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Review

Clinical significance of autoantibodies in autoimmune hepatitis

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ABSTRACT

The accurate diagnosis and classification of autoimmune hepatitis (AIH) rely upon the detection of characteristic autoantibodies. Positivity for anti-nuclear (ANA) and/or anti-smooth muscle (SMA) autoantibodies defines AIH type 1 (AIH-1), whereas anti-liver kidney microsomal type 1 (anti-LKM1) and/or anti-liver cytosol type 1 (anti-LC1) define AIH type 2 (AIH-2). ANA and SMA, and less commonly anti-LKM1, have also been detected in *de-novo* autoimmune hepatitis developing after liver transplantation, a condition that may affect patients transplanted for non-autoimmune liver disease. The diagnostic autoantibodies associated with AIH-1 are also detected in the paediatric AIH/sclerosing cholangitis overlap syndrome, referred to as autoimmune sclerosing cholangitis (ASC). ASC, like adult primary sclerosing cholangitis, is often associated with atypical perinuclear anti-neutrophil cytoplasmic autoantibodies (p-ANCA), although p-ANCA are also detected in other autoimmune liver diseases. These associations highlight the necessity for simple and prompt diagnostic autoantibody testing, and the requirement for the accurate interpretation of the results of the tests in the clinical context. Fine-mapping of antigenic autoantibody targets has facilitated the development of rapid molecular assays that have the potential to revolutionise the field if properly standardised and when used in combination with classical immunofluorescence. Despite their diagnostic significance, the pathogenic role of the various autoantibodies and the mechanisms by which they can potentially inflict damage onto the liver cell remain a topic for further research.

1. Introduction

Autoantibody profiles facilitate the accurate diagnosis of the main autoimmune liver diseases (AILDs) – namely autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) – while enabling identification of distinct disease subtypes [1,2]. Moreover, the presence of particular autoantibodies aids the diagnosis of overlap syndromes, such as paediatric autoimmune sclerosing cholangitis (ASC) [3], and of AIH arising *de-novo* following liver transplantation (LT) [4].

There are two common serological autoantibody profiles associated with AIH; anti-nuclear (ANA) and/or anti-smooth muscle (SMA) autoantibodies define AIH type-1 (AIH-1), and anti-liver kidney microsomal type-1 (anti-LKM1) and/or anti-liver cytosol type-1 (anti-LC1) autoantibodies characterise AIH type-2 (AIH-2) [2]. These profiles are usually mutually exclusive. The accurate detection of these autoantibodies is of great importance, given their link to response to treatment and overall prognosis [2]. AMA, SMA and, less commonly anti-LKM1, have also been described in *de-novo* AIH appearing after transplantation [4]. In the paediatric setting, ANA and SMA are also associated with ASC, which, in appearance, differs from AIH-1 only by the presence of the radiological changes characteristic of sclerosing cholangitis [3].

The diagnostic hallmark of primary biliary cirrhosis (PBC), another autoimmune liver disease, is the presence of anti-mitochondrial autoantibodies (AMA) specific predominantly for the E2 subunit of the pyruvate dehydrogenase complex. AMA are highly specific for PBC, and over 95% of patients are seropositive [5]. Other autoantibodies associated with PBC are the PBC-specific ANAs [6].

Atypical perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA), which is the most commonly detected autoantibody in adult primary sclerosing cholangitis (PSC), is also frequently present in ASC [7]. However, p-ANCA has low specificity, as it can be detected also in AIH-1 [1].

In this review we critically discuss the diagnostic and prognostic significance of autoantibody seropositivity in AIH, outlining key unresolved issues along the way. We describe recent advances

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in the field of autoantibody detection, driven largely by the development of assays utilising recombinant or purified molecular autoantibody targets.

2. Autoantibody testing

Immunofluorescence (IFL) is the most commonly used diagnostic autoantibody screening technique [1,2]. The methodology is almost identical to that introduced by Weller and Coons in 1954, which utilises unfixed, air-dried tissue sections, incubated with potentially reactive serum [8]. The unbound serological constituents are removed by washing, and any bound antibody is detected using a fluorochrome labelled second antibody, raised in animal and specific for human immunoglobulin [2]. Characteristic antibody staining patterns are subsequently recognised by ultraviolet microscopy.

Recognition and interpretation of immunofluorescence patterns are not always straightforward. This is especially true given the lack of standardisation between tests and the heavy operator-dependency. Because of this, reporting errors are not infrequent, particularly when considering more uncommon specificities, such as anti-LKM1. Moreover, problems do exist between laboratory reporting and clinical interpretation of the results, which can also partly be accounted for by a degree of unfamiliarity of some clinicians with the disease spectrum of AILD [1,9].

A consensus statement compiled in 2004 by the Committee for Autoimmune Serology of the International Autoimmune Hepatitis Group (IAIHG) provided guidelines for appropriate and effective autoantibody testing in AIH, concluding that indirect IFL on fresh, multi-organ (liver, kidney, stomach) rodent (usually rat) sections should constitute first line screening [2]. The recommendations of the Committee include detailed guidelines for the preparation of substrate, application of the test serum samples, optimal dilution of samples and fluorochrome-labelled revealing agents, selection of controls and identification of diagnostically relevant staining patterns. The use of the three tissues enables the simultaneous detection of virtually all autoantibodies relevant to liver disease, namely SMA, ANA, anti-LKM1, anti-LC1 and AMA (Table 1) [2].

Positive sera should be titrated to extinction and the pattern of nuclear staining for ANA positive subjects may be further characterised by the use of human epithelial type 2 (HEp2) cells [2]. Since anti-LKM1 and AMA both stain renal tubules, the plan of tissue section and orientation of the kidney are of particular importance. This enables the distinction of characteristic staining patterns, revealed only in sections containing both proximal and distal tubules; while AMA preferentially stains the mitochondria-rich distal tubules, anti-LKM1 characteristically stains the third portion of the proximal tubules [2]. Important is also the use of multi-tissue substrate containing the stomach since AMA stains the gastric parietal cells, while anti-LKM1 does not. Other factors to take into account are the quality and source of the tissue sections utilised. These should be preferably dried in air and used without further fixation. Commercially available sections are of variable quality because they are treated with fixatives to lengthen shelf-life, which inevitably results in enhanced background staining that may hinder the recognition of diagnostic autoantibodies, especially when these are present at low titre [2].

Since healthy adults can show positivity at the conventional starting serum dilution of 1/10, the arbitrary dilution of 1/40 has been designated clinically significant by the IAIHG. Healthy children, on the other hand, are rarely reactive; therefore titres of 1/20 for ANA and SMA and 1/10 for anti-LKM1 are considered clinically relevant [2,3]. Hence, the laboratory should report any level of positivity from 1/10, and the attending physician should interpret the result within the clinical context, accounting for the age of the patient [9].

3. Autoantibodies in autoimmune hepatitis

3.1. Anti-nuclear antibodies

ANA, the first AIH-associated autoantibody [10], is readily detectable and gives a nuclear staining in the kidney, stomach and liver sections (Fig. 1). In most cases, its pattern, especially in

Table 1
Autoantibodies and their targets in autoimmune liver diseases.

Autoantibody	Target antigen(s)	Liver disease	Value in AIH	Conventional method of detection	Molecular based assays
ANA	Chromatin Histones Centromeres Cyclin A Ribonucleoproteins Double stranded DNA Single stranded DNA	AIH PBC PSC Drug-induced Chronic hepatitis C Chronic hepatitis B NAFLD	Diagnostic of AIH-1	IIF	ELISA, IB, LIA
SMA	Microfilaments (filamentous actin) Intermediate filaments (vimentin, desmin)	Same as ANA	Diagnostic of AIH-1	IIF	ELISA
Anti-LKM1	Cytochrome P4502D6	AIH-2 Chronic hepatitis C	Diagnostic of AIH-2	IIF	ELISA, IB, LIA, RIA
Anti-LC1	Formimino-transferase cyclodeaminase	AIH-2 Chronic hepatitis C	Diagnostic of AIH-2 Prognosis of severe disease	IIF, DID, CIE	ELISA, LIA, RIA
SLA/LP	$tRNP^{(ser)sec}$	AIH Chronic hepatitis C	Diagnostic of AIH Prognostic of severe disease, relapse and treatment dependence	Inhibition ELISA	ELISA, IB, RIA
pANNA	Nuclear lamina proteins	AIH PSC/ASC	Point towards diagnosis of AIH	IIF	N/A
AMA	E2 subunits of 2-oxoacid dehydrogenase complexes, particularly PDC-E2	PBC	Against diagnosis of AIH	IIF	ELISA, IB, RIA

ANA, anti-nuclear antibodies; SMA, anti-smooth muscle antibodies; anti-LKM1, anti-liver kidney microsomal antibody type 1; anti-LC1, anti-liver cytosol antibody type 1; SLA/LP, soluble liver antigen/liver pancreas; pANNA, peripheral anti-nuclear neutrophil antibodies; AMA, anti-mitochondrial antibodies; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; NAFLD, non-alcoholic fatty liver disease; IIF, indirect immunofluorescence; DID, double-dimension immune-diffusion; CIE, counter-immune-electrophoresis; ELISA, enzyme-linked immunosorbent assay; IB, immunoblot; LIA, line-immuno-assay; RIA, radio-immune-precipitation assay; N/A, not applicable.

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