

DETECTION OF AUTOANTIBODIES TO THE NUCLEAR PORE COMPLEX gp210 IN PRIMARY BILIARY CIRRHOSIS PATIENTS BY A NEW ELISA

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ABSTRACT

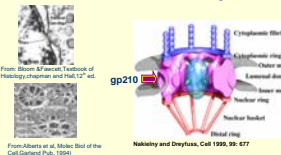
Objective: Evaluate the performance of a newly developed ELISA to detect autoantibodies to the nuclear pore complex gp210 protein in patients with primary biliary cirrhosis, viral hepatitis, and healthy control patients.

Methods: A new standardized ELISA (INOVA Diagnostics, San Diego, CA) was used to detect anti-gp210 antibodies in 174 patients with primary biliary cirrhosis, 20 HBV, 20 HCV, and 44 healthy controls. The results of the gp210 ELISA test were compared to those obtained using a western blot assay for detection of anti-gp210 antibodies. Sera were also tested by immunofluorescence assay (IFA) on rodent kidney-stomach-liver slides, soluble liver antigen/liver pancreas (SLA) ELISA (INOVA Diagnostics), and for antibodies to components of the 2-oxo acid dehydrogenase complexes by western blot.

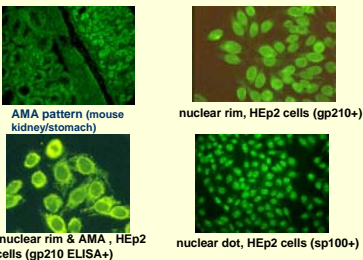
Results: Anti-gp210 antibodies were detected in 45(25.9%) of the PBC patients by gp210 ELISA and in 44 (25.2%) by western blot assay. Four patients were gp210 ELISA+/blot- and 3 patients were gp210 ELISA-/blot+. Of 35 PBC patients who were AMA negative, 10(28.6%) were positive for anti-gp210 antibodies by both the ELISA and blot assays. Six of these 10 patients were negative for anti-PDC-E2 by blot. One patient was found to be strongly positive for SLA, indicating a possible PBC/AIH overlap variant.

Conclusions: While the presence of anti-gp210 antibodies had a relatively low sensitivity for PBC (26%), detection of anti-gp210 antibodies by ELISA was 100% specific for PBC. In addition, these antibodies were found in some PBC patients negative for other conventional antibody markers of PBC. The anti-gp210 ELISA is a highly reproducible, standardized, and automatable assay. Unlike western blot, it is not dependent on subjective interpretation and provides comparable performance. The widespread availability of the gp210 ELISA assay will allow more extensive assessment of the diagnostic and prognostic roles of anti-gp210 antibodies in PBC.

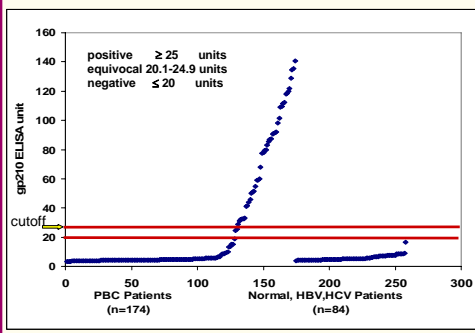
Nuclear Pore Complex



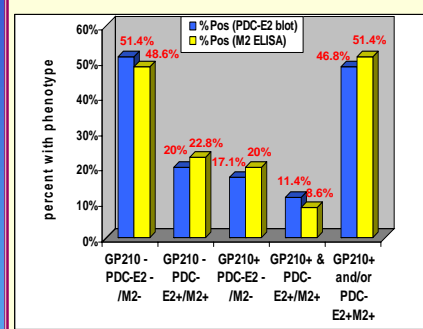
IFA Patterns Characteristic of PBC



Performance of Quanta Lite™ gp210 ELISA



AMA-negative PBC Patients (n=35)



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.

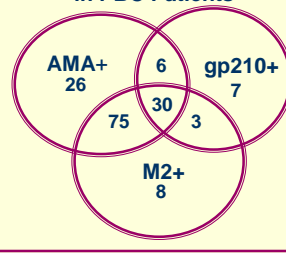
Despite the sensitivity of IFA and ELISA assays for AMA, at least 5-10% of PBC patients test negative for AMA. The failure to find AMA or other markers of PBC can contribute to a delay in the diagnosis of PBC and the possibility of additional liver damage.

About 50% of sera from PBC patients contain antinuclear antibodies (ANA). One ANA pattern associated with PBC is a punctuate nuclear rim/membranous staining characteristic of the nuclear pore membrane protein gp210. While gp210 antibodies have a relatively low sensitivity for PBC, in the range of 25%, they are extremely specific. gp210 antibodies have been reported in 10-50% of AMA-negative PBC patients and may identify a subgroup of patients with a more severe disease course. Like AMA, gp210 antibodies may precede the development of symptomatic disease.

The rim pattern associated with gp210 is difficult to read as a result of concomitant AMA or ANA staining and the pattern is not always specific for gp210. Detection of anti-gp210 by western blot is labor intensive, technically demanding, and relies on subjective interpretation of band intensity.

Studies have identified immunodominant epitopes of gp210 protein. Using this information, we have developed a new standardized commercial ELISA for detection of gp210 antibodies. In the present study we report on the performance of this ELISA on a well characterized panel of clinical sera.

Overlap of Specificities in PBC Patients



Summary and Conclusions

- gp210 antibodies were detected in 26% and 25.3% of PBC patients by the gp210 ELISA or by western blot assay respectively
- The specificity of the gp210 ELISA was 100%
- Testing sera exclusively for AMA will miss some PBC patients positive for other PBC-related serological markers such as PDC-M2 and gp210.
- gp210 antibodies were found in 28.6% of the AMA negative PBC patients in this cohort
- gp210 antibodies were the only marker detected in 17.1% of the AMA negative patients
- The Quanta Lite™ gp210 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 this gp210 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™ gp210 ELISA is presently under review by the USA FDA for *in vitro* diagnostic use.

Methodology

QUANTA Lite™ gp210 ELISA
 This ELISA uses a highly purified peptide corresponding to an immunodominant portion of the gp210 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.

Western Blot
 Standard immunoblot methodology using rat liver nuclear envelope proteins was used for detection of anti-gp210 reactivity.

Normal Range
 A combined panel of 236 specimens collected from healthy individuals was tested with the gp210 ELISA kit to establish a normal range for the assay. The specificity of the assay was 100% (236/236). The average value for this population was 3.7 units, the median value was 3.4 units. With the exception of one specimen with a value of 16.5 units, the other 235 specimens all had values less than 9.2 units. The positive cutoff of the assay is 25 units.

Selected References

Kaplan MM. Primary biliary cirrhosis. *N Engl J Med* 1996;335:1570-80. Fritzer MJ, Manns MP. *Clin Appl Immunol Rev* 2002; 3:87-113; Miyakawa H. et al. *Hepatology* 2001;34:243-8; Worman HJ, Couvralin JC. *Autoimmun Rev* 2003;2:211-7; Muratori P. et al. *Am J Gastroenterol* 2003;98:431-7; Miyachi K. et al. *J Autoimmun* 2003;20:247-54; Bandini O. et al. *Hepatology* 1996;23:1020-4; Itoh S. et al. *J Gastroenterol Hepatol* 1998;13:257-65; Invernizzi P. et al. *J Hepatol* 2001;34:366-72; Miyachi K. et al. *Mod Rheumatol* 2002;12:246-249; Shibata M. et al. *Prog in Hepatology* 1999;5:125-133; Nickowitz RE. et al. *Gastroenterology* 1994;106:153-9; Terasakovsky F, Worman HJ. *Hepatology* 1995;21:495-500.