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**THE DIAGNOSIS OF AUTOIMMUNE LIVER DISEASES.** DR. DELGADO DE LA POZA
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# PERSPECTIVES IN AUTOIMMUNITY

## ISSUE 4. AUTOIMMUNE LIVER DISEASES

### INTRODUCTION

Autoimmune liver diseases are chronic inflammatory conditions of the liver that include autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). The incidence of these diseases is around one to four per 100,000 population per year. They are complex disorders that involve factors of genetic predisposition to autoimmunity combined with exposure to environmental, infectious and epigenetic agents that lead to loss of tolerance to one's own liver antigens, causing liver failure or bile duct lesion[1]. In AIH, the autoimmune response targets hepatocytes; in PBC and PSC, it targets the epithelial cells of the bile ducts. The responses of the corresponding targets to this attack by the immune system, along with the effects of cholestasis, are key to determining general condition and fibrosis rate[2]. Many autoantibodies have been isolated in serum samples from patients with liver diseases. Most are non-specific but of high diagnostic and prognostic value; indeed, their detection is included in the diagnostic criteria for AIH and PBC. However, in PSC, autoantibodies are present but less important for diagnosis[3].

## PRIMARY BILIARY CHOLANGITIS

PBC is an autoimmune cholestatic liver disease characterized by progressive destruction of small and mid-sized bile ducts. Histologically, PBC involves nonsuppurative cholangitis with destruction of the biliary epithelium and portal infiltration by inflammatory cells, slowly leading to fibrosis and cirrhosis. The disease occurs almost exclusively in middle-aged women, who may exhibit typical symptoms with a biliary pattern (jaundice, pruritus, abdominal pain and fatigue), although nearly half of patients are asymptomatic. It also presents with elevation of clinical-chemistry parameters indicative of cholestasis (alkaline phosphatase, gamma-glutamyl transferase) and, often, selective elevation of IgM[2, 4]. PBC is usually associated with other autoimmune diseases, such as Hashimoto's disease, systemic sclerosis and Sjögren's syndrome. More than 60 autoantibodies have been identified in PBC; some are disease-specific and useful not only for diagnosis, but also for evaluation of disease severity, clinical phenotype and long-term prognosis. Other autoantibodies are of little significance as they are seen in other autoimmune diseases (Sjögren's syndrome, systemic sclerosis and AIH) or have low sensitivity and specificity[4, 5].

### Antimitochondrial antibodies

Antimitochondrial antibodies (AMAs) are highly specific autoantibodies for PBC. Their role is essentially diagnostic rather than prognostic. They are found in 90%-95% of patients with PBC and less than 1% of the healthy population. AMAs represent a diagnostic criterion for the disease[6-8].

The target antigen is a component of 2-oxo-acid dehydrogenase complexes; specifically, they target three components of this family of complexes: the E2 subunit of the pyruvate dehydrogenase complex, which is the main antigen to which more than 90% of autoantibodies react; the branched-chain 2-oxo-acid dehydrogenase complex, to which 50%-80% of autoantibodies react; the oxoglutarate dehydrogenase complex, to which 20%-60% of autoantibodies react; and the E1a subunit of the pyruvate dehydrogenase complex, to which 5%-25% of autoantibodies react[8].

AMAs are generally detected in serum using indirect immunofluorescence techniques on rat kidney, stomach and liver sections and Hep-2 cells. They may also be detected with immunoassays containing purified or recombinant mitochondrial antigens (enzyme immunoassays, chemiluminescence and blots). With currently available techniques, the rate of AMA-negative patients with PBC is less than 5%-10%, and more than half of them are positive for at least one type of PBC-specific antinuclear antibodies (ANAs), mainly anti-gp210 or anti-sp100 antibodies[8].

AMA titres do not change over time, and therefore are not useful for disease follow-up. Despite conflicting evidence, AMAs are not associated with disease severity or progression, and the clinical course of PBC in AMA-positive patients is similar to that in AMA-negative patients, although the latter may have more progressive disease due to delays in diagnosis and treatment[9]. AMAs can be detected in serum years before clinical signs or clinical-chemistry abnormalities can be identified; therefore, AMA positivity in healthy subjects is a risk factor for developing PBC in the future[6].

## Antinuclear antibodies

ANAs are detected by indirect immunofluorescence on Hep-2 cells in 30%-50% of patients with PBC and also by antigen-specific immunoassays. Three patterns of ANAs, defined by the International Consensus on ANA Patterns[10], are associated with PBC[5]: punctate nuclear envelope (AC-12), whose main antigens form part of the nuclear pore complex; multiple nuclear dots (AC-6), whose main antigens target components of promyelocytic leukemia (PML) nuclear bodies; and centromere (AC-3), whose main antigens are centromere proteins.

### *Punctate nuclear envelope (AC-12)*

**Anti-gp210 antibodies**. Gp210, a 210-kDa glycoprotein of the nuclear pore complex, is the main antigen targeted by autoantibodies yielding the AC-12 pattern on immunofluorescence. Anti-gp210 antibodies are highly PBC-specific, as they are identified in 25% of patients. They are associated with a poor prognosis and are included in the diagnostic criteria for PBC[7, 11-12]. These antibodies may vary over the clinical course of PBC depending on disease activity and progression and are histologically associated with more severe hepatitis, lobular inflammation and ductular reaction. Furthermore, persistently positive anti-gp210 antibodies are a risk factor for progression to terminal liver failure, whereas if they are initially positive and then become negative after starting treatment with ursodeoxycholic acid (UDCA), these patients have a more favorable prognosis.

**Other antibodies**. Anti-p62 antibodies can be detected in serum in 23% of patients with PBC with high specificity. These antibodies are associated with higher levels of bilirubin and may be useful as a biomarker in patients with suspected PBC who test negative for AMAs, anti-gp210 antibodies and anti-SP100 antibodies[13]. Other antigens that yield this pattern are lamin B receptors, which are found in 9% of patients.

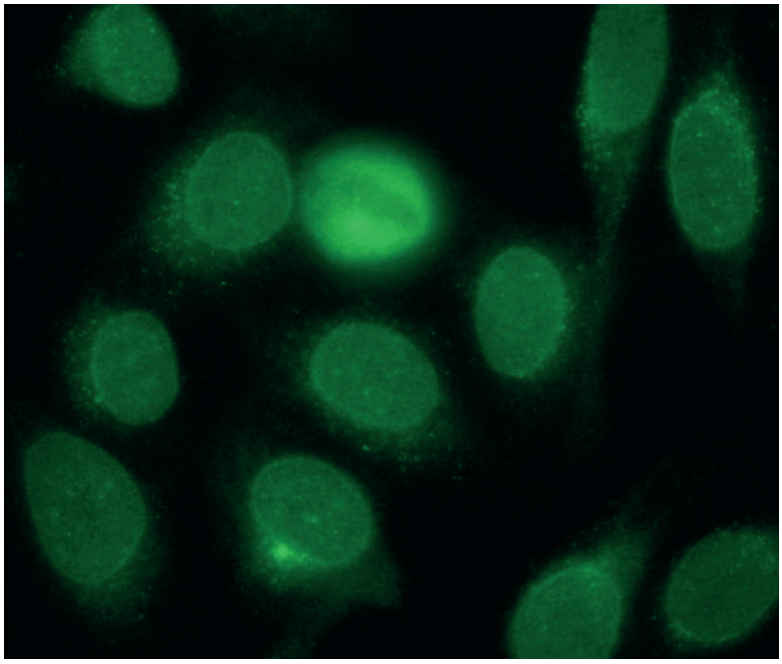

**Figure 1.** AC-12 Punctate nuclear envelope. Indirect immunofluorescence on HEp-2 cells (400X). Nuclear envelope reveals a punctate staining in interphase cells, with accentuation of fluorescence at the points where adjacent cells touch each other (ICAP).

### *Multiple nuclear dots (AC-6)*

**Anti-Sp100 antibodies**. Sp100, a nuclear protein, is a transcription factor collocated with the PML antigen, this being the main antigen that yields the nuclear dot pattern. Co-occurrence of anti-Sp140, anti-Sp100 and anti-PML antibodies and small ubiquitin-related modifiers (SUMOs) is common and suggests an autoimmune reaction against multiple nuclear body components in some patients with PBC[14, 15]. The prevalence of anti-Sp100 antibodies in patients with PBC is approximately 24%-45% with a specificity of 88%-99%. They are included in the diagnostic criteria for PBC and are particularly important in AMA-negative patients[7, 15]. Anti-Sp100 antigens are associated with urinary tract infections; this supports the hypothesis that some bacterial infections could be involved in PBC-specific autoimmunity induction[16].

**Other antibodies**. Sp140 was identified as a PML component that was characterized using serum from patients with PBC. These autoantibodies have a sensitivity of 18%-39% and a specificity of 94%-100%. The PML antigen was originally identified as an abnormally expressed protein in leukemia cells from patients with promyelocytic leukemia showing a sensitivity of 25%-47% and a specificity of 99%-100% in patients with PBC. SUMOs are covalently bound to Sp100 and PML, and anti-SUMO antibodies have been reported in patients with PBC with an AC-6 immunofluorescence pattern. The frequency of anti-SUMO-2 antibodies is 42% and that of anti-SUMO-1 antibodies is 15%, whilst anti-SUMO reactivity was not seen in cases of PBC that did not have this pattern.[15, 17]

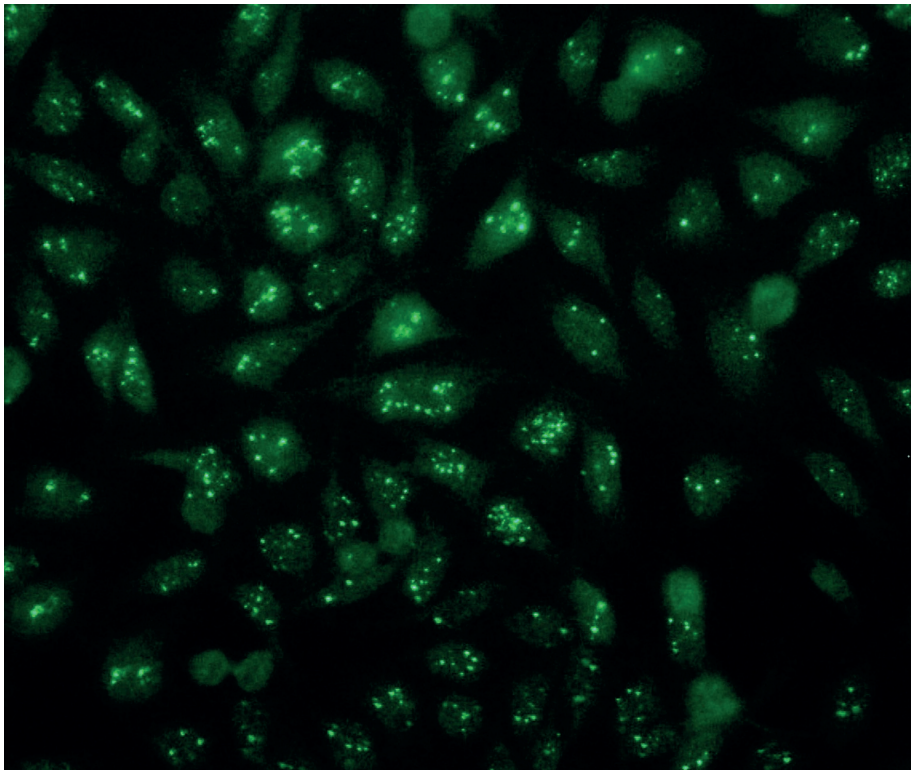

**Figure 2.** AC-6 multiple discrete nuclear dots. Indirect immunofluorescence on HEp-2 cells (400X). Countable discrete nuclear speckles: 6 to 20 nuclear dots/cell (ICAP).

### *Centromere (AC-3)*

Anti-centromere antibodies were first reported in patients with systemic sclerosis and yield the AC-3 nuclear dot pattern. Centromere proteins A, B and C are the main antigens targeted by these autoantibodies, which are detected in 10%-30% of patients with PBC with no apparent clinical signs of concomitant systemic sclerosis. Anti-centromere antibodies may appear before the onset of PBC, and titres are stable over time. They are associated with a high risk of progression to cirrhosis, portal hypertension and more severe ductular reaction[3].

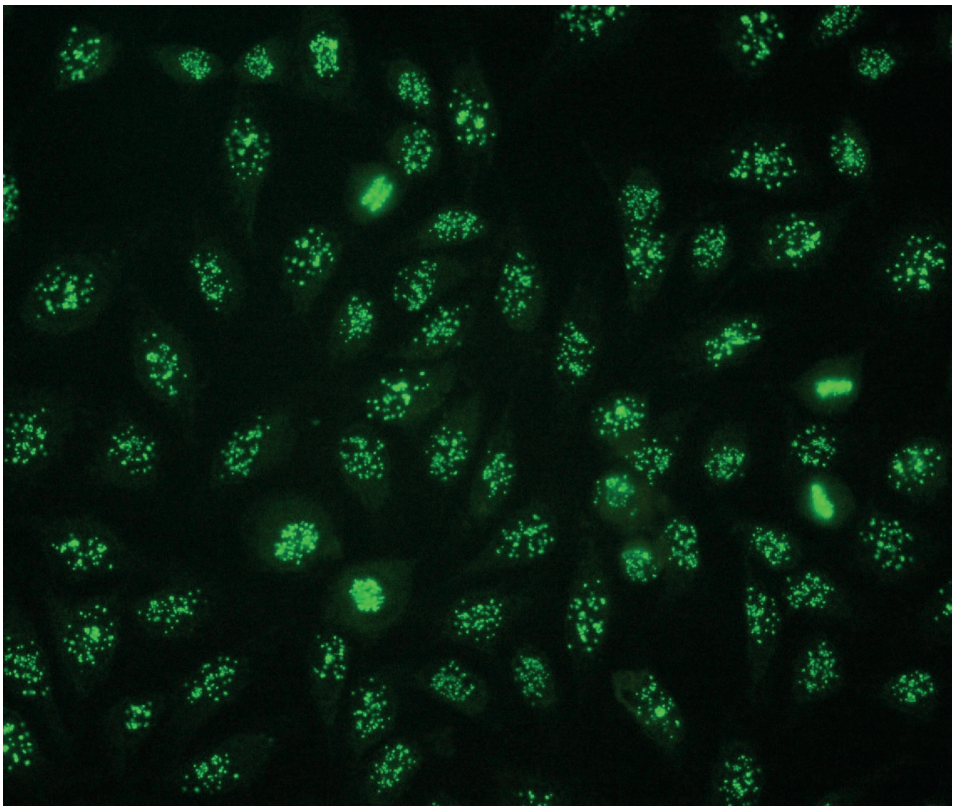

**Figure 3.** AC-3 centromere. Indirect immunofluorescence on HEp-2 cells (400X). Discrete coarse speckles (40-80/cell) scattered in interphase cells and aligned at the chromatin mass on mitotic cells (ICAP).

## Other antibodies

### *Kelch-like 12 (KLHL12) and hexokinase 1 (HK1) antibodies*

KLHL12 is a nuclear protein that is crucial for collagen export and ubiquitination. HK1 is an enzyme located in the outer membrane of mitochondria and is involved in glucose metabolism and apoptosis. These autoantibodies have a sensitivity of 23%-37% and 42%-46% in PBC, respectively; both have a specificity of 96%-97%[18-20]. They are detected in AMA-negative patients with PBC, and therefore could be useful for improving the diagnosis of these patients without having to resort to invasive techniques. Anti-HK1 antibodies are associated with a more severe PBC course; for anti-KLHL12 antibodies, the evidence is conflicting[18-20].

| Table 1. Autoantibodies in Primary Biliary Cholangitis (PBC) | | | | |
|---|---|---|---|---|
| **IIF pattern** | **Antigen** | **Sensitivity** | **Specificity** | **Clinical relevance** |
| **Anti-Mitochondrial antibodies** | Mitochondria | 90-95% | 90% | Diagnostic criteria for PBC |
| | PDC-E2 | 90% | | |
| | BCOADC-E2 | 50-80% | | |
| | OGDC-E2 | 20-60% | | |
| | PDC-E1a | 5-25% | | |
| **Punctate nuclear envelope (AC-12)** | gp210 | 25% | >95% | Diagnostic criteria for PBC<br>Poor prognosis |
| | p62 | 23% | | Poor prognosis |
| | Lamin B receptor | 9% | | |
| **Multiple nuclear dots (AC-6)** | Sp100 | 24-45% | >95% | Diagnostic criteria for PBC<br>Poor prognosis<br>Urinary tract infections |
| | Sp140 | 18-39% | | |
| | PML | 25-47% | | |
| | SUMO | 10-19% | | |
| **Centromere (AC-3)** | Centromere protein A, B, C | 10-30% | | Poor prognosis |
| **Others** | HK-1 | 42-46% | >95% | Poor prognosis |
| | KLHL12 | 23-37% | | Diagnostic value in anti-mitochondrial, gp210 and sp100 negative PBC |

**IIF**: Indirect immunofluorescence; **PDC-E2**: E2 subunit of the pyruvate dehydrogenase complex; **BCOADC-E2**: E2 subunit of the branched-chain 2-oxo-acid dehydrogenase complex; **OGDC-E2**: E2 subunit of the oxoglutarate dehydrogenase complex; **PDC-E1a**: E1a subunit of the pyruvate dehydrogenase complex; **PML**: promyelocytic leukemia; **SUMO**: small ubiquitin-like modifier; **HK-1**: hexokinase-1; **KLHL12**: Kelch-like 12

## AUTOIMMUNE HEPATITIS

AIH is a progressive inflammatory disease that targets hepatocytes, with a marked predominance in women. AIH may develop at any age, but its incidence peaks both in childhood and adolescence and at around 40 years of age. The most common clinical presentation consists of mild, non-specific symptoms, including fatigue, joint pain, general malaise, anorexia and weight loss. Serologically, aminotransferases and IgG are elevated and specific autoantibodies are detected. Histologically, it presents as interface hepatitis with periportal involvement, plasma cell infiltration and punched-out necrosis. Its diagnosis also requires all known causes of liver disease to be ruled out[2, 21-22]. AIH is associated with certain HLA polymorphisms in different populations depending on geographic location. In North America, it is associated with HLA-DRB1*03 and HLA-DRB1*04; in South America and Japan, it is associated with HLA-DRB1*04, DRB1*08 and HLA-DRB1*13; and in Europe, it is associated with HLA-DRB1*03, HLA-DRB1*04 and HLA-DRB1*07[23].

In 1993, to facilitate and standardize the diagnostic process, an expert group (the International Autoimmune Hepatitis Group) started to develop a diagnostic system with specific scores.

This system of diagnosis, revised in 1999 and again in 2008, assigns a numerical value to different elements that may be found in AIH; the sum of these numerical values yields a "definitive" or "probable" diagnosis of AIH or rules it out. It uses four parameters: positivity for non–organ-specific antibodies, serum IgG levels, the absence of viral hepatitis and histological assessment. This diagnostic system achieved a sensitivity of 81% or 88% and a specificity of 97% or 99%, depending on whether the cut-off point was 6 or 7, respectively[24].

Autoantibodies in AIH not only play a role in diagnosis, but also distinguish between two types of AIH. ANAs and anti–smooth-muscle antibodies (ASMAs) characterize AIH type 1 (AIH-1), whilst anti-liver kidney microsomal 1 (anti-LKM1) antibodies and anti-liver cytosol 1 (anti-LC1) antibodies characterize AIH type 2 (AIH-2). AIH-1 is much more common and affects both children and adults, whereas AIH-2 is primarily a pediatric disease[25]. AIH-2 occurs more often in children and young adults, has an acute or severe course, and features histologically advanced lesions at presentation, whilst treatment failure, relapse after suspending treatment and need for long-term treatment are common compared AIH-1.

## AIH-1 autoantibodies

### *ANAs*

ANAs were the first autoantibodies associated with AIH; however, their specificity is very low. Some 50%-75% of patients with AIH are ANA-positive. The ANA pattern in AIH is often punctate or homogeneous; its main targets are chromatin, histones, centromere, single- and double-chain DNA, cyclin A, and ribonucleoproteins. No antigen responsible for ANAs has been associated with a particular clinical course or higher diagnostic specificity for AIH[3, 25].

### *ASMAs*

ASMAs are associated with AIH-1 but may be detected in other liver diseases. ASMAs are detected in 85% of cases, and in 50% of cases they are found along with ANAs. In immunofluorescence on rat kidney, stomach and liver sections, ASMAs stain the smooth muscle of the muscularis mucosa of the stomach, the mesangium of the renal glomeruli and the artery walls. The pattern in the rodent kidney is of particular importance since it enables identification of a pattern that shows greater specificity for AIH, the TGV pattern: Tubular (positivity of tubular structures), Glomerular (positivity of glomerular mesangial cells) and Vascular (walls of the small and mid-sized arteries of the kidneys)[26]. In addition to the TGV pattern, immunofluorescence titres also add specificity for AIH. Some 80% of serum samples that show the TGV pattern react with actin filaments (F-actin) which are more specific for AIH. ASMA titres are correlated with disease activity [3, 25-26].

### *Antineutrophil cytoplasmic antibodies (ANCAs)*

Perinuclear antineutrophil cytoplasmic antibodies (p-ANCAs) may support the diagnosis of AIH, especially in the absence of other autoantibodies, although they may also be detected in chronic viral hepatitis, inflammatory bowel disease, PSC and vasculitis. These autoantibodies are found in 65%-81% of cases of AIH-1 and are rare in AIH-2. The p-ANCA pattern associated with AIH is the atypical pattern that recognizes beta tubulin isoform 5, which is part of the nuclear pore complex, and therefore this reactivity is also called the peripheral anti-

nuclear neutrophil antibody (p-ANNA) pattern. The prognostic value of atypical p-ANCAs remains a matter of debate, although it has been suggested that they are associated with a disease with a worse prognosis[3, 25, 27].

## AIH-2 autoantibodies

### *Anti-LKM antibodies*

Anti-LKM1 antibodies constitute a highly sensitive marker for AIH-2. In immunofluorescence on three rodent tissue sections, anti-LKM1 antibodies stain the cytoplasm of hepatocytes and proximal kidney tubules. The autoantigen recognized by anti-LKM1 antibodies is cytochrome P450 2D6. Anti-LKM1 antibodies can also be found in 13% of patients with chronic viral hepatitis C. Anti-LKM1 antibody titres are correlated with disease severity and activity[3, 25].

Anti-LKM3 antibodies may be found in 19% of patients with AIH, but also in patients with chronic viral hepatitis C or D. These antibodies recognize members of the uridine glycuronosyl transferase 1 family. Immunofluorescence to visualize anti-LKM3 antibodies require the use of human or primate substrates, where it stains the cytoplasm of hepatocytes and proximal kidney tubules[3, 25].

### *Anti-LC1 antibodies*

Anti-LC1 antibodies target the enzyme formiminotransferase cyclodeaminase. They are present in around 30% of patients with AIH-2, although they may also be seen in patients with hepatitis C. Anti-LC1 antibodies in three rodent tissue sections stain the cytoplasm of hepatocytes, but spare the centrilobular areas of the liver. In two thirds of patients with AIH-2, anti-LC1 antibodies co-occur with anti-LKM1 antibodies. Anti-LC1 antibodies appear to be correlated with disease activity and are associated with an unfavorable clinical course and more rapid disease progression[3, 25].

## Autoantibodies in AIH-1 and/or AIH-2

### *Anti-soluble liver antigen (SLA) antibodies*

Anti-SLA antibodies have the greatest specificity for AIH of all AIH-related antibodies (>95%); however, they are present in only around 20%-30% of patients. Anti-SLA antibodies cannot be detected by means of immunofluorescence and must be detected by resorting to specific antigen immunoassays. The antigen that they recognize is O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthetase. Anti-SLA antibodies are associated with a poor disease course, more severe histology, a longer time to disease remission and a higher relapse rate[3, 25].

### *Anti-asialoglycoprotein receptor (ASGPR) antibodies*

Anti-ASGPR antibodies can be detected in 15%-30% of patients with AIH. They target a specific membrane receptor in the liver and appear to be correlated with histological activity. However, anti-ASGPR antibodies are not disease-specific and may also be present in patients with chronic viral hepatitis or PBC[3, 28].

| Table 2. Autoantibodies in Autoimmune Hepatitis (AIH) | | | | |
|---|---|---|---|---|
| **AIH** | **Antigen** | **Prevalence** | **Clinical relevance** | **Simplified Score Diagnosis AIH** |
| **Type 1** | ANA | 50-75% | Diagnostic criteria for AIH | IIF ≥ 1/40 = 1 point<br>IIF ≥1/80 = 2 points |
| | SMA | 80-95% | Diagnostic criteria for AIH disease activity | IIF ≥ 1/80 = 2 points |
| | Atypical pANCA | 65-81% | Support diagnosis AIH | - |
| **Type 2** | LKM-1 | 2-4% | Diagnostic criteria for AIH poor prognosis disease activity | IIF ≥ 1/40 or positive = 2 points |
| | LC-1 | 1-2% | Poor prognosis | - |
| **Type 1 and/or Type 2** | SLA | 20-30% | Diagnostic criteria for AIH poor prognosis >95% specificity | Positive = 2 points |
| | ASGPR | 15-30% | - | - |

**ANA**: anti-nuclear antibodies; **SMA:** smooth muscle antibodies; **pANCA**: antineutrophil cytoplasmic antibodies; **LKM**: liver kidney microsomal; **LC-1**: liver citosol 1; **SLA**: soluble liver antigen; **ASGPR**: asialoglycoprotein receptor; **IIF**: indirect immunofluorescence

## PRIMARY SCLEROSING CHOLANGITIS

PSC is a chronic inflammatory disease of the biliary epithelium characterized by progressive destruction of the bile ducts, which are affected by obliterating concentric ("onion-skin") fibrosis leading to biliary stenosis. Unlike other types of AIH, PSC affects more men than women, and the mean age of onset is 41. Serologically, the most common clinical-chemistry abnormality is elevated alkaline phosphatase, but high titres of nonspecific autoantibodies (ANCAs, ANAs and ASMAs), elevated IgG and interface hepatitis may also be found. As it has characteristics similar to AIH, the diagnosis of PSC is based on cholangiography by magnetic resonance imaging which enables visualisation of multifocal stenosis of the intrahepatic and extrahepatic bile ducts with a sensitivity of 86% and a specificity of 94%[2, 29]. The most prevalent autoantibodies in patients with PSC are p-ANCAs (80%-93%), ANAs (8%-77%) and ASMAs (20%-83%); however, these autoantibodies have low specificity since they are often found in patients with ulcerative colitis, AIH or, to a lesser extent, PBC[3, 29].

### Atypical p-ANCAs

The pattern of these autoantibodies is the same as that described for AIH-1, and the main antigen responsible, as mentioned above, is beta tubulin isoform 5. Atypical p-ANCAs have been linked to unfavorable clinical outcomes in patients with PSC, although this is a matter of some debate[3, 30].

### Biliary epithelial cell antibodies

IgA isotype autoantibodies against biliary epithelial cells have been reported and are correlated with a worse disease course. They are not determined in routine clinical practice and may also be found in AIH[3].

### Anti-glycoprotein 2 antibodies

Anti-glycoprotein 2 antibodies have previously been reported in severe forms of Crohn's disease; however, these IgA isotype antibodies have recently been found in PSC. They are seen in 46.7%-71.5% of patients with PSC and are associated with large bile duct involvement, cholangiocarcinoma development and increased mortality. Therefore, anti-glycoprotein 2 antibodies could serve as a new biomarker for risk stratification in patients with PSC[3, 30].

**Table 3. Autoantibodies in Primary Sclerosant Cholangitis (PSC)**

| Antigen | Ig isotype | Prevalence | Clinical relevance |
|---|---|---|---|
| **Atypical pANCA** | IgG | 80-93% | Probable associated with poor prognosis |
| **BEC** | IgA | 63-65% | Poor prognosis |
| **GP2** | IgA | 47-72% | Poor prognosis. Cholangiocarcinoma |

**pANCA**: antineutrophil cytoplasmic antibodies; **BEC**: biliar epithelial cell; **GP2**: glicoprotein 2

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# TRENDING TOPICS

## THE DIAGNOSIS OF AUTOIMMUNE LIVER DISEASES

### AUTOANTIBODIES IN AUTOIMMUNE LIVER DISEASE-CLINICAL AND DIAGNOSTIC RELEVANCE.

Sebode M, Weiler-Normann C, Liwinski T, Schramm C.
Front Immunol. 2018;9:609. PMID: 29636752.

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### AUTOIMMUNE HEPATITIS: SERUM AUTOANTIBODIES IN CLINICAL PRACTICE.

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