Detection of primary biliary liver cirrhosis-associated antimitochondrial antibodies using an improved test system: Anti-M2-3E ELISA

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Introduction

Determination of anti-mitochondrial antibodies (AMA) is of particular significance for the diagnosis of primary biliary liver cirrhosis (PBC), an immune-mediated chronic inflammatory cholestatic liver disease of unknown aetiology. The most specific and sensitive diagnostic markers are antibodies against the M2 antigen. The molecular targets of these autoantibodies have been identified as members of the 2-oxoacid dehydrogenase complex family of enzymes within the mitochondrial respiratory chain. Among these enzyme components, the lipooyl binding domains (E2) are the major autoantigens in PBC.

Methods

Here we report on the development of a new ELISA (Anti-M2-3E ELISA) in which the native pyruvate dehydrogenase complex (PDH) and the designer antigen BPO are both used as target antigens. BPO combines all three E2 domains of branched-chain 2-oxo-acid dehydrogenase, PDH, and 2-oxo-glutarate dehydrogenase in one recombinant polypeptide. The new test system was evaluated with serum samples from patients suffering from PBC (n = 170), autoimmune hepatitis (AIH, n = 48), PBC/AIH overlap (n = 3), viral hepatitis (n = 200), systemic lupus erythematosus, Sjögren’s syndrome and rheumatoid arthritis (SLE, SS, RA, together n = 253) and from 400 healthy blood donors (HBD). It was also compared with other test systems for the detection of AMA.

Results

The sensitivity and specificity of the Anti-M2-3E ELISA were 93.1% (161/173) and 99.6% (245/246), respectively. In addition, none of the 400 healthy blood donors showed positive results using the Anti-M2-3E ELISA. Three sera from AIH patients, two from SLE patients and one Sjögren’s syndrome sample also exhibited high anti-M2 antibody concentrations in the Anti-M2-3E ELISA. Re-evaluation of their medical records revealed a co-existing PBC. In comparison, the ELISA systems using BPO individually or biochemically purified PDH (classic Anti-M2 ELISA) showed lower sensitivities, 90.2% (156/173) and 79.8% (138/173), respectively, whereas the specificities of all three test systems were almost identical (>98.8%). With the same cohort of sera IIF showed a sensitivity and specificity of 89.0% (154/173) and 98.8% (243/246), respectively, with an overall agreement in the group of PBC patients between the Anti-M2-3E ELISA and IIF of 94.3% (164/173).

Discussion

The combination of the artificial polypeptide BPO with native PDH as substrate in an Anti-M2 ELISA increases the sensitivity by 14% compared to the classic Anti-M2 ELISA while preserving an almost perfect specificity. The improved performance of the new test system is in good agreement with the hypothesis of non-shared epitopes. Moreover, the additional sensitivity of the Anti-M2-3E ELISA compared to the BPO-based ELISA indicates BPO-relevant epitopes other than linear structured ones in the core lipooyl domain. With the new test system even more PBC patients can be accurately identified than with the gold-standard technique IIF.