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Antimitochondrial and antinuclear antibodies in primary biliary cirrhosis**

Przeciwciała antymitochondrialne i antyjądrowe w pierwotnej żółciowej marskości wątroby

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S u m m a r y

Primary biliary cirrhosis (PBC) is a chronic liver disease of unknown etiology. It starts with inflammatory changes in the small biliary ducts and slowly develops pathological changes in the tissue, leading to complete liver cirrhosis. Serum autoantibodies are crucial tools for diagnosis of PBC. Antimitochondrial autoantibodies (AMA), directed against the 2-oxoacid-dehydrogenase complex of the inner mitochondrial membrane, are the most sensitive and specific immunologic markers of PBC. Additionally 50% of PBC patients have antinuclear antibodies (ANA). The nuclear envelope proteins have been identified as target of ANA reactivity. A part of PBC patients develops antibodies against constituents of the nuclear pore complex – gp210 protein, p62 nucleoporin and lamin B receptor (LBR). Antibodies to these antigens such as gp210 protein and p62 nucleoporin are highly specific for PBC and can aid in the serologic diagnosis, especially in cases in which antimitochondrial antibodies are not detectable. A review of several studies performed in various regions of the world showed wide differences in prevalence (9-45%) of antibodies against gp210 protein in PBC. There are reports indicating an association of positivity for antibodies against the nuclear pore complex with poor outcome of PBC. During the last years a number of another nuclear structures have been recognized as a specific targets of ANAs in PBC. These include Sp100 and promyelocytic leukemia proteins. Specific antinuclear antibodies will provide an important, novel clinical tool in diagnostic process of patients with PBC.

Key words: primary biliary cirrhosis, autoantibodies, liver, autoimmunity, nuclear pore complex

S t r e s z c z e n i e

Pierwotna żółciowa marskość (PBC) jest przewlekłą chorobą wątroby o nieznanej etiologii. Rozpoczyna sie od zmian zapalnych w małych kanalikach żółciowych, powoli doprowadzając do patologicznych zmian w tkance wątrobowej i całkowitej marskości wątroby. Przeciwciała występujące w surowicach pacjentów są przydatnym narzędziem uzupełniającym diagnozę choroby. Najbardziej czułym i swoistym markerem immunologicznym PBC są przeciwciała antimitochondrialne (AMA), skierowane przeciwko kompleksowi dehydrogenazy pirogronianowej, zlokalizowanemu w wewnętrznej błonie mitochondrialnej. Ponadto dla ok. 50% pacjentów z PBC charakterystyczne są przeciwciała przeciwjądrowe (ANA), gdzie głównymi autoantygenami są białka otoczki jądrowej: glikoproteina gp210, nukleoporyna p62 i receptor laminy B (LBR). Zwłaszcza przeciwciała skierowane przeciwko antygenom gp210 i p62 są bardzo specyficzne dla PBC i wykrywanie ich może być pomocne w diagnostyce serologicznej, zwłaszcza w przypadkach, w których nie wykryto przeciwciał przeciwmitochondrialnych. Istnieją doniesienia wskazujące na związek obecności przeciwciał skierowanych przeciwko białkom kompleksu porowego i gorszym rokowaniem w przebiegu PBC. Badania przeprowadzone w różnych regionach świata, wykazały też znaczne różnice w częstości występowania (9-45%) przeciwciał przeciwko białku gp210 w PBC. W ciągu ostatnich lat kolejne struktury jądrowe zostały zidentyfikowane jako istotne dla przeciwciał ANA w PBC. Należą do nich między innymi białko Sp100 i białka białaczki promielocytowej. Specyficzne przeciwciała przeciwjądrowe mogą stanowić nowe ważne, narzędzie w procesie diagnostycznym pacjentów z PBC.

Słowa kluczowe: pierwotna żółciowa marskość, wątroba, autoprzeciwciała, kompleks porowy

INTRODUCTION

Primary biliary cirrhosis is a chronic, slowly progressive cholestatic liver disease with features of an autoimmune disorder, with unknown etiology. It starts with inflammatory changes in the small biliary ducts and after developing pathological changes in the tissue, leading to complete liver cirrhosis and the necessity of liver transplantation (1-3). PBC

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occurs between the ages of 35-70 and affects predominantly women (1, 4).

Antimitochondrial antibodies (AMA), which are present in 90-96% of patients, are the most characteristic of this entity and can be observed long before the disease is clinically overt (5, 6). In addition, epitopes of T cells and B cells targeting mitochondrial autoantigens have been identified (7, 8).

Antinuclear antibodies may be also detected in about 50% of patients and they are relevant as a tool for diagnosis of PBC, specially in AMA-negative patients and their high specificity for PBC has been confirmed in several reports (9-11). They are directed against three components of the nuclear envelope (NE): the lamina, the pore complex (NPC) and the inner membrane (10-12). Remarkable is the high specificity of anti-NPCs antibodies for PBC, which appears to be greater than 95%. Therefore, the presence of anti-NPC antibodies can be used to confirm the diagnosis of PBC in atypical cases, especially when AMA are undetectable (11-13).

The large geographical differences in the frequency of PBC and regional difference in AMA and anti-nuclear antibodies prevalence are explained by distinct immunogenetic background in the population of PBC patients (14, 15).

ANTIMITOCHONDRIAL ANTIBODIES

The presence of autoantibodies in the serum of patients with primary biliary cirrhosis (PBC) were first suggested by Jan Mackay in 1958 (12). In the following years, PBC sera were found to manifest a characteristic pattern, when tested again animal tissues by indirect immunofluorescence (IIF) and the cytoplasmic target identified as the mitochondria. In the past 50 years an enormous number of experimental studies have focused on AMA, and numerous rewarding discoveries have been made.

There are nine subtypes of AMA, four of which have been involved in PBC, including anti-M2, anti-M4, anti-M8 and anti-M9. It has been demonstrated, that autoantigens recognized by anti-M2 are located in the inner membranes of mitochondria, whereas those recognized by anti-M4, anti-M8 and anti-M9 are located in the outer mitochondrial membranes. Anti-M9 can be detected in both anti-M2-positive and -negative PBC patients, while anti-M4 is only positive in the presence of anti-M2.

The target antigen M2 is attached to the inner mitochondrial membrane (13, 16, 17) and consists of five components (17). These components have been identified in the following years on molecular bases as subunits of the 2-oxo acid dehydrogenase complex of the inner mitochondrial membrane: the pyruvate dehydrogenase complex (PDC), the 2-oxoglutarate dehydrogenase complex and the branched-chain 2-oxo acid dehydrogenase complexes (18-20). Using an original expression vector, Gershwin first identified the cDNA encoding the 70 kDa mitochondrial antigen that led to the identification of E2 subunit of pyruvate dehydrogenase complex (PDC-E2) (21). Serum antimitochondrial antibodies react against members of the 2-oxo-acid dehydrogenase complexes (2-OADC) family, including PDC-E2, the E2 subunit of branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2), the E2 subunit of the oxoglutarate dehydrogenase complex (OGDC-E2),the dihydrolipoamide dehydrogenase (E3)-binding protein (E3BP), and the E1α subunit of pyruvate dehydrogenase complex (PDC-E1α) (22). Sera of histologically proven PBC cases react only with the antigens BCOADC or OGDC, but not with PDC-E2, in 4-13% (23,24). More recently, Gershwin, Leung, and colleagues created a recombinant fusion protein (MIT3), which includes the immunodominant portions of the 3 primary targets of AMA, E2 – subunits of the pyruvate dehydrogenase complex (PDC-E2), the branched-chain 2-oxo-acid dehydrogenase complex (BCOADC-E2) and the 2-oxo-glutarate dehydrogenase complex (OGDC-E2) (25).

The major M2-antigen which is recognized by nearly 90-96% of PBC sera is the E2 component of PDC.

By indirect immunofluorescence false-positive results are quite frequent and in many cases a more sensitive and specific confirmation test, such as immunoblotting or ELISA, is required. In studies of AMA reactivity in large numbers of sera, Leung et al. have demonstrated that recombinant antigens used for immunodiagnosis are strikingly specific for PBC. Specificity and sensitivity is dramatically increased with the use of ELISA and/or immunoblot assays compared with immunofluorescence (28). Recently, it has been suggested that Luminex and an ELISA using a mixture of purified PDC and MIT3 as antigenic targets may increase sensitivity of the AMA detection systems (26, 27).

AMA positivity is considered specific for PBC, indeed it is one of the three diagnostic criteria for the disease. No proof of a pathogenic role for AMA has been obtained thus far. It is not clear how autoantibodies directed against a ubiquitous antigen might in turn produce a highly tissue-specific autoimmune injury. Studies of AMA-M2-positive individuals with initially no evidence of cholestatic liver disease of whom the majority developed fully manifested PBC over time additionally indicated that these autoantibodies may indeed be sufficiently specific very early markers development of PBC (28-30). Metcalf et al. reported that about 75% of asymptomatic individuals with serum AMA eventually developed symptoms of PBC over a 10-25-year observation period (29). Serum AMA strongly suggest the diagnosis of PBC at early stages even before the appearing of biochemical cholestasis. Although highly specific, AMA do not predict the prognosis in patients with PBC (30). Study of stored sera of well-characterized PBC patients followed for 7-28 years indicate that AMA levels are not associated with disease severity and progression (28).

Furthermore, AMA will typically reappear in patients transplanted for PBC but histological features of PBC re-manifest in few cases (31).

M4 is a single antigen with molecular weight of 52 kDa. Anti-M4 is found predominantly in patients with histological features of chronic active hepatitis and PBC. Recent studies have identified the major proteins in the M4 fraction which is related to the PDC-E1 subunits and sulphite oxidase (32-37). Anti-M8 has been found only in coexistence with anti-M2, the presence of anti-M8 indicates progressive disease activity. On the other hand, not all anti-M2-positive patients have anti-M8 (34, 38). Anti-M9 antibody is detected predominantly in patients with asymptomatic and early PBC, and it also can be positive in anti-M2-negative PBC patients. Patients with only anti-M9 have all the typical biochemical features found in classic anti-M2-positive patients, but seem to have slower disease progression and benign outcome, whereas patients having complement-fixing antibodies against anti-M2, anti-M4, and anti-M8 seem to have more active disease and worse outcome (34, 36, 37), though this finding wasn't supported by a blinded study on Dutch PBC patients conducted by Vleggaar et al (39).

The exact role played by AMA in the immunopathology and pathogenesis of PBC remains elusive. However, current data indicate that the destruction of biliary cells is mediated by liver-infiltrating autoreactive T cells specific for the dominant PDC-E2 autoantigen (40-43).

AMA are non-organ- and non-species-specific, and contain IgA, IgG and IgM subclasses. Some studies have demonstrated that the different AMA IgG subclasses have different clinical significance (44).

Several possible mechanisms have been suggested regarding the generation of AMA, such as oxidative damage, molecular mimicry and changed biliary epithelial cell (BEC) apoptosis (45, 46).

Approximately 5% of well-documented PBC patients do not react with any of the mitochondrial antigens using currently available assays.

ANTINUCLEAR ANTIBODIES

Nuclear envelope protein autoantibodies

Multiple nuclear reactivities may be present in an individual patient. Although serum anti-NE are helpful diagnostic tools, their association with disease pathogenesis remains to be elucidated, generally they are believed to be non-pathogenic. Since around the 1990, a number of nuclear envelope structures have been recognized as specific targets of antinuclear autoantibodies (ANA) producing a rim-like staining (anti-NE) at indirect immunofluorescence. In PBC approximately 50% of sera manifest anti-NE positivity (47, 48) when tested by IIF using commercially available substrates such as HEp-2 cells. Although the fluorescent test is still the most widely used screening assay for anti-NE testing, new immunochemical tests based on ELISA and immunoblotting with purified antigens have been developed.

Components of the nuclear lamina were first identified as target antigens in 1975; proteins of the nuclear pore complex (NPC) in 1991, and most recently various proteins of the inner nuclear membrane.

The inner nuclear membrane contains a number of unique proteins that include the lamina – associated polypeptides LAP1 and LAP2 and the lamin B receptor (LBR).

The NPCs are 125 MDa supramolecular structures embedded in the bilayer nuclear membrane at sites where outer and inner nuclear membranes are joined. NPCs are composed of over a hundred proteins and two of them: glycoprotein 210 (gp210) and nucleoporin p62 have been identified as potential autoantigens.

Because some of the autoreactive T-cell clones specific for human pyruvate dehydrogenase complex (PDC)-E2 can cross-react to mimicry peptides derived from nuclear antigens such as human gp210, Nakamura et al. proposed the hypothesis that molecular mimicry can be operative in the diversification of autoantibody-repertoire from PDC-E2 to gp210 during the course of disease progression in PBC (9, 43).

Anti-gp210 antibodies are highly specific for PBC, the prevalence varies between 15 and 45% of all PBC patients and 10-50% of AMA-negative PBC patients (10, 47, 48) in different studies. Their specificity appears to be greater than 99% (47-49). Anti-gp210 antibodies can be detected by indirect immunofluorescence assay, but detection of the nuclear rim pattern associated with them is frequently difficult to interpret as a result of concomitant AMA or ANA staining and can also be the result of non-gp210 specificities (48). Detection of anti-gp210 by western blot is technically demanding and relies on subjective interpretation (47). Molecular studies have identified an immunodominant epitope of gp210. It is compose of an amino-terminal domain of 1,783 amino acids a 20-amino acid transmembrane segment and a cytoplasmic carboxy-terminal tail domain of 58 amino acid. The carboxyl terminus of gp210, with its domain oriented to the outside of the nuclear lumen and supporting the nuclear pore complex is thought to be particularly antigenic. Nickowitz and Worman studying the reaction of autoantibodies with different fusion proteins corresponding to the carboxy-terminal domain of gp210 have found that the epitope recognized by the autoantibodies was contained within a streth of 15 amino acids. This sequence was proposed as the real autoantibody – binding site in gp210, which, since has structure close to the epitope seems to be the best antigen for such test. Reliable enzyme-linked immunosorbent (ELISA) tests based on recombinant gp210 expressed in bacteria or polypeptides that have been chemically synthesized have been established and they are available for their detection (49, 50).

Nucleoporin p62 is another protein complex associated with the nuclear envelope, localized near the central gated channel of the NPC. It remains associated with the NPC-lamina fraction. p62 is a serine/ /threonine-rich protein of ~520 amino acids, with tetrapeptide repeats at the amino terminus and a series of alpha-helical regions with hydrophobic heptad repeats.

It is synthesized as a soluble cytoplasmic precursor of 61 kDa (51). Anti-p62 antibodies are detected in 20-50% of PBC sera. They also generate a perinuclear pattern in IIF (52, 53). They occur with a specificity for PBC 96-99%. Some studies have been reported that anti-p62 positive patients have higher levels of serum bilirubin and more marked inflammatory infiltrates on liver biopsy (11, 54).

Courvalin et al. have identified autoantibodies from patients with primary biliary cirrhosis that recognize the nuclear envelope of mammalian cells on indirect immunofluorescence microscopy. These antibodies bind to a 58-kD integral membrane protein (p58) of the turkey erythrocyte nuclear envelope, which has been previously identified as a membrane receptor for lamin B (54). The antibodies also bind to a 61-kD integral membrane protein (p61) of the rat liver nuclear envelope. Affinity-purified antibodies eluted from turkey p58 bind to rat p61, showing that two proteins share an epitope(s) and p61 is likely to be the rat liver lamin B receptor. Anti-LBR antibodies are sporadic and were reported in few percent of PBC (52, 54), although a higher frequency was reported in Japanese study (47).

Anti-LAP antibodies were detected in a variety of autoimmune conditions including PBC. Anti-LAP2 antibodies were reported to occur in 6-15% of PBC cases, but are not disease-specific (47).

In contrast to AMA, some of these ANA directed against NPC (anti-pg210, anti-p62) have been reported to be associated with disease severity and poor prognosis in PBC (10, 11, 47, 55, 56, 60). Some groups have recently found a strong association between antibodies directed against NPC proteins and more active liver disease. The anti-NPC-positive patients showed more marked alterations at liver function tests, and had more severe histological features of inflammation and hepatocellular damage (11, 52, 57, 61-63). The presence of anti-gp210 and/or anti-p62 can strengthen the diagnosis of PBC in cases where the clinical presentation may be unclear.

Antibodies to Sp100

Antibodies to Sp100 are directed against a nuclear protein that has a molecular mass of 95-100 kDa and a dot-like distribution within cell nuclei by indirect immunofluorescence (57, 58). Sp100 is localized to punctate domains in the nucleus nuclear dots or nuclear bodies. Indirect immunofluorescence of PBC patient sera produce a characteristic pattern called “nuclear dots pattern”, clearly different from the speckled pattern of anti-nuclear ribonucleoprotein (anti-RNP) antibodies and from the typical dot pattern of anti-centromere antibodies, which are also found in PBC patients (11, 60, 61, 62-65). They have high specificity for PBC among patients with chronic liver disease (65, 66). Its frequency was higher in PBC patients studied in Europe (27-30%) (66), than in patients from North America (20-25%) (68, 69). Within the appropriate clinical context of chronic liver disease, the performance parameters for anti-Sp100 (specificity, 94%) support their diagnostic role in PBC, especially in AMA-negative patients (46, 69-73). They occur in older patients with PBC.

Antibodies to PML

PML originally was identified as a protein aberrantly expressed in leukemic cells of patients with acute promyelocytic leukemia (72). Similar to Sp100, the PML protein appears to have transcription regulatory functions. Anti-PML antibodies are present in about 15-20% of PBC patients (70)and they are specific for PBC (71)

Antibodies to Sp140

These antibodies are found concurrently with anti-Sp100 in 90% of instances, and their independent diagnostic and prognostic roles have yet to. No association was found between anti-Sp140 and any clinical feature of PBC (74).

Anticentromere antibodies (ACA)

Nakamura et al. demonstrated for the first time that anticentromere antibodies are a significant predictive factor for the progression to developing a complication of portal hypertension (56). The target antigen of ACA in PBC patients is centromere protein B (CENP-B), an 80-kd polyprotein that interacts with centromeric heterochromatinin human chromosomes (75).

SUMMARY

The fact that a variety of autoantibodies have been detected in PBC suggests the disease has a complicated pathogenesis. The validated prognostic markers for PBC currently use are total serum bilirubin and the Mayo risk scores, which is calculated using a formula employing age, serum bilirubin level and a albumin level. Antibodies profiles could be used to predict prognosis in patients, first of all in patients with asymptomatic disease. AMAs are detected in most sera of PBC patients. AMA titers or patterns have no prognostic value, but their presence is diagnostic of PBC. They present many years before the manifestation of clinical and histological signs. ANA are found in about 50% of PBC patients. Anti-gp210, anti-Sp100 and anti-p62 are highly specific for PBC. They have been reported to be associated with disease severity and poor prognosis in PBC. The detection of novel PBC-specific antinuclear antibodies can provide novel tools in diagnosis and prognosis in PBC patients.

Multicentre long-term cohort study conducted on relatively large group of PBC patients should be conducted, what is most important, to demonstrate prognostic significance of these antinuclear antibodies in PBC.

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