Line immunoassay for parallel detection of 9 different autoantibodies in the serological differential diagnosis of PBC

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Introduction

Primary biliary liver cirrhosis (PBC) is associated with different serum autoantibodies against antigens found in mitochondria, nuclear dots and nuclear membrane. Analysis of these antibodies helps to discriminate between PBC and autoimmune hepatitis (AIH). We evaluated a new robust multiparametric test system for diagnosing autoimmune liver diseases.

Methods

A line immunoassay was created using native full-size AMA-M2 and the following recombinant proteins: M2-3E (synonym: BPO; a fusion protein of the immunogenic lipoic domains of branched-chain oxoacid dehydrogenase, pyruvate dehydrogenase and oxoglutarate dehydrogenase), Sp100 (spot-pattern 100kDa protein) and PML (promyelocytic leukaemia protein), both representing antigens from nuclear dots, gp210 (glycoprotein 210, part of the nuclear pore complex), LC-1 (liver-kidney microsomes), LKM-1 (liver-kidney microsomes), SlA/LP (soluble liver antigen/liver-pancreas antigen) and Ro-52 (anti-Ro-52 antibodies in combination with anti-SLA/LP are discussed as potential markers for an unfavourable disease course).

This profile was used to screen for antibodies in sera of 170 patients with clinically characterised PBC, 49 with AIH, 200 with viral hepatitis (HBV or HCV) and 50 healthy blood donors. Prevalence and intensity of the bands were automatically evaluated using a commercial computer programme (EUROLineScan, EUROIMMUN, Germany).

Results

In 94% of the PBC sera, antibodies against at least one of the antigens AMA-M2, M2-3E, Sp100, PML, gp210 were detected with a specificity of 99% as referred to the panels of viral hepatitis and blood donors. Four out of six positive samples in the AIH group could be attributed to PBC/AIH overlap patients after re-evaluation of the medical records.

Discussion

Using the new comprehensive profile, a serological diagnosis of PBC can be made with a yet unequalled sensitivity of 94%. Autoantibodies against the designer antigen M2-3E and native AMA-M2 play a predominant role in the majority of patients, with M2-3E contributing the most to the sensitivity of the assay. However, in 6% of the cases immune reactivities were exclusively directed against Sp100, PML and/or gp210. These patients would not have been detected by immunoassays using AMA-M2 as the single antigen. In sum, the novel line immunoassay represents an outstanding diagnostic tool for PBC. It exceeds all previous detection methods with regard to sensitivity and specificity, including testing by indirect immunofluorescence.

Scientific presentation at the 10th International Workshop on Autoantibodies and Autoimmunity (Guadalajara, Mexico, March 2008)