



## A NEW ELISA SIMULTANEOUSLY DETECTS PRIMARY BILIARY CIRRHOSIS-SPECIFIC MITOCHONDRIAL AND NUCLEAR AUTOANTIBODIES

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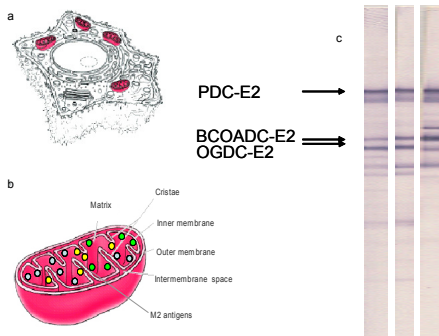
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### Background (1)

Primary biliary cirrhosis (PBC) is an immune-mediated chronic cholestatic disease characterised by specific anti-mitochondrial antibody (AMA) responses (Fig.1a) directed against members of the 2-oxo-acid dehydrogenase complexes (M2 antigen) of the inner mitochondrial membrane (Fig.1b) and in particular the inner lipoyl domains of the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), the branched chain oxoacid dehydrogenase complex (BCOADC-E2) and the oxoacid dehydrogenase complex (OGDC-E2). (Fig.1c)



**Figure 1** (a) Structure of a mitochondrion; (b) Components of the 2-oxo-acid dehydrogenase multienzyme complexes (M2 antigen) are located in the inner mitochondrial membrane; (c) the major mitochondrial antigens are the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), the branched chain oxoacid dehydrogenase complex (BCOADC-E2) and the oxoacid dehydrogenase complex (OGDC-E2)

### Background (2)

AMA are routinely detected by indirect immunofluorescence (IFL), using kidney/stomach/liver rodent tissue sections or immobilized HEp2 cells; AMA stain the renal tubuli with a clear strengthening of the staining at the level of the distal, smaller, tubules. AMA also stain the gastric parietal cells of the stomach, and the dual renal-gastric positivity gives a clear, unmistakable immunofluorescent picture.

IFL is labor intensive and highly dependent on the skills and the experience of the observer; still, in 5-10% of the PBC patients AMA are undetectable by IFL.

Molecular based assays such as ELISAs based on a purified mitochondrial fraction designed as 'M2 antigen' have allowed more objective detection of AMA but as by IFL, a significant proportion of PBC patients is still seronegative for anti-M2 antibodies.

A recombinant fusion protein (MIT-3) which includes the immunodominant portions of the 3 major mitochondrial antigens i.e PDC-E2, BCOADC-E2 and OGDC-E2 has been developed by the group of EM Gershwin (at Davis, California) and a new standardized commercially available ELISA based on the MIT-3 has been manufactured by INOVA Diagnostics (San Diego, California).

### Background (3)

Although AMA are considered to be the hallmark of PBC, they are not the only disease-specific autoantibodies. Previous studies have shown that two other sets of anti-nuclear antibodies (ANA) against the nuclear body sp100 antigen giving a multiple nuclear dot (MND) pattern by IFL and the nuclear pore gp210 giving a rim-like/membranous (RL/M) pattern by IFL are specifically present in up to 50% of PBC patients and may have prognostic significance. ELISAs detecting autoantibody reactivity to sp100 and gp210 epitopes have also been developed by INOVA.

### Material and Methods

ELISA's using the mixture (PBC screen) or individual MIT3, gp210 and sp100 ELISAs were used to test:

98 patients with PBC;

104 pathological controls (32 autoimmune hepatitis, 12 primary sclerosing cholangitis, 25 chronic hepatitis B infected patients and 35 chronic hepatitis C patients) and 80 healthy controls.

Pathological and healthy controls were negative by individual ELISA's for AMA(MIT3), gp210 and sp100 antibodies.

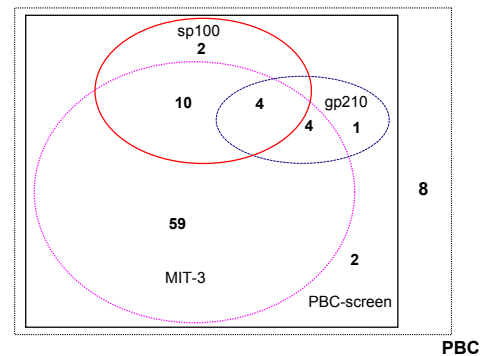
### Aim

To evaluate the performance of a newly developed ELISA (PBC screen) based on an antigenic mixture of the triple hybrid (MIT3), gp210 and sp100 epitopes compared to the performances of individual MIT3, gp210 and sp100 ELISAs.

### Results

Using the PBC screen ELISA, a positive result was obtained in all 80 PBC patients positive for AMA, sp100 or gp210 by individual ELISA's and in 2/10 PBC patients negative by all individual ELISA's.

None of the controls has shown a positive result using the PBC screen ELISA.



### Conclusions

•Our results indicate that the PBC screen ELISA based on an antigenic mixture of the major mitochondrial and nuclear antigenic targets performs as well as ELISA's based on individual antigens.

If similar data are confirmed on larger numbers of PBC patients and pathological controls, the new PBC screen ELISA can be used for the screening of PBC-autoantibody serology instead of a combination of individual ELISA's

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