Diagnostic Role of Specific Liver Autoantibodies In Autoimmune Liver Diseases

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Abstract

Background: Autoimmune liver diseases (AILD) are part of a spectrum of autoimmune diseases primarily involving the liver and include autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). These diseases usually occur in isolation but sometimes their features may overlap, which is a distinct entity with different therapy known as overlap syndrome. Autoimmune hepatitis (AIH) is a chronic inflammatory condition of the liver due to an autoimmune attack against hepatocytes. AIH is characterized clinically by female preponderance and variable presentation, biochemically by high serum levels of transaminases, serologically by elevated immunoglobulin G (IgG) and positive circulating autoantibodies, and histologically by interface hepatitis. A key diagnostic criterion for all AIH scoring systems is the detection of autoantibodies, which not only assists in the diagnosis but also allows differentiation of AIH types. ANA and SMA characterize AIH-1, while anti-LKM-1 and anti-LC-1 define AIH-2, though occasionally ANA or SMA can coexist with anti-LKM-1 or anti-LC-1, the clinical course in these cases being similar to that of AIH-2

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Autoimmune liver diseases (AILD) are part of a spectrum of autoimmune diseases primarily involving the liver and include autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and

primary sclerosing cholangitis (PSC). These diseases usually occur in isolation but sometimes their features may overlap, which is a distinct entity with different therapy known as overlap syndrome (1)

Genetic predisposition, environmental factors and defects in immune regulation underlie the induction and perpetuation of autoimmunity. AIH, PSC and PBC share common pathways of immune-mediated liver injury, involving the hepatic recruitment of CD4+ and CD8+ T cells, which display cytotoxicity against liver or biliary cells, leading to liver fibrosis, cirrhosis and liver failure (2)

An imbalance between effector and regulatory T cells appears to underlie the loss of immune tolerance to self-antigens in many autoimmune diseases with a poorly understood pathway. Several studies have provided evidence of viral or bacterial triggers in AILD etiology, suggesting that autoimmunity may result from immune recognition of microbial peptides that display sequence similarity to auto antigenic peptides, called molecular mimicry (3)

It is also possible that AILD results from the modification of self-antigens by drugs or micro-organisms, making them immunogenic, or from the aberrant exposure of normally sequestered liver antigens to the immune system as a result of liver damage. The observations that particular MHC class II alleles predispose individuals to developing AILD provide a strong argument that antigen presentation to CD4+ T cells is a central event in the pathogenesis. Although the three diseases exhibit similarities in their pathogeneses, they differ in their patterns of liver injury (4)

Autoimmune hepatitis:

Autoimmune hepatitis (AIH) is a chronic inflammatory condition of the liver due to an autoimmune attack against hepatocytes. AIH is characterized clinically by female preponderance and variable presentation, biochemically by high serum levels of transaminases, serologically by elevated immunoglobulin G (IgG) and positive circulating autoantibodies, and histologically by interface hepatitis (5)

The initial perception of AIH as a chronic inflammatory liver dysfunction which mainly affects young women has been amplified to both sexes of all age groups and all ethnic societies worldwide . AIH can be asymptomatic or present in various forms from subclinical disease to acute liver failure and end-stage liver disease (6)

Specific diagnostic criteria and scoring systems have been established which include analysis of autoantibodies (ANA, SMA, anti-LKM1, and anti SLA), immunoglobulins (IgG), viral markers (IgM anti-HAV, HBsAg, HBV DNA, and HCV RNA) and histological findings (7)

Auto immune hepatitis (AIH) is subdivided into two types according to the serological profile: type 1 (AIH-1) is characterized by anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (SMA), whereas type 2 (AIH-2) is characterized by anti-liver-kidney microsomal antibody type 1 (anti-LKM1) and/or by anti-liver cytosol type 1 antibody (anti-LC1) (8)

Epidemiology:

AIH occurs worldwide, with a variable clinical phenotype and a disparity in age, gender, ethnicity, and geography related incidence and prevalence. Although uncertain, phenotypic variations and changes may in part rely on environmental, infectious, microbial, and genetic factors (9)

The geographical differentiation can be explained by the "hygiene hypothesis" which proposes high sanitation standards, lack of microbial exposure, and hence altered microbiome compositions as the underlying cause of increased systemic immune and autoimmune responses within the population (10)

Risk factors:

Female sex is a clear risk factor for AIH, in all populations; three quarters of AIH patients are female. This feature is shared with the majority of autoimmune diseases. AIH was first reported in young women, leading to consider it a disease of this age group, but from the late 90s AIH onset after the age of 60 has increasingly been reported in several populations and geographic areas, therefore AIH should always be suspected also in elderly patients with acute or chronic liver disease (11)

Viral infections have been reported to be a risk factor for AIH, providing an insight in the pathogenetic mechanism of molecular mimicry, whereby immune responses to pathogens are redirected towards structurally similar self-antigens. This has been best described in AIH-2, in which an amino acid sequence of the target auto antigen CYP2D6 is shared in common with sequences of hepatitis C virus proteins, and other viruses belonging to the herpes virus family. Moreover, anti-LKM1 is detected in up to 10% of HCV infected subjects, with a tendency to disappear after HCV clearance (12)

Genetic predisposition is conferred mainly by polymorphisms in human leukocyte antigen (HLA) alleles, as shown by early studies and later confirmed by a genome-wide association European study. However, this is not sufficient to trigger the disease since HLA predisposing to AIH are found in up to 30% of the healthy general Caucasian population (13)

Pathophysiology:

Although all pathophysiologic mechanisms of AIH are not fully understood, there is growing evidence that a genetic predisposition, molecular mimicry, and an imbalance between effector and regulatory immunity in a particular autoimmune ecosystem are key pathologic factors for disease development (6)

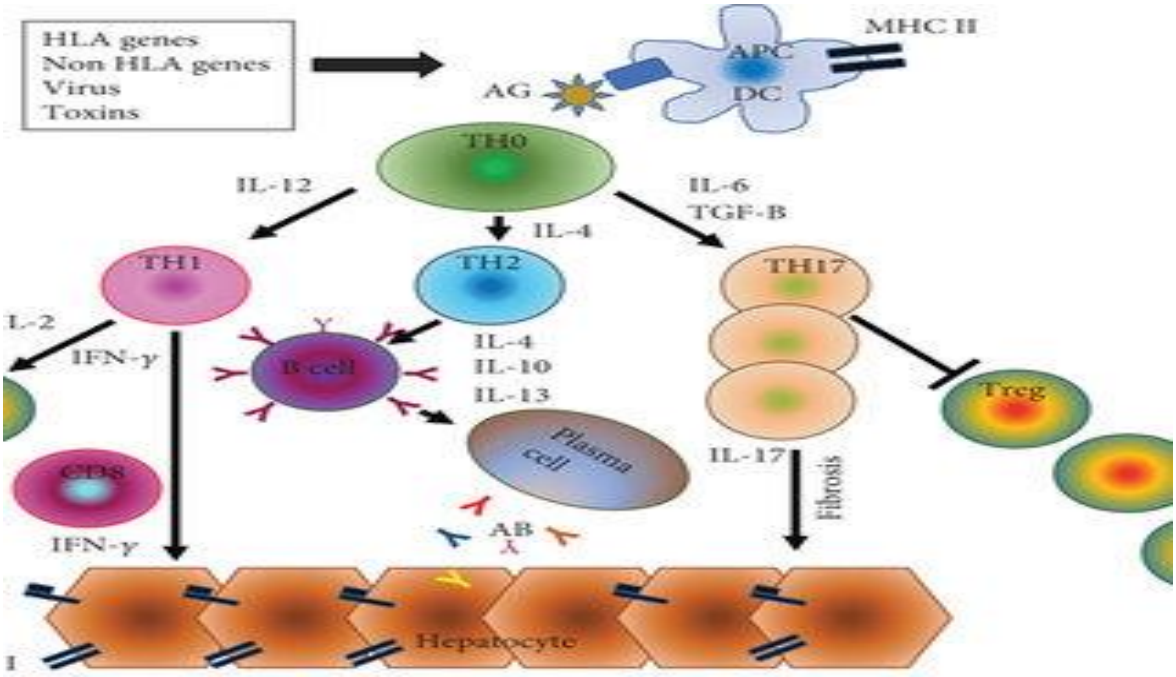

Figure 1: Pathogenesis of autoimmune hepatitis.

Autoimmune hepatitis is characterized histologically by a dense infiltrate of lymphocytes, macrophages and plasma cells in the liver. Despite the presence of circulating autoantibodies and plasma cell liver infiltration, AIH is considered a T cell disease, since B cell activation is a T cell dependent event. The key pathogenic role of T cells in AIH is mirrored by the disease predisposition conferred by HLA class II polymorphisms (8)

Human leucocytic antigen (HLA) and non-HLA molecules as well as environmental triggers such as viruses, toxins, and the microbiome have been suggested as key components for a T cell-mediated immune response. The presentation of auto antigenic peptide (AG) to naïve CD4+ T helper cells (TH0) by antigen-presenting cells (APC, dendritic cells (DC)) leads to a secretion of pro inflammatory cytokines (IL-12, IL-6, and TGF-B) who give rise to the development of Th1, Th2, and TH17 cells (6)

Outside the HLA system, genetic studies identified several single nucleotide variants in the coding regions of tumor necrosis factor-induced protein 3 (TNFIP3) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), which have also been associated with the development of auto immune disease (14)

Further putative triggers (e.g., viruses) for AIH have also been linked to the hypothesis of molecular mimicry and cross-reactivity between foreign epitopes and hepatic antigens. This includes hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis E virus (HEV), measles, Epstein-Barr virus (EBV), and herpes simplex virus (15)

Molecular mimicry is furthermore posed as a possible key element for microbiome-associated and drug-induced intestinal autoimmunity. Changes in the composition of the microbiome may

lead to increased intestinal permeability, which subsequently facilitates transition of bacteria into the portal circulation (16)

Clinical features and diagnosis:

Clinical presentation:

The clinical presentation of AIH in adults is very heterogeneous, ranging from asymptomatic cases to acute liver failure. The proportion of asymptomatic patients varies between studies from one in six to one in three. They are identified when liver function tests are performed for check-ups or insurance purposes. These patients have similar liver histology to symptomatic subjects, and need to be treated in order to avoid disease progression (17)

The most common clinical presentation is one of mild non-specific symptoms, including fatigue, arthralgia, malaise, anorexia, weight loss. In young females, amenorrhea is a typical presenting symptom, AIH can present during or shortly after pregnancy. Extra hepatic autoimmune diseases affect 20–50% of AIH patients, and may be the leading clinical manifestation at diagnosis, autoimmune thyroid disease being the most common one (18)

Diagnosis:

There is no single diagnostic test for AIH and diagnosis is based on a combination of clinical, biochemical, immunological and histological indices, and the exclusion of other known causes of liver disease that may share serological and histological features with AIH (e.g. hepatitis B, C and E, Wilson disease particularly in young patients, non-alcoholic steatohepatitis and DILI). Liver biopsy is mandatory in the diagnostic work up of AIH **(European Association for the Study of the Liver et al., 2015).**

Diagnostic scoring systems have been developed by the IAIHG for adult patients that published a simplified scoring system based on autoantibodies, IgG, histology, and exclusion of viral hepatitis that is better suited to clinical application **(Lv et al., 2019).**

In the setting of severe acute AIH the simplified IAIHG scoring system has a low diagnostic performance, as patients may have normal IgG levels and negative autoantibodies **(Muratori et al., 2021).**

Autoantibodies:

A key diagnostic criterion for all AIH scoring systems is the detection of autoantibodies, which not only assists in the diagnosis but also allows differentiation of AIH types. ANA and SMA characterize AIH-1, while anti-LKM-1 and anti-LC-1 define AIH-2, though occasionally ANA or SMA can coexist with anti-LKM-1 or anti-LC-1, the clinical course in these cases being similar to that of AIH-2 (19)

A major target of SMA is the actin of smooth muscle, whereas the molecular target of LKM-1 is CYP2D6 and of anti-LC-1 is FTCD. In the IAIHG scoring systems extra points are allocated to higher titers of ANA, SMA, anti-LKM-1 and anti-LC-1 as measured by indirect immunofluorescence (IIF) using rodent stomach, kidney and liver as substrate (20)

Anti-LC-1 can be present on its own, but frequently occurs in association with anti-LKM-1. This co-occurrence can go unnoticed because anti-LKM-1 obscures the anti-LC-1 pattern. Anti-LC-1 can also be detected by commercial tests (ELISAs, line blots and immunoblots) (21)

Positivity for autoantibodies is not sufficient for the diagnosis of AIH since they can be present, usually at low titer, in other liver disorders such as viral hepatitis, Wilson disease and non-alcoholic steatohepatitis (20)

Anti-SLA, originally described as the hallmark of a third type of AIH, is also found in up to 50% of patients with AIH-1, AIH-2 or ASC, where it defines a more severe course. Anti-SLA is not detectable by conventional immunofluorescence, but the definition of its molecular target as SEPSECS has enabled the establishment of molecularly based diagnostic assays (22)

Anti-SLA is highly specific for AIH, but currently available immunoassays have low sensitivity. There is a small proportion of patients with AIH without detectable autoantibodies. This condition, which responds to immunosuppression like the sero-positive form, represents sero-negative AIH (22)

Liver histology:

Liver biopsy is necessary to establish the diagnosis of AIH, the typical histological picture being a dense mononuclear and plasma cell infiltration of the portal areas, which expands into the liver lobule leading to damage of the hepatocytes at its periphery with erosion of the limiting plate (‘interface hepatitis’) (23)

Hepatocytes surrounded by inflammatory cells become swollen and undergo pyknotic necrosis. Plasma cells are usually abundant at the interface and within the lobule, but even their presence in low number is compatible with the diagnosis of AIH. When AIH presents acutely or at the time of relapse, pan lobular hepatitis with connective tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule (‘bridging collapse’) is often observed (24)

Treatment:

The aim of treatment is to achieve biochemical remission, defined as normal serum transaminase and IgG levels(17).Biochemical remission parallels improvement of histological activity and its maintenance prevents disease progression, AIH-related symptoms also disappear on biochemical remission (25)

Standard treatment

Standard treatment includes prednisolone and azathioprine, and is effective in 80–90% of the patients. Corticosteroids are the backbone of AIH treatment, in both children and adults; they are very effective in the vast majority of patients in achieving biochemical remission (26).Glucocorticoids act by binding to their cognate receptor, the glucocorticoid receptor (GR), leading to induction or repression of thousands of genes. Their anti-inflammatory effect is mediated by T cell signaling and down regulation of pro-inflammatory cytokine production. Moreover, they stimulate the proliferation of T-regs (27)

Primary biliary cirrhosis

Primary biliary cholangitis, formerly known as primary biliary cirrhosis, is a chronic inflammatory autoimmune cholestatic liver disease characterized by the destruction of small intrahepatic bile ducts, leading to fibrosis and eventually cirrhosis and its complications (28)

Epidemiology:

Primary biliary cholangitis is mainly diagnosed in women (92% of patients), with a female: male ratio of about 10:1, and a mean age at presentation of 55 years. Some reports show an increasing incidence over time. The prevalence of the disease ranges from 1.9 to 40.2 per 100,000 people, and has also grown over time. The increased prevalence is probably attributable to a combination of better disease recognition and data collection, and increased survival after the introduction of ursodeoxycholic acid (UDCA) (29).PBC is characterized by a significant geographical discrepancy, suggesting a possible role of environmental triggers in the development of disease (30)

Pathogenesis

Primary biliary cholangitis seems to be related to complex interactions between genetic predisposition and environmental triggers. Geographical clustering of cases has been reported, and different factors have been associated with the disease, such as infectious agents, hair dyes, nail polish and cigarette smoking, even if their role has not been yet defined (31)

Clinical presentation:

Fatigue and pruritus are the most common symptoms in PBC patients, and often have a quite negative effect on quality of life. Patients with pruritus might have excoriations or bleeding as a result of chronic scratching. Melanin deposition may occur, causing hyperpigmentation of the skin in up to 50% of cases (28)

Long-term complications of the disease include osteopenia and osteoporosis, hyperlipidemia and vitamin deficiencies. In late stages, typical signs of cirrhosis and portal hypertension (spider nevi, palmar erythema, ascites, splenomegaly and muscle wasting) might be present, as well as an increased risk of hepatocellular carcinoma (HCC) (32)

Diagnosis

Primary biliary cholangitis patients typically present with a chronic cholestatic biochemical profile: alkaline phosphatase (ALP) levels are increased to 2 or more times the upper limit of normal, and this increase is usually sustained for 6 months or longer, similarly γ-glutamyl transferase levels are increased to 5 or more times the upper limit of normal. Total bilirubin is usually normal in early stages, while abnormal values should raise concern for advanced disease. Serum aminotransferases might be slightly elevated (33)

The serological hallmark of PBC is the presence of anti-mitochondrial antibodies (AMA), highly disease-specific antibodies identified in about 95% of PBC patients (34)

AMA in a patient with raised ALP is diagnostic of PBC, after the exclusion of other intrahepatic and extrahepatic causes of cholestasis. AMA-negative PBC occurs in 5% of patients with an otherwise typical clinical, biochemical and histological profile. The presence of isolated AMA positivity in the absence of clinically apparent liver disease or abnormal liver function tests is seen in up to 0.5% of otherwise healthy individuals. This finding may be a hallmark of preclinical disease, and up to 17% will develop PBC over 5 years (32)

Other autoantibodies are often identified in PBC patients, particularly antinuclear antibodies (ANA). Anti-Sp100 and anti-gp210 ANA have a high specificity for PBC, and could be of help in the diagnosis when AMA is negative (29)

Moreover, the presence of anti-Sp100 or anti-gp210 is associated with a more clinically aggressive disease. IgM levels are often high, and this is useful in the differential diagnosis with autoimmune hepatitis (AIH) in which high IgG levels are typical, while IgM levels are low. Hyperlipidemia is also a common finding. PBC may coexist with other liver autoimmune disorders, particularly AIH (Overlap syndrome) (30)

Primary sclerosing cholangitis

Primary sclerosing cholangitis is a chronic cholestatic liver disease in which inflammation and fibrosis lead to multifocal biliary strictures and progression to end-stage liver disease. Intra- and extra hepatic bile ducts are primarily affected. The close association with inflammatory bowel diseases (IBDs) is a hallmark of the condition, affecting about two-thirds of the patients (28)

Clinical presentation and diagnosis:

Primary sclerosing cholangitis is insidious and about half of patients do not have symptoms. Abnormal liver function tests are often the only signs at diagnosis, while hepatomegaly is present in 44% of patients and splenomegaly in 39% of them (28)

Primary sclerosing cholangitis is suspected when increased ALP levels persist for more than 6 months, and diagnosis relies on cholangiographic findings of bile-duct strictures detected by either

magnetic resonance cholangio-pancreatography or endoscopic retrograde cholangio-pancreatography(35)

"Overlap" variants

PBC-AIH variant:

About 10-20% of PBC patients may present AIH, either simultaneously or consecutively, and a small proportion of patients with well-established AIH may develop PBC (32).This condition was formerly called "PBC-AIH overlap syndrome", but the use of the term "overlap" has been discouraged, since not all the patients concurrently present both diseases at the same time, and replaced with "variant", which seems more proper to describe all the possible clinical scenarios. However, the term "overlap" is still very popular in the routine practice (36).

References:

1. **Kathuria P., Arora S., Karna R., Kumar N., Kumar S., and Kar P. (2021).** An Interesting Case of Autoimmune Liver Disease. *Annals of the National Academy of Medical Sciences (India)*; *57*(01): 62-64.
2. **Invernizzi F., Cilla M., Trapan S., Guarino M., Cossiga V., Gambato M., and Floreani A. (2022).** Gender and Autoimmune Liver Diseases: Relevant Aspects in Clinical Practice. *Journal of Personalized Medicine*; *12*(6): 925.
3. **Liu Q., Li Y., Ma X., and Tang R. (2019).** Epigenetics of autoimmune liver diseases: Current progress and future directions. *J. Bio-X Res*; 2: 46–55.
4. **Deneau M., Jensen M.K., Holmen J., Williams M.S., Book L.S., and Guthery S.L. (2013).** Primary sclerosing cholangitis, autoimmune hepatitis, and overlap in Utah children: Epidemiology and natural history. *Hepatology*; 58: 1392–1400.
5. **Terziroli Beretta-Piccoli B., Mieli-Vergani G., and Vergani D. (2022).** Autoimmmune hepatitis. *Cell Mol Immunol* ;19 (2):158-176.
6. **Sucher E., Sucher R., Gradistanac T., Brandacher G., Schneeberger S., and Berg T. (2019).** Autoimmune hepatitis—immunologically triggered liver pathogenesis—diagnostic and therapeutic strategies. *Journal of immunology research.*
7. **Floreani A., Restrepo-Jiménez P., Secchi M.F. (2018).** Etiopathogenesis of autoimmune hepatitis. *Journal of Autoimmunity*; 95: 133–143.
8. **Mieli-Vergani G., Vergani D., Czaja A.J, Manns M.P, Krawitt E.L., Vierling J.M. (2018).** Autoimmune hepatitis. *Nat Rev Dis Prim.*;4:18017
9. **vanGerven N.M.F., Verwer B.J., Witteetal B.I. (2014).** Epidemiology and clinical characteristics of autoimmune hepatitis in the Netherlands. *Scandinavian Journal of Gastroenterology* ; 49(10) : 1245–1254.
10. **Clark A., and Mach N. (2016).** Role of vitamin D in the hygiene hypothesis: the interplay between vitamin D, vitamin D receptors, gut microbiota, and immune response. *Frontiers in Immunology* ; 7 : 627.

11. **Lamba M., Ngu J.H., Stedman C.A.M. (2021).** Trends in incidence of autoimmune liver diseases and increasing incidence of autoimmune hepatitis. *Clin Gastroenterol Hepatol Clin Pract J Am Gastroenterol Assoc* ;19:573.
12. **Terziroli Beretta-Piccoli B., Di Bartolomeo C., Deleonardi G., Grondona A.G., Silvestri T., and Tesei C. (2019).** Autoimmune liver serology before and after successful treatment of chronic hepatitis C by direct acting antiviral agents. *J Autoimmun.*;102:89–95.
13. **Engel B., Laschtowitz A., Janik M.K., Junge N., Baumann U., and Milkiewicz P. (2021).** Genetic aspects of adult and pediatric autoimmune hepatitis: a concise review. *Eur J Med Genet*;104:214.
14. **Xu E., Cao H., Lin L., and Liu H. (2017).** Polymorphism in the tumor necrosis factor-α inducible protein 3 (TNFAIP3) gene is associated with type-1 autoimmune hepatitis risk in Chinese Han population. *PLoS One* ; 12 (4) : article e0176471.
15. **Czaja A. J. (2016).** Factoring the intestinal microbiome into the pathogenesis of autoimmune hepatitis. *World Journal of Gastroenterology* ; 22 (42) :9257–9278.
16. **Mack C.L., Adams D., Assis D.N., Kerkar N., Manns M.P., and Mayo M.J. (2020).** Diagnosis and management of autoimmune hepatitis in adults and children: 2019 Practice Guidance and Guidelines From the American Association for the Study of Liver Diseases. *Hepatol Baltim Md* ;72:671–722.
17. **Chinese Society of Hepatology, Chinese Society of Gastroenterology & Chinese Society of Infectious Diseases, Chinese consensus on the diagnosis and management of autoimmune hepatitis. (2017).** *J Dig Dis* ;18: 247–264.
18. **Wong G.W, Yeong T., Lawrence D., Yeoman A.D., Verma S., Heneghan M.A. (2017).**Concurrent extra hepatic autoimmunity in autoimmune hepatitis: implications for diagnosis, clinical course and long-term outcomes. *Liver Int J Int Assoc Study Liver* ;37 :449–457.
19. **Santos B.C., Guedes L.R., Faria L.C, and Couto C.A. (2019).**Wilson's disease presentation resembling autoimmune hepatitis. *BMJ Case Rep* ;12.
20. **Terziroli Beretta-Piccoli B., Ripellino P., Gobbi C., Cerny A., Baserga A., Bartolomeo C.D. (2018).**Autoimmune liver disease serology in acute hepatitis E virus infection. *J Autoimmun*.
21. **Costaguta A., González A., Pochettino S., Trotta L., Vicentín R., and Wagener M. (2018).** Incidence and clinical features of autoimmune hepatitis in the Province of Santa Fe (Argentina). *J Pediatr Gastroenterol Nutr*;67 : 107–110.
22. **Miao Q., Bian Z., Tang R., Zhang H., Wang Q., and Huang S. (2015).** Emperipolesis mediated by CD8 T cells is a characteristic histopathologic feature of autoimmune hepatitis. *Clin Rev Allergy Immunol*; 48:226–235.
23. **Tiniakos D.G., Brain J.G., and Bury Y.A. (2015).** Role of histopathology in autoimmune hepatitis. *Dig Dis Basel Switz* ;33 :53–64.

24. **Mieli-Vergani G., Vergani D., Baumann U., Czubkowski P., Debray D., and Dezsofi A. (2017).** Diagnosis and management of paediatric autoimmune liver disease: ESPGHAN Hepatology Committee Position Statement. *J Pediatr Gastroenterol Nutr.*
25. **European Association for the Study of the Liver, EASL Clinical Practice Guidelines: Autoimmune hepatitis. (2015).** *J Hepatol* ;63:971–1004.
26. **Ramamoorthy S., and Cidlowski J.A. (2016).** Corticosteroids: mechanisms of action in health and disease. *Rheum Dis Clin North Am* ;42:15–31.
27. **Sarcognato S., Sacchi D., Grillo F., Cazzagon N., Fabris L., Cadamuro M., and Guido M. (2021).** Autoimmune biliary diseases: primary biliary cholangitis and primary sclerosing cholangitis. *Pathologica*;*113*(3) : 170.
28. **European Association for the Study of the Liver (EASL). (2017).** Clinical practice guidelines: the diagnosis and management of patients with primary biliary cholangitis. *J Hepatol* ; 67:145-172.
29. **Carey E.J., Ali A.H., and Lindor K.D. (2015)** . Primary biliary cirrhosis. *Lancet*; 386: 1565-1575.
30. **Tsuneyama K., Baba H., and Ogawa H. (2017).** Primary biliary cholangitis: its pathological characteristics and immunopathological mechanisms. *J Med Invest* ; 64:7-13.
31. **Dalekos G.N., and Gatselis N.K. (2019).** Variant and specific forms of autoimmune cholestatic liver diseases. *Arch Immunol Ther Exp (Warsz)* ;67:197-211.
32. **Zen Y., Hübscher S.G., and Nakanuma Y. (2018).** Bile duct diseases. In: Burt A.D., Ferrell L.D, Hübscher S.G., eds. *MacSween's pathology of the liver. 7th ed. Philadelphia, PA: Elsevier* ; 515.
33. **Lazaridis K.N., and LaRusso N.F. (2015).** The cholangiopathies. *Mayo Clin Proc* ; 90:791-800.
34. **Lazaridis K.N., and LaRusso N.F. (2016).** Primary sclerosing cholangitis. *N Engl J Med* ; 375:1161-1170.
35. **Zhang W., De D., Mohammed K.A., Munigala S., Chen G., Lai J.P., and Bacon B.R. (2018).** New scoring classification for primary biliary cholangitis–autoimmune hepatitis overlap syndrome. *Hepatology communications* ; *2*(3): 245-253.