Autoantibodies in liver autoimmunity: diagnostic, clinical and prognostic significance

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Abstract

Liver autoantibodies represent a heterogenous group of organ and non-organ specific autoantibodies which are currently used for the diagnosis of autoimmune liver diseases (AiLD). AiLD affect either the hepatocyte or the biliary epithelial cell. In the former cases, the affected patients are suffering from autoimmune hepatitis (AIH), while in the latter the corresponding diseases are either primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). Most liver autoantibodies are used for the confirmation of the disease at presentation. Only few of those may have prognostic significance and are tested at various time points as indicators of response to treatment. The loss of tolerance to organ-specific autoantibodies noted in AiLD is an indirect hint of the role of the respective autoantigens in the pathogenesis of the disease. This chapter discusses major issues regarding the diagnostic, clinical and pathogenic significance of liver autoantibodies

Abbreviations

Autoimmune hepatitis (AIH), Antimitochondrial antibody (AMA), Anti-nuclear antibody (ANA), Antineutrophil cytoplasmic antibody (ANCA), Asialoglycoprotein Receptor (ASGPR), Cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA), Cytochrome (CYP), Human epithelioma 2 (HEp-2) cells, International Autoimmune Hepatitis Group (IAIHG), Indirect immunofluorescence (IFL), Filamentous actin

(F-actin), Formiminotransferase cyclodeaminase (FTCD), Liver kidney microsomal type 1 (LKM1), Liver cytosol type 1 (LC1), Perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), Primary biliary cirrhosis (PBC), Primary sclerosing cholangitis (PSC)

Introduction

Most studies referring to autoimmune liver disease (AiLD)-associated autoantibodies are concentrated on those directly related to the diagnosis of the disease, based on the established criteria for AiLD. Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis are the main three forms of AiLD and autoantibodies immediately related to these diseases have largely been studied.

The diagnosis of AIH recognized in two forms, AIH-1 and AIH-2, is largely based on the serological diagnosis of anti-nuclear antibodies (ANA) and smooth muscle antibodies (SMA) which are present in AIH-1, as well as, the prompt recognition of anti-liver kidney microsomal antibodies type 1 (anti-LKM1) and anti-liver cysotolic type 1 (anti-LC1) which characterize AIH-2. AIH-1 outnumbers AIH-2, with over 80-95% of AIH cases being AIH-1.

The diagnosis of PBC is confirmed - without a doubt- if in addition to clinical, serological and histological evidence of the disease, anti-mitochondrial antibodies (AMA) and/or disease-specific anti-nuclear antibodies (ANA) are present in the serum of the affected individuals.

The diagnosis of PSC solely based on serological markers is difficult to make. Most patients have anti-neutrophic cytoplasmic antibodies (ANCA) and clinical, biochemical as well as radiological features compatible with this disease. Amongst the three AiLD, PSC is the disease which is currently missing molecular antigen serological tests which without a doubt could pin-point towards a definite diagnosis of the disease. Thus, serological tests such as AMA or anti-LKM1 antibodies have a remarkable positive predictive value (PPV) for AIH-2 and PBC, respectively; however, no test so far has been found to have significant PPV for PSC.

Also, autoantibody testing can help to distinguish one AiLD from another i.e AIH from PBC or PBC from PSC. It also facilitates diagnosis of overlapping conditions, as patients with overlapping AIH/PBC or AIH/PSC features are found in a considerable.

Large epidemiological studies have clearly shown an increase of the incidence and prevalence of AiLD worldwide. Environmental, genetic and epigenetic factors have been accused for the induction of the diseases and the increase of cases over the years in industrial as well as the recently reported increase in developing countries (1-2). It has also become clear that an increase of the prevalence can be attributed to a parallel increase of the use of diagnostically relevant tests, including (by far) the wide incorporation of routine testing for liver autoantibody testing in individuals with abnormal liver tests and a clinical suspicion of AiLD (3) . The pathogenic significance of these autoantibodies is a matter of debate (4-6) and our laboratory is heavily interested on the role played by molecular mimicry in their induction (6-10) .

A synopsis of the diagnostic and clinical relevance of some of the so-called classical autoantibodies seen in patients with AiLD is provided. However, the main tasks will be to highlight the clinical utility of autoantibodies which are not always tested in routine laboratories using antibodies against the asialoglycoprotein receptor (ASGPR) as an example (11-12). It becomes apparent that some of the unconventional autoantibodies related to AiLD may not lack clinical usefulness after all. We will also address issues related to emerging points which are important for proper interpretation of liver autoantibody testing.

Autoimmune hepatitis

Laboratorians as well as clinicians are well aware that AIH-1 shows a female preponderance and can be diagnosed at any age, while the diagnosis of AIH-2 is virtually made only in children and especially in girls. Usually the two patterns of AIH-related autoantibodies are mutually exclusive, but if the serology includes positive tests for autoantibodies of both AIH types, the clinical disease manifests closely to that noted in children with AIH-2 (13).

The results of autoantibody testing for ANA, SMA, anti-LKM1, and anti-LC1 by IIF are widely used as a part of the "simplified" criteria of the International Autoimmune Hepatitis Group (IAIHG) for the routine diagnosis of AIH-1 or AIH-2 (14).

ANA testing in AIH

In general, ANA are the most frequently detected autoantibodies and inevitably the best studied so far in terms of their antigenic specificities and their relevance to autoimmune diseases (15). In relation to AIH, ANA are present in 30-70% of patients with AIH-1. However, meticulous assessment of ANA in this disease has failed to identify a single antigen being the sole marker for AIH-1. In other words, physicians should be aware that ANA that have a disease-specific pattern or occur only in AIH do not exist.

Testing of ANA in AiLD has traditionally been based on indirect immunofluorescence (IIF) using the triple rodent liver, kidney and stomach tissues in accordance with the guidelines issued by IAIHG. At a first glance this sounds paradoxical, as routine testing of ANA is by large based on IIF using human epithelioma cells (HEp)-2, the most prominent and efficient cell substrate for ANA testing. HEp-2's advantages for proper ANA testing have been discussed in great detail over the years (15).

These autoantibodies are frequently present in autoimmune rheumatic diseases such as systemic lupus erythematosus, Sjögren's syndrome and systemic sclerosis. An important point which needs to be underlined is that the co-existence of autoimmune rheumatic diseases in patients with AIH (as well as patients with PBC) is a frequent phenomenon. Thus, patients with AIH may indeed have ANA with antigenic specificity indistinguishable to that seen in patients with autoimmune rheumatic diseases. The best example to illustrate this is the presence of SS-A autoantibodies directed against Ro-52 which are frequently seen in patients with AIH in isolation or in combination with other autoantibodies (16).

Smooth muscle autoantibodies (SMA)

Their predominant target is filamentous actin (17). However, several serum samples positive for SMA do not recognize filamentous actin, suggesting that autoantigens other than that target are also present (18). Their prognostic significance is questionable, though studies in children have shown that their titre behavior can be used as an indirect indicator of response to immunosuppression.

Antibodies to soluble liver antigen (anti-SLA)

Anti-SLA is an organ specific autoantibody mainly seen in patients with AIH-1. This autoantibody can be the sole marker of AIH. Heated debate exists as to whether anti-SLA are only found in AIH-1 or can also be present in AIH-2 (13, 19-20). There is a consensus amongst authors that the presence of this autoantibody identifies patients with a more severe disease, who fail to respond to treatment (21). This makes anti-SLA

testing clinically meaningful and its testing is highly recommended, not only at presentation but also over time (21).

Diagnostic relevance of anti-ASGPR antibodies

High titre anti-ASGPR antibodies have predominantly been detected in patients with chronic active hepatitis, the disease currently known as AIH. It was also found that t the titres of these ASGPR autoantibody titres sharply decrease as soon as corticosteroids are initiated to patients (12, 22). The former finding is of importance because largely explains the differences found amongst studies regarding the prevalence of anti-ASGPR in AIH cohorts including mixed sera from patients at diagnosis and during treatment. Direct comparisons can be made only between studies referring to sera from naïve (untreated patients tested at diagnosis) AIH patients (23). The prevalence rates in children (24) and adult patients (25) with AIH-1 appear to be comparable, with anti-ASGPR antibodies being detectable in 75-82% AIH-1 patients. Anti-ASGPR antibodies appear less prevalent in children with AIH-2 (around 24-40%) (24, 26).

The diagnostic value of anti-ASGPR antibodies is underlined by studies suggesting that up to 80% of AIH patients, who are seronegative for conventionally-tested autoantibodies such as ANA, SMA, anti-LKM1 (an even anti-SLA), may have anti-ASGPR antibodies (27-28). In practical terms, these findings suggest that the autoantibody serological profile of patients with this disease may have been missed, if anti-ASGPR antibodies are not incorporated in the routine autoantibody testing (29) of patients with suspected AIH. This has led some expert reports to underline the importance of anti-ASGPR antibody testing (30-31). However, the guidelines of the diagnostic criteria for AIH do not include anti-ASGPR antibodies in the autoantibodies that are included in the scoring for the probable or definite diagnosis of AIH (32-33).

In view of the lack of commercially available assays, testing for anti-ASGPR antibodies has been limited to few university laboratories using laborious *in house* assays for research purposes. The reports so far published have also indicated that the specificity of anti-ASGPR for AIH is not as good as that seen for other autoantibodies, like anti-LKM1, anti-SLA and anti-SMA filamentous actin. Anti-ASGPR have been reported in more than 10% of patients with chronic hepatitis B or C, patients with PBC, and patients with alcoholic hepatitis (23, 34-38). The inconsistent results may be due to the lack of a standardized assay. In recent years, a new commercially-available ELISA based on purified rabbit ASGPR has been developed and a study has been published reporting on the sensitivity and the specificity of the assay. The authors of this study reported the presence of anti-ASGPR antibody in 70% of naïve (untreated) AIH patients and in less than 30%, who have been tested under immunosuppressive treatment (22). Detectable anti-ASGPR antibodies were found in approximately 10% patients with chronic hepatitis B or C (22). Anti-ASGPR antibodies were practically absent in other pathological controls (including patients with PBC and alcoholic hepatitis) (22). The interesting finding of this study was the striking specificity of anti-ASGPR for AIH (up to 100%) compared to PBC. Such findings have not been reported previously.

Clinical utility of anti-ASGPR antibodies

An early study testing anti-ASGPR antibodies in AIH-1 patients from USA noted more frequent relapses upon treatment cessation in anti-ASGPR antibody positive AIH patients compared to anti-ASGPR antibody negative (25). Serum imumunoglobulin levels appeared higher in those with anti-ASGPR antibodies (25). The association of disease activity indices or response to treatment and the presence of anti-ASGPR antibodies has been noted by others (24-25, 39-40). The recently published study employing the new anti-ASGPR antibody ELISA has found a correlation between anti-ASGPR and transaminasemia in patients followed for long period of time (22). Intriguing is the finding suggesting that anti-ASGPR antibodies may precede the onset of elevated transaminases. That finding and the fact that anti-ASGPR antibodies are

decreased over the course of immunosupression indicate that this autoantibody may be a prognostic marker of disease activity (23, 35-36). A recent report not published *in extenso* has also found that anti-ASGPR antibodies decrease during immunosupression (41-42).

It is of interest that most work regarding the pathogenic significance of liver autoantibodies is focused on those rarely seen in patients with AIH (particularly in AIH-2). There are several explanations for this, the main reason being that such autoantibodies are organ specific and their pathogenic potential is easier to speculate. Molecular mimicry mechanisms involving AIH-2 autoantigens and viruses has been the focus of increasing interest and ongoing investigation (43).

The role of autoantibodies in autoimmune cholestatic liver diseases is also discussed, as these autoantibodies are diagnostically relevant and may relate to the prognosis of the disease.

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