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Narrative Review

Serology in autoimmune hepatitis: A clinical-practice approach

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ABSTRACT

Serology is key to the diagnosis of autoimmune hepatitis (AIH). Clinicians need to be aware of which tests to request, how to interpret the laboratory reports, and be familiar with the laboratory methodology. If correctly tested, > 95% of AIH patients show some serological reactivity. Indirect immunofluorescence on triple rodent tissue is recommended as first screening step, since it allows the detection of all liver-relevant autoantibodies, except for anti-soluble liver antigen (SLA) antibody, which needs to be detected by molecular based assays. The threshold of immunofluorescence positivity is a titer equal or exceeding 1/40, but for patients younger than 18 years even lower titers are clinically significant. Anti-nuclear antibody (ANA) and/or anti-smooth muscle (SMA) antibody characterize type 1 AIH. ANA in AIH typically shows a homogeneous staining pattern on HEp2 cells, without any specific target antigen. Anti-SMA displays different staining patterns on indirect immunofluorescence: the vascular/glomerular (VG) and the vascular/glomerular/tubular (VGT) patterns are considered specific for AIH, whilst the V pattern can be found in a variety of diseases. Type 2 AIH, which is rare and affects mostly children/adolescents, is characterized by anti-liver kidney microsomal 1 and/or anti-liver cytosol 1 antibodies. The presence of anti-neutrophil cytoplasmic antibody (ANCA), particularly atypical p-ANCA (pANNA), points to the diagnosis of AIH, especially in absence of other autoantibodies. Since it is associated with sclerosing cholangitis and inflammatory bowel disease, these conditions have to be ruled out. The only antibody specific for AIH is anti-SLA, which is associated with a more severe disease course.

1. Introduction

The presence of autoantibodies is key to the diagnosis of autoimmune hepatitis (AIH), as stated by the diagnostic scoring systems issued by the International Autoimmune Hepatitis Group (IAIHG) [1–3]. The IAIHG diagnostic criteria are widely accepted [4,5] and have received extensive external validation [6–8]. In addition to the diagnostic importance, autoantibody specificities allow the classification of AIH in type 1 and type 2. The first is the most common type, affecting both children and adults, and is characterized by positive anti-nuclear (ANA) and/or anti-smooth muscle (SMA) antibodies. AIH type 2 accounts for one third of the AIH cases in children and adolescents, is rare in adults, has a more aggressive course, including more frequent fulminant presentation, and is characterized by the presence of anti-liver kidney microsomal 1 (LKM1) and/or anti-liver cytosol 1 (LC1) antibodies [9]. According to the most recently published IAIHG simplified scoring system, which aims at being clinical-practice friendly, a definite diagnosis of AIH cannot be reached in absence of a positive serology, though if autoantibodies are not detected in the initial work-up of a patient with liver disease of unknown origin, it is important to repeat the serological tests during the disease course, since autoantibodies may appear later, particularly if AIH presents acutely [10]. The clinician should be aware of which serological tests to request and be able to interpret the results within the clinical context. It would also help the clinician to understand the essential principles of the laboratory techniques used, as they play an important role in the interpretation of the results.

In this context, it is of interest that in a paper published in the New England Journal of Medicine in 2000 the authors cast doubts on the usefulness of testing for ANA and SMA in the diagnostic work-up of patients with elevated transaminase levels, owing to a perceived inaccuracy of the methodology used in the States at the time [11,12]. However, if the appropriate methodology is used, the detection of autoantibodies is of great clinical significance, as ANA and/or SMA are positive in > 95% of type 1 AIH patients [13].

The present paper provides a clinical-practice oriented overview on liver autoimmune serology focusing on AIH (Table 1).

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Table 1
Clinically relevant features of autoantibodies in autoimmune hepatitis [13,15,24].

	Target antigen	Frequency in AIH		Method of detection	Specificity for AIH	Peculiarity
		Type 1	Type 2			
ANA	histones, centromere, chromatin, double- and single-stranded DNA, cyclin-A and ribonucleoproteins. Undefined in 1/3 of AIH patients	75%	Rare	IIF	No	Homogeneous IIF pattern in 2/3 of the cases
SMA	Actin components	95%	Rare	IIF	Yes for VG and VGT pattern	Different IIF staining patterns on kidney tissue (V, VG, VGT)
Anti-actin	Filamentous actin	75%	Unknown	Molecular assays	Not entirely	Usually coexists with SMA in AIH1
Anti-LKM1	CYP2D6	absent	At least 2/3	IIF	Yes	Detected in up to 11% of hepatitis C cases
Anti-LC1	formiminotransferase cyclodeaminase	Very rare	2/3	IIF (masked if concomitant anti-LKM)	Yes	Rarely detecteced in hepatitis C
Anti-SLA/LP	O-phosphoseryl-tRNA:selenocysteine-tRNA synthase	20–50%	20–50%	Molecular assays	Yes	Rarely detected in hepatitis C
pANNA	Unknown	50–96%	Very rare	IIF	No	Associated with IBD and ASC/PSC

ANA, antinuclear-antibody; AIH, autoimmune hepatitis; IIF, indirect immunofluorescence; SMA, smooth-muscle antibody; VG, vessel glomerular; VGTvessel glomerular tubular; LKM, anti-liver kidney microsomal; LC, liver microsomal; SLA/LP, soluble liver antigen/liver pancreas; pANNA, peripheral anti-nuclear neutrophil antibodies; IBD, inflammatory bowel disease; ASC, autoimmune sclerosing cholangitis; PSC, primary sclerosing cholangitis.

2. Methodology

Indirect immunofluorescence (IIF) is the gold standard for the routine testing of liver autoantibodies, as it enables the simultaneous detection of virtually all the specificities relevant to autoimmune liver disease [4], including, besides ANA, SMA, anti-LKM1 and anti-anti-LC1, autoantibodies pointing to the diagnosis of primary biliary cholangitis (PBC), i.e. anti-mitochondrial antibody (AMA) and PBC specific ANA, making it highly relevant in the diagnostic work-up of liver disease of unknown origin [14]. In 2004, the Committee for Autoimmune Serology of the IAIHG published a consensus paper detailing guidelines for autoantibody testing in AIH, including comprehensive technical instructions as well as guidance on the interpretation of the IIF patterns [14]. According to this consensus paper, patient sera should be incubated with fresh triple rodent tissue, namely liver, kidney and stomach, and subsequently washed to remove non-adherent serological constituents. A fluorochrome-labeled antiserum directed to human immunoglobulins is then used to detect tissue bound antibody by ultraviolet microscopy [14], each specificity giving a characteristic pattern. The interpretation of the immunofluorescence reactivity is operator-dependent and requires sound expertise. The quality of the rodent substrate is also an important issue, as commercially available sections may be treated with fixatives leading to background staining and thus impairing the correct identification of antibodies [15]. Though these hurdles could be overcome by employing solid phase assays, based on the use of specific antigens, for several autoantibodies the specific target antigen is unknown (see below), making solid-phase assays only complementary to the screening by IIF [16]. If positive nuclear immunofluorescence is detected, sera must be titrated to extinction and IIF on human epithelial type 2 (HEp2) cells should be performed to characterize the pattern of nuclear staining, taking advantage of the large nuclei of this tumour cell line. HEp2 cells should not be used at the screening stage, because low ANA titers are frequently detected on HEp2 cells in healthy subjects [17,18].

IIF on ethanol-fixed human neutrophils allows the detection of anti-neutrophil cytoplasmic antibodies (ANCA), whereby atypical p-ANCA (p-ANNA) may support the diagnosis of AIH, particularly in absence of other autoantibodies [14].

As mentioned above, the molecular targets for some of the autoantibodies relevant to autoimmune liver disease have been identified, leading to the development of molecular tests for the detection of AMA, anti-LKM1, anti-LC1, and partially for ANCA [13]. Other antibodies relevant to the diagnosis of AIH, namely anti-soluble liver antigen/liver pancreas (SLA/LP), are not detectable by IIF, and their identification relies on molecular tests. Solid phase assays are commercially available and widely used, despite lack of standardization [19]. However, except for anti-SLA/LP, they should not be used as first-line screening [4].

3. Anti-nuclear antibodies

ANA was first reported in 1954 and was named “anti-nuclear factor” [20]. This reactivity was found to be responsible for the so called lupus erythematosus (LE) cells in the blood of LE patients. LE cells are neutrophils that have phagocytosed denatured nuclei of damaged cells, the phagocytosis being mediated by the presence of ANA [21]. LE cells were subsequently described in the ascites of patients with hypergammaglobulinemic chronic hepatitis, leading to the name “lupoid hepatitis”, the original name for AIH type 1 [22]. ANA lacks disease specificity, it is found in a wide range of autoimmune, infectious, toxic, and malignant diseases, but also, at low titers, in healthy adults, its prevalence increasing with age [17,18]. ANA molecular targets are only partially known, making IIF the recommended screening test, in order to avoid false negative results in patients who do not have antibodies reacting to known antigens covered by the commercially available molecular-based assays [16,23] (Fig. 1). In AIH, ANA characterizes type 1 disease, together with SMA, and is present in 75% of patients [15,24].