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# Evaluation of the primary biliary cholangitis-related serologic profile in a large cohort of Belgian systemic sclerosis patients

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## Abstract

**Background:** Systemic sclerosis (SSc) and primary biliary cholangitis (PBC) are autoimmune diseases that may occur concomitantly and are both strongly associated with disease-specific autoantibodies. This study investigated the prevalence and fine specificity of PBC-specific serology (PBC-Ab) and associations with the SSc-subtypes and SSc-specific antibodies as well as the association with cholestatic liver enzymes. Furthermore, three different techniques for the detection of PBC-Ab were compared.
**Methods:** Serum of 184 Belgian SSc patients with a known SSc-antibody profile, was analyzed for PBC-Ab (anti-mitochondrial antibodies [AMA], anti-Gp210, anti-Sp100 and anti-PML) using indirect immunofluorescence (IIF) analysis on human epithelioma-2000 (HEp-2000) cells (ANA-IIF, Immunoconcepts) and liver-kidney-stomach tissue sections (IIF-LKS) (Menarini), and a line immunoblot (LB) (EuroImmun). Alkaline phosphatase/γ-glutamyl transferase (ALP/GGT) were evaluated at time of first sampling (t0) and after 3 years of follow-up (t3).
**Results:** PBC-Ab were present in 13% of patients and significantly correlated with centromere antibodies (anti-CENP-B), but not correlated with the limited cutaneous SSc subgroup (lcSSc). The most frequent reactivities were AMA (11%, with 9% AMA-M2) and Sp-100 antibodies (5%), showing a major overlap. There was no relevant association between the presence of PBC-Ab and ALP or GGT elevation at t0 nor at t3. Detection of AMA with IIF-LKS is comparable to LB. ANA-IIF screening was less sensitive compared to LB.
**Conclusions:** A wide range of PBC-Ab is detectable in SSc in the absence of cholestatic liver enzyme elevations, even after 3 years of follow-up. However, as these antibodies may precede PBC-disease up to 10 years further prospective follow-up of our cohort will be necessary.

**Keywords:** alkaline phosphatase; antimitochondrial antibodies; primary biliary cholangitis; systemic sclerosis.

## Introduction

Systemic sclerosis (SSc) and primary biliary cholangitis (PBC) are chronic auto-immune diseases that may occur concomitantly. SSc is a rare connective tissue disease characterized by a triad of fibrosis of the skin and internal organs, vasculopathy and immunological abnormalities. The disease has a very heterogeneous clinical presentation and patients are classically subclassified based on the extent of skin thickening into limited cutaneous SSc (lcSSc), affecting skin distal from the elbow and knee joints, and diffuse cutaneous SSc (dcSSc), affecting skin of the face, trunk and both proximal and distal extremities [1]. PBC is a cholestatic liver disease with chronic destructive non-suppurative cholangitis of the intrahepatic bile ducts, which may progress, via a fibrotic stage, to cirrhosis in a minority of patients. Both diseases are strongly associated with the presence of disease-specific autoantibodies [2, 3].

In SSc, antinuclear antibodies (ANA) are present in about 95% of patients [4]. In routine practice, serum is

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screened for the presence of ANA by indirect immunofluorescence (IIF) on human epithelioma-2(000) (HEp-2(000)) cells. After a positive ANA IIF result, further elaboration for the specific antigenic targets (anti-ENA) mostly reveals one of the following antibodies: anti-centromere (anti-CENP-B), anti-DNA topoisomerase I (anti-Scl70), anti-fibrillarin, anti-PM/Scl or anti-RNA polymerase III (anti-RNAPIII). These autoantibodies are highly SSc-specific and mutually exclusive. Furthermore, these antibodies are of prognostic importance due to their association with specific disease manifestations, i.e. anti-CENP-B positivity is linked to lcSSc and pulmonary arterial hypertension, anti-Scl70 positivity is linked to dcSSc and interstitial lung disease and anti-RNAPIII is linked to dcSSc, malignancies and scleroderma renal crisis [5]. Today, several commercial multiparameter assays exist to detect these SSc-specific antibodies (SSc-Ab).

Antimitochondrial antibodies (AMA) are the serological hallmark of PBC, with mostly AMA-M2 type (targeting the proteins of the alpha-keto acid dehydrogenase complexes) considered clinically relevant. AMA occur in 90–95% of PBC patients and may be present years before disease onset. Furthermore, 90% of asymptomatic patients positive for AMA express histological features compatible with PBC [6, 7]. The guidelines of the European Association for the Study of the Liver (EASL) prescribe that patients with AMA are at risk for developing PBC and recommend yearly follow-up of the cholestatic liver tests alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) for these patients [3]. In routine practice, screening for AMA is mostly performed by IIF on liver-kidney-stomach (LKS) tissues. In the case of a characteristic fluorescence pattern, the presence of AMA-M2 is confirmed with a specific immunoassay. Additionally, the presence of AMA may be suspected if a coarse granular filamentous staining throughout the cytoplasm is observed in the ANA IIF analysis (ICAP pattern AC-21) [8]. Other PBC-specific antibodies (PBC-Ab) include antibodies against Sp100, Gp210 and PML [9]. Sp100 and PML are nuclear proteins associated with a "multiple nuclear dots" pattern when examined by IIF on HEp-2(000) cells (ICAP pattern AC-6) [8]. Gp210 may be visible as a membranous pattern (ICAP pattern AC-12) [8]. Similar as for serological diagnosis of SSc, different commercial multiparameter immunoassays are available to detect the different PBC-Ab. Moreover, some of these assays detect both SSc-and PBC-specific ANA in parallel.

The association between PBC and SSc is described repeatedly in the literature. The prevalence of SSc in PBC-patients is estimated at around 2–18%, while the frequency of PBC disease in SSc-patients varies around 2–10% [10–15]. The reported coexistence of SSc and PBC-specific autoantibodies is higher and fluctuates between 13% and 21% [13, 16–18]. Although liver disease is not a characteristic finding for the disease, it is not a rare observation in SSc patients, with PBC considered the main cause of liver diseases in SSc patients [15]. Table 1 gives an overview of the different studies published regarding the prevalence and characteristics of PBC-Ab and PBC disease in SSc patients.

Historically, AMA detection was often not included in the serological routine work-up of SSc patients' normal liver function tests. However, more recently, since the introduction of multiparameter assays with parallel detection of SSc- and PBC-specific ANA, AMA-M2 are frequently detected in SSc patients (even in the absence of a characteristic IIF pattern on LKS or HEp-2[000]). Nevertheless, until today, it is unclear if this PBC-specific serology in SSc patients is predictive for the development of PBC disease and which SSc patients have a higher risk for developing PBC. This knowledge would be of particular importance to improve timely diagnosis and guide early treatment of SSc-patients with ursodeoxycholic acid (UCDA) [3].

The aim of our study was to investigate the prevalence and fine specificity of PBC-specific serology as well as associations with the SSc-subtypes and SSc-specific antibodies in a large Belgian cohort of SSc patients. Associations between the presence of PBC-specific serology and biochemical markers for cholestatic liver disease were also evaluated (up to 3 years after detection of the antibodies). Furthermore, we compared three different techniques for the detection of PBC-Ab: IIF on HEp-2000 cells, IIF on LKS tissue sections for AMA and a line immunoassay for AMA-M2 and the other PBC-specific auto-antibodies Gp210, Sp100 and PML.

## Materials and methods

### Patients and samples

All consecutive SSc patients included in the SSc unit of Ghent University Hospital (Ghent, Belgium) having a minimal of 3 years' follow-up data available after the time of serum sampling or who were still in follow-up at the Rheumatology department were included (n = 184). All patients fulfilled the preliminary classification criteria of the American College of Rheumatology (ACR) and/or the LeRoy and Medsger criteria for early SSc and/or the new 2013 ACR/European League Against Rheumatism (EULAR) SSc classification criteria [19–21]. Approval was obtained by the Ghent University Hospital Ethics Committee (amendment 2018/0353) and all patients signed informed consent. The diagnosis of PBC was made according to the EASL

**Table 1:** Overview of different publications regarding the prevalence and characteristics of PBC antibodies in SSc patients.

| | Study | Number of SSc patients | PBC antibody assays | Prevalence of PBC disease and/or antibodies in the SSc cohort | Statistical associations and other findings |
|---|---|---|---|---|---|
| 1. | Assassi et al. [12] | 817 | AMA: IIF HEp-2 and MIT3 ELISA<br>Anti-Sp100:ELISA<br>Anti-Gp210: ELISA | **2% (16/817) PBC disease+**<br>**12% (101/817) PBC antibody+**<br>2% (16) AMA IIF+<br>7% (56) MIT3+<br>3% (26) Sp100+<br>0.4% (3) Gp210+ | – Patients with PBC were significantly older and had longer disease duration<br>– PBC-disease subgroup significantly associated with anti-CENP-B positivity, but not with lcSSc subgroup<br>– Association of anti-Sp100 with CENP-B antibodies<br>– Association of anti-CENP-B and anti-Scl70 with higher levels of ALP |
| 2. | Norman et al. [17] | 52 | AMA: MIT3 ELISA<br>Anti-Sp100: ELISA<br>Anti-Gp210: ELISA | **15% (8/52) PBC antibody+**<br>13% (7) MIT3+<br>2% (1) Sp100+ | – No association of PBC-antibodies with gender, age, SSc subgroup, SSc antibodies, prevalence of pruritus, the presence of other autoimmune conditions or liver parameters<br>– No anti-Gp210 positivity |
| 3. | Mytilinaiou et al. [16] | 37 | AMA: IIF HEp-2, LKS en MIT3 ELISA<br>Anti-Sp100:ELISA | **13% (5/37) PBC antibody+**<br>13% (5) MIT3+ | – No elevated liver parameters in the five PBC antibody positive subjects<br>– five subjects were only MIT3 positive, no positivity for anti-Sp100 or AMA on IIF HEp-2 or LKS |
| 4. | Imura-Kumada et al. [13] | 225 | AMA: MIT3 ELISA<br>Anti-Sp100:ELISA<br>Anti-Gp210: ELISA | **10% (22/225) PBC disease+**<br>**21% (48/225) PBC antibody+**<br>16% (37) MIT3+<br>6% (13) Sp100+<br>1% (3) Gp210+ | – 6% (13/225) suspected of PBC disease but PBC antibody negative<br>– patients with PBC were associated with CENP-B and lcSSc |
| 5. | Cavazzana et al. [18] | 201 | AMA: IIF LKS and MIT3 ELISA<br>Anti-Sp100:ELISA<br>Anti-Gp210: ELISA | **4% (8/201) PBC disease+**<br>**21% (43/201) PBC antibody+**<br>18% (36) MIT3+<br>3% (5) Sp100+<br>0.5% (1) Gp210+ | – Association of PBC-antibodies with lcSSc, anti-CENP-B and elevated ALP levels<br>– AMA detected by IIF on LKS showed lower sensitivity but higher specificity compared to MIT3 ELISA |
| 6. | Mari-Alfonso et al. [15] | 1572 | / | **4% (67/1572) PBC disease+** | – No antibody detection |

ALP, alkaline phosphatase; AMA, anti-mitochondrial antibodies; IIF, indirect immunofluorescence; LKS, liver kidney stomach tissue sections; lSSc, limited systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; PBC, primary biliary cholangitis; SSc, systemic sclerosis.
Bold text PBC disease+ indicates PBC disease present; Bold text PBC antibody+ indicates PBC-specific serology positive.

practice guidelines [3]. None of the patients were on UCDA treatment at time of serum sampling (t0). Three years after sampling (t3), one patient received UCDA treatment.

## Auto-antibody detection

Serum of all patients was analyzed for SSc- and PBC-specific antibodies. IIF on HEp-2000 cells (Immunoconcepts, Sacramento, CA, USA) was used to screen for ANA (ANA-IIF) and was considered positive at a titer ≥1:40. Interpretation of ANA patterns occurred following the International Consensus on ANA Patterns (ICAP) guidelines [8]. IIF on rodent LKS tissue sections (IIF-LKS) was performed for the detection of AMA using a serum screen dilution of 1:40 (Menarini, Florence, Italy). The presence of SSc-specific antibodies was evaluated with the Euroline SSc profile (IgG) immunoblot (EuroImmun, Lübeck, Germany), which detects antibodies directed against Ro52, anti-Scl70, CENP-A, CENP-B, RNA polymerase III subunits 11 and 155 (anti-RNAPIII/11 and anti-RNAPIII/155), fibrillarin, Th/To and PM-Scl

subunits 75 and 100 (anti-PM/Scl75 and anti-PM/Scl100). In addition, all samples were analyzed for PBC-Ab with the Euroline Profile Autoimmune Liver Diseases (IgG) line immunoblot (EuroImmun), which detects following antibodies: AMA-M2, M2-3E, Sp100, PML and Gp210. This line immunoblot (LB) combines both the native 74 kDa E2-antigen of the M2-pyruvate dehydrogenase complex (PDC) (most targeted antigen in PBC), as well as the recombinant M2-3E fusion protein, which contains the immunogenic domains of E2-subunits of the 3 M2-enzymes PDC, branched chain oxo-acid dehydrogenase complex (BCOADC) and the oxoglutarate dehydrogenase complex (OGDC), in order to increase the sensitivity for AMA-M2 antibodies. M2-3E is similar to the hybrid MIT3 antigen ELISA used in previous publications [12, 17, 18]. Results of the LBs were digitized using a calibrated flatbed scanner, and absolute signal intensities were imported by a computer program for further analysis (EUROLineScan, EuroImmun). Interpretational criteria as proposed by Bonroy et al. were applied for the SSc-specific antibody analysis [2]. Interpretation of the PBC antibody LB was performed according to manufacturer's instructions (positive if signal intensity >10 units [U]).

### Other laboratory analyses

The relationship between the presence of PBC-Ab and ALP and GGT levels at the time of sampling (t0) as well as 3 years after sampling (t3) were investigated (t3 data available in 138/184 patients). ALP and GGT levels were defined as elevated when concentrations were above the (gender-dependent) upper limit of normal (ULN). For statistical processing of data, SPSS Statistics 25 (SPSS Inc.) was used. Continuous variables were converted into z-scores in case gender-dependent differences were expected [22]. Statistical significance was accepted at $p < 0.05$.

# Results

## Patients and samples

In total, 184 SSc patients were included in this study (33 dcSSc, 124 lcSSc and 27 limited SSc [lSSc] patients). Seventy-five percent of patients were female and the mean age was 58 years (range 21–86).

## Prevalence and fine specificity of PBC-Ab and relation with SSc-subtype and SSc-specific antibodies

Antibodies against at least one PBC-specific antigen (detected by LB or by IIF-LKS) were found in 13% of SSc patients (23/184). AMA (detected on LB and/or IIF-LKS) were present in 11% (20/184) of sera, with AMA-M2 (including M2-3E reactivity) observed in 9.2% (17/184) (for more details see comparison LKS and LB). Anti-Sp100 was detected in 5% of patients (9/184) and the antibodies against Gp210 and PML were only detected in one patient each (<1%). Multiple reactivities were present in up to 30% of PBC-serology positive cases, with 66% of the anti-Sp100 positive cases also showing AMA-M2 or M2-3E reactivity (see Figure 1). A more detailed overview of the reactivities on LB is given in Supplementary File 1. Chi-square testing showed no statistically significant differences for gender and age between both PBC-Ab positive and negative subgroups (Table 2). In addition, no statistical difference could be shown when comparing patients with dcSSc and lcSSc (Fisher exact, $p = 0.569$). However, there was a significant association between PBC-Ab positivity and the presence of anti-CENP-B antibody (Fisher exact, $p = 0.042$).

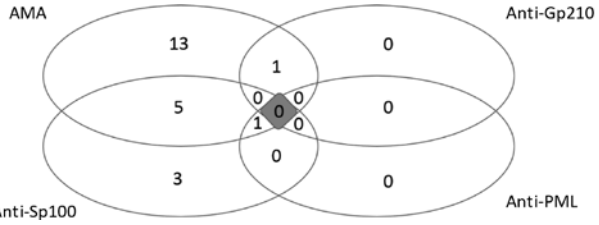


**Figure 1:** Number of patients out of a total of 184 SSc patients that tested positive for one or more of the following PBC-specific antibodies: AMA, anti-Gp210, anti-Sp100 and anti-PML.

**Table 2:** Comparison of demographic and laboratory data in SSc patients negative and positive for PBC-specific autoantibodies.

| | PBC-specific autoantibodies | | p-Value |
|---|---|---|---|
| | Negative | Positive | |
| Mean age, years (SD) | 57.2 (13.4) | 60.9 (11.0) | 0.202 |
| Gender (M/F) | 1/3 | 1/11 | 0.054 |
| SSc type[a] | | | 0.569 |
| dcSSc | 30/33 (91%) | 3/33 (9%) | |
| lcSSc | 106/124 (86%) | 18/124 (14%) | |
| lSSc | 25/27 (93%) | 2/27 (7%) | |
| SSc-specific antibodies[b] | | | **0.042** |
| No SSc-specific Ab | 61/67 (91%) | 6/67 (9%) | |
| Anti-Scl70 | 28/29 (97%) | 1/29 (3%) | |
| **Anti-CENP-B** | 66/80 (83%) | 14/80 (17%) | |
| Anti-PM/Scl | 1/1 (100%) | 0/1 (0%) | |
| Anti-RNAPIII | 5/6 (83%) | 1/6 (17%) | |
| Anti-Scl70 and anti-CENP-B | 0/1 (0%) | 1/1 (100%) | |

[a]Only dcSSc and lcSSc were included for statistical analysis (Fisher exact test). p-Values in bold are considered statistically significant ($p < 0.05$); [b]Comparison of patients with and without anti-CENPB; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; lSSc, limited systemic sclerosis.

## Relation between PBC-Ab and cholestatic liver enzymes

The presence of PBC-Ab was correlated with the cholestatic liver enzymes ALP and GGT (paired analysis available for n = 136). We observed no statistical difference in ALP levels between the PBC-Ab positive and negative subgroup (Mann-Whitney U, p = 0.098) at the time of sampling (t0), nor 3 years thereafter (t3) (Mann-Whitney U, p = 0.220) (see Figure 2A). In addition, paired analysis of t0 and t3 data of PBC-Ab positive patients showed no significant increase in ALP levels (Wilcoxon signed rank test, p = 0.163) (Figure 3A). Two of the patients with PBC positive serology showed elevated ALP levels at t0 (ALP titers 1.3× ULN and 1.05× ULN). At t3, ALP levels normalized for one patient without PBC diagnosis (from 1.05× ULN towards 0.9× ULN), whereas for the other patient (with AMA positivity detected by IIF-LKS and LB [also positive for anti-SP100, anti-PML and anti-M3-3E]), ALP levels remained elevated (1.5× ULN). In that patient, PBC was diagnosed but he was not treated with UCDA at the moment of sampling.

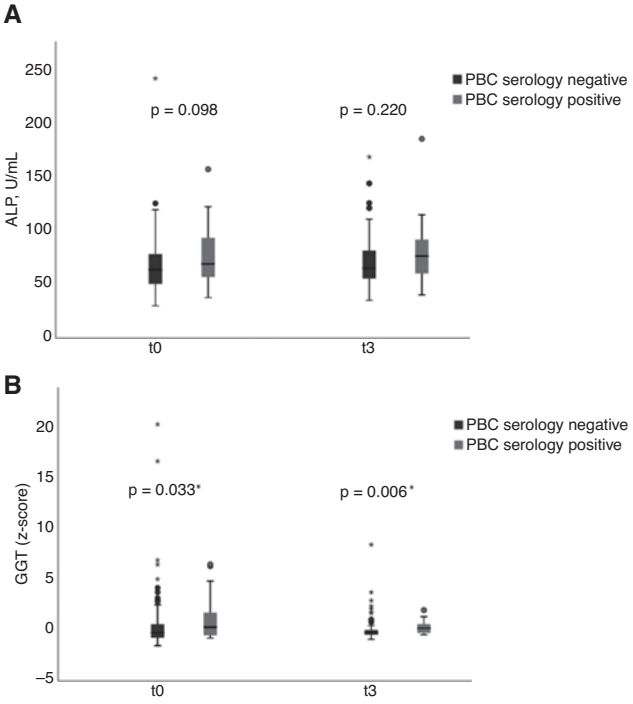


**Figure 2:** Box plot of the liver enzymes ALP (A) and GGT (B) for PBC-antibody positive and negative subgroups at the time of sampling (t0) and after 3 years of follow-up (t3).
A statistical difference in GGT (expressed as z-scores) (panel B) but not in ALP levels (panel A) between both subgroups at both time points was observed (Mann-Whitney U testing, *p-values <0.05 were considered statistically significant).

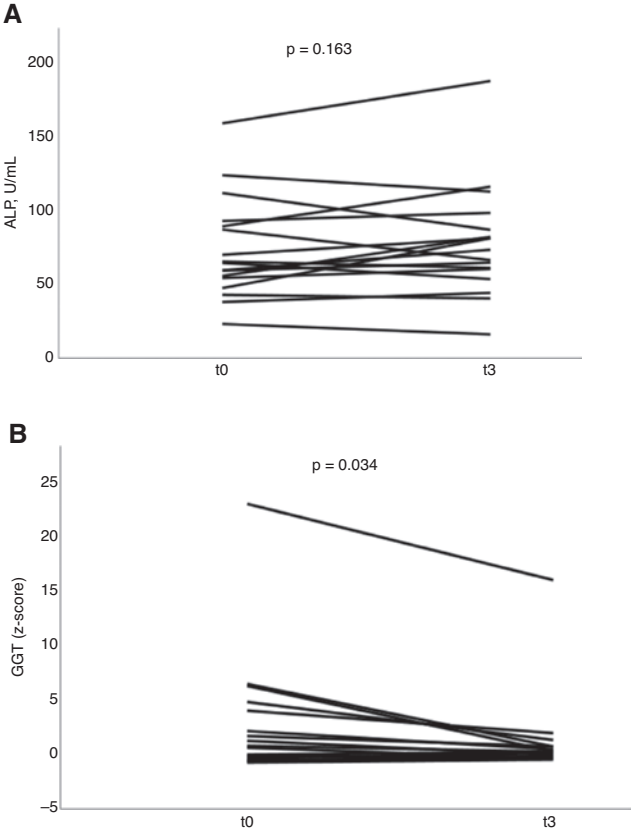


**Figure 3:** Line chart for ALP (A) and GGT (B) levels from PBC antibody positive patients at time of sampling (t0) and after 3 years (t3).
Paired analysis of t0 and t3 data of PBC-serology positive patients showed a significant difference in GGT levels (expressed as z-scores) (panel B) but not in ALP levels (panel A) (Wilcoxon signed rank test, *p-values <0.05 were considered statistically significant).

In contrast, for GGT, we observed a statistical difference in levels between the PBC-Ab positive and negative subgroup at t0 (Mann-Whitney U, p = 0.033), and at t3 (Mann-Whitney U, p = 0.006) (Figure 2B). In addition, paired analysis of t0 and t3 data of PBC-serology positive patient showed a significant difference in GGT levels (Wilcoxon signed rank test, p = 0.034) (Figure 3B). Nevertheless, this is due to a lowering of GGT levels after 3 years of follow-up and thus is not clinically relevant in the development of PBC disease. Detailed analysis of PBC serology positive patients showed four cases with elevated GGT levels at t0, with normalization in one of them at t3. However, elevation of GGT may be seen in a number of different diseases and is not considered specific enough for diagnosis of PBC [3].

## PBC-specific serology detected with different methods

A comparison between the results for AMA as detected by IIF-LKS and LB showed a good agreement with a kappa

**Table 3:** 2×2 contingency table for detection of antimitochondrial antibodies (AMA) by indirect immunofluorescence (IIF) on rodent liver-kidney-stomach (LKS) tissue sections and by line immunoblot (LB).

| | IIF on LKS tissue sections | | |
|---|---|---|---|
| | **AMA present** | **AMA absent** | **Total** |
| LB[a] | | | |
| AMA present | 13 | 4 | 17 |
| AMA absent | 3 | 164 | 167 |
| Total | 16 | 168 | 184 |

[a]Presence of AMA defined by reactivity for AMA-M2 and/or M2-3E.

value of 0.77 (95% confidence interval 0.60–0.93). The contingency table is given in Table 3. Four samples were positive for AMA on LB but negative on IIF-LKS. All four samples had reactivity against AMA-M2 (range signal intensities 17–72 U), while only two showed reactivity against M2-3E (52 and 56 U). The three LB negative/IIF LKS positive samples showed weak to moderate intensity scoring on IIF.

We also evaluated the performance of IIF on HEp-2000 cells for detection of the different PBC-Ab. The typical AMA pattern (AC-21) was only observed in 25% of AMA positive samples (LKS and/or LB positive), but when it was observed it was highly specific (100%). In contrast, we observed no multiple nuclear dots (AC-6) or nuclear membrane pores (AC-12) patterns in our cohort, hence presence of antibodies against Sp100, PML or Gp210 could not be predicted.

## Discussion

The prevalence of PBC-Ab in SSc disease varies according to the literature from 15 to 21% [13, 16–18]. Depending on the study, only AMA or AMA in combination with the other PBC-Ab were evaluated in SSc patients [2, 10, 12, 13, 15]. In our very SSc cohort study, using an LB that detects AMA, Sp100, Gp210 and PML reactivities, we found a slightly lower prevalence of 13%. This discrepancy may be due to differences in techniques used to detect PBC-Ab or population-based differences. Indeed, in contrast to previous studies, we included an lSSc-subgroup. When this subgroup was excluded, PBC-antibody positivity increased to 15%. AMA were most frequently detected (in 11% of patients), followed by anti-Sp100 (5%), anti-Gp210 (0.5%) and anti-PML (0.5%), which is similar to previous publications [13, 16–18].

A statistically significant association was demonstrated between PBC positive serology and the presence of anti-CENP-B. However, we observed no statistical association between the presence of PBC-Ab and the lcSSc subtype. These findings are in agreement with what was previously published by Assassi et al. [12]. Other studies, based on smaller cohorts, reported an association with both CENP-B antibodies as well as the lcSSc-subgroup [13, 18]. In line with the findings of Norman et al., we also observed no association between age and gender and the presence of PBC-Ab [17].

In both SSc and PBC, the appearance of disease specific antibodies may precede disease development [6, 23]. Within this context, we evaluated the relationship between the presence of PBC-Ab and cholestatic liver enzymes both at the time of serum sampling, as well as 3 years thereafter. To the best of our knowledge, this was the first study to investigate the liver parameters in SSc patients after multiple years of follow-up. We could not demonstrate a statistically significant difference for ALP levels between both subgroups at both time points, nor in the paired analysis between t0 and t3 for the PBC serology positive samples. However, as AMA may be present up to 10 years before development of PBC, it may be necessary to repeat this statistical analysis at a later time point. In contrast, we observed a statistically significant difference for GGT levels, both at time of sampling and after 3 years, with slightly higher levels in the PBC antibody positive subgroup. However, according to the EASL guidelines, GGT is not specific enough for diagnosis of PBC as different other liver diseases may provoke a GGT elevation [3]. Earlier studies of Norman et al. and Mytilinaiou et al. did not find a significant difference between PBC serology and the liver enzymes ALP and GGT [16, 17]. In contrast, the study of Cavazzana et al. showed that there was an association between the presence of PBC-Ab and elevated ALP levels [18]. None of these studies however investigated liver parameters multiple years after diagnosis.

As today, the classical sequence of screening for PBC-Ab by LKS followed by confirmation using a specific assay is often abandoned in routine practice, we performed a comparison between both methods (LB and LKS). We observed a good agreement between both for AMA, an observation in line with the data of Han and colleagues [24]. Nevertheless, it must be mentioned that in about 4% of our cohort (or 35% within the positive samples) PBC-specific serology was only detected in one of both strategies. Moreover, the finding of LB positive samples without LKS IIF positivity (23% on LKS) suggests that LKS IIF cannot be used as screening. As we observed no association between the specific cholestatic liver enzyme ALP (both at t0 and t3) and the presence of PBC-Ab detected by either of the techniques, we cannot make firm

conclusions about the preferable first line method or the need for parallel analysis with both techniques. The LB for detection of PBC-Ab contains both the native E2-antigen of the M2-PDC enzyme, which is the most targeted antigen in PBC, and the recombinant M2-3E fusion protein, which contains the immunogenic domains of E2-subunits of the three M2-enzymes PDC, BCOADC and OGDC, in order to increase the sensitivity for AMA-M2 antibodies. However, taken into account the M2-3E fusion protein, LB identifies only one extra patient (1/20) (see Supplementary File 1), suggesting no major added value in including this fusion protein in the LB. Our data showed that different PBC-specific reactivities may occur concomitantly in SSc patients. Considering this overlap, we found that the additional detection of antibodies targeting Sp100, Gp210, PML and M2-3E retrieved five extra patients compared to single detection of AMA-M2 (2.7% within the SSc cohort, 25% of all LB positive samples).

Additionally, we investigated if IIF on HEp-2000 cells could be used as a screening technique for PBC-Ab. We found an excellent specificity (100%) but a poor sensitivity 25%) for the detection of AMA. None of the samples with Gp210, Sp100 or PML antibodies were detected by IIF (n = 7). Therefore, IIF does not seem appropriate as a screening technique for PBC-Ab. Muratori et al. showed that ANA detected by IIF were present in half of PBC patients, but this study included also other fluorescence patterns not specific for PBC disease (e.g. anti-SSA pattern, anti-CENP-B pattern, etc.) [25].

Our study has some limitations. We did not perform echography or liver biopsy, nor did we correlate PBC serology with clinical symptoms for all samples, thereby making it impossible to make a final diagnosis of PBC disease in the whole SSc cohort. As mentioned, as AMA may be present up to 10 years before development of PBC, the follow up time of 3 years in this study is limited. As this is a prospective cohort, we plan to repeat the analysis after 6 or 10 years of follow-up.

We conclude that, in our SSc study cohort, PBC-Ab were present in 13% and significantly correlated with anti-CENP-B positivity. The most frequent reactivities were AMA (11%, with 9% AMA-M2) and Sp-100 antibodies (5%), showing a major overlap. We observed comparable detection of AMA on IIF and LB with no added value for the detection of anti-M2-3E, while ANA IIF screening was less sensitive compared to LB for the detection of the other PBC-Ab (Gp210, Sp100 and PML). There was no relevant association between presence of PBC-specific serology and cholestatic liver enzymes up to 3 years follow up. However, as AMA may precede PBC-disease up to 10 years, further prospective follow-up of our cohort will be necessary.

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