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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.

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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.

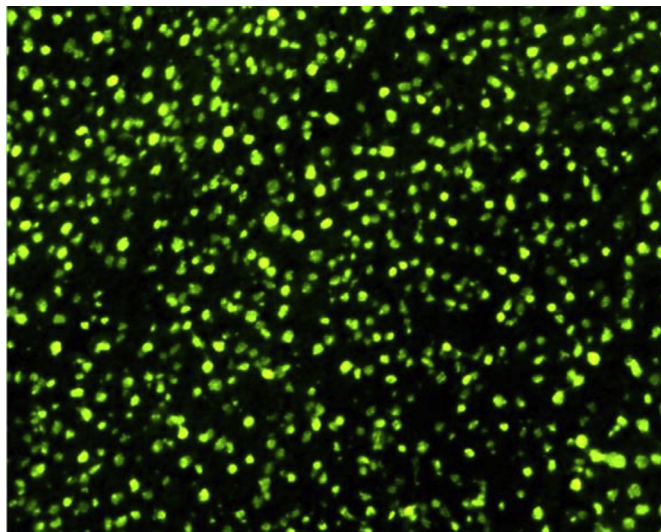


Fig. 1. Antinuclear antibodies in autoimmune hepatitis. Immunofluorescence pattern of antinuclear autoantibodies on rodent liver section.

the liver, is homogeneous, with a coarsely or finely speckled pattern observed less frequently [11].

HEp2 cells, characterised by prominent nuclei, should be used for a clearer definition of the nuclear pattern (Fig. 2); however, they should not be used for screening purposes due to a high positivity rate in adult and paediatric healthy populations [12,13]. In AIH, ANA target antigens are heterogeneous and not completely defined. ANA have been found to be reactive with single- and double-stranded deoxyribonucleic acid (DNA), small nuclear ribonucleoproteins (sn-RNPs), centromeres, histones, chromatin and cyclin A [14–16]. The advent of new techniques using recombinant nuclear antigens and immuno-assays will hopefully lead to a better definition of ANA target antigens, meanwhile assessing their specificity for diagnosis and their possible role in the pathogenesis of AIH-1 [1].

The mechanism leading to ANA production in AIH is not clear, though it has been related to the release of nuclear components following hepatocyte injury and/or a loss of B cell tolerance to several nuclear components [17]. In AIH, ANA titre is clinically relevant if higher than 1/40 in adults and higher than 1/20 in children [2], in whom it correlates with disease activity [18].

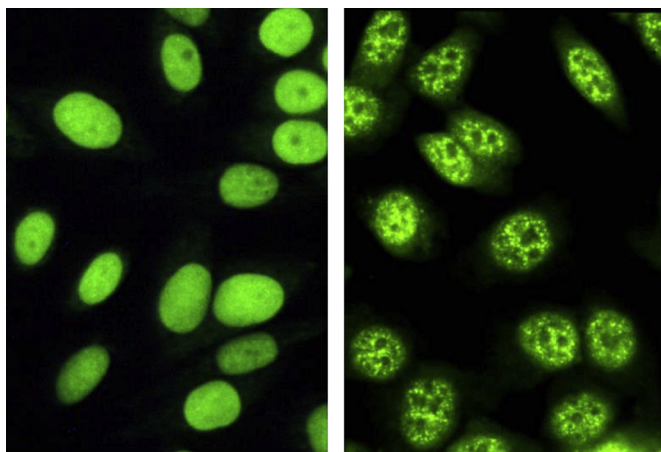


Fig. 2. Anti-nuclear antibody immunofluorescence pattern on human epithelial type 2 cells. The homogeneous pattern (left) is the most common in autoimmune hepatitis (AIH). The speckled pattern (right) is less common in AIH.

ANA have been also identified in up to 52% of patients with PBC [19], where they are associated with the characteristic immunofluorescence patterns of multiple nuclear dot or rim-like membranous appearance on HEp-2 or HeLa cells [20–22]. ANA are present not only in other autoimmune disorders such as systemic lupus erythematosus (SLE), Sjögren syndrome and systemic sclerosis [23] but also, at low titres, in patients with viral hepatitis, drug-induced hepatitis, and alcoholic and non-alcoholic fatty liver disease [19,24]; thus, though considered diagnostic markers, ANA are not specific for AIH.

3.2. Anti-smooth muscle antibodies

SMA antibodies are detected in kidney, stomach and liver sections, where they stain the walls of the arteries. In the stomach they also stain the muscularis mucosa and the lamina propria. On the renal substrate, it is possible to visualise the V (vessels), G (glomeruli) and T (tubules) patterns [25] (Fig. 3). The VG and VGT patterns are more specific for AIH than the V pattern. The VGT pattern corresponds to the “F-actin” or microfilament (MF) pattern observed when cultured fibroblasts are used as a substrate [26]. Neither the VGT nor the anti-MF patterns are, however, entirely specific for AIH. SMA are directed against constituents of the cytoskeleton such as actin and non-actin components including tubulin, vimentin, desmin, and skeletin [19].

The first recognised AIH-specific SMA target antigen was revealed after preincubation of serum with thrombosthenin (platelet actomyosin) or with purified thrombosthenin-A (the actin fraction of thrombosthenin). This observation suggested that SMA was directed against actin constituents [27]. More specifically, the use of vinblastine-arrested cultured fibroblasts as a substrate led to the demonstration that AIH-1-specific SMA VGT-positive sera stain predominantly the microfilaments. In contrast, non-AIH-1 SMA V-positive sera react with nonactin-containing intermediate filaments [28–30]. The AIH-1-specific target of SMA responsible for the VGT pattern remains elusive [31]. However, many studies suggest that actin, in its polymerised filamentous form, rather than its monomeric G actin form may be the target of SMA with the VGT pattern. Despite this, approximately 20% of AIH-specific SMA-positive cases fail to react with purified F-actin, and anti-F-actin positivity is reported in diseases distinct from AIH-1 [2,32,33].

In AIH, SMA usually occur at titres higher than 1/80, though in very young patients with AIH-1 the titres may be as low as 1/20 [34]. Like ANA, SMA are not specific for AIH, since their presence has also been reported in advanced liver disease of other aetiologies, infectious diseases and rheumatic disorders [35].

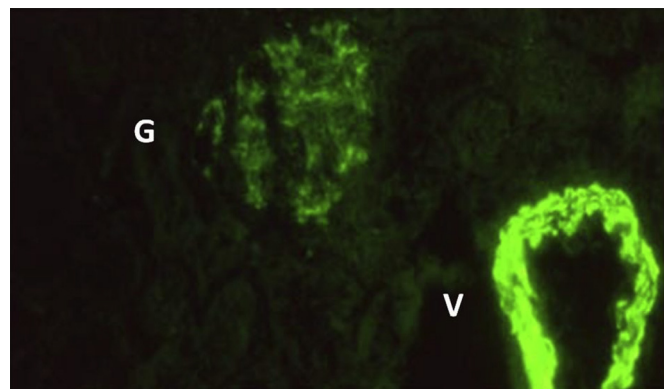


Fig. 3. Anti-smooth-muscle antibodies in autoimmune hepatitis. Immunofluorescent pattern of anti-smooth-muscle antibodies (SMA) on rodent renal section. SMA stains arterial vessels (V) and glomeruli (G) (VG pattern).

3.3. Anti-liver–kidney–microsomal type 1 antibodies

Anti-LKM1 antibodies, the hallmark of AIH-2, stain the hepatocellular cytoplasm and the P3 portion of the renal tubules. As alluded to above, some confusion can occur between the immunofluorescence patterns of anti-LKM1 and AMA because both autoantibodies stain the liver as well as the kidney [1]. However, AMA stain the liver more faintly than anti-LKM1, and mark the renal tubules more diffusely, while accentuating the distal tubules (Fig. 4). Importantly, AMA stain gastric parietal cells while anti-LKM1 do not [36].

The identification of the molecular targets of anti-LKM1 – cytochrome P4502D6 (CYP2D6) –, and AMA – enzymes of the 2-oxo-acid dehydrogenase complexes –, has led to the establishment of immune-assays based on the use of recombinant or purified antigens. Commercially available enzyme-linked immunosorbent assays (ELISAs) are accurate for detection of anti-LKM1, at least in the context of AIH-2, and reasonable for the detection of AMA. Thus, if immunofluorescence remains doubtful, the use of molecularly based immune-assays may be of help [37]. Anti-LKM1 titre is clinically relevant if equal or higher than 1/40 in adult patients and 1/10 in subjects up to the age of 18 years [2].

Intriguingly, anti-LKM1 are also present in up to 10% of patients with chronic hepatitis C virus (HCV) infection, and their titre has been found to be associated with disease activity [38]. HCV infection may play a role in the initiation of the autoimmune process. Interestingly, reactivity against CYP2D6_{193–212}, identified as a major B-cell epitope in AIH-2, is also found in 50% of HCV infected patients with seropositivity for anti-LKM1 antibodies [39]. Cross-reactivity between antibodies directed against homologous regions of HCV (NS5B HCV_{2985–2990}) and cytomegalovirus (exon CMV_{130–135}) has

been demonstrated in HCV-positive/anti-LKM1-positive patients [40]. Moreover, Manns et al. reported a sequence homology between CYP2D6_{254–271} and the amino acid (aa) sequence 310–324 of E1 HCV and the aa sequence 156–170 of IE1 75 Herpes Simplex Virus 1 (HSV-1) [41]. As anti-LKM1 antibodies cross-react with homologous regions of CYP2D6, HCV, CMV and HSV, a “multi-hit” mechanism for the generation of these antibodies and possibly of AIH-2 may be envisaged [39,41]. According to this model, sequential exposure to common viral pathogens like CMV or HSV may establish permissive immunologic conditions by priming a cross-reactive subset of T cells in a genetically predisposed host. Depending on the level of exposure and the degree of genetic susceptibility, a minority of recurrently infected individuals may progress to autoimmune disease. It is therefore conceivable that an yet unidentified virus infection may be part of the origin of the autoimmune attack in AIH [9]. Support to this model has been provided by an interesting case-report describing a 10-year-old girl who acquired HCV infection following liver transplant for end-stage liver disease caused by α 1-anti-trypsin deficiency. Two weeks after HCV infection IgM anti-LKM1 appeared, followed by IgG anti-LKM1, a finding suggesting HCV as a trigger of a primary anti-LKM1/anti-CYP2D6 autoimmune response [42].

A second type of anti-LKM antibodies (anti-LKM-2), nowadays of historical interest, was associated with hepatitis induced by ticrynafen, a uricosuric diuretic, withdrawn from clinical use in 1980 because of its severe hepatotoxicity. Anti-LKM-2 antibodies were found to react most intensely with the cells in the initial third of the proximal tubule and their target antigen was defined as cytochrome P4502C9 (CYP2C9) [19]. Anti-LKM-3, which target members of the uridine glucuronosyltransferases (UGTs) family 1, give an immunofluorescent pattern similar to anti-LKM1, though

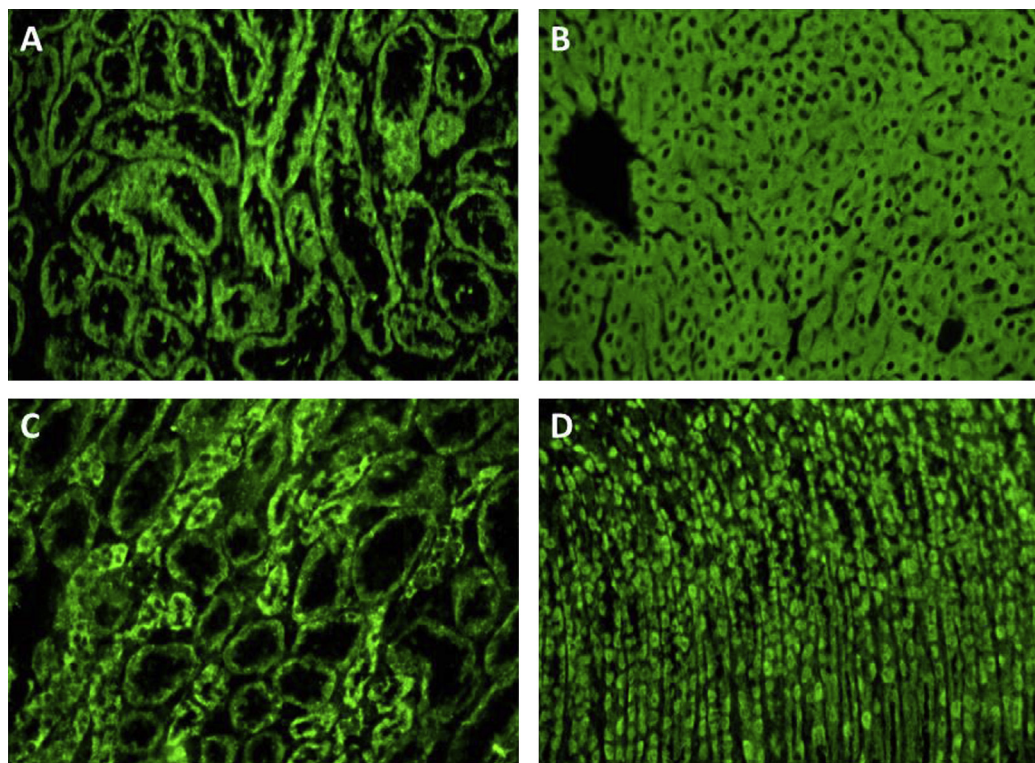


Fig. 4. Immunofluorescence pattern of anti-liver kidney microsomal type 1 and anti-mitochondrial antibodies. Anti-liver kidney microsomal type 1 (anti-LKM1) antibodies (A&B) stain mainly the proximal tubules (A), while anti-mitochondrial (AMA) antibodies (C&D) stain the smaller, distal tubules (C) of rodent kidney. Since these specificities are sometimes confused, the use of rodent liver (B) and stomach (D) sections is recommended to discriminate between the two reactivities; AMA typically stain the gastric parietal cells while anti-LKM1 stain the liver but not the stomach.

they occur mainly in hepatitis delta (D) patients; their presence, however, has also been reported in up to 19% of patients with AIH-2 [43–45].

Lastly, antibodies to CYP1A2, which were first described in patients with AIH induced by dihydralazine, were detected in patients with a form of AIH resembling AIH-2 as part of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome [46]. APECED is a monogenic autosomal recessive disorder caused by homozygous mutations in the autoimmune regulator (AIRE) gene and characterised by a variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis [47,48]. Heterozygous AIRE mutations have been reported in 3 children with severe AIH-2 and extrahepatic autoimmune manifestations [49] and in 4 children with AIH-1 and a family history of autoimmune disease [50].

3.4. Anti-liver cytosol type 1 antibodies

Anti-LC1 was originally described, both in combination with anti-LKM1 and in isolation, to define a clinical entity resembling AIH-2 [51]. Later, anti-LC1 was also infrequently found in association with the serological markers of AIH-1 [52] and in patients with chronic hepatitis C virus infection [53]. Anti-LC1 can be detected by indirect immunofluorescence using the standard tissue panel composed of rodent liver, kidney and stomach. It stains the cytoplasm of liver cells with relative sparing of the centrilobular area (Fig. 5), but is usually obscured by the concurrent presence of anti-LKM1 [2]. In the presence of anti-LKM1, anti-LC1 can be detected by the use of liver cytosol in double-dimension immunodiffusion, or counterimmunoelectrophoresis, and a positive reference serum [54]. In Western blot, anti-LC1 reacts with a 58–60 kDa protein when human liver cytosolic fraction is used as a substrate. The molecular target has been identified as the formimino-transferase cyclodeaminase, an enzyme involved in folate metabolism and most highly expressed in the liver [55]. The presence and titre of anti-LC1 antibodies have been found to correlate with disease activity and represent a potentially useful marker of residual hepatocellular inflammation in AIH [56]. The presence of anti-LC1 in

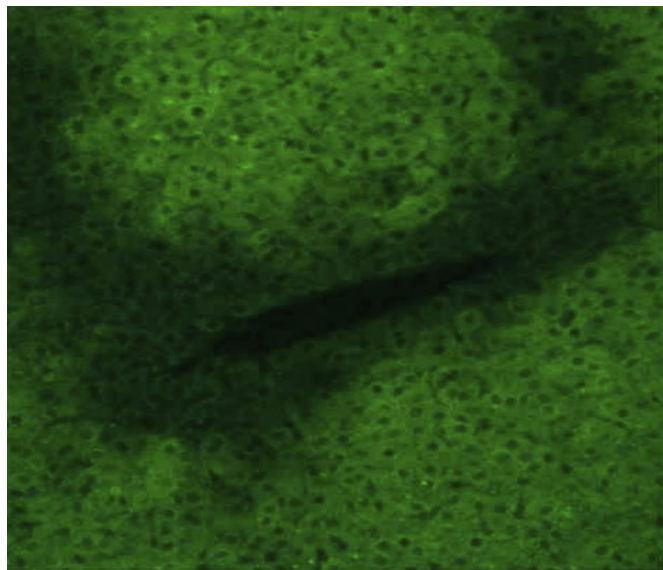


Fig. 5. Anti-liver cytosol type 1 antibodies. Immunofluorescence pattern of anti-liver cytosol type 1 (anti-LC1) antibodies on a rodent liver section: they stain the cytoplasm of hepatocytes with a weakening of the stain around the central vein.

isolation scores positively towards a diagnosis of AIH-2, allowing prompt initiation of treatment [9].

3.5. Anti-soluble liver antigen/liver–pancreas antigen antibodies

Anti-SLA and anti-LP, originally described as individual antibodies, share the same antigenic target and have been subsequently identified as the same autoantibody [57]. Anti-SLA/LP are detected by radioimmunoassay and ELISA but cannot be detected by immunofluorescence [9]. The presence of anti-SLA/LP in the absence of conventional antibodies, has led to the proposed definition of a third group of AIH (AIH-3) [58]. However, early reports used a cut-off point for conventional autoantibodies levels higher than those currently used for the diagnosis of AIH; therefore, this entity has not been accepted by the IAIHG. Anti-SLA/LP is highly specific for the diagnosis of AIH, and its detection at the time of diagnosis identifies patients with a more severe disease and a worse outcome [59].

Screening of cDNA expression libraries identified a UGA tRNA suppressor-associated antigenic protein (tRNP^{(ser)sec}), more precisely O-phosphoserine-tRNA:selenocysteine-tRNA synthase (Sep-SecS), as the anti-SLA target [60]. Molecularly based diagnostic assays have become available, but their full evaluation is still underway [9].

3.6. Anti-neutrophil cytoplasmic antibodies

ANCAs react to cytoplasmic components of neutrophils, giving a perinuclear (pANCA) or cytoplasmic (cANCA) pattern [9,61]. c-ANCA mainly reacts with proteinase 3 and is found in Wegener's granulomatosis; whereas p-ANCA reacts with myeloperoxidase and is frequently detected in microscopic polyangiitis. pANCAs are frequently present in AIH-1, akin to PSC and inflammatory bowel disease (IBD), however the staining pattern is atypical, being associated with peripheral nuclear membrane components, hence the name of peripheral anti-nuclear neutrophil antibody (pANNA) [31]. The description of a 50 kDa neutrophil-specific nuclear protein of the nuclear pore complex as the target antigen of pANNA was followed by its tentative identification as tubulin b chain 5 [1].

In contrast to AIH-1, pANNA is virtually absent in AIH-2. Detection of pANNA can aid the diagnosis of AIH, particularly in the absence of other autoantibodies [2].

3.7. Anti-asialoglycoprotein receptor antibodies

Attempts to identify putative auto-antigens specifically expressed on the hepatocyte surface in AIH have led to the description of a crude liver extract preparation known as the liver specific protein (LSP) and its major component, the asialoglycoprotein receptor (ASGPR) [62]. ASGPR, also designated as hepatic lectin, is a type II transmembrane glycoprotein. It is the only known liver-specific auto-antigen, and is constitutively expressed on the hepatocellular membrane. Antibodies to ASGPR are observed in 88% of all patients with AIH and can co-exist with ANA, SMA and anti-LKM1 [19]. However, they are not disease specific, as they can also be found in viral hepatitis, drug-induced hepatitis and PBC [63]. In AIH the levels of anti-ASGPR correlate with inflammatory disease activity and may be used as an additional marker to monitor treatment efficacy [64].

Anti-ASGPR antibody detection requires either purified or recombinant antigen. The lack of disease specificity coupled with the difficulty in developing a reliable molecular assay for the detection of anti-ASGPR has limited its wider applicability in diagnostic practice [31].

4. Diagnostic relevance of autoantibodies in autoimmune liver disease

A panel of autoantibodies of diagnostic relevance to liver disease should be investigated in all patients with abnormal liver function tests and/or liver disease of unknown aetiology [2,65]. However, negative or low autoantibody titre on a single test cannot exclude the diagnosis of AILD because autoantibody titres usually vary during the course of the disease. Repeated tests potentially allow autoantibody detection and correct disease classification. Conversely, in the absence of any other clinical and laboratory features suggestive of AILD, the presence of autoantibodies even at high titres is insufficient to accurately make a diagnosis [31].

In AIH, autoantibody positivity assists diagnosis while also enabling the differentiation of AIH into the two disease subtypes; AIH-1 and AIH-2. Positivity for ANA and/or SMA characterises AIH-1, whereas anti-LKM1 defines AIH-2 [2]. Positivity for antibodies associated with each subtype tends to be mutually exclusive. Antibodies to LC1, SLA/LP, actin, and neutrophil cytoplasmic antigens can assist diagnosis in cases negative for the classical AIH-associated autoantibodies; for this reason, they were also included in the original and revised IAIHG diagnostic scoring systems [66,67].

Approximately 15% of AIH-1 patients are ANA positive, 35% are SMA positive and 60% are positive for both autoantibodies [7]. Approximately 75% of ANA positive patients show a homogenous IFL staining pattern, while the remainder display a speckled pattern [31]. Laboratory, clinical, and histological indices are similar in AIH-1 regardless of positivity for ANA and/or SMA [7]. ASC patients have a serological autoantibody profile indistinguishable from that of AIH-1; ANA and/or SMA are present in almost all cases [3].

As mentioned above, ANA and/or SMA can be detected in other liver diseases, such as chronic viral hepatitis B, C and D, acute liver failure, drug-induced hepatitis, non-alcoholic steatohepatitis, alcohol-induced liver disease and hepatocellular carcinoma [19,24]. However, the titre is low and the patterns differ from those associated with AIH-1 [1,7].

In adult PSC, ANA and/or SMA are detected in approximately 20% of patients [68]. The possibility these patients have an adult form of ASC remains to be formally investigated.

All AIH-2 patients are seropositive for anti-LKM1 and/or anti-LC1, these reactivities supporting the diagnosis of AIH-2, particularly in the absence of HCV infection [34,67,69,70]. Though less than one-third of AIH-2 patients are solely anti-LC1 positive [51], it is important to test for this reactivity to reach a diagnosis [67] and initiate promptly immunosuppressive treatment [9].

Autoantibody positivity can also arise after liver transplantation for various diseases, the most frequently reported autoantibodies being ANA, followed by SMA [71,72]. Anti-LKM1 has also been described after transplantation, but its fluorescence pattern is often atypical, reacting with an unidentified cytosolic antigen [71,73]. In patients transplanted for non-autoimmune liver disease, *de-novo* AIH, characterised by positive autoantibodies and interface hepatitis, has been identified as a cause of graft dysfunction [71,74]. Though autoantibody positivity is relatively frequent after liver transplantation, and is not *per se* diagnostic of *de-novo* AIH, it is prudent to monitor autoantibodies regularly, particularly in the presence of abnormal liver function tests. Autoantibody seropositivity, in combination with high IgG and transaminase levels is an indication for prompt histological assessment [4].

5. Prognostic relevance of autoantibodies in autoimmune hepatitis

ANA and SMA titres tend to decline or disappear during effective immunosuppressive therapy in the majority of AIH-1 patients [1,7].

However, neither the titre at diagnosis nor the extent of fluctuations over time are thought to predict the disease course and outcome [7], though a positive correlation between the SMA titre and transaminase levels over time was observed in one study focused upon paediatric AIH-1 cases [18], suggesting a potential use of these antibodies, together with IgG levels, to monitor disease activity in children. Czaja and colleagues suggest that adult anti-actin positive AIH-1 patients have an earlier age at disease onset [32]. They also report that these patients respond less well to corticosteroid treatment, progressing to liver failure or liver transplantation more frequently compared to those seronegative [32].

The presence of double stranded DNA (dsDNA) autoantibodies is associated with higher IgG levels and elevated rates of relapse during immunosuppressive treatment compared to seronegative cases [75]. Additionally, persistence or re-appearance of inflammatory activity on histology – indicative of unresponsiveness to immunosuppressive treatment or relapse after treatment withdrawal – is associated with positivity for anti-ASGPR autoantibodies [63,76]. Moreover, anti-SLA antibodies denote patients with a particularly severe course of AIH and a propensity for relapse after corticosteroid withdrawal [58,59,77].

Anti-LKM1 positive paediatric patients tend to present at a younger age than ANA/SMA positive patients. They have higher bilirubin and transaminase levels at diagnosis and more commonly present with fulminant hepatic failure [34]. Additionally, they more frequently have partial IgA deficiency. Excluding patients with

Table 2

Diagnostic and prognostic significance of autoantibodies in autoimmune hepatitis.

Autoantibody	Diagnostic significance	Prognostic significance
ANA	Diagnostic of AIH-1	Not associated with disease course or outcome
SMA	Diagnostic of AIH-1	Not associated with disease course or outcome
Anti-LKM1	Diagnostic of AIH-2	Associated with younger age at presentation More frequently associated with fulminant hepatic failure More frequently associated with partial IgA deficiency
Anti-LC1	Diagnostic of AIH-2	Associated with severe liver inflammation and rapid progression to cirrhosis
SLA/LP	Diagnostic of AIH Present when other markers are absent	Associated with more severe disease course, treatment dependence, relapse after drug withdrawal and need for transplantation
pANNA	Points towards the diagnosis of AIH	Associated with development of AIH-sclerosing cholangitis overlap syndrome
Anti-actin	Points towards the diagnosis of AIH	Associated with younger age at disease onset Associated with treatment dependence in children Predicts progression to liver failure and need for transplantation
Anti-ASGPR	Points towards the diagnosis of AIH	Associated with more severe interface hepatitis Predicts relapse after drug withdrawal
Anti-dsDNA	No reported association	Associated with higher IgG level Predicts relapse during immunosuppressive treatment

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; Anti-ASGPR, anti-asialoglycoprotein receptor; anti-dsDNA, anti-double stranded DNA antibodies; Ig, immunoglobulin; AIH, autoimmune hepatitis.

fulminant hepatic failure, a severely impaired hepatic synthetic function – assessed by the presence of both prolonged prothrombin time and hypoalbuminemia – is more common in ANA/SMA-positive patients [34]. The severity of interface hepatitis at diagnosis appears similar in both types of AIH, although cirrhosis on initial biopsy is more frequent in AIH-1, suggesting a more chronic course of disease [34]. Akin to anti-LKM1, AIH-2 patients with anti-LC1 antibodies have histologically more severe disease compared to those seronegative [51,56,70] (Table 2).

6. Concluding remarks

Accurate and prompt detection of autoantibody seropositivity is critical for the diagnosis of autoimmune liver disease. Autoantibodies discriminate between distinct subtypes of AIH and facilitate the diagnosis of overlap syndromes.

Routine autoantibody testing by indirect immunofluorescence has recently been complemented by molecular assays based on purified or recombinant antigen. Although the specific molecular targets of anti-LKM1 and anti-LC1 in AIH-2 have been clearly identified, those of AIH-1, namely ANA and SMA, require better definition.

Despite their importance in diagnosis and classification, the pathogenic role the autoantibodies and the mechanisms by which they may potentially contribute to liver damage remain a topic for further research.

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