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REVIEW

Diagnostic autoantibodies for autoimmune liver diseases

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Autoimmune liver diseases are conditions of low prevalence that comprise the triad of autoimmune hepatitis, primary biliary cholangitis (cirrhosis) and primary sclerosing cholangitis and their poorly characterised overlapping syndromes. Diagnostic autoantibodies are associated with autoimmune hepatitis and primary biliary cholangitis but not with primary sclerosing cholangitis. Autoantibodies are useful disease markers that facilitate early diagnosis of autoimmune hepatitis and primary biliary cholangitis and allow for therapeutic intervention to prevent progression to liver cirrhosis and associated complications. Adult onset type 1 autoimmune hepatitis is associated with F-actin reactive smooth muscle autoantibody, antinuclear autoantibody in 60% of patients, and autoantibody to SLA/LP in 15–20%. Juvenile onset type 2 autoimmune hepatitis is associated with LKM-1 and LC-1 autoantibodies. Primary biliary cholangitis is associated with a mitochondria-associated autoantibody designated M2 in >90% of patients and with disease-specific antinuclear autoantibodies in 50% that bind to antigens in the nuclear core complex and in multiple nuclear dots. Autoantibodies to the nuclear core complex target gp210, nucleoporin p62 and nuclear lamin B receptor. Autoantibodies to multiple nuclear dots target Sp100 and PML antigens. Liver autoantibodies in asymptomatic patients with normal liver function may precede the subsequent development of overt autoimmune liver disease. For routine diagnostic immunology laboratories, initial screening for liver autoantibodies by immunofluorescence remains the method of choice with confirmation for reactivity with their target antigen by enzyme-linked immunosorbent assay (ELISA) or line blot when required.
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INTRODUCTION

Autoimmune liver diseases comprising the triad of autoimmune hepatitis, primary biliary cholangitis (PBC) (cirrhosis) and primary sclerosing cholangitis and their overlap syndromes are uncommon. The nomenclatures for primary biliary cirrhosis have recently been changed to PBC largely because patients with this disease do not necessary have cirrhosis at the time of clinical presentation.[1] The prevalence of autoimmune hepatitis varies from 0.1 to 1.9/100 000 among Caucasian populations[2] and that of PBC is similar at 2.3/10 000.[3] Nonetheless early diagnosis is essential because if untreated, the diseases progress to liver cirrhosis and death from liver failure, whereas early therapeutic intervention by immunosuppression for autoimmune hepatitis[4] and by ursodeoxycholic acid (UDCA) for PBC[5] can control disease progression. Liver autoantibodies play a key role in early identification of these diseases as they may occur in asymptomatic subjects before the development of overt disease.[5]

DIAGNOSTIC AUTOANTIBODIES FOR AUTOIMMUNE HEPATITIS

Codified criteria for the diagnosis of autoimmune hepatitis have been developed by the International Autoimmune Hepatitis group.[6] The criteria comprise compatible liver histopathology including interphase hepatitis, elevated serum IgG, liver autoantibodies, elevated serum transaminases and negative serology for viral hepatitis. Interphase hepatitis is characterised by lymphocytic infiltration with or without plasma cells with associated hepatocyte cell death (piecemeal necrosis) at parenchymal-connective tissue junctions (interphases) around portal tracts.

Classification

Autoimmune hepatitis is divided into type 1 and type 2, distinguished by autoantibody profile and by age of onset, with type 1 in adults, and type 2 in children, but with indistinguishable clinical presentation. Patients who are asymptomatic at presentation have a good prognosis and may not require immunosuppressive therapy. On the other hand, cirrhosis on initial liver biopsy carries a poor prognosis.[7] DRB1*04:01 positivity has been identified in association with a favourable clinical outcome.[8]

F-actin-specific smooth muscle autoantibody, antinuclear autoantibody and autoantibody to SLA/LP segregates with type 1 autoimmune hepatitis

Smooth muscle antibody with specificity for F-actin microfilaments is the prototype autoantibody that segregates with type 1 autoimmune

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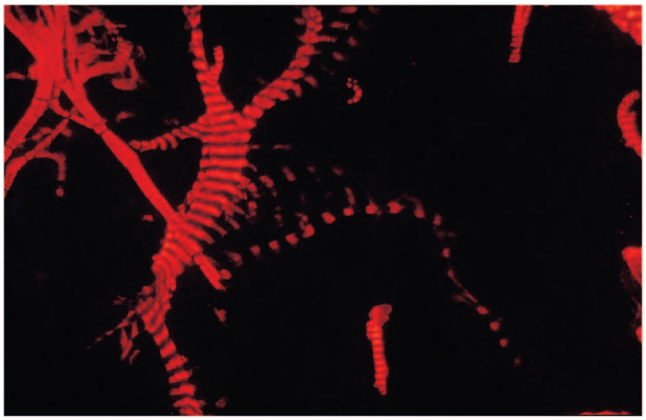

Figure 1 F-actin-specific SMA-T smooth muscle antibody binds skeletal muscle striations by immunofluorescence.

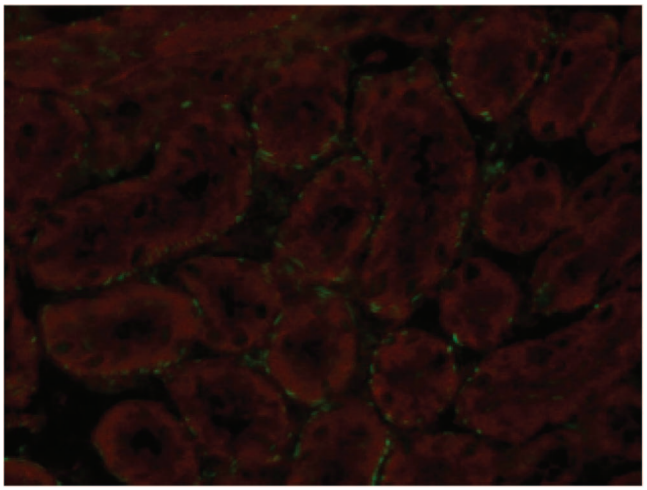

Figure 2 F-actin-specific SMA-T smooth muscle antibody binds by immunofluorescence contractile fibrils around renal tubules in a 'picket-fence' pattern.

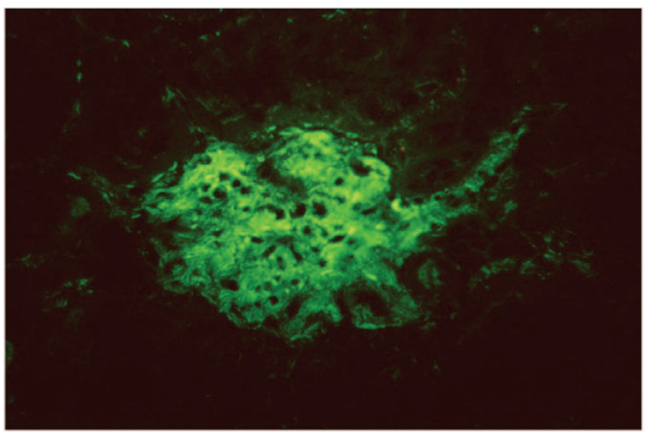

Figure 3 F-actin-specific SMA-G smooth muscle antibody gives immunofluorsence staining of the mesangium of renal glomeruli.

hepatitis and a serological marker for histological and biochemical disease activity.[9] It is found in about 60% of patients.[10] Designated smooth muscle autoantibody because of its reactivity with smooth muscle[11,12] has since been found to react with F-actin microfilaments in skeletal muscle[13] (Figure 1), cardiac muscle[14] and non-muscle cells that include gastric parietal cells[15] and brain synapses.[16] Specificity for actin was first demonstrated by Gabbiani *et al.*[17] by immunoabsorbtion with platelet actin. In routine diagnostic laboratories, smooth muscle autoantibody is recognised by the immunofluorescence (IF) staining of the gastric muscularis externa, muscularis mucosa and smooth muscle fibres that extend from the muscularis mucosa into the lamina propria. F-actin-specific smooth muscle autoantibody is recognised by the additional characteristic pattern of IF staining of contractile fibrils around renal tubules (Figure 2) and designated as 'SMA-T' autoantibodies, frequently with staining of the mesangial cells of renal glomeruli (SMA-G) (Figure 3) together with staining of blood vessels of renal blood vessels (SMA-V). The original subclassification of smooth muscle antibody into SMA-V, SMA-G and SMA-T introduced by Botazzo remains useful to this day.[18] The classic 'picket-fence' staining around renal tubules of SMA-T is diagnostic when it is clearly visualised (Figure 2). However this pattern of staining may be difficult to identify with low-titre autoantibodies; hence it is prudent to confirm SMA-T autoantibody by using as substrate cultures of cell lines, which then display characteristic 'actin cables' by immunofluorescence[19] (Figure 4). Despite its initial promise, an ELISA developed for F-actin[20] may generate false positive results in sera that are classical SMA-V and not SMA-T.[19] Thus, while the ELISA may be used for screening, it is not suitable for confirmation (Figure 4). One possible explanation may be the depolymerisation of filamentous F-actin to monomeric globular G-actin as antibody to G-actin is not specific for type 1 autoimmune hepatitis. In contrast to SMA-T autoantibody directed against F-actin microfilaments, SMA-V autoantibody is typically directed to vimentin intermediate filaments that can be seen with a variety of viral infections.[21–23] Smooth muscle autoantibody is thus a heterogeneous set of autoantibodies that react with various molecular targets of the cytoskeleton.[24]

Autoantibody to soluble liver antigen/liver pancreas (SLA/LP) is an additional, more recent and less frequent diagnostic marker for type 1 autoimmune hepatitis[25] that, occurring either alone or together with autoantibody to Ro52, carries a poor prognosis.[8,26,27] The target antigen has been identified as UGA suppressor tRNA-associated protein,[28] a serine tRNA protein complex implicated in cotranslational incorporation of selenocystine into cells[29] It is present in 15–20% of patients.[30] SLA/LP autoantibodies are major risk factors for a poor short- and long-term outcome. These patients are in need of high surveillance. The presence of anti-SLA autoantibody conferred 3.1-fold increased risk of hepatic death in AIH patients. The remission rates were comparable between anti-SLA seropositive and seronegative AIH patients, while anti-SLA positivity was associated with nearly two-fold increased risk of relapse after drug withdrawal. Human leukocyte antigen (HLA) allele DR3 was positively associated with anti-SLA autoantibody.[26]

Antinuclear autoantibody giving specked or homogeneous nuclear staining by immunofluorescence along with autoantibodies to nucleoli[31] frequently segregates with F-actin-specific SMA-T autoantibody and is found in about 60% of patients.[10] The presence of Lupus Erythematosus (LE) cells was the basis for the outdated nomenclature of 'Lupoid Hepatitis'.[32] The designation'chronic active hepatitis' first applied to a novel liver disease in the 1950s was replaced by the designation 'Autoimmune Hepatitis' in 1965.[33]

Autoantibody to LKM-1 and LC-1 segregates with type 2 autoimmune hepatitis

Autoantibody to Liver Kidney Microsomes-1 (LKM1) is the signature antibody of type 2 autoimmune hepatitis. Its major target antigen is

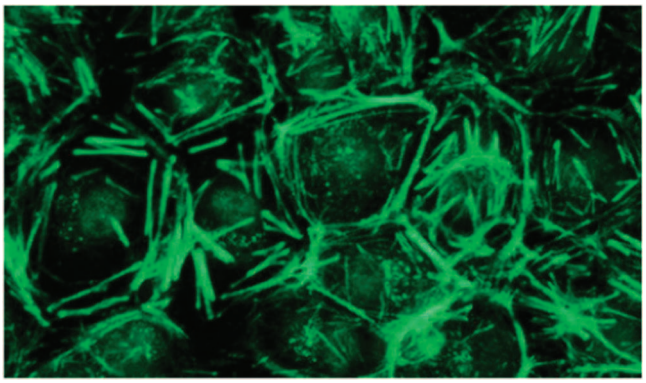

Figure 4 F-actin-specific SMA-T smooth muscle antibody gives immunofluorescence staining of 'actin cables' in a rat epithelial cell line.

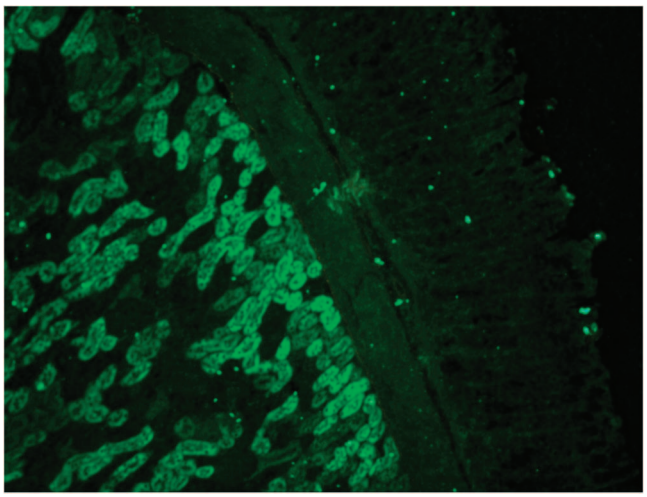

Figure 5 LKM-1 antibody shows staining of proximal renal tubules by immunofluorescence.

Cytochrome P4502D6 (CYP2D6).[34,35] Anti-LKM autoantibody gives characteristic staining of the proximal renal tubules (Figure 5) and hepatocytes (Figure 6) by immunofluorescence. LKM-1 autoantibody is also found in up to 10% of patients with hepatitis C virus infections

Autoantibody to LC-1 stains the hepatocytes by immunofluorescence but spares cells around the central vein (Figure 7).[36] The target antigen has been identified as formiminotransferase cyclodeaminase,[37,38] a 62 kDa cytosolic protein. LC-1 antibody is found together with LKM autoantibody in 30% of cases, and in 10% of cases it is the sole autoantibody. In contrast to autoantibody to LKM, anti-LC-1 autoantibody parallels liver disease activity.[39] Its presence is associated with a unfavourable clinical course and a more rapid disease progression[40]

DIAGNOSTIC AUTOANTIBODIES FOR PBC

PBC is a progressive disease of insidious onset resulting in destruction of epithelial cells of small intrahepatic bile ducts leading to cholestasis and cirrhosis. As with autoimmune hepatitis, early diagnosis is essential as it can be controlled by UDCA.[5] It is characterised by elevated serum alkaline phosphatase, diagnostic autoantibodies to mitochondria and PBC-specific anti-nuclear autoantibodies and liver histopathology of granulomas around the bile ducts[41,42]

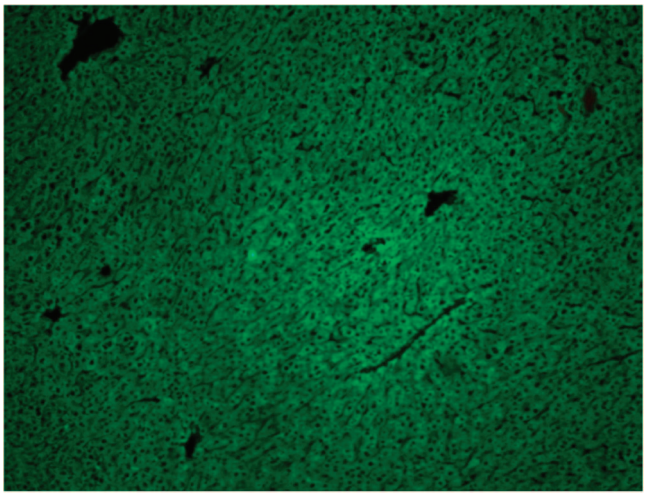

Figure 6 LKM-1 antibody shows staining of hepatocytes by immunofluorescence.

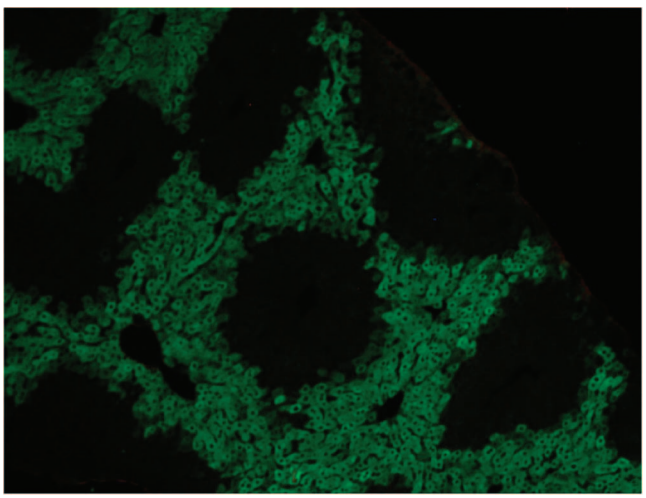

Figure 7 LC-1 antibody showing staining of hepatocytes but not those around the central vein.

Autoantibodies to mitochondria, designated M2 diagnostic for PBC are found in 95–98% of patients. The cDNA encoding the target antigen was molecularly cloned by Gershwin *et al.*[43] and identified as the inner lipoyl domain of the E2 subunit of pyruvate dehydrogenase complex.[44] In routine diagnostic laboratories, it is identified by distinctive immunofluorescence staining of distal renal tubules, gastric parietal cells and liver hepatocytes. In doubtful instances, the presence of the antibody can be confirmed by line blots with the target M2 antigen.

Autoantibodies to PBC-specific anti-nuclear autoantibody

PBC-specific anti-nuclear autoantibody (ANA) comprises antibody to the nuclear pore complex that targets gp210 and nucleoporin p62[45,46] as well as antibody to multiple nuclear dots that target Sp100 and PML, and are found in about 50% of patients with PBC.[47] PML is a transformation and cell growth suppressing protein expressed in promyelocytic leukaemia cells that is co-localised with Sp100 in nuclear dots.[48] Sp100 may function as a nuclear hormone transcriptional coactivator.[49] Autoantibody to the nuclear pore complex that

Table 1 Diagnostic autoantibodies in autoimmune hepatitis and primary biliary cholangitis

Type 1 autoimmune hepatitis	F-actin reactive smooth muscle autoantibody
	Anti-nuclear autoantibody
	SLA/LP autoantibody
Type 2 autoimmune hepatitis	LKM-1 autoantibody
	LC-1 autoantibody
Primary biliary cholangitis	M2 mitochondria autoantibody
	Nuclear core complex autoantibody directed to gp230, nucleoporin p62 and nuclear lamin B receptor
	Muliple nuclear dots autoantibody directed to sp100 and PML

gives punctate nuclear rim immunofluorescence staining accounts for about 25% of PBC-specific ANA,[50] binds to the lamin B receptor[51] and to gp210 that binds preferentially to the N terminus of gp210[52] and is a poor prognostic marker.[53] Autoantibody to centromeres, although not specific to PBC may also be found in this disease.[54] PBC-specific ANA may be particularly useful in M2-negative primary biliary cirrhosis.

PRIMARY SCLEROSING CHOLANGITIS

Primary sclerosing cholangitis is a cholestatic liver disease characterised by inflammation and fibrosis of both intrahepatic and extrahepatic bile ducts that leads to multifocal bile duct strictures and eventually to cirrhosis[55]. Although a number of autoantibodies of low prevalence and low titres have been reported in primary sclerosing cholangitis, none are diagnostic of this disease.[56] Inflammatory bowel disease is associated in up to 80% of patients.[57] pANCA (anti-neutrophil cytoplasmic antibody) has been proposed as a marker of ulcerative colitis and primary sclerosing cholangitis,[58] but it has been questioned whether the association is an epiphenomenon.[59] There is no effective treatment besides liver transplantation.

OVERLAP SYNDROMES

Some patients have a hepatitic and cholestatic profile in association with autoantibodies that segregate with autoimmune hepatitis and PBC. These are a poorly characterised set of patients.[60,61] Nonetheless It has been reported that 2–19% of patients with primary biliary cirrhosis and 7–14% of patients with primary sclerosing cholangitis have features that overlap with autoimmunue hepatitis.[61]

PREDICTIVE VALUE OF DIAGNOSTIC AUTOANTIBODIES FOR ASYMPTOMATIC AUTOIMMUNE LIVER DISEASESS

Autoantibodies in asymptomatic subjects may precede overt autoimmune disease by many years. This is certainly the case for autoantibodies associated with asymptomatic PBC patients followed up for 19 years.[62] Liver biopsies of asymptomatic subjects with mitochondria antibody and normal liver function has shown the histological hallmarks of PBC.[63] Autoantibodies to F-actin-specific smooth muscle antibody may also be found in asymptomatic subjects with normal liver function.[64] A 12-year follow-up has reported the rare occurrence of development of overt autoimmune hepatitis in asymptomatic subjects with F-actin antibody and normal liver function.[65]

CONCLUDING REMARKS

F-actin reactive smooth muscle autoantibody, antinuclear autoantibody and autoantibody to SLA/LP are diagnostic for type 1 autoimmune hepatitis, while LKM-1 and LC-1 autoantibody are diagnostic for type 2 autoimmune hepatitis. M2 mitochondria autoantibody, nuclear pore complex autoantibody directed to gp210, nucleoporin p62 and nuclear lamin B receptor and autoantibody to multiple nuclear dots that target Sp100 and PML antigens are diagnostic for PBC. Table 1 provides a summary of diagnostic autoantibodies in autoimmune hepatitis and PBC. There are no diagnostic antibodies for primary sclerosing cholangitis.

CONFLICT OF INTEREST

The author declare no conflict of interest.

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