



# The Prevalence of PBC-Specific Autoantibodies In Patients with Systemic Sclerosis

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## Abstract

### Background

The prevalence of clinically significant primary biliary cirrhosis (PBC) in patients with systemic sclerosis (SS) is estimated to be 2.5%. It has also been shown that more than 90% of clinically and biochemically asymptomatic subjects who are positive for AMA express histological features compatible with PBC. Early detection of PBC is of clinical importance as timely introduction of treatment with ursodeoxycholic acid (UDCA) makes the prognosis in this condition comparable to the general population. Detection of AMA has been recently improved by introduction of a new assay utilizing the Gershwin-Leung recombinant MIT3 antigen containing the 3 major epitopes for AMA autoantibodies. ELISA tests for detection of 2 other PBC-specific autoantibodies (AAB), gp210 and sp100 have also recently become available.

### Study aims

Assess the prevalence of PBC-specific MIT3 IgG-AMA, gp210 and sp100 AAB, as well as SS-associated centromere and Scl-70 AAB in SS patients. Compare clinical and biochemical parameters in AMA positive and negative SS patients.

### Materials and Methods

Fifty-two consecutive patients with SS referred to the Rheumatology Dept were included. Thirty one suffered from limited skin SS (lcSS) and 20 from diffuse SS (dcSS). Only one patient in this cohort was diagnosed with PBC before inclusion into the study. MIT3 AMA, gp210, sp100, centromere A&B, Scl-70, and Jo-1 AAB were detected by ELISA (INOVA Diagnostics, San Diego). Appropriate statistical tests were used for the comparison between analyzed groups.

### Results

Eight (15%) patients with SS tested positive for PBC specific AAB (7 were MIT3 IgG and 1 sp100 positive). No statistically significant differences were observed between PBC-specific AAB positive and negative subjects in terms of gender, age at the diagnosis, SS presentation (lcSS vs dcSS), prevalence of pruritus or liver biochemistry. A clear, but not statistically significant trend towards increased prevalence of chronic fatigue in PBC-specific AAB positive patients was observed (63% vs 37%). AAB to centromere or Scl-70 were detected in 57.7% of the SS patients. No patients were positive for both. Of the 8 SS patients positive for PBC-specific AAB, 2 were also positive for centromere and 3 were positive for Scl-70 AAB.

### Conclusions

In patients with systemic sclerosis, PBC-specific AAB are detected in 15% of subjects and thus occur 6 times more commonly than clinically recognized PBC. As it is very likely that these patients indeed suffer from PBC and early treatment with UDCA may improve their prognosis, screening for these autoantibodies should be considered as a part of their routine assessment.

## Introduction

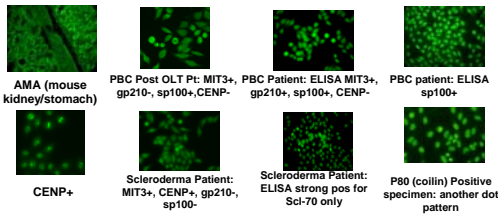
Systemic sclerosis (SSc) and primary biliary cirrhosis (PBC) are both chronic, presumed autoimmune conditions usually affecting middle age females. The prevalence of clinically significant PBC in patients with systemic sclerosis is estimated to be 2.5%. Anti-mitochondrial antibodies (AMA) are serological markers of PBC and occur in up to 90-95% of patients. Early detection of PBC is of clinical importance as timely introduction of the treatment with ursodeoxycholic acid (UDCA) makes the prognosis in this condition comparable to the general population. In many patients with liver disease, detection of AAB by IFA can be complicated by the presence of multiple AAB. This can make interpretation difficult and lead to both false positive and negative results. New ELISA assays utilizing the molecular targets of specific autoantibodies have recently been developed. In the case of PBC, a new assay utilizing the patented Gershwin-Leung recombinant MIT3 antigen containing the 3 major epitopes for AMA autoantibodies has demonstrated significant improvement in sensitivity over previous assays. ELISA tests for detection of 2 other PBC-specific autoantibodies (AAB), gp210 and sp100, have also recently become available. In the present study, we have applied these new PBC assays to assess the frequency of PBC-specific antibodies in a well-defined cohort of patients with SSc.

Assay	Target Antigen
QUANTA Lite™ M2 EP(MIT3) ELISA	Recombinant fusion protein MIT3
QUANTA Lite™ sp100 ELISA	peptide incorporating immunodominant portions of sp100 protein
QUANTA Lite™ gp210 ELISA	peptide incorporating immunodominant portions of gp210 protein
QUANTA Lite™ SLA (soluble liver antigen) ELISA	recombinant soluble liver antigen
QUANTA Lite™ Chromatin ELISA	highly purified calf thymus chromatin (depleted of histone H1 and non-histone proteins)
QUANTA Lite™ Centromere ELISA	recombinant CENP-A & B proteins
QUANTA Lite™ Scl-70 ELISA	native topoisomerase I
QUANTA Lite™ Jo-1 ELISA	purified Jo-1
QUANTA Lite™ F-Actin IgG ELISA	polymerized f-actin
QUANTA Lite™ SSA-52/60 (Ro-52/60)	recombinant Ro-52/native Ro-60
QUANTA Lite™ SSA-52 (Ro-52)	recombinant Ro-52

All assays are FDA 510(k)-cleared

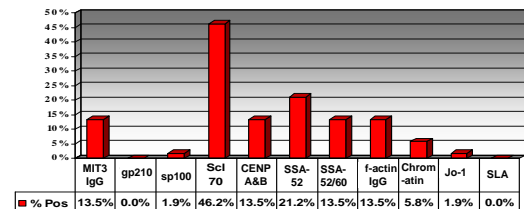
## Immunofluorescence Assay - Dots, Dots, and more Dots

Presence of antibodies to multiple targets can result in mixed patterns, masking, and challenging interpretation

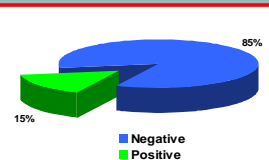


With exception of first photo, substrate is HEp-2 (INOVA Diagnostics), 1:40 dilution

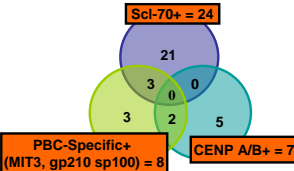
## Frequency of Autoantibodies in Scleroderma Cohort



## Frequency of Scleroderma Patients with PBC-specific Autoantibodies



## Overlap of Conventional SSc and PBC-Specific Antibodies



## Clinical & Laboratory data on Patients

	PBC-specific autoantibodies		P
	Positive (n=8)	Negative (n=44)	
Age (yrs)	59±13	54±11	0.3
Gender (M/F)	2/6	5/38	0.3
SSc type (localized/generalized)	5/3	26/17	0.9
Age at the diagnosis (yrs)	54±9	51±11	0.4
Pruritus (%)	13	2	0.3
Chronic fatigue (%)	63	39	0.18
AST (U/L)	21±6	22±9	0.9
ALT (U/L)	17±5	18±10	0.8
ALP (U/L)	79±18	72±32	0.6
GGT (U/L)	21±11	28±25	0.4
BILIRUBIN (mg/dl)	0.4±0.2	0.4±0.3	0.9
CHOLESTEROL (mg/dl)	233±45	225±37	0.6
TRIGLYCERIDES (mg/dl)	184±79	163±124	0.6
ALBUMIN (g/dl)	42±6	42±5	0.9

## Results

- Eight (15%) patients with SS tested positive for PBC specific autoantibodies (7 were M2 EP(MIT3) IgG and 1 was sp100 autoantibody positive).
- No statistically significant differences were observed between PBC-specific autoantibody positive and negative subjects in terms of gender, age at the diagnosis, SSc presentation (lcSS vs dcSS), prevalence of pruritus or liver biochemistry.
- A trend towards increased prevalence of chronic fatigue in PBC-specific AAB positive patients was observed (63% vs 37%, p=0.18).

## Conclusions

1. PBC-specific and scleroderma-associated autoantibodies can be easily detected with new ELISA assays. Uncertainty often encountered with IFA examination of sera with multiple reactivities can therefore be avoided
2. PBC-specific autoantibodies occur 6 times more commonly than clinically recognized PBC
3. Because early introduction of UDCA improves the prognosis of patients with PBC detected at the early stage, screening for these autoantibodies should be considered as a part of the routine assessment of SSc patients.

Selected Ref: Gabeta et al, J.Clin.Immunol 2007 in press; Nakamura et al, Hepatol 2007; Rigamonti et al, Gut 2007;