



Value of "new" autoantibody testing in the differential diagnosis of autoimmune liver conditions

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ABSTRACT

Background. Making a clinical diagnosis in patients with apparent autoimmune liver disease when all the classical criteria are not fulfilled can be a challenge. Newly developed autoantibodies may potentially be useful in establishing the diagnosis in this situation.

AIM. To evaluate the performance of newly developed ELISAs to detect the following autoantibodies: anti-gp210, anti-sp100, anti-M2 (MIT3) IgG, anti-M2(MIT3) IgA, anti-centromere antibodies, anti-SLA, and anti-chromatin in patients with various autoimmune liver disorders.

Methods. The study group comprised 323 patients including 34 pts with AIH, 10 pts with AIH and (+)ve AMA, 8 pts with AIH/PSC overlap, 46 pts with PSC, 11 pts with unknown cholangiopathy, 57 pts with AMA (-)ve PBC and 157 pts with AMA (+)ve PBC. The diagnosis of AMA (+)ve PBC was established in patients with AMA by M2 ELISA, elevated ALP and typical histology. The diagnosis of AMA (-)ve PBC was established in patients AMA(-)ve by M2 ELISA, elevated ALP, histology typical for PBC, and with a normal MRCP. The diagnosis of AIH was confirmed using the AIH scoring system. The diagnosis of large duct PSC was based on (+)ve MRCP or ERCP. Unknown cholangiopathy was diagnosed in patients with duct injury and or ductopenia who lack typical histological features of AIH, PBC, or PSC, and had (-)ve standard serological markers of PBC or AIH. M2 ELISA was analyzed both at TWH and at INOVA Diagnostics laboratories. Other autoantibodies were analyzed at INOVA Diagnostics laboratories.

Results. Fifteen out of 57 patients with PBC who were AMA (-)ve by M2 ELISA tested AMA (+)ve with the MIT3 (Gershwin-Leung antigen)-based M2 ELISA. Utilizing a panel of 3 tests: M2(MIT3) IgG, gp210 and sp100 allowed serological confirmation of PBC in 20 out of 57 (35%) of patients initially labeled as AMA (-) PBC. The prevalence of gp210 and chromatin antibodies was significantly higher in patients with PBC and clinically bad outcome. Five out of 11 patients with unknown cholangiopathy tested (+)ve for M2(MIT3) IgG antibodies. All patients with histological features of AIH and positive AMA by M2 ELISA were also AMA (+)ve by M2(MIT3)IgG ELISA, 50 % of whom were also positive for gp210 or sp100. Approximately one fifth of patients with PSC tested (+)ve for chromatin antibody. With the exception of one PSC patient, all the others tested negative for all other antibodies analyzed in this study.

Conclusion. Newly developed ELISA tests for anti-mitochondrial and anti-nuclear antibodies may play a significant role in confirming/establishing clinical diagnosis in patients with chronic autoimmune liver disorders, especially in those patients lacking conventional AMA or having unknown cholangiopathies.

BACKGROUND

• In chronic autoimmune liver conditions autoantibodies reflect an underlying autoimmune reaction. Usually they are not directly involved in the pathogenesis of these conditions and are often considered to be surrogate markers of an ongoing autoimmune process

• Unfortunately, clinical practice clearly shows that a significant proportion of patients with autoimmune liver conditions do not suffer from clear-cut autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), or primary biliary cirrhosis (PBC). A degree of overlap between these conditions does exist and, despite several attempts, no consensus criteria have yet been established to classify these patients and thus clinicians can be faced with important therapeutic dilemmas.

• In order to facilitate clinical diagnosis and to help with therapeutic decisions, a series of ELISAs to detect new autoantibodies have been recently developed. These include anti-chromatin antibody, anti-centromere antibody, anti-soluble liver antigen (SLA) antibody, anti-nuclear pore complex protein gp210, and anti-nuclear body associated protein sp100. In addition, a novel ELISA test based on the patented Gershwin-Leung MIT3 recombinant molecule which contains the 3 major mitochondrial antigens recognized by PBC sera has been recently developed and demonstrated to be more sensitive and specific for detection of AMA than immunofluorescence (IF) technique or conventional M2 ELISA.

AIM OF THE STUDY

To assess the prevalence of a variety of autoantibodies in a large cohort of patients with different types of autoimmune liver disease and evaluate their value in uncertain cases.

MATERIALS AND METHODS

Patients Studied

- REB approval obtained
- Study conducted in a single center (Table 1)
- Serum samples from consecutive clinic patients and PBC serum bank

Methods

Standard screening M2 ELISA

- Varellisa M2 kit from Pharmacia Diagnostics

QUANTA Lite™ M2 EP(MIT3) IgG ELISA

- utilizes recombinant fusion protein MIT3

QUANTA Lite™ M2 EP(MIT3) IgA ELISA

- utilizes recombinant fusion protein MIT3

QUANTA Lite™ sp100 ELISA

- utilizes peptide incorporating immunodominant portions of sp100 protein

QUANTA Lite™ gp210 ELISA

- utilizes peptide incorporating immunodominant portions of gp210 protein

QUANTA Lite™ SLA (soluble liver antigen) ELISA

- utilizes recombinant soluble liver antigen

QUANTA Lite™ Chromatin ELISA

- utilizes highly purified calf thymus chromatin (depleted of histone H1 and non-histone proteins)

QUANTA Lite™ Centromere ELISA

- utilizes recombinant CENP-A and CENP-B proteins

For all QUANTA Lite™ ELISAs: 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.

Note: As of 27 Oct 2005, all the Quanta Lite ELISA kits utilized in this study are FDA 510(k)-cleared.

Table 1. Diagnostic Criteria

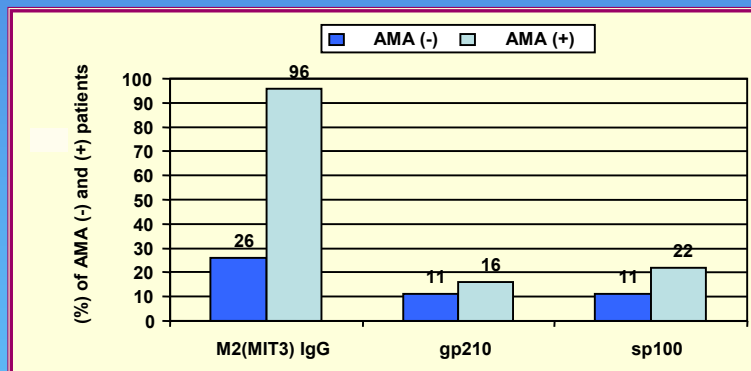
Diagnosis	Criteria
Autoimmune hepatitis (AIH)	1. International Hepatitis Group Criteria
AMA positive AIH (AIH+AMA group)	1. International Hepatitis Group Criteria 2. Lack of histological features suggesting PBC 3. Positive AMA by routine ELISA
AIH/PSC Overlap	1. International Hepatitis Group Criteria 2. ERCP/MRCP diagnostic for PSC
PSC	1. ERCP/MRCP diagnostic for PSC
Unknown Cholangiopathy	1. Elevated ALP and/or GGT 2. Histology: a) bile duct injury or ductopenia b) (without diagnostic features for PBC or PSC) 3. Normal ERCP/MRCP 4. History excluding other potential causes such as drugs or herbs
PBC, AMA negative	1. Elevated ALP and/or GGT 2. Histology compatible with PBC 3. Negative AMA by routine ELISA
PBC, AMA positive	1. Elevated ALP and/or GGT 2. Histology compatible with PBC 3. Positive AMA by routine ELISA
PBC bad outcome group (Serum bank)	1. Elevated ALP and/or GGT 2. Histology compatible with PBC 3. Negative or positive AMA by routine ELISA 4. Necessity of liver transplantation or death due to liver disease

RESULTS

Table 2. Prevalence of autoantibodies in autoimmune liver disease

Diagnosis/number of patients	AMA Routine n (%)	AMA M2(MIT3) IgG n (%)	AMA M2(MIT3) IgA n (%)	gp210 n (%)	sp100 n (%)	Centromere n (%)	SLA n (%)	Chromatin n (%)
AIH (n=34)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	6 (18%)	4 (12%)	13 (38%)
AIH/PSC (n=8)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (25%)
PSC (n=46)	0 (0%)	2 (4%)	1 (2%) – low titer	0 (0%)	0 (0%)	1 (2%)	1 (2%)	9 (20%)
AIH (AMA(+))ve (n=10)	10 (100%)	10 (100%)	6 (60%)	1 (10%)	4 (40%)	2 (20%)	2 (20%)	3 (30%)
PBC(AMA (-))ve (n=57)	0 (0%)	15 (26%)	8 (14%)	6 (11%)	6 (11%)	11 (19%)	0 (0%)	8 (14%)
PBC(AMA (+))ve (n=157)	157 (100%)	150 (96%)	118 (75%)	26 (16%)	35 (22%)	16 (10%)	1 (1%)	11 (7%)
Unknown Cholangiopathy (n=11)	0 (0%)	5 (41%)	2 (17%)	0 (0%)	0 (0%)	2 (18%)	0 (0%)	3 (27%)

Figure 1. Proportion of AMA M2(MIT3) IgG (+), gp210 (+) and sp100 (+) in AMA (-) and AMA (+) patients (with routine ELISA)



Specific Markers in AMA negative patients with PBC

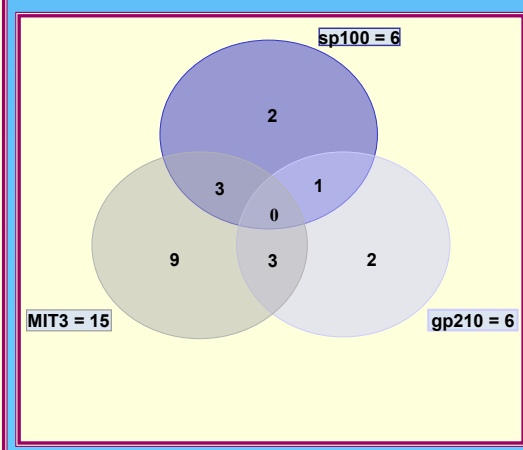


Figure 2. AMA testing in PBC and "Unknown" Cholangiopathy: Results according to test type

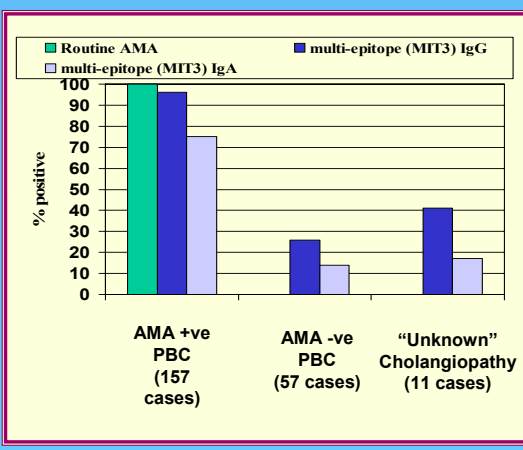


Figure 3. sp100 and gp210 in PBC and "Unknown" Cholangiopathy

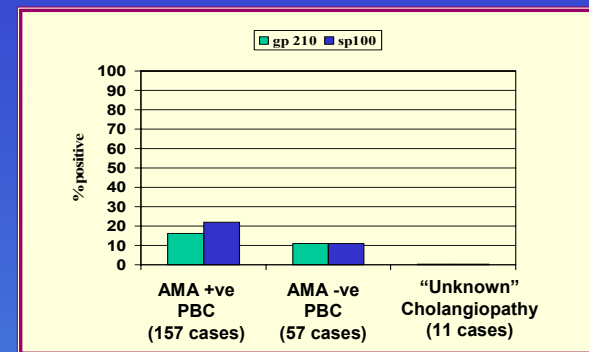


Table 3. Antibody Results in PBC: Alive vs. Dead/Tx

Autoantibody	PBC – dead or Tx n=38	PBC - alive n=176	P bad outcome vs. others
Routine AMA ELISA	30 (79%)	127 (72%)	NS
MIT3 IgG AMA	34 (89%)	131 (74%)	NS
gp210	14 (37%)	18 (10%)	0.0002
sp100	8 (21%)	33 (19%)	NS
centromere	6 (16%)	21 (12%)	NS
SLA	0 (0%)	1 (0.6%)	NS
chromatin	6 (16%)	13 (7%)	0.003

Table 4. PBC: Do M2(MIT3) IgG distinguish a different population?

Autoantibody	All PBC n=214	AMA MIT3 IgG positive n=167	AMA MIT3 IgG negative n=47	P AMA (+) vs. (-)
gp210	32(15%)	28 (17%)	4 (8%)	NS
sp100	41 (19%)	35 (21%)	6 (11%)	NS
centromere	27 (13%)	19 (11%)	8 (15%)	NS
SLA	1 (0%)	1 (1%)	0 (0%)	NS
chromatin	19 (9%)	12 (7%)	7 (13%)	NS

CONCLUSIONS

1. The MIT3 IgG ELISA greatly facilitates diagnosis of previously "Unknown" Cholangiopathy
2. gp210 and chromatin antibodies may be markers for more severe disease

Summary Autoantibody Testing in AILD

- M2 ELISA -ve PBC: 26 % MIT3 +ve
- "Unknown" Cholangiopathy: 41 % MIT3 +ve
- Severe PBC: gp210/chromatin +ve
- AIH (AMA +ve): overlapping antibody pattern
- PSC ± AIH: featureless serological pattern