4.
24. Lassoued K, Brenard R, Degos F, Courvalin JC, Andre C, Danon F, et al. Antinuclear antibodies directed to a 200-kilodalton polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patients with primary biliary cirrhosis. Gastroenterology 1990;99:181-6.
25. Nickowitz RE, Wozniak RW, Schaffner F, Worman HJ. Autoantibodies against integral membrane proteins of the nuclear envelope in patients with primary biliary cirrhosis. Gastroenterology 1994;106:193-9.
26. Nakamura M, Kondo H, Mori T, Komori A, Matsuyama M, Ito M, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. Hepatology 2007;45:118-27.
27. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
28. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med 2005;353:1261-73.
29. Moteki S, Leung PS, Coppel RL, Dickson ER, Kaplan MM, Munoz S, et al. Use of a designer triple expression hybrid clone for three different lipoyl domain for the detection of antimitochondrial autoantibodies. Hepatology 1996;24:97-103.
30. Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum 2005;35:35-42.
31. Arnett FC. HLA and autoimmunity in scleroderma (systemic sclerosis). Int Rev Immunol 1995;12:107-28.
32. Assassi S, Arnett FC, Reveille JD, Gourh P, Mayes MD. Clinical, immunologic, and genetic features of familial systemic sclerosis. Arthritis Rheum 2007;56:2031-7.
33. Gourh P, Tan FK, Assassi S, Ahn CW, McNearney TA, Fischbach M, et al. Association of the PTPN22 R620W polymorphism with anti-topoisomerase I- and anticentromere antibody-positive systemic sclerosis. Arthritis Rheum 2006;54:3945-53.
34. Reveille JD, Fischbach M, McNearney T, Friedman AW, Aguilar MB, Lisse J, et al. Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants. Semin Arthritis Rheum 2001;30:332-46.
35. Whyte J, Hough D, Maddison PJ, McHugh NJ. The association of primary biliary cirrhosis and systemic sclerosis is not accounted for by cross reactivity between mitochondrial and centromere antigens. J Autoimmun 1994;7:413-24.
36. Gabeta S, Norman GL, Liaskos C, Papamichalis PA, Zografos T, Garagounis A, et al. Diagnostic relevance and clinical significance of the new enhanced performance M2 (MIT3) ELISA for the detection of IgA and IgG antimitochondrial antibodies in primary biliary cirrhosis. J Clin Immunol 2007;27:378-87.
37. Nakamura M, Shimizu-Yoshida Y, Takii Y, Komori A, Yokoyama T, Ueki T, et al. Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis. J Hepatol 2005;42:386-92.
38. Itoh S, Ichida T, Yoshida T, Hayakawa A, Uchida M, Tashiro-Itoh T, et al. Autoantibodies against a 210 kDa glycoprotein of the nuclear pore complex as a prognostic marker in patients with primary biliary cirrhosis. J Gastroenterol Hepatol 1998;13:257-65.
39. Invernizzi P, Podda M, Battezzati PM, Crosignani A, Zuin M, Hitchman E, et al. Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. J Hepatol 2001;34:366-72.
40. Muratori P, Muratori L, Ferrari R, Cassani F, Bianchi G, Lenzi M, et al. Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. Am J Gastroenterol 2003;98:431-7.