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RESEARCH ARTICLE

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Clinical impact of antibodies to Sp100 on a bacterial infection in patients with primary biliary cholangitis

Takashi Himoto[1] | Shuhei Yamamoto[1] | Kaho Morimoto[1] | Satoshi Tada[1] | Shima Mimura[2] | Koji Fujita[2] | Joji Tani[2] | Asahiro Morishita[2] | Tsutomu Masaki[2]

[1]Department of Medical Technology, Kagawa Prefectural University of Health Sciences, Takamatsu, Japan

[2]Department of Gastroenterology and Neurology, Kagawa University School of Medicine, Takamatsu, Japan

Correspondence
Takashi Himoto, Department of Medical Technology, Kagawa Prefectural University of Health Sciences, 281-1, Hara, Mure-Cho, Takamatsu, Kagawa 761-0123, Japan.
Email: himoto@chs.pref.kagawa.jp

Abstract

Background: A specific antinuclear antibody for primary biliary cholangitis (PBC) is anti-Sp100, which was recognized as a serological marker of concurrent urinary tract infection. We sought to determine the clinical characteristics of PBC patients who had anti-Sp100.

Patients and Methods: Fifty-one patients with PBC and 10 healthy controls (HCs) were enrolled. Anti-Sp100 were determined with an ELISA method. Lipopolysaccharide-binding protein (LBP) was measured as a serological hallmark for bacterial infection. The correlations of anti-Sp100 with demographic, laboratory, and pathological parameters were investigated.

Results: Six of the 51 (11.8%) PBC patients had anti-Sp100, whereas none of the HCs did. There was no significant difference in the frequency of antimitochondrial antibodies (AMAs) between PBC patients with and without anti-Sp100 (67% vs. 82%, $p = 0.5839$). Biochemical and immunological parameters were not associated with the emergence of anti-Sp100 in these patients. The clinical stage by Scheuer classification was not correlated with the existence of anti-Sp100. No significant difference in the serum LBP levels was found between PBC patients with and without anti-Sp-100, although serum LBP levels were significantly higher in PBC patients with anti-Sp100 than in HCs (8.30 ± 2.24 ng/ml, vs. 5.12 ± 2.48 ng/ml, $p = 0.0022$). The frequency of granuloma formation was higher in the liver specimens of PBC patients with anti-Sp100 than in those without anti-Sp100 (67% vs 29%, $p = 0.0710$).

Conclusion: anti-Sp100 does not become a complementary serological marker for PBC in AMA-negative patients. A bacterial infection may trigger the production of anti-Sp100. Another factor is required to initiate the autoantibody production.

KEYWORDS
antibodies to Sp100, bacterial infection, granuloma, lipopolysaccharide-binding protein, primary biliary cholangitis

1 | INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease, characterized by the progressive destruction of the intrahepatic bile ducts via autoimmune responses, leading to liver cirrhosis and subsequent liver failure. Antimitochondrial antibodies (AMAs) are extremely useful for the diagnosis of PBC, because the autoantibodies are detected in up to 95% of the patients.[1] In addition, antibodies to gp210 (anti-gp210) and Sp100 (anti-Sp100), which give a rim-like membrane pattern and a multiple nuclear dots (MND) pattern on HEp2 cells, respectively, by an indirect immunofluorescence (IIF) method, have been identified as PBC-specific antinuclear antibodies (ANAs).[2] Anticentromere antibodies, characterized by a discrete speckled pattern on HEp2 cells, are also regarded as a complementary serological hallmark for the clinical diagnosis of PBC, especially in AMA-negative PBC patients.[3,4]

The major target antigens of ANAs with the multiple dots pattern have been recognized as Sp100 and promyelocytic leukemia (PML) nuclear body protein. Sp100 is a 53-kDa nuclear protein with transcription activity, which has an aberrant electrophoretic mobility to 100 kDa.[5]

The reported prevalence of anti-Sp100 in PBC patients ranged from 8.7% to 40.0% in PBC patients,[6-15] and it has been speculated that the detection of these antibodies might be useful for the diagnosis of PBC, especially in the patients who were seronegative for AMAs.[16,17] Faster disease progression was observed in PBC patients with anti-Sp100 compared to PBC patients without anti-Sp100.[5,10] Likewise, PBC patients who were seropositive for anti-Sp100 showed a close association with unfavorable prognosis.[11,18] A decline in the titer of anti-Sp100 might reflect a favorable response to the treatment with ursodeoxycholic acid (UDCA).[18]

There was an interesting report that anti-Sp100 could become a predictive candidate for recurrent urinary tract infection in PBC patients.[19] It is widely recognized that several bacterial infections, including *Esherichia coli (E. coli)* and *Lactobacillus delbrueckii*, *and Mycobacterium gordonae*, may trigger cross-reactive immune responses between microbial and human mitochondrial antigens.[20-23] On the other hand, epithelioid granulomas are frequently observed in the liver tissues of PBC patients. The granuloma formation is likely to be derived from an immune response by a certain microbial agent such as *Propionibacterium acnes (P. acnes* [24]).

The primary purpose of this study was to investigate the clinical variables associated with the emergence of anti-Sp100, including demographic, biochemical, and histological parameters in the enrolled PBC patients. We also explored whether the occurrence of anti-Sp-100 was associated with a bacterial infection in such patients.

2 | MATERIALS AND METHODS

2.1 | Patients

Fifty-one patients were randomly selected from the patients who were diagnosed as PBC at the Hospital of Kagawa University School of Medicine from 2005 to 2015. The clinical diagnosis of PBC was based on the internationally accepted criteria[25,26]: biochemical evidence of cholestasis with elevation of alkaline phosphatase (ALP), the presence of AMA, or histopathological evidence of nonsuppurative cholangitis and the destruction of small- or medium-sized bile ducts. Ten healthy control (HC) subjects were also enrolled as a comparison group. This clinical study was approved by the ethical committees of Kagawa Prefectural University of Health Sciences and Kagawa University School of Medicine. Fully informed consent was obtained from each participant.

2.2 | Laboratory assessments

Total bilirubin (T-Bil), alanine aminotransferase (ALT), ALP, and immunoglobulin M (IgM) levels were measured by the standard laboratory techniques. AMAs were determined by an indirect fluorescent method using a rat stomach and kidney as a substrate. We consider 1:20 or higher titers as seropositive for AMA. Antibodies to Sp100 in each participant's serum were assayed with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Inova Diagnostics). The cut-off value of antibodies to Sp-100 was set at 25 Units according to the manufacturer's instructions. Serum lipopolysaccharide-binding protein (LBP) levels were also measured as a serological hallmark for a bacterial infection[27] with a commercially available ELISA kit (Hycult Biotechnology). All samples were frozen at −80°C and tested immediately after defrosting.

2.3 | Histological assessments

Liver tissue specimens were obtained by liver biopsy under the guidance of ultrasound, using 16-gauge needles. This tissue samples were fixed in 10% formalin and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin. The histological evaluation of PBC was based on Scheuer's classification system.[28] The frequencies of chronic nonsuppurative destructive cholangitis (CNSDC) and the formation of epithelioid granuloma were also examined in the enrolled patients.

2.4 | Statistical analyses

The data are presented as the mean ± SD. The Mann–Whitney *U* test and the Bonferroni/Dunn method were applied for the comparison of two and three groups, respectively. Fisher's exact probability test was used to compare the differences in frequencies. p-values < 0.05 were considered significant.

3 | RESULTS

3.1 | Patients' characteristics

The patients' clinical, laboratory, and histological characteristics are summarized in Table 1. Four male and 47 female PBC patients, aged 19–74 years, were enrolled. Concomitant extrahepatic

TABLE 1 Patients characteristics

Age (y.o.)	56.3 ± 11.7 (19–74)
Gender (Male/Female)	4/47
T-Bil (mg/dl)	0.9 ± 1.0 (0.3–6.4)
ALT (IU/L)	56 ± 45 (12–263)
ALP (U/L)	577 ± 344 (142–1,811)
IgM (mg/dl)	441 ± 362 (77–2275)
Seropositivity for AMA (%)	41 (80%)
Scheuer's classification (n = 47) (I/II/III/IV)	22/12/8/5
Concurrent autoimmune diseases	
CREST syndrome	8 cases
Sjögren's syndrome	6 cases
Hashimoto's disease	1 case
Rheumatoid arthritis	1 case

diseases were CREST syndrome in eight patients, Sjögren's syndrome in six patients, Hashimoto's disease in one patient, and rheumatoid arthritis in one patient. AMA and anti-Sp100 were present in the sera of 41 patients (80%) and six patients (12%), respectively. Forty-seven patients were histologically confirmed. We classified these patients into four categories of the Sheuer's classification: 22 patients in stage 1, 12 in stage 2, eight in stage 3, and five in stage 4.

3.2 | Demographic and laboratory factors associated with anti-Sp100

Figure 1 illustrates the distribution of anti-Sp100 titers in the PBC patients and HCs. Six of the 51 (12%) PBC patients had anti-Sp100 in their sera, and the titers ranged from 29.1 to 115.8 U, whereas anti-Sp100 was not present in the sera of any of the ten HCs. All six patients with anti-Sp100 gave the MND pattern on HEp-2 cells (Figure 2).

We next compared the demographic factors, including age at entry, gender, and concomitant extrahepatic autoimmune diseases between the PBC patients who were seropositive and those who were seronegative for anti-Sp100. As shown in Table 2, there was no significant between-group difference in age or gender, and no specific autoimmune diseases were associated with the emergence of anti-Sp100 among the PBC patients.

With respect to laboratory assessments, no significant differences in serum T-Bil, ALT, ALP, or IgM levels were detected between the anti-Sp100-seropositive and -seronegative groups. The frequency of AMA in the PBC patients with anti-Sp100 was approximately equivalent to the frequency in the PBC patients without anti-Sp100, indicating that anti-Sp100 cannot be a complimentary hallmark for PBC patients who are seronegative for AMA (Table 2).

Figure 3 depicts the serum LBP level in each category. The serum LBP levels were significantly higher in the PBC patients with anti-Sp100 compared to the HCs (8.30 ± 2.24 vs. 5.12 ± 2.48 ng/ml, p = 0.0002). However, there was no significant difference in the serum LBP levels between the PBC groups with and without anti-Sp100 (8.30 ± 2.24 vs. 7.10 ± 1.73 ng/ml, p = 0.1560).

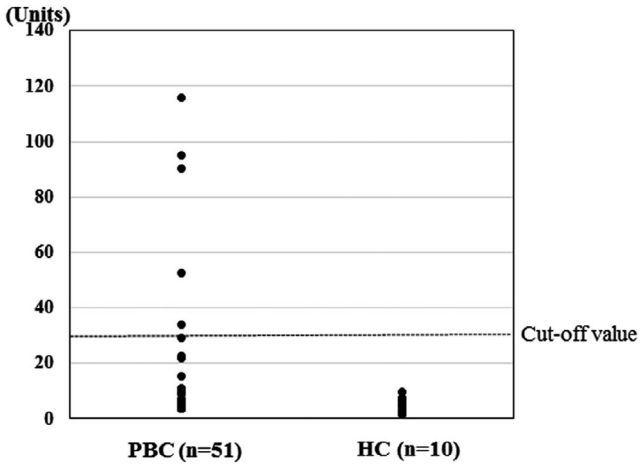

FIGURE 1 Distribution of anti-Sp100 titers in PBC patients and HCs

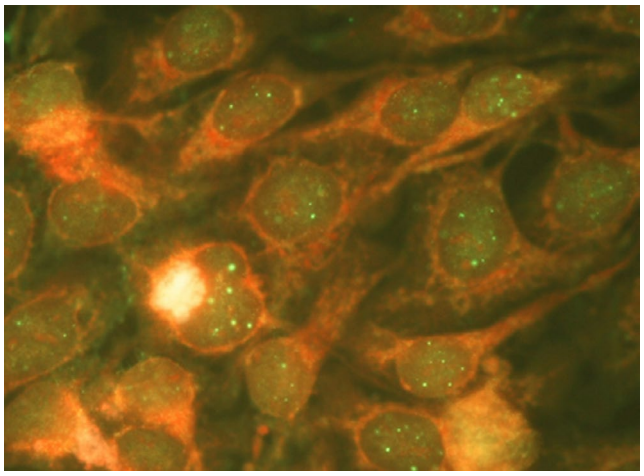

FIGURE 2 Representative of the immunofluorescence pattern of anti-Sp100 antibodies on HEp-2 cells (1:40 dilution)

TABLE 2 Comparisons of demographic, laboratory, and histological findings between the PBC patients who were seropositive and seronegative for anti-Sp100

	Seropotive for anti-Sp100 ($n = 6$)	Seronegative for anti-Sp100 ($n = 45$)	p-value
Age (y.o.)	62.8 ± 8.1	55.6 ± 11.9	0.1951
Gender (Male/Female)	1/ 5	3/42	0.9999
T-Bil (mg/dl)	1.5 ± 1.8	0.8 ± 0.9	0.1764
ALT (IU/L)	87 ± 101	53 ± 35	0.1147
ALP (U/L)	338 ± 124	603 ± 351	0.1034
IgM (mg/dl)	302 ± 314	456 ± 367	0.3708
Seropositivity for AMA (%)	4 (67%)	37 (82%)	0.5839
Scheuer's classification (I/II/III/IV) ($n = 47$)	2/1/1/2	20/11/7/3	0.2671
Frequency of CNSDC (%)	4 (67%)	24/41 (58%)	0.7047
Frequency of granuloma formation (%)	4 (67%)	12/41 (29%)	0.071
Concurrent autoimmune diseases			
CREST syndrome	1 case		
CREST syndrome	8 cases		
Sjögren's syndrome	6 cases		
Hashimoto's disease	1 case		
Rheumatoid arthritis	1 case		

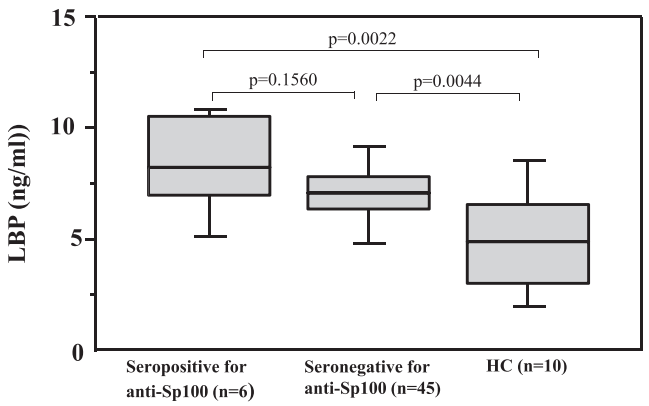

FIGURE 3 Comparison of serum LBP level in each category. The boxes represent the values within 25th and 75th percentiles. The horizontal bars represent the medians

3.3 | Histological parameters associated with anti-Sp100

We explored the correlation between the emergence of anti-Sp100 and histological progression (Scheuer's classification), or the presence of CNSDC and the formation of epithelioid granuloma among the 47 patients with histologically confirmed PBC. The emergence of anti-Sp100 was not correlated with the histological progression status. In addition, anti-Sp100 did not depend on the presence of CNSDC. However, the PBC patients who were seropositive for anti-Sp100 tended to have a higher frequency of epithelial granuloma formation than those who were seronegative for anti-Sp100 (67% vs. 29%, $p = 0.0710$, Table 2). No significant difference in serum LBP level was found between PBC patients with and without granuloma formation (7.37 ± 1.90 vs. 7.21 ± 1.90 ng/ml, $p = 0.8399$).

4 | DISCUSSION

We confirmed that the prevalence of anti-Sp100 antibodies in PBC patients was within the previously reported ranges,[6-15] but no significant difference in the frequency of anti-Sp100 was not detected between the AMA-positive and AMA-negative PBC patients, indicating that anti-Sp100 cannot become a complementary serological marker of PBC in AMA-negative patients. Our analyses also did not elucidate that anti-Sp100 was associated with the disease severity, which did not support the previous studies.[5,11,18]

Our findings revealed that the PBC patients with anti-Sp100 had significantly higher serum LBP levels than HCs, implying that a bacterial infection might trigger the production of antibodies to Sp100 in patients with PBC. LPB is a 50-kDa polypeptide that is abundantly synthesized in the liver as an acute-phase protein.[29] The measurement of blood lipopolysaccharide (LPS) is considered to be difficult because of its short half-life and inconsistency with the currently available methods.[30] Therefore, instead of measuring LPS directly, serum LBP levels can be used as a hallmark of endotoxemia caused by a gram-negative bacillary infection.

Several putative mechanisms by which *E. coli* infection may evoke autoimmune responses have been proposed in susceptible individuals who develop PBC.[23,31] Shimoda et al.[32] suggested that

PBC-specific ANA reactivity was the final step of inter-molecular spreading, initially involving mitochondrial antigens and subsequently expanding to nuclear mimics. On the other hand, the sequence similarities between epitope regions of *E. coli* and human Sp100 might trigger a direct cross-reactive immunity to the targets.[19] Consequently, *E. coli* infection was likely to initiate a loss of tolerance to Sp100 and subsequently leads to the production of antibodies to Sp100 in susceptible individuals who develop PBC.[33]

Several bacterial agents are potentially involved in the pathogenesis of PBC.[20-24] However, it was *P. acne* alone that was associated with the granuloma formation in the liver tissues of such patients.[24] In the patients with Crohn's disease, *E. coli* infection also played a crucial role in granuloma formation.[34] We thus hypothesized that a gram-negative bacillary infection may be also responsible for the granuloma formation in the liver specimens of PBC patients. However, our present study did not reveal a close correlation between the serum LBP level and the granuloma formation in the PBC patients. All four PBC patients who had anti-Sp100 and granuloma formation did not necessarily show higher serum LBP levels. Indeed, we could not support the hypothesis. The reason for a higher frequency of granuloma formation in the PBC patients with anti-Sp100 remains uncertain. Further examinations are required to clarify that.

There are several study limitations to consider. The clinical trial was conducted in a single center cohort study, and thus, the sample numbers were limited, although we acquired valuable results in this study. A large-scale cohort study is required to confirm the present results.

We also did not examine other parameters which suggest a bacterial infection, including the peripheral white blood cell count, erythrocyte sedimentation rate (ESR), or the serum C-reactive protein (CRP), procalcitonin, and interleukin-6 (IL-6) levels. According to a previous study by Tsalkidou et al,[27] however, serum LBP levels were the most reliable hallmark of urinary tract infection among these parameters. We thus investigated the serum LBP levels as a serological hallmark of the bacterial infection.

In addition, a longitudinal trial was not performed, and the alternation in the titers of anti-Sp100 antibodies before and after the treatment with UDCA was thus not monitored in the enrolled patients. Indeed, we were unable to investigate the correlation between the titer of anti-Sp100 antibodies and responsiveness to UDCA in these patients at all.

The prevalence of urinary tract infection at entry could not be investigated, because this study was retrospective. Urine culture in the enrolled patients at entry would be useful to reveal the correlation between the emergence of anti-Sp100 antibodies and the urinary tract infection in such patients. Further prospective studies are required to clarify the correlation.

In summary, the emergence of anti-Sp100 cannot be used as a complementary serological hallmark of PBC in AMA-negative patients. Bacterial infection may trigger the production of anti-Sp100 in patients with PBC. However, another factor is probably necessary to initiate the autoantibody production.

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None

CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data presented in this article are available on demand from the corresponding author.

ORCID

Takashi Himoto https://orcid.org/0000-0003-3860-2202

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