

ENHANCED DETECTION AND CHARACTERIZATION OF PRIMARY BILIARY CIRRHOSIS PATIENTS WITH NEW M2 EP(MIT3), gp210, AND sp100 ELISA ASSAYS

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Abstract

Objective: Evaluate the combined and individual performances of 3 newly developed ELISAs (INOVA Diagnostics, San Diego, CA) to detect IgG autoantibodies to primary biliary cirrhosis (PBC)-specific gp210, sp100 antigens, and mitochondrial autoantigens in serum samples from patients with PBC, pathological and healthy controls.

Methods: A combined cohort of 771 subjects, including 168 definite PBC, 2 PBC/AIH, 4 probable PBC, 35 AIH, 2 PSC, 11 liver disease of uncertain diagnosis, 29 disease controls, and 520 healthy control subjects from several clinical centers were evaluated for gp210 and sp100 autoantibodies by ELISA (INOVA Diagnostics, San Diego, CA). Specimens were also tested for AMA antibodies with two M2 ELISAs (INOVA Diagnostics, San Diego, CA), one prepared using conventional antigen and one prepared using a patented recombinant MIT3 triple hybrid antigen developed by Gershwin and Leung.

Results: Overall, AMA antibodies were detected in 88.2% (150/170) of the definite PBC or PBC/AIH patients by the new M2 EP(MIT3) ELISA compared to 76.5% (130/170) by the conventional M2 ELISA using pyruvate dehydrogenase complex antigen. Frequency of AMA antibody detection with the M2 EP(MIT3) ELISA varied in the cohorts examined from different centers, ranging from 85 to 94.5%. This result highlights the requirement to evaluate the relative performance of different assays on the same specimens, not just the same clinically-defined group. Anti-sp100 antibodies were detected in 26.5% and gp210 antibodies were detected 21.2% of the PBC patients. While M2 EP(MIT3), gp210, and sp100 reactivity overlapped in many cases, 3 PBC sera had only gp210, 2 PBC sera had only sp100 antibodies and one specimen had both. Testing specimens for M2 EP(MIT3), gp210, and sp100 identified 156/170 (91.8%) of the PBC specimens with at least one positive result. Among 15 patients with suspected liver disease, 2 were negative by the conventional M2 assay but strongly positive using the new M2 EP(MIT3) assay. Specificity of the M2 EP(MIT3) assay was 97.8% (584/597). One MIT3 positive "healthy control" had strong AMA reactivity on IFA testing. The gp210 and sp100 ELISAs demonstrated 100% specificity.

Conclusions: The new M2 EP(MIT3) ELISA using the triple hybrid MIT3 antigen offers significantly improved sensitivity compared to the conventional M2 ELISA. gp210 and sp100 ELISAs can detect PBC-specific antibodies in AMA negative PBC specimens. Use of the new M2 EP(MIT3), gp210, and sp100 ELISAs will decrease the number of PBC patients falling into the "AMA-negative" group and may aid in accelerating their diagnosis and institution of treatment.

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), detected by immunofluorescence assay (IFA) on kidney/stomach/liver (KSL) or HEp-2 substrates, are the classic serological markers of PBC and can be found in up to 90-95% of PBC patients. IFA is labor intensive and highly dependent on skill and experience of the observer for interpretation of the IFA patterns. Liver disease patients often have antibodies to multiple targets and this can make interpretation difficult and lead to both false positive and negative results. ELISA assays developed using a purified mitochondrial antigen fraction, originally designated "M2" after the nomenclature in Berg and Klein's 1986 paper have permitted more sensitive and objective detection of AMA antibodies.

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 can contribute to a delay in the diagnosis of PBC and the possibility of additional liver damage. Some of these "AMA-negative" specimens have been shown to have antibodies to other PBC-specific antigens such as gp210 and sp100. The presence of persistently high levels of gp210 antibodies despite ursodeoxycholate therapy has been associated with increased risk of liver failure.

In 2000 Gershwin and Leung patented a recombinant fusion protein (MIT3) which includes the immunodominant portions of the 3 primary targets of AMA. This antigen has shown to have a higher diagnostic sensitivity than conventional ELISAs for AMA. We now report on the development of a new standardized commercial ELISA utilizing the patented MIT3 antigen.

In the present study, we have examined a panel of 771 sera by gp210, sp100, and the new MIT3-based ELISA. The new MIT3-based assay is significantly more sensitive than the conventional M2 ELISA. The combined use of the gp210 and sp100 assays results in detection of PBC patients who might be missed or fall into the clinically uncertain group of "AMA-negative" patients.

Methodology

QUANTA Lite™ M2 EP(MIT3) ELISA

- Utilizes recombinant fusion protein MIT3 QUANTA Lite™ sp100 ELISA (510(k) cleared)

- Utilizes peptide incorporating immunodominant portions of sp100 protein QUANTA Lite™ gp210 ELISA (510(k) cleared)

- Utilizes peptide incorporating immunodominant portions of gp210 protein

Antigens are bound to color-coded 96 microwell polystyrene plates. Patient specimens are run at a 1:101 dilution. All assays use pre-diluted controls, single-point antigen specific calibration, 30 minute room temperature incubations, ready-to-use conjugate, and single vial TMB substrate solution. Results expressed in arbitrary units.

NOVA Lite™ HEp-2 IFA

Patient sera run at 1:40 dilution.

Cutoff Establishment for M2 EP (MIT3) ELISA

A panel of 520 specimens collected from healthy individuals and 77 specimens from patients with a variety of non-PBC diseases was tested with the M2 EP (MIT3) ELISA kit to establish the cutoff for the assay. The specificity of the assay was 97.8% (584/597) for healthy controls and non-PBC disease sera. Excluding 5 AIH patients which might have an undiagnosed PBC overlap syndrome and 1 healthy control who was AMA+, the specificity would be 98.8% (584/591).

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+)



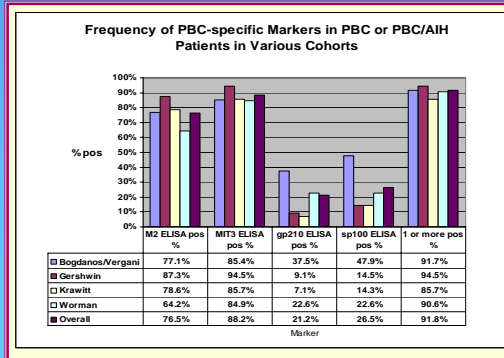
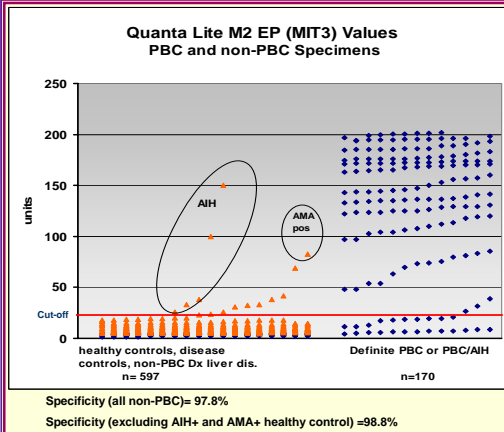
Nuclear Dot Patterns - not PBC specific



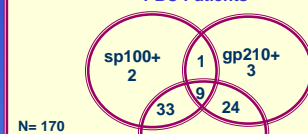
P80 (collin) approx 1-4 dots, HEp-2 cells



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



Overlap of PBC-specific Markers in PBC Patients



- 11.8% (20/170) of the total cohort of definite PBC specimens were AMA (MIT3) negative
- 30% (6/20) of the AMA(MIT3)-negative PBC patients were positive for gp210 and/or sp100 antibodies (3 pts gp210+, 2 pt sp100+, 1 pt both gp210 and sp100+)
- Combined testing for the 3 markers identified 91.8% (156/170) of the PBC patients

Summary and Conclusions

- The new M2 EP(MIT3) ELISA was on average about 12% more sensitive than the conventional M2 ELISA on the cohorts tested in this study (sensitivity M2 EP(MIT3)= 88.2%, M2 ELISA=76.5%).
- sp100 antibodies were found in 26.5% of PBC specimens.
- gp210 antibodies were found in 21.2% of PBC patients. In addition to being a PBC-specific marker in AMA(MIT3)-negative PBC, gp210 antibodies may have prognostic significance identifying patients with increased risk of severe liver disease.
- 30% of the AMA(MIT3)-negative specimens were positive for gp210 and/or sp100 antibodies.
- Combined testing for the 3 markers identified 91.8% (156/170) of the PBC patients
- Specificity of the M2 EP(MIT3) ELISA was 97.8% for all specimens not definitely diagnosed as PBC (includes disease controls, other liver diseases, and healthy controls). If 5 AIH patients (who might have an undiagnosed PBC/AIH overlap syndrome) and 1 healthy control who was AMA+ are excluded, the specificity is 98.8% (584/591).
- Commercial availability of the new MIT3-based ELISA, the gp210 ELISA, and the sp100 ELISAs provides clinicians with additional serological markers for detection of PBC and may help earlier identification, diagnosis, and treatment of patients negative for conventional markers of PBC.
- Availability of the assays in ELISA format presents an alternative to IFA, which is labor-intensive, subjective, and requires highly trained personnel for interpretation.
- The Quanta Lite M2 EP(MIT3) ELISA has been submitted to FDA for review and 510(k) clearance.