PRESENTED AT ANNUAL MEETING OF ASSOCIATION OF MEDICAL LABORATORY IMMUNOLOGISTS, AUGUST 29004

DETECTION OF AUTOANTIBODIES TO sp100 IN PRIMARY BILIARY CIRRHOSIS PATIENTS BY A NEW ELISA

Gary L. Norman[1], Zakera Shums[1], Dimitrios-Petrou Bogdanos[2], Howard J. Worman[3], Carol L. Peebles[1] , Lesley Lee[1], Andrew J. Lassen[1], Carlos von Muhlen[4], Edward L. Krawitt[5], Diego Vergani[2] , [1]INOVA Diagnostics, San Diego, CA; [2]Institute of Liver Studies, Kings College Hospital, London, UK, [3]Columbia University, New York, NY, [4]Hospital Sao Lucas, Pontificia Universidade Catolica do Rio Grande do Sul, Porto Alegre, Brazil, [5] University of Vermont, Burlington, VT.

ABSTRACT

Objective: Evaluate the performance of newly developed ELISA to detect autoantibodies to sp100, the major antigen responsible for the nuclear dot pattern seen in some patients with primary biliary cirrhosis.

Methods: A new ELISA (INOVA Diagnostics, San Diego, CA) was used to detect anti-sp100 antibodies in a combined cohort of 273 patients with primary biliary cirrhosis (PBC), PBC/AIH, or suspected PBC and in 177 normal and disease control patients from several clinical centers. Sera from one center (HJW) were also screened for the presence of gp210 antibodies (Quanta Lite™ gp210 ELISA, INOVA Diagnostics, San Diego, CA) and anti-mitochondrial antibodies (AMA) by IFA. In addition, a cohort of 51 sera (CVM) identified with nuclear dot-like patterns consistent with sp100 or p80 (coilin) were tested for sp100 and gp210 antibodies.

Results: Anti-sp100 antibodies were detected in approximately 27% (74/273) of the PBC patients by sp100 ELISA and in 1.7% (3/178) of the non-PBC patients. All 3 reactive non-PBC patients were clinically diagnosed SLE patients. Determination of the presence of AMA, sp100, and gp210 antibodies in one cohort, identified PBC patients who were positive for only one of the 3 analytes. Twelve of 51 sera with nuclear dot-like patterns identified by routine screening were found to be sp100 positive. One of the 12 positive sp100 specimens was also positive for AMA and M2 antibodies and one had M2 (but no AMA) antibodies. Follow-up of these patients found that patients had been diagnosed with a variety of conditions, but none specifically with PBC.

Conclusions: While the presence of anti-sp100 antibodies had a relatively low sensitivity for PBC (27%), detection of anti-sp100 antibodies by ELISA was highly specific (98.3%) for PBC. The only non-PBC disease sera showing significant sp100 reactivity was SLE (8.3%, 3/36). The use of sp100 and gp210 ELISAs identified PBC patients which were negative for AMA antibodies. The availability of the sp100 ELISA assay will allow more extensive assessment of the diagnostic and prognostic role of anti-sp100 antibodies in PBC.

Background

Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by the destruction of the small intrahepatic bile ducts. Progressive duct destruction leads to increasing functional impairment of the liver and, over time, can lead to liver failure and the necessity of liver transplantation.

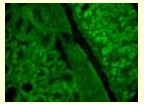
Serological assays are important aids for recognition and diagnosis of PBC. Anti-mitochondrial antibodies (AMA) are the classic serological markers of PBC and are found in up to 90-95% of PBC patients. Identification of pyruvate dehydrogenase complex- E2 as the primary target of AMA reactivity permitted the development of ELISA assays which are more sensitive than IFA for detection of AMA.

Despite the sensitivity of IFA and ELISA assays for AMA, at least 5-10% of PBC patients test negative for AMA. The failure to find AMA or other markers of PBC can contribute to a delay in the diagnosis of PBC and the possibility of additional liver damage.

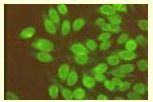
About 50% of sera from PBC patients contain antinuclear antibodies (ANA). One ANA pattern specifically associated with PBC is a speckled pattern of approximately 10-20 nuclear dots (multiple nuclear dot pattern). The major antigen responsible for the nuclear dot pattern has been identified as the sp100 protein. Sp100 antibodies, like antibodies to the nuclear pore complex protein gp210, have a relatively low sensitivity for PBC (in the range of 25%), but are extremely specific. Both sp100 and gp210 antibodies have been reported in AMA-negative PBC patients. There have been reports suggesting the presence of these antibodies may be associated with an adverse disease course.

The dot (speckled) pattern associated with sp100 can be difficult to read as a result of concomitant AMA , ANA, or other dot-like staining. Utilizing information on the immunodominant epitopes of the sp100 protein, we have developed a new standardized commercial ELISA for detection of sp100 antibodies. In the present study we report on the performance of this ELISA on a well-characterized panel of clinical sera.

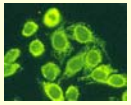
IFA Patterns Characteristic of PBC



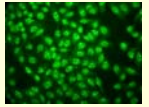
AMA pattern (mouse kidney/stomach)



nuclear rim, HEp-2 cells (gp210+)

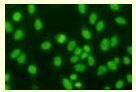


nuclear rim & AMA , HEp-2 cells (gp210 ELISA+)

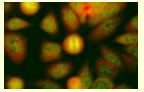


nuclear dot, approx 10-20 dots, HEp-2 cells, (sp100+)

Nuclear Dot Patterns – not PBC specific



P80 (coilin) approx 1-4 dots, HEp-2 cells



Centromere antibodies - approx 40 dots, HEp-2 cells,metaphase

Methodology

QUANTA Lite™ sp100 ELISA

This ELISA uses a highly purified peptide corresponding to immunodominant portions of the sp100 protein bound to color-coded 96 microwell polystyrene plates. Patient specimens are run at a 1:101 dilution. The ELISA assay uses pre-diluted controls, single-point antigen specific calibration, 30 minute room temperature incubations, ready-to-use conjugate, and single vial TMB substrate solution. Results are expressed in arbitrary units.

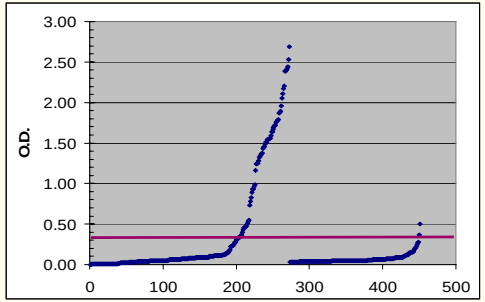
NOVA Lite™ HEp-2 IFA

Patient sera run at 1:40 dilution.

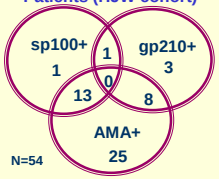
Cutoff Establishment

A combined panel of 177 specimens collected from healthy individuals (62) and from patients with a variety of non-PBC diseases (115) was tested with the sp100 ELISA kit to establish the cutoff for the assay. The specificity of the assay was 100% (62/62) for normal individuals and 98.3% (174/177) for healthy plus disease state sera.

Performance of Quanta Lite™ sp100 ELISA



Overlap of Specificities PBC Patients (HJW cohort)

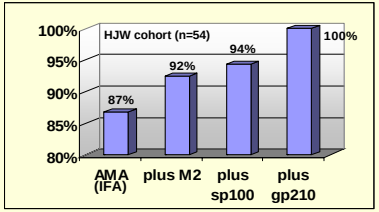


- **testing for gp210 and sp100 identifies 5 patients who would be missed if only AMA reactivity was assessed by IFA (3 pts gp210+, 1 pt sp100+, 1 pt both gp210 and sp100+)**
- **Combined testing for the 3 markers identified 94.4% (51/54) of the PBC patients**
- **Use of M2 ELISA would identify the 3 remaining specimens (see following panel)**

Summary of Quanta Lite™ sp100 ELISA Performance

Clinical group (n=450)	n=	Pos	% pos
Primary biliary cirrhosis(PBC),PBC/AIH,PBC,PBC/AIH?	273	74	27.1%
Autoimmune Hepatitis (AIH)	44	0	0.0%
Primary sclerosing cholangitis (PSC),PSC?	3	0	0.0%
Hepatitis C Virus (HCV)	6	0	0.0%
Hepatitis B Virus (HBV)	4	0	0.0%
SLE	36	3	8.3%
Rheumatoid arthritis	3	0	0.0%
Suspected liver disease, diagnosis unclear	3	0	0.0%
Misc specimens positive for: Sm(1), RNP(1),SS-B(1), SCL-70(1), Jo-1(1),Ribosome P(1), Chromatin(2), Centromere(1), ASCA) (2), histone(3),GBM (2)	16	0	0.0%
healthy, asymptomatic individuals	62	0	0.0%

Cumulative Detection of PBC Patients with Sequential Testing for Additional Antibodies



Retrospective Analysis of Clinical Reference Laboratory Specimens identified with p95 (sp100) or p80 (coilin) IFA patterns

A variety of specimens with patterns consistent with p80 or sp100 were assessed for sp100 and gp210 antibodies by ELISA.

p80 pattern = 41 specimens : 8 specimens sp100 ELISA positive
p95 (sp100) = 10 specimens: 5 specimens sp100 ELISA positive

Summary and Conclusions

- sp100 antibodies were detected in approximately 27% of the PBC patients by the sp100 ELISA
- The specificity of the sp100 ELISA was 100% for healthy controls (62/62) and 98.3% (174/177) for all non-PBC specimens (normals + disease sera)
- Testing sera exclusively for AMA will miss some PBC patients positive for other PBC-related serological markers.
- In one of the cohorts examined in this study, 100% of the PBC patients were found to be positive for at least one of the PBC-related antibodies (AMA, M2, gp210, sp100)
- The use of specific ELISA assays can clarify the significance of IFA patterns
- The Quanta Lite™ sp100 ELISA offers a sensitive, specific, reproducible, objective, and automatable alternative to conventional IFA or western blot methodologies which are labor-intensive and require subjective interpretation of staining patterns by highly experienced personnel.
- The commercial availability of the sp100 ELISA will provide clinicians with an additional serological marker for detection of PBC and may help earlier identification, diagnosis, and treatment of patients negative for conventional markers of PBC.
- The Quanta Lite™ sp100 ELISA is presently being prepared for submission to the FDA for review.

Selected References: Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1996;335:1570-80; Fritzler MJ, Manns MP. Clin Appl Immunol Rev 2002; 3:87-113; Miyakawa H. et al. Hepatology 2001;34:243-8.; Worman HJ, Courvalin JC. Autoimmun Rev 2003;2:211-7; Szostecki C. et al., Clin. Exp Immunol. 68:108-116., Bluther M et at. Autoimmunity 1999; 29:33-42, Zuchner D. et al, Hepatology 1997; 26:1123-1130.