

Laboratory diagnosis of autoimmune hepatitis

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Abstract

Autoimmune liver diseases include autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). There is no specific diagnostic test for autoimmune hepatic diseases. Diagnosis is based on careful exclusion of other causes of chronic liver disease like viral, metabolic, genetic and toxic aetiologies of chronic hepatitis or hepatic injury, together with the finding of several suggestive laboratory markers. The paper describes major autoantibodies and serological diagnostic strategies in patients with autoimmune hepatitis.

Key words: autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoantibodies, diagnostics.

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Autoimmune liver diseases include 3 distinct disease entities: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) [1]. In most cases each condition is associated with distinct serological profile, morphological changes, clinical course and response to therapy although overlapping syndromes also have been described [2]. Autoimmune hepatitis is a disease characterised by inflammation and destruction of the hepatic parenchyma which shows a good response to immunosuppressive therapy. PBC and PSC are primarily autoimmune diseases of the biliary tract [3, 4]. In PBC, damage is restricted to the small intralobular bile ducts whereas, in PSC the extrahepatic and/or intrahepatic ducts may be affected. Autoimmune etiology of these diseases is proved by the presence of hypergammaglobulinemia and of a variety of autoantibodies. Additionally, up to 70% of patients with these conditions have at least one concomitant immunological disorder, most frequently Sjögren's syndrome, rheumatoid arthritis, thyroiditis, diabetes or ulcerative colitis [1]. Although familial AIH, PBC, or PSC is rare, there appears to be an underlying genetic susceptibility to each condition as evidenced by well established associations with various HLA haplotypes or allotypes, several of which are also associated with other autoimmune disorders [1, 5].

There is no specific diagnostic test for autoimmune hepatic diseases. Diagnosis is based on careful exclusion

of other causes of chronic liver disease like viral, metabolic, genetic and toxic aetiologies of chronic hepatitis or hepatic injury, together with the finding of several suggestive laboratory markers like autoantibodies, some of which, such as antimitochondrial antibodies (AMA) in PBC, are relatively disease-specific but most others, such as antinuclear antibodies, are non-specific markers. The diseases can be confirmed by histological examination revealing specific morphological changes [1, 4].

Autoantibodies contribute to the diagnosis of AIH and have led to the serological subclassification into three distinct types [1, 4]. Based on these results, a serological subclassification of AIH has been developed [6-8]. Although this classification remains controversial, it has resulted in the delineation of three subtypes which can be distinguished on the basis of the presence of different autoantibodies (Figure 1). The classical form of AIH (type 1) displays antinuclear antibodies (ANA.) This form of AIH represents 80% of all cases of AIH, making it the most prevalent subclass. Antinuclear antibodies show specificity for both functional and structural proteins in the cell nucleus. They can be directed against nuclear membranes, proteins or DNA [8-13]. Unfortunately the target antigens are heterogeneous and incompletely defined even within a single disease such as AIH. For the diagnostic work-up of AIH the molecular characterization of target antigen specificity does not supply important additional information to increase diagnostic pre-

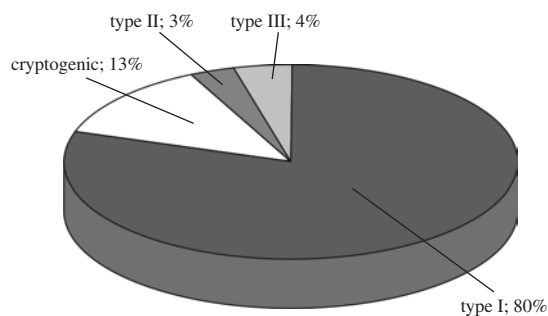


Fig. 1. The frequency of AIH subtypes

cision [8]. Screening determinations of ANA are routinely performed by indirect immunofluorescence on cryostat sections of rat liver and on Hep 2 cell slides [8]. Most commonly, a homogeneous or speckled immunofluorescence pattern is demonstrable. Antinuclear antibodies have been found to be reactive with an array of antigens. They represent the most common autoantibody in AIH and occur in high titres usually exceeding 1 : 160. However, the titre does not correlate with the course of disease, prognosis, progression, requirement of transplantation or disease activity [8]. Anti-smooth muscle antibodies (SMA) are directed against cytoskeletal proteins such as actin, troponin and tropomyosin [14, 15]. They frequently occur in high titres in association with ANA. They are not highly specific for AIH and have been shown to occur in advanced liver diseases of other etiologies, in infectious diseases and in rheumatic disorders. In these cases titres are often lower than 1 : 80. SMA autoantibodies are also determined by indirect immunofluorescence on cryostat sections of rat stomach. In paediatric patients, SMA autoantibodies may be the only marker of AIH type 1. When present in very young patients with AIH type 1 the titres may be as low as 1 : 40. The patients with type 2 of AIH have autoantibodies directed against antigens expressed in the endoplasmic reticulum (ER) termed anti-microsomal autoantibodies, these were shown to possess specificity against cytochrome P450 monooxygenases expressed in the ER. Anti-microsomal antibodies are directed against CYP2D6 – expressed not only in the liver but also in the kidney (liver/kidney microsomal antibodies LKM) [8, 16-18]. The third serological group of AIH (type 3) present with an antibody directed against a protein present in the 100 000 g supernatant of liver homogenate [8, 17]. This was termed anti-soluble liver antigen; it was independently described as anti-liver pancreas autoantibody (SLA/LP) [6, 18-20]. SLA/LP autoantibodies have recently been shown to be directed against a UGA repressor t-RNA-associated protein [21-24]. These two antibodies have been reported in only about 30% of patients, and some of these patients do not have ANA, SMA, or anti-LKM1.

There is some evidence that anti-SLA and anti-LP are one and the same autoantibody. These findings represent a major advance in the study of autoimmune hepatitis [25]. Investigators claim that anti-SLA/LP has 100% specificity (with 30% sensitivity) for autoimmune hepatitis. Cloning of SLA/LP provided the opportunity for the development of a simple and widely available test for the antibody. Type 3 shares clinical similarity with AIH type 1; because of this similarity, it is believed by some researchers not to represent a distinct subclass of its own [4, 26]. 70-80% of patients with AIH present with high titres ANA, SMA, SLA/LP or anti-LKM, but diagnosis can be difficult in the 20–30% who do not have these markers [6, 25]. Many such patients have other autoantibodies, including those showing perinuclear staining on neutrophils (pANCA) and antibodies reacting with the hepatocyte-specific asialoglycoprotein receptor (ASGPR) [6, 25]. However, testing for anti-ASGPR is not yet widely available and neither this antibody nor pANCA is exclusive to autoimmune hepatitis. single underlying mechanism but is most likely a group of diseases with a similar clinical presentation. In view of this, it is interesting to note that AIH occurs in 10% of patients with autoimmune polyglandular syndrome type 1 (APS-1) [6, 26]. Autoimmune polyglandular syndrome type 1 combines hypoparathyroidism, mucocutaneous fungal infections, adrenal insufficiency and a number of other immune-mediated symptoms such as nail dystrophy, vitiligo and alopecia. In patients with APS-1 who develop AIH, autoantibodies are present which are also directed against CYP proteins, namely, CYP1A2 and CYP2A6 [6, 27].

Primary sclerosing cholangitis (PSC) is a chronic and progressive cholestatic liver disease, which is characterized by inflammation and fibrosis of mainly the large bile ducts leading to biliary cirrhosis in a high percentage of patients [28, 29]. Primary sclerosing cholangitis usually involves both the intrahepatic and extrahepatic biliary tree. It is often associated with underlying inflammatory bowel disease, especially ulcerative colitis [28]. After secondary causes of sclerosing cholangitis are eliminated, biochemical tests, clinical presentation, and histology suggest the diagnosis [28, 29]. In PSC multiple non-specific autoantibodies, which are rather epiphenomena to chronic inflammation, can be found including ANA in 7-77%, anticardiolipin antibodies in 4–66%, SMA antibodies in 13-20%, anti-thyroid peroxidase (TPO) antibodies in 16% and rheumatoid factor in 15% [28, 29]. Atypical perinuclear-staining, antineutrophil antibodies (p-ANCA) can be found in 60-93% of patients with PSC but also in patients with AIH. Anti-neutrophil antibodies associated with PSC are distinct from c-ANCA and classical p-ANCA which are commonly used as diagnostic and therapeutic seromarkers for Wegeners granulomatosis and microscopic polyangiitis, respectively. Primary sclerosing cholangitis, ulcerative colitis and autoimmune hepatitis are associated with atypical p-ANCA which has a distinct staining pattern on indirect immunofluorescence

microscopy [30]. Although a few studies have demonstrated some correlation between particular clinical parameters and the presence of autoantibodies, there is presently insufficient evidence to make use of any of them in determining prognosis. Most are present at low prevalence and at relatively low titres [28, 29].

Primary biliary cirrhosis (PBC) is a chronic liver disease characterised by cholestasis, antimitochondrial antibody (AMA) and lymphocyte-predominant portal inflammation with a variable degree of fibrosis. While its pathogenesis remains obscure, considerable progress has been made in characterizing the autoantibody response that occurs in PBC. Seropositivity for AMA antibody is not specific for the disease, but remains highly sensitive (98%) [31]. The targets of the AMA response are enzymatic members of the family of the 2-oxo-acid dehydrogenase complexes [31, 32]. Approximately 90–95% of PBC serum samples react against the pyruvate dehydrogenase E2 complex. Antimitochondrial antibody are very heterogeneous with respect to antigen specificity and polyclonality of the B-cell response leading to their formation [32]. These factors, combined with antibody avidity and expression of immunoglobulin isotypes and subclasses, can account for considerably variable inter-laboratory sensitivity and specificity in the detection of AMA. Antimitochondrial antibody can be detected by IIF, complement fixation, immunodiffusion, radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), immunoblotting (IB), and a novel functional assay that depends on demonstrating inhibition of the catalytic activity of the respective enzyme [32]. For IIF, some laboratories employ commercially prepared HEp-2 substrates, although other laboratories use the conventional cryopreserved rodent kidney substrates [32]. A shortcoming of IIF techniques is that, compared to ELISA, they lack sensitivity and the ability to distinguish autoantibodies to specific molecular targets [32]. An advantage of IIF, especially when HEp-2 substrates are used, is the ability to detect other relevant autoantibodies such as those directed against nuclear pore complexes, centromere and other intracellular antigens that may have diagnostic and prognostic importance in PBC. In the near future, new test systems and assays are emerging and they include bead technology and other solid phase antigen arrays [32]. Each assay has limitations when determining the sensitivity, specificity and the positive predictive value for the detection of AMA. For most clinical cases, the detection of pyruvate dehydrogenase complex-E2 (PDC-E2) alone may be sufficient to ensure the diagnosis [5, 32]. However, to achieve acceptable diagnostic relevance (i.e., positive predictive value) more than one assay may be required [32]. The simultaneous detection of autoantibodies to numerous antigens in a single assay, such as the microarray technique, may provide useful alternatives to current protocols. Although AMA is considered the humoral hallmark of PBC, antibodies against various mitochondrial enzymes can be

frequently detected in patients with infectious liver diseases [32]. Depending on the assay used, up to 15% of PBC patients have been found to be AMA-negative. Sera from a subgroup of patients, including some AMA-negative patients, are positive for antibodies to nuclear components including Sp100, promyelocytic leukemia proteins, and two components of the nuclear pore complex. Antinuclear antibodies are also detectable in approximately 50% of subjects with PBC. Two particular autoantibodies, those that recognize nuclear pore membrane protein gp210 and those against nuclear body protein sp100, appear to be highly specific and detectable in approximately 25% of individuals with PBC. ANA against other nuclear envelope and nuclear body proteins also occur less frequently but appear to be highly specific for PBC [33]. Most clinical laboratories use indirect immunofluorescence microscopy to detect ANA and two labeling patterns that predominate in PBC are punctate nuclear rim and multiple nuclear dots. Antibodies giving these patterns most often recognize nuclear pore membrane protein gp210 and nuclear body protein sp100, respectively. These ANA are highly specific for PBC and detected in approximately 25% of patients [33]. Less frequently, ANA apparently unique to PBC recognize other proteins of the nuclear envelope and nuclear bodies. The antibodies against gp210, sp100 and some other nuclear proteins are very specific to PBC and may therefore be useful diagnostic markers. The clinical significance of ANA in PBC has been widely investigated and data indicate that, unlike AMA, they are not associated with disease severity and may be present many years before other clinical, biochemical, or histological manifestations occur. Antinuclear antibody and smooth muscle antibody arise in 35% and 66% of patients with primary biliary cirrhosis, respectively [32, 33]. Serum anticentromere antibodies in patients affected by the CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasias) are noted in 10–15% of instances. Other autoantibodies in association with the disease include

Table 1. Typical autoantibodies in autoimmune liver diseases

Autoimmune liver disease	Typical autoantibodies
AIH type I	ANA, SMA
AIH type II	LKM
AIH type III	SLA/LP, pANCA, anti-ASGPR
AIH cryptogenic	Autoantibodies not detected
PBC	AMA, anti-Sp100; anti-leukemia proteins, anti-membrane proteins gp210
PSC	No typical autoantibodies ANCA, ANA, anticardiolipin, anti-TPO and Rheumatoid factor may be present

rheumatoid factor (70%) and antithyroid (antimicrosomal, antithyroglobulin) antibodies (40%) [33].

Although much progress has been made in the understanding of the clinical expression of autoimmune hepatitis, its pathogenesis remains obscure despite more than 30 years of research [6]. There is considerable evidence pointing to a genetic susceptibility to the disease, [1, 5, 6] which may be related to one or more defects in the control of liver autoreactivity [25]. A characteristic feature of AIH is the fact that none of the major autoantigens detected by common autoantibodies in AIH are liver-specific, and not all are disease-specific. The diagnosis of AIH therefore rely on autoantibodies as a single positive identification marker. It is rather a diagnosis reached by the exclusion of other factors leading to chronic hepatitis, which include viral, toxic, genetic and metabolic aetiologies. The diagnosis of AIH is a probability best reflected in the AIH diagnostic score [4]. The most typical constellations of the antibodies for autoimmune liver diseases are presented in Table 1.

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