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STATE UNIVERSITY OF MEDICINE AND PHARMACY
NICOLAE TESTEMITANU

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AUTOIMMUNE HEPATITIS

Guidelines for students

CHISINAU
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ABBREVIATIONS

AIH	–	autoimmune hepatitis
ALT	–	alanine aminotransferase
AMA	–	antimitochondrial antibodies
ANA	–	antinuclear antibody
AP	–	alkaline phosphatase
APECED	–	autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
ASGPR	–	antibody to asialoglycoprotein receptor
AST	–	aspartate aminotransferase
ELISA	–	enzyme-linked immunosorbent assays
F-actin	–	antibodies to actin
FTCD	–	formiminotransferase cyclo-deaminase
HAV	–	hepatitis A virus
HBV	–	hepatitis V virus
HCC	–	hepatocellular carcinoma
HCV	–	hepatitis C virus
HLA	–	human leukocyte antigen
IBD	–	inflammatory bowel disease
Ig G	–	immunoglobulin G
LC-1	–	liver cytosol type 1
LKM-1	–	liver/kidney microsomal antibody type 1
LM	–	liver microsome antibody
NKT	–	Natural killer T
pANCA	–	perinuclear anti-neutrophil cytoplasmic antibodies
PBC	–	primary biliary cirrhosis
PDH-E2	–	the E2 subunits of the pyruvate dehydrogenase complex
PSC	–	primary sclerosing cholangitis
SEPSECS	–	Sep [O-phosphoserine] tRNA synthase) Selenocysteine Synthase
SLA	–	soluble live antigen
SLA/LP	–	soluble liver antigen/liver pancreas
SMA	–	smooth muscle antibodies
TPMT	–	thiopurine methyltransferase
UDP	–	glucuronosyltransferases
UGT	–	uridine diphosphate glucuronosyltransferase
ULN	–	upper limit of normal range

DEFINITION

Autoimmune hepatitis (AIH) may be defined as a self-perpetuating inflammation of the liver of more than 6 months duration and unknown etiology characterized by the elevation of serum transaminases, hypergammaglobulinemia, high titres of serum autoantibodies and histological changes in the liver mainly consisting in interface hepatitis and portal plasma cell infiltration associated with variable degree of fibrosis [9]. The disease has a progressive course to cirrhosis without treatment. It is considered to be an autoimmune disease because of its common association with autoimmune extrahepatic disorders and the efficacy of immunosuppressive therapy in inducing clinical, biochemical and histological remission in most cases [9].

HISTORY

Autoimmune hepatitis (AIH) was first described by Waldenström in 1950 as a form of chronic hepatitis in young women showing jaundice, elevated gamma globulins and amenorrhea, which eventually led to liver cirrhosis. He also first described a beneficial effect of steroids in the patient cohort he reported on and thereby laid the groundwork for the first chronic liver disease found to be curable by drug therapy [22].

In 1956 Mackay et al. proposed the term lupoid hepatitis owing to the association of AIH with antinuclear antibodies (ANA) and its similarity with systemic lupus erythematosus. The syndrome has since been described under various names [64].

The term autoimmune hepatitis was introduced in 1993 by the International Autoimmune Hepatitis Group, which defined the diagnostic criteria [27]. Systematic evaluations of the cellular and molecular immunopathology, of the clinical symptoms and of laboratory features has subsequently led to the establishment of autoimmune hepatitis as a separate clinical entity which is serologically heterogeneous, treated by a specific therapeutic strategy [50]. An established (Alvarez 1999) and recently simplified (Hennes 2008) revised scoring system allows for a reproducible and standardized approach to diagnosing AIH in a scientific context [3, 21]. Today, AIH is a treatable chronic liver disease in the majority of cases. Much of the same initial treatment strategies of immunosuppression still represent the standard of care. The largest challenge regarding treatment is the timely establishment of the correct diagnosis.

EPIDEMIOLOGY AND NATURAL HISTORY OF AUTOIMMUNE HEPATITIS

The reported prevalence of AIH ranges from 10 to 17 per 100 000 in Europe and appears to be similar to that of primary biliary cirrhosis [19,36]. No published prevalence data are available from the UK. AIH accounted for two of 121 patients presenting to a UK hospital with jaundice [19].

The prevalence of AIH is estimated to range between 50 and 200 cases per 1 million in Western Europe and North America among the Caucasian population [17,64]. The prevalence of AIH is similar to that of systemic lupus erythematosus, primary biliary cirrhosis and myasthenia gravis, which also have an autoimmune etiology [17]. Among the North American and Western European Caucasian population AIH accounts for up to 20% of cases with chronic hepatitis [36]. In Africa and Asia the prevalence is lower than in white populations.

The mean annual incidence of AIH among white Northern Europeans is 1.9 per 100,000, and its point prevalence is 16.9 per 100,000 [19,29]. It accounts for 2.6% of the transplantations in Europe and 5.9% in the United States [44].

Racial, sexual, and age-related differences in incidence

The disease is most common in whites of northern European ancestry with a high frequency of HLA-DR3 and HLA-DR4 markers. The Japanese population has a low frequency of HLA-DR3 markers [56]. In Japan, autoimmune hepatitis is associated with HLA-DR4.

AIH may present at any age in either sex, although it occurs most frequently in women between the ages of 10 and 30 years and during postmenopause [11]. Women are affected more often than men (70-80% of patients are women) [19].

Autoimmune hepatitis has a bimodal age distribution, with a first peak of incidence at age 10-20 years and a second at age 45-70 years. Approximately one half of affected individuals are younger than 20 years; incidence peaks in premenstrual girls. Patients with AIH-2 tend to be younger; 80% of patients with AIH-2 are children [11].

However, autoimmune hepatitis may occur in people of any age, including infants and older adults. The diagnosis should not be overlooked in individuals older than 70 years [11]. Men may be affected more commonly than women in older age groups.

Natural history

A prospective study has indicated that as many as 40% of patients with untreated severe disease die within 6 months of diagnosis [36].

Cirrhosis develops in at least 40% of survivors; 54% develop esophageal varices within 2 years after cirrhosis; and 20% of individuals with esophageal varices die from hemorrhage [37]. Sustained serum aminotransferase levels of more than 10-fold normal or more than 5-fold normal in conjunction with serum gama-globulin concentrations at least 2-fold normal identify patients with early mortality [37].

Bridging necrosis or multiacinar necrosis on histologic examination progresses to cirrhosis in 82% of patients within 5 years, and mortality is 45% [11]. Patients with less severe laboratory and histologic findings fare better, but cirrhosis still develops in 49% within 15 years and death from hepatic failure occurs in 10% [11].

An acute onset of illness is common (40%), and a fulminant presentation, characterized by hepatic encephalopathy within 8 weeks of disease onset, is possible [37]. Three randomized, controlled treatment trials published between 1971 and 1974 have established that prednisone alone or in combination with azathioprine improves symptoms, laboratory tests, histologic findings, and immediate survival [19].

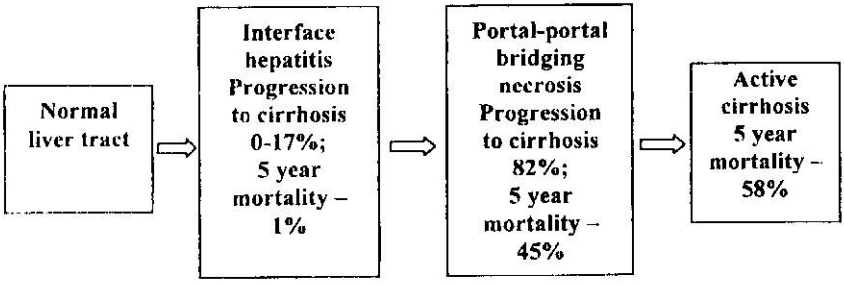

Figure 1. **Natural history of autimmune hepatitis** [Czaja A. 2005].

PATHOPHYSIOLOGY OF AUTOIMMUNE HEPATITIS

Autoimmune hepatitis reflects a complex interaction between triggering factors, autoantigens, genetic predispositions, and immunoregulatory networks.

The pathogenesis of AIH involves dynamic interplay of genetics, environmental exposures, the immune repertoire and dysfunction of immunoregulation (*Fig. 2*) [22]. In the absence of a defined etiology, pathogenetic mechanism(s) or a disease-specific diagnostic laboratory test, AIH has been classified into two types on the basis of its autoantibody profiles [36].

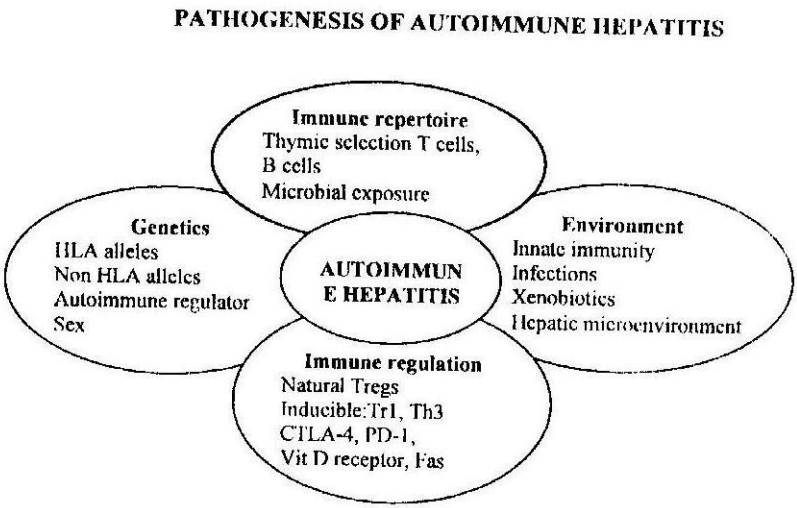

Figure 2. **Interactive factors of genetics, immune repertoire, immune regulation, and environment involved in the pathogenesis of autoimmune hepatitis.**

Aetiology

The aetiology of autoimmune hepatitis is (as yet) unresolved. The immunologic tolerance of the body's own cell structures can be disturbed as a result of:

- immunogenetic abnormalities in the MHC system (major histocompatibility complex, i.e. the main complexin the HLA system, classes I and II);

- clonal deletion (removal of so-called "mobile" genes from plasmids) as well as suppressor defects;
- and molecular mimicry (partial correspondence of the molecular structure of a foreign antigen with a certain body-own protein structure).

Trigger factors

AIH must be triggered by an antigen. Trigger factors include environmental noxae, medication, toxins, bacteria (e.g. salmonella antigen), hepatitis viruses HAV, HBV, HCV, Epstein-Barr, lymphochoriomeningitis virus and measles viruses as well as the Herpes simplex virus (type 1 of which is mainly responsible for AIH type 2) [11, 53]. The viral trigger hypothesis has recently aroused great interest, since various viruses (e.g. HAV, measles, Epstein-Barr) are known to persist "unnoticed" for years, e.g. in lymphocytes.

Sometimes the disease may be precipitated by a drug. Several cases of AIH have been associated with minocycline, interferon α, nitrofurantoin and, recently, with infliximab [19]. There are anecdotal reports of associations with many other drugs including ezetimibe, interferon β, ornidazole, diclofenac, indomethecin, terbinafine, methyldopa, ranitidine, atorvastatin, fluvastatin, fibrates, adalimubab and after hepatitis A vaccination [5, 11, 19]. AIH has also been reported after herbal medicines [11]. The development of AIH has also been precipitated by interferon therapy in chronic hepatitis B and C even in children [5].

Pathogenesis

Current evidence suggests that liver injury in a patient with autoimmune hepatitis is the result of a cell-mediated immunologic attack. Aberrant display of human leukocyte antigen (HLA) class II on the surface of hepatocytes facilitates the exposure of normal liver cell membrane constituents to antigen-presenting cells (APCs). APCs present hepatic antigens to uncommitted helper T lymphocytes (T_H0). APCs and helper T lymphocytes interact at the ligand-ligand level, which, in turn, activates T_H0. This activation is followed by functional differentiation into helper T cell 1 (T_H1) or helper T cell 2 (T_H2), according to the cytokines prevailing in the tissue and the nature of the antigen. T_H1 primarily secretes interleukin 2 (IL-2) and interferon gamma, which

activate macrophages and enhance expression of HLA classes I and II, thus perpetuating the immune recognition cycle [7, 9, 32].

T_H2 cells primarily produce interleukins 4, 5, and 10, which stimulate autoantibody production by B lymphocytes.

The reasons for the aberrant HLA display are unclear. It may be initiated or triggered by genetic factors, viral infections (eg, acute hepatitis A or B, Epstein-Barr virus infection), and chemical agents (eg, interferon, melatonin, alpha methyldopa, oxyphenisatin, nitrofurantoin, tienilic acid). The asialoglycoprotein receptor and the cytochrome monooxygenase P-450 IID6 are proposed as the triggering autoantigens [55].

Physiologically, T_H1 and T_H2 cells antagonize each other. Regulatory mechanisms strictly control the autoantigen recognition process; their failure perpetuates an autoimmune attack. Liver cell injury can be caused by the action of cytotoxic lymphocytes that are stimulated by IL-2, complement activation, engagement of natural killer lymphocytes by the autoantibody bound to the hepatocyte surface, or reaction of autoantibodies with liver-specific antigens expressed on hepatocyte surfaces [33].

Autoantibody-coated hepatocytes from patients with autoimmune hepatitis are killed when incubated with autologous allogenic lymphocytes. The effector cell was shown to be an Fc receptor-positive mononuclear cell. It was shown that T-cell clones from liver biopsy specimens in children with autoimmune hepatitis who express the γ/δ T-cell receptor are preferentially cytotoxic to liver-derived cells.

Autoantigens

The autoantigens associated with AIH include various cytoplasmic enzymes (Table 1). The autoantigen of type 2 AIH is the cytochrome monooxygenase CYP2D6, and it has homologies with hepatitis C virus, cytomegalovirus, and herpes simplex virus type 1 [9]. Homologies between human CYP2D6 and viral proteins might underlie the pathogenesis of AIH in some patients whose immune system recognizes viral antigen as self antigen (molecular mimicry). Repeated exposures to the same or similar viral proteins might thereby break self tolerance.

Many other substrates are recognized by autoantibodies associated with AIH. The transfer ribonucleoprotein complex $tRNP^{(Ser)Sec}$, renamed SEPSECS (Sep [O-phosphoserine] tRNA:Sec [selenocysteine] tRNA synthase), is recognized by anti-SLA, formiminotransferase

cyclodeaminase is recognized by anti-LC1, and UGT are recognized by anti-1.KM3 [9]. Antibodies to liver microsomes primarily bind to cytochrome CYP1A2, and they are associated with the autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy (APECED) syndrome and the presence of AIH [9].

Antibodies to CYP1A2 were first described in patients with AIH induced by dihydralazine and with AIH that later was recognized as the APECED syndrome, a genetic disorder caused by a point mutation in a transcription factor expressed in dendritic and medullary epithelial cells of the thymus. CYP1A2, which is only expressed in the liver, appears to be an autoantigen in at least 2 different types of liver disease. In the drug-induced liver disease, CYP1A2 might have become antigenic by binding a metabolite of dihydralazine; in APECED, CYP1A2 might have become antigenic through the disrupted function of the *autoimmune regulator* (*AIRE*) and the escape of autoreactive lymphocytes into the peripheral circulation.

Molecular Mimicry

In molecular mimicry, multiple antigens with the same or similar epitopes can activate CD4+ T cells because of incomplete specificity of T-cell antigen receptors *(Tab. 1)* [7,9]. This activation leads to expansion of liver-infiltrating cytotoxic T cells that can cause liver injury and antigen-sensitized plasma cells that produce autoantibodies. Humoral cross-reactivity (cross-reacting antibodies) has been described in autoimmune conditions, but cellular cross-reactivity (cross-reacting lymphocytes) has been difficult to demonstrate. Because of molecular mimicry, different environmental agents, drugs, and viruses might produce AIH. Molecular mimicry might also underlie the development of recurrent or de novo AIH after liver transplantation and cause multiple autoimmune diseases to occur in the same patient [55]. Cross-reactivity has been shown between hepatitis C virus antigens and host-derived smooth muscle and nuclear antigens, and HLA B51 has been associated with cross-reactive immune responses between viral and microsomal antigens [7,8]. Molecular mimicry is a concept that is key to the pathogenesis of AIH and has contributed to the development of animal models of the human disease [9,55].

Genetic Factors

The MHC controls the presentation of antigens to the immune system and thereby immune activation. DRβ is a polypeptide chain of the class II MHC molecules, which present antigen to CD4+ T lymphocytes. The *DRB* alleles *DRB1*0301*, *DRB1*0401*, *DRB1*0404*, and *DRB1*0405* encode the same or similar 6 amino acid sequences (LLEQKR or LLEQRR) at positions 67 to 72 in the antigen-binding groove, and these are the alleles that affect susceptibility to type 1 AIH [9, 55].

Similarly, *DRB1*0701* is also believed to affect the antigen-binding groove and promote presentation of antigens that induce type 2 AIH. *DQB1*0201* is in strong linkage disequilibrium with *DRB1*07* and *DRB1*03*, so it may be the principal genetic determinant of anti-LKM1–associated AIH [9]. *DRB1*1301*, the principal susceptibility allele in South America, is associated with protracted infection with hepatitis A virus. This allele might allow individuals (especially children, who are frequently infected with hepatitis A virus infection in this region) to develop AIH as a consequence of protracted exposure to viral and liver antigens.

Various polymorphisms or point mutations in genes outside the *MHC* region probably contribute to AIH phenotypes *(Tab. 1)*. In white North American and northern European patients, a polymorphism of the *cytotoxic T-lymphocyte antigen-4* (*CTLA-4*) gene is associated with increased incidence of AIH; the same polymorphism has been found in patients from this population with primary biliary cirrhosis. The absence of this association in South American and Japanese patients reveals the challenge to extending observations from one ethnic group to another. A polymorphism in *tumor necrosis factor* α (*TNFA*2*) is associated with highly inducible and constitutive levels of TNF-α in serum and occurs mainly in young white patients with AIH who respond less well to corticosteroid therapy than patients without the polymorphism [9, 11].

Other polymorphisms in immune modulators associated with AIH are in *Fas*: *tumor necrosis factor receptor superfamily* (*TNFRSF*) at position -670 (*TNFRSF6*); *interleukin (IL)-2*, *-4*, and *-6*; and *vitamin D receptor* (*VDR*) [55]. A point mutation in *tyrosine phosphatase CD45* has also been associated with AIH. The number of these genetic modulators will increase as analyses of the human genome continue using microarray technology and genome-wide DNA microsatellite techniques.

Table 1

Advances in Understanding of Pathogenic Mechanisms

Advance	Nature	Principal features
Characterization of target antigens	Cytochrome 2D6 tRNP$^{(Ser)Sec}$ Formiminotransferase cyclodeaminase UGT Cytochrome 1A2	Target antigens for anti-LKM1, SLA, LC1, LKM3 dihydralazine-induced hepatitis, and APECED
Clarification of molecular mimicry	Homologous amino acid sequences between genomes of hepatitis C virus, cytomegalovirus and herpes simplex type 1 viruses and cytochrome 2D6, hepatitis C virus, and SMA and ANA	Cross-reacting autoantibodies Concurrent immune diseases
Identification of genetic risk factors inside the MHC	*DRB1*0301*, *DRB1*0401* (North America, northern Europe) *DRB1*0404*, *DRB1*0405* (Mexico, Japan, China) *DRB1*1301* (South America) *DRB1*07*, *DQB1*0201* (type 2 AIH)	Influence susceptibility, phenotype, and severity in different ethnic groups
Identification of genetic risk factors outside the MHC	*CTLA-4* gene polymorphism *TNFA*2* gene polymorphism *TNFRSF6* gene polymorphism *Tyrosine phosphatase CD45* mutation *Vitamin D receptor* polymorphism	No disease specific; affect clinical phenotype; ethnic variablity
Recognition of deficiencies in cellular regulators	Regulatory $CD4^{+}CD25^{+}$ T cells NKT cells	Deficient number and function impair suppression of CD8+ T cells

Alterations in Immunocyte Populations

Regulatory T cells (CD4+CD25+Treg cells) modulate CD8+ T-cell proliferation by suppressing production of interferon gamma and increasing secretion of *IL-4, IL-10*, and *transforming growth factor β* (*Tab. 1*) [9,33]. There is a subpopulation of Treg cells that lacks CD127; these CD4+CD25+CD127- cells might have greater regulatory activity than CD4+CD25+Treg cells. The number and functions of Treg cells are decreased in AIH [33]. These deficiencies might arise from genetic factors, because the siblings and children of patients with primary biliary cirrhosis have deficient Treg cell functions [50]. Treg cells can be

expanded or freshly generated in culture, and deficiencies in their number and function might be corrected by the adoptive transfer of new cells.

Natural killer T (NKT) cells regulate the immune response; their activities might also be deficient in patients with AIH. They are present in normal liver and regulate cytokine levels by inducing apoptosis of altered hepatocytes. NKT cells are constitutively cytotoxic–they contain granzymes and perforin that can induce apoptosis – but they can also have anti-inflammatory and immune suppressive actions by producing cytokines such as IL-4 *(Fig. 3)* [9]. NKT cells have counterbalancing inhibitory and stimulatory receptors, and their activities depend on intrinsic factors that control signaling through specific receptors. The purinergic receptor (P2X7) on NKT cells responds to purine-based danger signals and can inhibit or stimulate NKT cell activity, depending on whether the danger signal is received by naive or activated NKT cells. Furthermore, NKT cells regulate differentiation of Treg cells, another mechanism by which they might affect immune reactivity [9]. Although patients with AIH have fewer intrahepatic NKT cells than patients with primary biliary cirrhosis, animals that are deficient in NKT cells do not develop experimental immune-mediated liver disease. The multiplicity of NKT cell functions and the diversity of conditions that affect their activities suggest that they have a role in the pathogenesis of AIH.

Plasma cells that stain positive for IgG4 were detected in 35% of liver tissue specimens from patients with AIH [9]. These patients also had higher serum levels of IgG than patients without the hepatic infiltrate and faster and more durable responses to corticosteroid therapy. Patients with AIH cannot, however, be distinguished by serum IgG4 levels (which tend to be normal) and can be identified only by staining plasma cells for IgG4 in liver tissue [4]. IgG4 binds complement with low affinity and is not believed to promote the pathogenesis of hepatocyte injury. Nevertheless, it might be worthwhile to investigate the type 2 cytokine pathway of immunocyte differentiation in the development of AIH.

Pathogenesis conclusions

Fundamentally, AIH is caused by a loss of immunological self-tolerance. The reasons for this, however, remain incompletely understood. A genetic component to susceptibility is suggested by the preponderance

of patients who are female, in addition to the findings of specific allotypes within the major histocompatability complex alleles. These genetic associations vary between racial and geographical populations; in Northern America and Northern Europe, the human leucocyte antigen allotypes DRB1*0301 and DRB1*0401 are associated with AIH.

Molecular mimicry by viral agents has been suggested as a possible mechanism of loss of self-tolerance. Cross-reactive antibodies to hepatic antigens can be found in patients with AIH, cytomegalovirus, herpes simplex virus and hepatitis C. Similarly, multiple drugs have been implicated in the pathogenesis in the genetically susceptible patient including minocycline, nitrofurantoin and atorvastatin.

Recent evidence also suggests that the adaptive and humoral immune response is deregulated in AIH. A key area of investigation concerns regulatory T-cells (CD4+ CD25+), which promote self-tolerance by suppressing proliferation of auto-reactive T cells. These have been shown to be deficient both in number and function in AIH patients.

Evidence for an autoimmune pathogenesis includes the following:

- Hepatic histopathologic lesions composed predominantly of cytotoxic T-cells and plasma cells.
- Circulating autoantibodies (ie, nuclear, smooth muscle, thyroid, liver-kidney microsomal, soluble liver antigen, hepatic lectin).
- Association with hypergammaglobulinemia and the presence of a rheumatoid factor.
- Association with other autoimmune diseases.
- Response to steroid and/or immunosuppressive therapy.

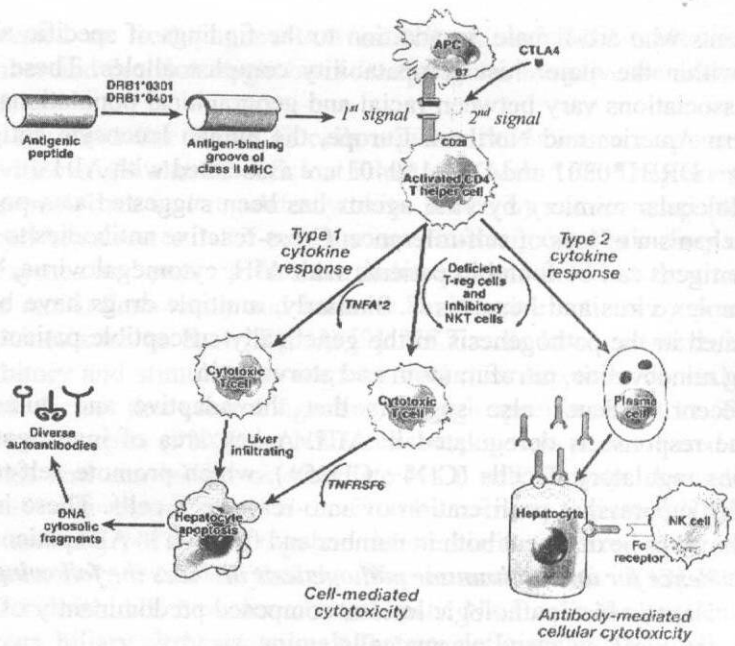

Figure 3. **Putative pathogenic pathways of AIH [by Czaja AJ, Manns MP, 2010].**

*The antigenic peptide (a self-antigen or foreign antigen that resembles a self-antigen) is displayed in the antigen-binding groove of a class II molecule of the MHC. Genetic factors, especially DRB1*0301 and DRB1*0401 in white North American and northern European adults, encode the structure of the antigen-binding groove and affect the nature of the antigen that can be accommodated. Recognition of the antigen display on the surface of the antigen-presenting cell (APC) by the CD4+ T-cell completes the first costimulatory signal necessary for immunocyte activation (1st signal). Ligation of a B7 molecule on the surface of the APC with the CD28 molecule on the surface of the CD4+ T cell completes the second costimulatory signal necessary for immunocyte activation (2nd signal). The activated CD4+ T-cells produce signature cytokines that facilitate the clonal expansion of liver-infiltrating cytotoxic T-cells (type 1 cytokine response) or plasma cells that produce Ig (type 2 cytokine response). Deficiencies in the number and function of the Treg cells and NK cells and genetic polymorphisms in TNFA*2 and TNFRSF6 increase the type 1 cytokine response and proliferation of liver-infiltrating cytotoxic T cells. Hepatocyte apoptosis is accomplished by binding of the Fas ligand of the cytotoxic T cell to the Fas molecule on the hepatocyte surface (cell-mediated cytotoxicity). The Igs produced by the expanded clone of plasma cells, possibly as a consequence of deficient Treg and NKT cell function, bind to normal constituents of the hepatocyte membrane and attract NK cells with Fc receptors. The hepatocytes undergo cytolysis (antibody-mediated cellular cytotoxicity). IgG4 staining can be used to characterize some of the plasma cells.*

MORPHOLOGY

Liver biopsy examination at presentation is recommended to make the diagnosis and to guide the treatment decision. Autoimmune hepatitis has no distinctive histology. The pattern resembles that of chronic active hepatitis: portal and periportal infiltration from some plasma cells as well as a high number of lymphocytes are evident *(Figs. 4, 5)* [30]. The lymphocytes are mainly of the T-cell type, where by the ratio of subtypes CD4:CD8 is about 1:1. The lymphocytes reveal emperipolesis (capble of infiltrating and surrounding other cells). Hepatocytes often show hydropic swelling and ballooning. Inflammatory activity varies, where by some areas are near normal. In the lobule, there are infiltrates of lymphocytes with differing density together with activated Kupffer cells. As the degree of infiltration increases, with inflammatory activity varying from portal zone to portal zone, *piecemeal necroses* (interphase hepatitis) and *bridging necroses* (between adjacent vascular structures) as well as liver *cell rosettes* (hepatocytes surrounding a prominent canaliculus in a circular fashion) become more distinctive histologically [30]. Whereas lytic necrosis leaves a "blank" in the trabecular texture, acidophilic necrosis shows residues containing Councilman bodies. Early on in this process, it may be possible to detect pronounced fibrosis.

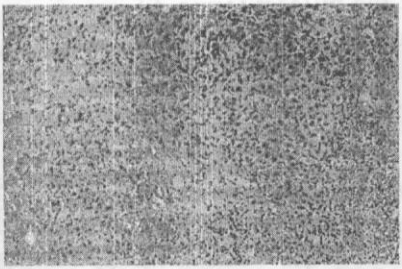

Figure 4. **Acute AIH type 1 (ANCA +) with pronounced centrilobular parenchymal loss.**

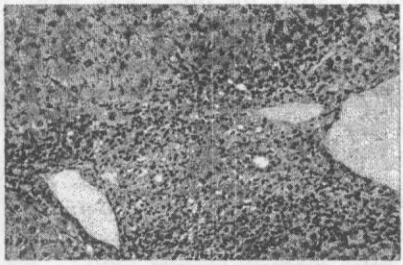

Figure 5. **Lymphoplasmocytic interface hepatitis in autoimmune hepatitis type 1 (HE).**

AIH is thus characterized by signs of portal, periportal, and lobular hepatitis. Due to the development of cirrhosis, both the entire liver structure (blood vessels, lymph vessels, bile ducts) and the parenchyma are transformed.

In acute presentation unavailability of liver biopsy should not prevent from start of therapy. Interface hepatitis is the histological hallmark (*Fig. 6*), and plasma cell infiltration is typical (*Fig. 7*) [36].

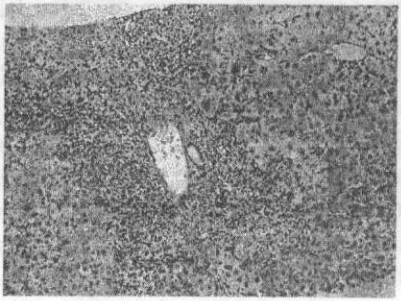

Figure 6. **Interface hepatitis. The limiting plate of the portal tract is disrupted by a lympho-plasmacytic infiltrate.**

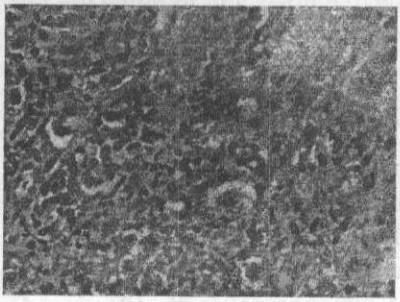

Figure 7. **Plasma cell infiltration. Plasma cells, characterized by cytoplasmic halo about the nucleus, infiltrate the hepatic parenchyma.**

Neither histological finding is specific for AIH, and the absence of plasma cells in the infiltrate does not preclude the diagnosis. Eosinophils, lobular inflammation, bridging necrosis, and multiacinar necrosis may be present [36]. Granulomas rarely occur. The portal lesions generally spare the bile ducts. In all but the mildest forms, fibrosis is present and, with advanced disease, bridging fibrosis or cirrhosis is seen. Occasionally, centrizonal (zone 3) lesions exist, and sequential liver tissue examinations have demonstrated transition of this pattern to interface hepatitis in some patients.

The histological findings differ depending on the kinetics of the disease. Compared to patients with an insidious onset, patients with acute severe hepatic failure exhibit more interface and lobular hepatitis, lobular disarray, hepatocyte necrosis, central necrosis and submassive necrosis, but less fibrosis and cirrhosis.

CLINICAL ASPECTS

Clinical symptoms

AIH has diverse presentations, and it is important to diagnose it during the early stages of disease. The main early symptoms are fatigue and arthralgia, but 25% to 34% of patients are asymptomatic at diagnosis,

including some with cirrhosis [1, 9, 11]. Clinical features of autoimmune hepatitis widely vary. Most cases have an insidious onset. Patients may be asymptomatic or have nonspecific symptoms (eg, fatigue, anorexia, weight loss, behavioral changes, amenorrhea). Nausea is often a prominent symptom and amenorrhoea is common. Systemic or cutaneous abnormalities occur in 25% of patients. Joint pains, sometimes flitting, are reported in 30-60% of patients, although joint swelling is uncommon [19]. Rarer features include a maculopapular skin rash and unexplained fever [1, 19]. Epistaxis, bleeding gums, and bruises with minimal trauma are frequent complaints.

Autoimmune hepatitis may present as acute hepatitis, chronic hepatitis, or well-established cirrhosis. Autoimmune hepatitis rarely presents as fulminant hepatic failure [22, 28].

Up to 50% of patients, even with an insidious onset of disease, may be clinically jaundiced or report previous episodes of icterus. About 30% of patients have cirrhosis at presentation so some patients (especially the elderly) may present with ascites, suggesting liver decompensation and/or a variceal bleed [16].

Approximately one third of patients present with symptoms of acute hepatitis marked by fever, hepatic tenderness, and jaundice. In some patients, the acute illness may appear to resolve spontaneously; however, patients invariably develop signs and symptoms of chronic liver disease. Other patients experience rapid progression of the disease to acute liver failure, as marked by coagulopathy and jaundice. Ascites and hepatic encephalopathy also may ensue.

When AIH presents as acute hepatitis the liver histology may be atypical: lobular hepatitis and centrilobular necrosis are common and cirrhosis less common [25]. Furthermore, serum autoantibodies are sometimes absent initially but develop later. Other causes of acute hepatitis (viral, drug-induced, Wilson's disease) need to be carefully excluded. In these sometimes very ill patients a definitive diagnosis of AIH and institution of immunosuppressive treatment should not await demonstration of 'chronicity' by monitoring liver tests over weeks/months. Patients with liver failure should be referred to a liver transplant centre [16, 20].

The chronic hepatitis associated with autoimmune hepatitis may range in severity from a subclinical illness without symptoms and with abnormal results on liver chemistries to a disabling chronic liver disease.

Symptoms and physical examination findings may stem from the various extrahepatic diseases associated with autoimmune hepatitis. Common symptoms include the following:

• Fatigue • Upper abdominal discomfort • Mild pruritus • Anorexia • Myalgia • Diarrhea • Cushingoid features	• Arthralgias • Skin rashes (including acne) • Edema • Hirsutism • Amenorrhea • Chest pain from pleuritis • Weight loss and intense pruritus (unusual)

Many patients have histologic evidence of cirrhosis at the onset of symptoms. This is true both for patients with an initial presentation of acute hepatitis and for patients with chronic hepatitis. Thus, subclinical disease often precedes the onset of symptoms.

As many as 20% of patients present initially with signs of decompensated cirrhosis [16]. In other patients, chronic hepatitis progresses to cirrhosis after years of unsuccessful immunosuppressant therapy marked by multiple disease relapses. This is said to occur in 20-40% of patients. Patients with cirrhosis may experience classic symptoms of portal hypertension, namely variceal bleeding, ascites, and hepatic encephalopathy. Patients with complications of cirrhosis should be referred for consideration of liver transplantation.

Physical Examination

Common findings on physical examination are as follows:

- Hepatomegaly (83%).
- Jaundice (69%).
- Splenomegaly (32%).
- Spider angiomata (58%).
- Ascites (20%).
- Encephalopathy (14%).

All of these findings may be observed in patients with disease that has progressed to the point of cirrhosis with ensuing portal hypertension. However, hepatomegaly, jaundice, splenomegaly, and spider angiomata also may be observed in patients who do not have cirrhosis.

Complications may include the following:

- Cirrhosis and complications of cirrhosis (eg, ascites, coagulopathy, hepatic coma).
- Portal hypertension.
- Esophageal varices.
- Malnutrition (with poor growth in children).

Gastrointestinal tract bleeding as a complication of portal hypertension is usually rare.

Associated disease

Autoimmune hepatitis, especially type 2, is associated with a wide variety of other disorders. Involvement of other systems may present at disease onset or may develop during the course of active liver disease. Most of these conditions are immunologic in origin.

Patients may present with manifestations of the following hematologic disorders:

- Hypersplenism.
- Autoimmune hemolytic anemia.
- Coombs-positive hemolytic anemia.
- Pernicious anemia.
- Idiopathic thrombocytopenic purpura.
- Eosinophilia.

Gastrointestinal disease associated with autoimmune hepatitis includes inflammatory bowel disease, which is seen in 6% of cases [19]. The presence of ulcerative colitis in patients with autoimmune hepatitis should prompt performance of cholangiography to exclude primary sclerosing cholangitis (PSC). A study of 140 pediatric patients with autoimmune hepatitis, autoimmune cholangitis, and overlap syndrome identified 23 patients with celiac disease.

Associated endocrinologic conditions include Graves disease (6%) and autoimmune thyroiditis (12%) [59].

Associated rheumatologic complications include the following:	Other associated conditions are as follows:
• Rheumatoid arthritis and Felty syndrome • Sjögren syndrome • Systemic sclerosis • Mixed connective-tissue disease • Erythema nodosum • Leukocytoclastic vasculitis (patients may present with leg ulcers)	• Proliferative glomerulonephritis • Fibrosing alveolitis • Pericarditis and myocarditis • Febrile panniculitis • Lichen planus • Uveitis

Pediatric presentation

In 1997, *Gregorio et al* published a series of 52 cases of autoimmune hepatitis in children (32 children with autoimmune hepatitis type 1 [AIH-1] and 20 children with autoimmune hepatitis type 2 [AIH-2]) [18]. Median patient ages were 10 years for AIH-1 and 7.4 years for AIH-2. Other autoimmune disorders occurred in 20% of patients and 40% of their relatives; these included autoimmune thyroiditis, celiac disease, inflammatory bowel disease, diabetes mellitus, and other disorders. AIH-2 can be part of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), an autosomal recessive genetic disorder in which liver disease is reportedly present in about 20% of cases.

In 50% of the children, acute presentation mimicked acute viral hepatitis (ie, abdominal discomfort, vomiting, nausea, jaundice). Fulminant hepatic failure occurred in 11% of the children and was more common in patients with AIH-2. Insidious presentation was characterized by intermittent jaundice or nonspecific symptoms. Routine blood analysis revealed incidental findings of abnormal liver enzymes. Patients with autoimmune hepatitis developed cirrhosis and portal hypertension.

In 2005, *Oettinger et al.* published a series of 142 cases in children with autoimmune hepatitis [34]. Clinical findings included the following:

- Jaundice (58%).
- Nonspecific weakness (57%).
- Anorexia (47%).
- Abdominal pain (38%).
- Paleness (26%).

AIH-1 was found in 73% of the children, AIH-2 was found in 25% of the children, and 4 children could not be classified. Liver biopsy showed active hepatitis (52%), cirrhosis (38%), and mild inflammatory activity (10%).

Additional autoimmune disorders often occur in children with autoimmune hepatitis.

In children with AIH-1, associated autoimmune disorders include the following:	In children with AIH-2, associated autoimmune disorders include the following:
• Ulcerative colitis • Sclerosing cholangitis • Arthritis • Vasculitis • Glomerulonephritis • Diabetes mellitus	• Polyendocrinopathy • Alopecia areata • Diabetes mellitus • Thyroiditis

Acute liver failure occurs primarily between the ages of 13 months and 4 years in children with AIH-2. It typically occurs after puberty in patients with AIH-1.

DIAGNOSIS

Laboratory studies

Laboratory findings in autoimmune hepatitis include the following:

Elevated serum aminotransferase levels (1.5-50 times reference values). Even at the subjective, asymptomatic stage, the transaminases are elevated to differing degrees. With the onset of symptomatic or acute hepatitis, high values can be detected for AST, ALT and GDH (3 to 10 times, and more, above normal levels). Serum aminotransferases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) are elevated in 100% of patients at initial presentation, with average values of 200-300 U/L.

Aminotransferase values correlate poorly with the degree of hepatic necrosis; however, values in the thousands may indicate acute hepatitis or a severe flare of preexisting disease. Continued elevation of the aminotransferases in the face of ongoing therapy is a reliable marker for ongoing inflammatory activity in the liver. Normalization of the aminotransferase levels during therapy is an encouraging sign, but active liver

inflammation is present in more than 50% of patients with normalized liver chemistries. Indeed, biochemical remission may precede true histologic remission by 3-6 months [23]. Worsening of aminotransferase levels in a patient undergoing treatment or in a patient who is in remission may signal a resurgence of disease activity.

Elevated serum immunoglobulin levels, primarily immunoglobulin G (IgG). A sharp rise in γ-globulin (1.5 to 2.0 times above normal) is a characteristic feature. This is almost solely due to an increase in the Ig G fraction. Increased serum γ-globulin and immunoglobulin (Ig) G levels are found in about 85% of patients.

Ig A can be decreased. Patients with AIH-2 commonly have partial immunoglobulin A (IgA) deficiency An increase in serum IgA levels suggests steatohepatitis (alcoholic or non-alcoholic) or drug-induced liver injury rather than AIH, whereas an increase in IgM levels is more characteristic of primary biliary cirrhosis (PBC).

Immunoglobulin levels typically return to normal during treatment. The gamma globulin or the IgG level may be followed on a regular basis as a marker of disease responsiveness to therapy.

Mild to moderately elevated serum bilirubin.

Serum alkaline phosphatase is normal or only mildly raised; a more than two fold elevation suggests an alternative or additional diagnosis. A sharp increase in the alkaline phosphatase values during the course of autoimmune disease may reflect the development of primary sclerosing cholangitis (PSC) or the onset of hepatocellular carcinoma as a complication of cirrhosis. The γ-GT value is only moderately or not at all increased. Cholinesterase is often decreased. Cholestasis-indicating enzymes (γ-GT, AP, LAP) are rarely elevated; clearly raised levels point to an overlap syndrome rather than to a (very infrequent) cholestatic form of autoimmune hepatitis.

Hypoalbuminemia and prolongation of prothrombin time are markers of severe hepatic synthetic dysfunction, which may be observed in active disease or decompensated cirrhosis.

Hyperprotcinaemia (>8 g/dl) ensues. The blood sedimentation rate is greatly increased.

Other hematologic abnormalities may include the following:

- Mild leukopenia.
- Normochromic anemia.

- Coombs-positive hemolytic anemia.
- Thrombocytopenia.
- Elevated erythrocyte sedimentation rate.

Eosinophilia is uncommon, but counts ranging from 9% to 48% are described. Autoimmune hepatitis has even been described as the sole presenting feature of idiopathic hypereosinophilic syndrome.

At this point, the serology of viral hepatitis (HBV, HCV, possibly HDV) has to be clarified.

Autoantibodies (ANA, SMA, LKM, SLA/LP,) and their titres are determined. When the presence of an overlap syndrome is suspected, the determination of specific autoantibodies is required (e.g. AMA with subtypes, LP, pANCA, anti-histone 2B, antinuclear antibodies).

HLA typing (e.g. -A1, -B8, -DR3, -DR4) completes the diagnosis. For the diagnosis of AIH, the score system of F. Alvarez et al. (1999) and E.M. Hennes et al. (2005) may be helpful *(Tab. 4-6)*.

Imaging techniques

Imaging studies, in general, are not helpful in reaching a definitive diagnosis of autoimmune hepatitis; however, the presence of heterogeneous hepatic echotexture on abdominal ultrasound or abnormal contrast enhancement on abdominal CT imaging may suggest the presence of active inflammation or necrosis. The appearance of an irregular nodular liver may confirm the presence of cirrhosis. Furthermore, these imaging studies may be used to rule out the presence of hepatocellular carcinoma, a potential complication of autoimmune hepatitis–induced cirrhosis.

A transition to cirrhosis can only be seen using sonography. With the help of elastography, it is possible to detect stiffening of the liver (fibrosis? cirrhosis?). A striking finding is the sonographic determination of enlarged abdominal (or periportal) inflammatory lymph nodes *(Fig. 8)*.

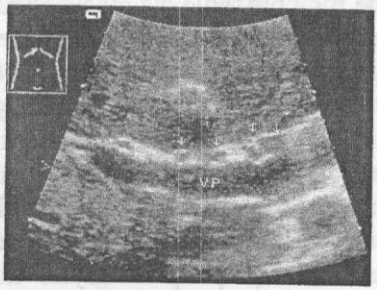

Figure 8. **Active autoimmune liver cirrhosis type 1 with enlarged periportal inflammatory lymph nodes (arrows) (VP - portal vein).**

Liver Biopsy

Liver biopsy is the most important diagnostic procedure in patients with autoimmune hepatitis. This procedure can be performed percutaneously, with or without ultrasound guidance, or by the transjugular route. The latter is preferred if the patient has coagulopathy or severe thrombocytopenia. A transjugular liver biopsy also may be preferable if ascites is present or if the liver is small, shrunken, and difficult to reach percutaneously.

Liver biopsy routinely is performed in the outpatient setting to investigate abnormal liver chemistries. Liver biopsy should be performed as early as possible in patients with acute hepatitis who are thought to have autoimmune hepatitis. Confirmation of the diagnosis enables initiation of treatment at an early stage in the disease process.

The role of biopsy in patients presenting with well-established cirrhosis secondary to autoimmune hepatitis is less clear. As an example, the initiation of treatment in a patient with cirrhosis, normal aminotransferase levels, and a minimally elevated gamma globulin level is not expected to influence the disease outcome.

Liver histology

The role of liver biopsy in the diagnosis of AIH has been affirmed by the IAIHG with regard to both the revised and simplified criteria. Biochemical and immunological blood tests are insufficiently specific on their own for a definite diagnosis of AIH. For example, 20% of patients with biopsy-proven nonalcoholic fatty liver disease meet the criteria for a probable diagnosis of AIH prior to liver biopsy. Thus, liver biopsy is recommended in all patients with suspected AIH unless there is the histology by an experienced liver histopathologist is recommended.

Interface hepatitis, formerly called piecemeal necrosis (inflammation of hepatocytes at the junction of the portal tract and hepatic parenchyma), is a typical feature of AIH. It occurs in 84-98% of patients but may also be seen in patients with drug-induced, viral and other hepatitides. Additionally, the presence of periportal lymphocytic or plasma cell-rich lymphoplasmacytic inflammation, hepatocyte swelling and necrosis is common. However, 34% of patients with AIH have few or no portal or acinar plasma cells. A more diffuse or panacinar hepatitis is less common; it may occur in either AIH of acute onset or in disease that has relapsed following treatment withdrawal. Occasionally the abnormalities are mainly in the centrolobular zone.

Pyknotic cell necrosis and ballooning degeneration of hepatocytes are present in 39% of all patients with AIH. Other liver biopsy findings in AIH include perivenular/zone 3 necrosis with or without portal-based inflammation and giant multinucleated hepatocytes.

Granulomatous inflammation, cholangitis, siderosis, copper deposition and steatosis or steatohepatitis are sometimes seen but, if prominent, make a diagnosis of AIH less likely and receive a negative rating in the IAIHG classification. However, lymphocytic cholangitis and/or a mixed inflammatory infiltrate encircling and infiltrating bile ducts has recently been described in 10% of patients with AIH.

Liver biopsy also provides information on prognosis. One-quarter to one-third of patients have cirrhosis at presentation, although cirrhosis is uncommon in patients with drug-related AIH. Patients with cirrhosis and those with bridging necrosis at diagnosis have a poorer prognosis than those without. Despite this, patients with cirrhosis and AIH usually have steroid-responsive disease and warrant proactive treatment.

Serological Assessment

The conventional serologic markers of AIH (antinuclear antibodies (ANA), smooth muscle antibodies (SMA), and antibodies to liver/kidney microsome type 1 (LKM-1) are used for diagnosis. Other antibodies have been characterized that have prognostic connotations, and they have an emerging ancillary function (*Tab. 2*) [9].

ANA, SMA, anti-LKM1, and anti-LC1 constitute the conventional serological repertoire for the diagnosis of AIH *(Fig. 9)* [3, 12, 36]. In North American adults, 96% of patients with AIH have ANA, SMA, or both, and 4% have anti-LKM1 and/or anti-LC1 [7, 36]. Anti-LKM1 are deemed more frequent in European AIH patients and are typically unaccompanied by ANA or SMA. They are possibly underestimated in the United States [36].

Anti-LKM1 are detected by indirect immunofluorescence, but because they may be confused with antimitochondrial antibody (AMA) using this technique, they can be assessed by measuring antibodies to cytochrome P4502D6, the major molecular target of anti-LKM1, using commercial enzyme-linked immunosorbent assays (ELISA).

Autoantibodies are not specific to AIH and their expressions can vary during the course of the disease [8]. Furthermore, low autoantibody titers do not exclude the diagnosis of AIH, nor do high titers (in

the absence of other supportive findings) establish the diagnosis. Seronegative individuals may express conventional antibodies later in the disease or exhibit nonstandard autoantibodies [8].

Table 2

Diagnostic and Prognostic Serologic Markers

Autoantibodies	Antigen(s)	Principal features
ANA	Diverse nuclear proteins: chromatin, ribonucleoproteins ribonucleoprotein complexes	Conventional diagnostic marker of type 1 AIH
SMA	Actin and nonactin components (vimentin, skeletin)	Conventional diagnostic marker of type 1 AIH
Anti-LKM1	CYP2D6	Diagnostic marker of type 2 AIH
Anti-SLA	$tRNP^{(Ser)Sec}$	High specificity; Predictor of relapse and severity; Present in 20%–26% of cases of cryptogenic hepatitis; Associated with HLA-DRB1*03.
Anti-actin	Polymerized F-actin	Assay-dependent correlations; Associated with early age of onset and poorer outcome than ANA in some assays; Could define a subset of patients with SMA and poor prognosis.
Anti-α-actinin	Component of actin associated with anti-ssDNA	"Double reactivities" of anti-α-actinin with anti-actin or anti-ssDNA; Associated with severe clinical and histologic disease.
Anti-LC1	Formiminotransferase cyclodeaminase	Detection of anti-LKM1, Fluctuates with disease activity; Associated with early age of onset, concurrent immune diseases; rapid progression to cirrhosis; Rare in the United States; Antigen has been used to develop models of disease.
pANCA (atypical)	Nuclear lamina proteins	Diagnosis of type 1 AIH; Reclassification of cryptogenic chronic hepatitis as type 1 AIH.

Anti-LKM3	UGT	Present in 8% of patients with type 2 AIH and 6% with chronic hepatitis D; Might be the only marker detected; Rare in fulminant hepatitis.
ASGPR	Asialoglycoprotein receptor	Prognostic implications; Severe Disease; Histological activity; Relapse.
LM	Cytochrome P450 1A2	Diagnosis of APECED hepatitis

Autoantibody titers in adults only roughly correlate with disease severity, clinical course, and treatment response. In pediatric populations (patients aged ≤ 18 years), titers are useful biomarkers of disease activity and can be used to monitor treatment response. When tested on rodent tissues, an autoantibody titer of 1:40 is significant in adults, whereas in children titers of 1:20 for ANA and SMA, and 1:10 for antiLKM1, are clinically relevant, because autoantibody reactivity is infrequent in healthy children [3]. If present in high titer, anti-LKM1 strongly support the diagnosis of AIH, even if liver biopsy is precluded by other clinical considerations.

The mainstay technique for autoantibody screening is indirect immunofluorescence on composite sections of freshly frozen rodent stomach, kidney and liver. This technique not only permits the detection of ANA, SMA, anti-LKM1, and AMA but also suggests the presence of other autoantibodies of an evolving clinical importance, such as antibody to liver cytosol type 1 (anti-LC1) and antibody to liver kidney microsome type 3 (anti LKM-3). Confirmation of the presence of the latter autoantibody is obtained with assays detecting antibodies to their molecular targets, formiminotransferase cyclo-deaminase (FTCD) and family 1 UDP-glucuronosyl-transferases (UGT1A), respectively (*Tab. 2*).

Other autoantibodies that may be useful in classifying patients who lack the conventional serological findings are anti-SLA and atypical perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) [35, 36]. Atypical pANCA, originally considered specific for PSC and inflammatory bowel disease (IBD), are frequently present in patients with AIH, and occasionally can be the only autoantibodies detected. ANCA typically do not coexist with anti-LKM1. Recent evidence indicates that the target of atypical pANCA is located within the nuclear membrane. For this reason, a more suitable designation may be peripheral anti-neutrophil nuclear antibody (pANNA) [36].

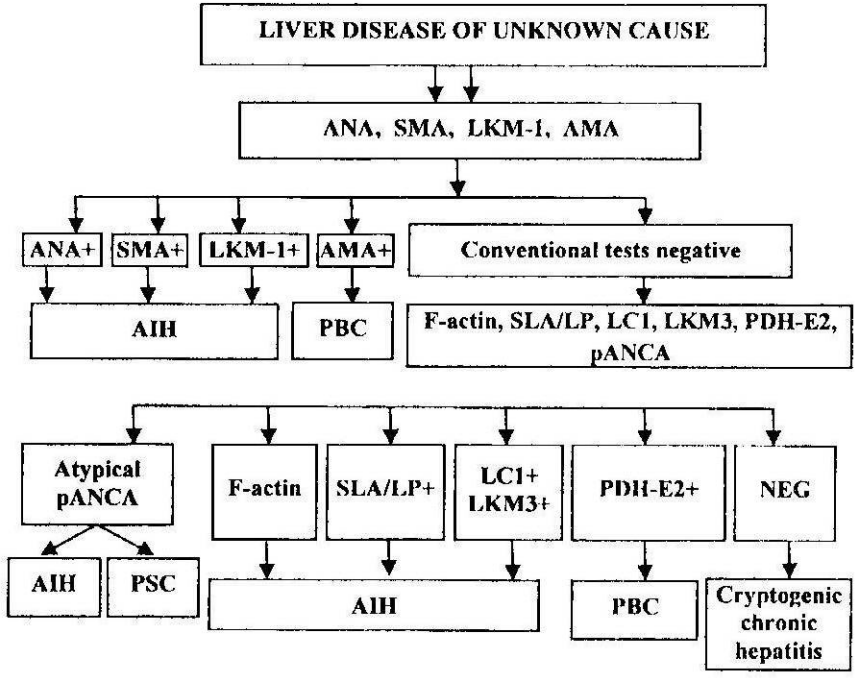

Figure 9. **The use of serological tests assisting in the diagnosis of AIH.**

Serological tests in the evaluation of acute or chronic hepatitis of undetermined cause. The initial serological battery includes assessments for ANA, SMA, LKM-1, and AMA. The results of these conventional tests then direct the diagnostic effort. If one or more tests are positive, the diagnosis of autoimmune hepatitis (AIH) or primary biliary cirrhosis (PBC) should be pursued. If these tests are negative, other serological assessments are appropriate, including tests for antibodies to actin (F-actin), SLA/LP, LC-1, UDP-glucuronosyltransferases (LKM-3), the E2 subunits of the pyruvate dehydrogenase complex (PDH-E2), pANCA. The results of these supplemental tests may suggest other diagnoses, including primary sclerosing cholangitis (PSC), or cryptogenic chronic hepatitis.

Anti-SLA (soluble liver antigen) and anti–liver-pancreas (anti-LP), originally described as separate autoantibodies in AIH, were later found to target the same antigen and to represent a single serological entity [35]. These antibodies are now referred to as anti-SLA or anti-SLA/LP.

Their molecular target is a transfer ribonucleoprotein. SLA has recently been renamed SEPSECS (Sep [O-phosphoserine] tRNA synthase) Selenocysteine Synthase. Anti-SLA are occasionally found in patients with AIH who are negative for ANA, SMA, and anti-LKM1, but are more commonly found in association with the conventional autoantibodies, especially if sensitive immunoassays are used [36]. Anti-SLA are highly specific for the diagnosis of autoimmune liver disease, and their detection may identify patients with more severe disease and worse outcome. Commercial ELISAs are available for their detection.

Genetic Considerations

Multiple genetic associations with AIH have been described in different ethnic groups. The primary genetic association is with the major histocompatibility complex locus, and associations of HLA alleles with disease predisposition, clinical phenotype, response to therapy, and outcome have been studied [13]. AIH is a complex polygenic disorder unlikely to be transmitted to subsequent generations; thus, routine screening of patients or family members for genetic markers is not recommended. AIH may be present in patients with multiple endocrine organ failure, mucocutaneous candidiasis, and ectodermal dystrophy. Such patients have the rare genetic disorder autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), caused by a single-gene mutation located on chromosome 21q22.3 that affects the generation of the autoimmune regulator (AIRE) protein. AIRE is a transcription factor expressed in epithelial and dendritic cells within the thymus that regulates clonal deletion of autoreactive T-cells (i.e., negative selection). APECED has an autosomal recessive pattern of inheritance and lacks HLA DR associations and female predilection. The liver autoantigens associated with APECED are cytochrome P450 1A2 (CYP1A2), CYP2A6 in addition to CYP2D6 [36]. Antibodies to cytochrome P450 1A2 were previously called anti liver microsomal (anti-LM) antibodies. This is the only syndrome involving AIH that exhibits a Mendelian pattern of inheritance, and genetic counseling for the patient and family members are warranted.

Autoantibody Classification

In the serum of most patients with AIH there are detectable non-organ-specific autoantibodies such as antinuclear antibody (ANA) and anti-smooth muscle antibody (ASMA), although the exact function of

these antibodies remains unknown. AIH has been categorised into two distinct disease subtypes based on these antibody profiles.

Type 1 AIH is associated with the presence of either ANA or ASMA in the serum and accounts for about 75% of patients [2,59]. The ANAs react with histones and DNA and typically show a homogenous staining pattern on immunofluorescence, similar to that seen in systemic lupus erythematosus. Speckled and nuclear work patterns are also seen but are not specific for AIH; they are also found in PBC. Although other staining patterns are seen, these are not known to be of significance. Serum antibodies to double-stranded DNA are found in 15% of patients with AIH. When present, they are highly specific for either AIH or systemic lupus erythematosus.

Smooth muscle antibodies react to several cytoskeletal elements including F-actin. Although titres of autoantibodies fluctuate during treatment, disease activity does not correlate closely with titres.

In addition, 10-30% of patients with AIH will have detectable antibodies to soluble liver antigen or liver pancreas antigen; these were shown to be the same antigen which is now designated SLA/LP [36]. These antibodies are specific for AIH, so may also be a useful adjunct in the diagnosis of type 1 AIH when conventional autoantibodies are negative. Controversy has existed in relation to the existence of a third subtype of AIH defined by the presence of these anti-SLA/LP antibodies, but these patients display the typical clinical and pathological hallmarks of type 1 AIH and should be treated as such.

Although antibodies to actin and atypical peripheral antineutrophilic cytoplasm (p-ANCA) are also frequently seen in type 1 AIH, their applicability is limited by their lack of specificity.

Type 2 AIH is associated with the presence of either anti-liver kidney microsomal-1 (LKM-1) or anti-liver cytosolic-1 (LC-1) antibodies [3, 27]. Anti-LKM-1 antibodies target several epitopes of hepatic cytochromes, specifically cytochrome P-450 2D6 (CYP2D6). Moreover, cross-reactivity has been demonstrated between a number of viruses known to infect humans, including hepatitis C virus (HCV). The implications of these findings are that viruses may mimic self and, by cross reactivity with P450 epitopes, trigger hepatic autoimmunity. Type 2 AIH accounts for less than 10% of all cases in northern Europe and North America but is commoner in southern Europe. The clinical phenotypes of disease associated with type 1 and type 2 AIH are summarised in table 3.

Table 3

Classification of autoimmune hepatitis (AIH) based on autoantibody profiles of patients

Feature	*Type 1 AIH*	*Type 2 AIH*
Characteristic autoantibodies	ANA, ASMA, Anti-actin antibody; Anti-SLA/LP antibodies in 25% of ANA negative patients	Anti-LKM-1 antibody Anti-LC-1 antibody
Geographical variation	Worldwide	Worldwide
Age at presentation	All ages	Usually childhood and young adulthood
Sex (F:M)	3:1	10:1
Clinical phenotype	Variable	Generally severe
Associated diseases	Thyroiditis, ulcerative colitis, synovitis	Vitiligo, type 1 diabetes
Histopathological features at presentation	Broad range: mild disease to cirrhosis	Generally advanced, ↑ inflammation/cirrhosis
Treatment failure	Rare	Common
Relapse after drug withdrawal	Variable	Common
Need for long-term maintenance	Variable	Approximately 100%

Anti-mitochondrial antibodies are occasionally identified in patients with AIH. Previously it was thought that poor interpretation of staining patterns on immunofluorescence of proximal renal tubules and on liver sections was responsible for the interpretation of LKM-1 positivity as detectable mitochondrial antibodies, although it is clear that such patients do exist. In large series, 8-12% patients with AIH had detectable antimitochondrial antibodies throughout their AIH disease course, without any evidence of PBC on serial liver biopsies [4].

Non-organ-specific autoantibodies are not specific to AIH. They are found in a minority of patients with PBC and with primary sclerosing cholangitis; 20-40% of patients with alcoholic liver disease have low ANA or ASMA titres [37]. In patients with non-alcoholic fatty liver disease, 25% have serum positive for ASMA or ANA and 20% meet the IAIHG criteria for probable or definite AIH prior to biopsy [19]. ANA positivity may also occur in hepatocellular carcinoma.

Table 5

Modified diagnostic criteria for the diagnosis of autoimmune hepatitis (AIH)

Parameter/feature	Score	Parameter/feature	Score
Female sex	+2	Other autoimmune disease(s) In either patient or first-degree relative	+2
ALP:AST(or ALT) ratio		Average alcohol intake	
<1.5	+2	< 25 g/day	+2
1.5-3.0	0	> 60 g/day	-2
>3.0	-2		
Serum globulins or IgG above normal		Liver histology	
		Interface hepatitis	+3
>2.0	+3	Lymphoplasmacytic infiltrate	+1
1.5-2.0	+2	Rosetting of liver cells	+1
1.0-1.5	+1	None of the above	-5
<1.0	0	Biliary changes	-3
		Atypical features	-3
ANA, SMA or LKM-1		Optional additional parameters	
>1:80	+3	Seropositivity for other	
1:80	+2	defined antibodies	+2
1:40	+1	HLA DR3 or DR4	+1
<1:40	0		
AMA positive	-4	Response to therapy	
		Remission alone	+2
		Remission with relapse	+3
Hepatitis viral markers		***Interpretation of aggregate***	
Positive	-3	***scores***	
Negative	+3	***Pretreatment***	
		Definite AIH	***>15***
		Probable AIH	***10 - 15***
Drug history		***Posttreatment***	
Positive	-4	***Definite AIH***	***>17***
Negative	+1	***Probable AIH***	***12 - 17***

A simplified scoring system has been proposed recently to ease clinical application and is based on the presence and level of autoantibody expression by indirect immunofluorescence, serum immunoglobulin G

(IgG) concentration, compatible or typical histological features, and the absence of viral markers *(Tab. 6)* [21]. In three recent retrospective studies, the simplified scoring system performed with high sensitivity and specificity in the diagnosis of AIH, but it has yet to be validated in prospective studies.

Table 6

Simplified diagnostic criteria for the diagnosis of autoimmune hepatitis (AIH): adapted from Hennes et al 2008 [21]

Feature/parameter	Discriminator	Score
ANA or SMA+	≥1:40	+1*
ANA or SMA+	≥1:80	+2*
Or LKM+	≥1:40	
Or SLA+	Any titre	
IgG or immunoglobulin level	> Upper limit of normal > 1.1 x Upper limit	+1 +2
Liver histology	Compatible with AIH Typical of AIH	+1 +2
Absence of viral hepatitis	No Yes	0 +2

*Addition of points achieved for all antibodies (maximum 2 points).
≥ 6 points: probable AIH; ≥ 7 points: definite AIH.

Differential Diagnosis

Because autoimmune hepatitis is a potentially treatable condition, a missed diagnosis can have serious consequences. The diagnosis should be considered in all patients with hepatitis, especially females. Untreated autoimmune hepatitis can result in death due to liver failure.

Similarly, a wrong diagnosis of autoimmune hepatitis can expose the patient to unnecessary complications of immunosuppressant therapy, which can be serious and life threatening.

The differential diagnosis of AIH includes various forms of acute and chronic hepatitis. These include toxin-induced hepatitis, hepatotropic (hepatitis A–E viruses) and nonhepatotropic viral hepatitis (cytomegalovirus, Epstein-Barr virus, herpes simples virus, and varicella virus) infections, metabolic liver disorders such as NASH, and inherited liver disorders such as hemochromatosis, Wilson disease, and α 1-antitrypsin deficiency.

Autoimmune hepatitis must also be differentiated from autoimmune polyendocrine syndrome type I (APS-1), autoimmunity in hepatitis C virus (HCV) infection, immune-mediated drug-induced hepatitis, cryptogenic hepatitis, and overlap syndrome.

Overlap syndromes

The term 'overlap syndrome' has been introduced to the field of hepatology to describe variant forms of autoimmune hepatitis (AIH) which present with characteristics of AIH and primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). Diagnostic criteria of AIH, PBC, and PSC include clinical, biochemical, histopathological, and cholangiographic findings *(Tab. 7) [51]*. Overlap syndromes are characterized by fulfilling criteria of different autoimmune hepatopathies, but standardization of diagnostic criteria for the overlap syndromes is still lacking. Although AIH, PBC, and PSC all have some characteristic features, patients within each of these disorders can present with a spectrum of clinical, biochemical, serological, and histological findings. Practically all of these findings may overlap with those of one of the other disorders, and the boundaries between the classical conditions are therefore not always distinct.

Table 7

Clinical, biochemical, histologic, and cholangiographic criteria of autoimmune liver diseases. 'Overlap syndromes' show characteristics of both, autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), or autoimmune cholangitis (AIC)

	AIH	PBC	PSC	AIC
Female: male	4:1	9:1	1:2	9:1
Predominant serum liver test elevation	ALT, AST	AP, γ-GT	AP, γ-GT	AP, γ-GT
Serum Ig elevation	IgG	IgM	IgG, IgM	IgM
Autoantibodies	ANA, ASMA, LKM, SLA, p-ANCA	AMA, AMA-M2	p-ANCA	ANA, ASMA
HLA association	A3, B8, DR3, DR4	DR8	DR52	B8, DR3, DR4

Histology	Lymphocytic interface hepatitis (moderate/severe)	Florid bile duct lesion	Fibrosing bile duct lesion	Florid bile duct lesion
Diagnosis	AIH score > 15	AMA-M2; cholestatic serum enzyme pattern; compatible histology	Bile duct stenoses/dilatations (cholangiography); cholestatic serum enzyme pattern; inflammatory bowel disease; p-ANCA	Cholestatic serum enzyme pattern; AMA neg.; ANA or ASMA pos.; histology compatible with PBC
First line medical therapy	Corticosteroids + azathioprine	UDCA	UDCA	UDCA

There is at present no unequivocal definition of overlap syndromes (OLS), or "atypical manifestations of autoimmune hepatitis" as overlap syndrome is also known. The term overlap syndrome indicates that the characteristics of two different autoimmune diseases are present in one liver but that the true overlap syndrome possibly has a single cause [50, 51].

AIH patients may demonstrate serological features that suggest another diagnosis. AMA occur in about 5% of AIH patients in the absence of other biliary features ("serological overlap"), and their presence may confound the clinical diagnosis [42]. AMA may disappear or persist as long as 27 years without an evolution into PBC. The revised original scoring system can render a diagnosis of "probable AIH" in these patients, if other features of AIH are sufficiently strong.

The prevalence of overlap syndromes among patients with AIH, primary sclerosing cholangitis (PSC), and primary biliary cirrhosis (PBC) has been estimated at about 18% (*fig. 10*).

Other acute and chronic liver diseases of diverse etiologies that can have serological features of AIH include alcoholic and nonalcoholic fatty liver disease, acute and chronic viral hepatitis, and drug-induced hepatitis. The two most common variants are AIH-PBC and AIH-PSC overlaps.

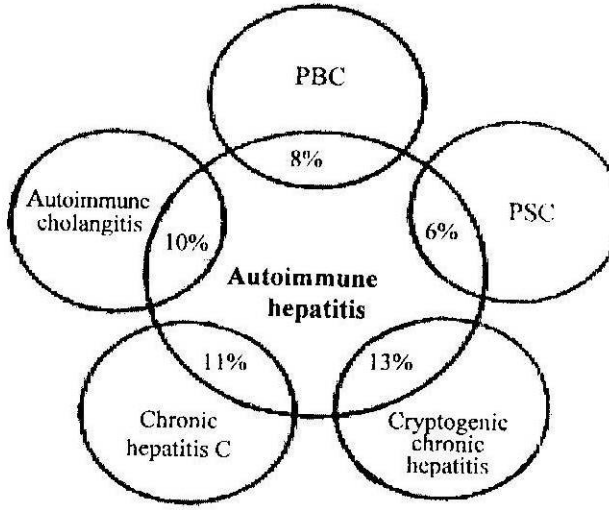

Figure 10. **Prevalence of serological and morphological features common to chronic liver disease.**

AIH-PBC Overlap

In the majority of these patients, serologic evaluations show the presence of antimitochondrial antibodies (AMAs) but histologic characteristics of AIH are present [47]. Characteristic features of patients with AIH-PBC overlap syndrome include elevation of serum transaminases, markers of cholestasis, and immunoglobulins M and G, the presence of AMA-M2, and histological findings compatible with AIH including moderate to severe interface hepatitis *(Tab. 8)*. There are no controlled studies of the management of AIH/PBC overlap. In most reports patients received conventional treatment of the dominant diseased – UDCA for PBC and prednisolone (with or without azathioprine) for AIH. The incidence of variceal bleeding, liver failure and liver transplantation may be higher in overlap than in PBC alone or AIH alone [2, 47]. For this reason, diagnosis and proactive treatment of the AIH component is important and liver biopsy should be considered in patients with PBC but in whom serum transaminases persistently exceed 100 U/l [19].

A second group of patients frequently classified as AIH-PBC overlap are negative for AMA, positive for ANA or ASMA, or both, but have histologic findings consistent with PBC. This entity has been termed

autoimmune cholangitis or AMA – negative PBC [2,47]. Autoimmune cholangitis (AIC) shares many features with PBC including the female preponderance, typical symptoms of fatigue and pruritus, cholestatic serum enzyme pattern, and 'florid lesions' of small ductules leading to fibrosis and cirrhosis of the liver in the long term *(Tab. 8)* [47].

These patients are usually treated with ursodeoxycholic acid. Treatment with UDCA at doses identical to those administered in PBC (13–15 mg/kg/d) appears justified [51]. The benefit of corticosteroids in this setting is controversial.

Table 8

Characteristics of the overlap syndromes of AIH/PBC and AIH/PSC and AMA-negative PBC

	AIH/PBC	AIH/PSC	AMA – negative PBC
Proportion of women	87%	57%	>90%
Peak age	Middle age	Young age	Middle age
Liver enzymes	ALT,AST, γ-GT, AP, bilirubin ↑	ALT,AST, γ-GT, AP, bilirubin ↑	γ-GT, AP, bilirubin ↑
Immunoglobulins	IgG, IgM ↑	IgG, IgM ↑	IgM ↑
Antibodies	ANA, SMA, AMA	ANA, SMA, pANCA	ANA, SMA
Histology	Interface hepatitis, bile duct lesions	Interface hepatitis, fibrosing obliterative cholangitis	Granulomatous and lymphocytic infiltration of the bile ducts, ductopenia
IBD	+/-	+	+
Therapy	UDC +/- glucocorticoids	UDC +/- glucocorticoids	UDC

AIH-PSC Overlap

Patients with this overlap syndrome have serologic findings suggestive of AIH (presence of ANA or ASMA, or both) but radiographic findings suggestive of PSC [6]. This entity should be suspected in patients with a clinical diagnosis of AIH who present with pruritus and have radiographic or histologic findings of PSC *(tab. 7)*, known inflammatory bowel disease (specifically ulcerative colitis), and elevated alkaline

phosphatase *(Tab. 8)*. Treatment of these patients is difficult. Corticosteroids alone or in combination with azathioprine have not been shown to be very effective. Most patients with AIH/PSC overlap have been treated with prednisolone and azathioprine with or without UDCA [19]. Falls are usually seen in serum transaminases but not in the serum alkaline phosphatase level.

Acute Severe Presentation

AIH can have an acute severe presentation that can be mistaken for a viral or toxic hepatitis [28]. Sometimes autoimmune hepatitis may present as acute liver failure. Corticosteroid therapy can be effective in suppressing the inflammatory activity in 36%-100% of patients, whereas delay in treatment can have a strong negative impact on outcome [10, 24]. In addition, unrecognized chronic disease can exhibit a spontaneous exacerbation and appear acute. If extrahepatic endocrine autoimmune features are present in children with severe acute presentation the APECED syndrome must be excluded [18].

THERAPY OF AUTOIMMUNE HEPATITIS

Treatment indications

Absolute indications for treatment

Definitive indications for treatment have been developed on the basis of three randomised control trials that demonstrated features associated with poor outcome [31]. These trials have demonstrated that patients with serum AST levels of at least 10-fold the upper limit of the normal range (ULN) or more than five-fold ULN in conjunction with a serum gama-globulin level more than two-fold ULN have a high mortality (60% at 6 month) if untreated [36,63]. Furthermore, histological findings of bridging necrosis or multilobular necrosis at presentation progress to cirrhosis in 82% of untreated patients and are associated with a 5-year mortality of 45% [46]. These laboratory and histological findings of disease severity at presentation are absolute indications for corticosteroid treatment (*Tab. 9*) [36]. Incapacitating symptoms associated with hepatic inflammation, such as fatigue and arthralgia, are also absolute indications for treatment regardless of other indices of disease severity (*Tab. 9*).

Table 9

Indications for Immunosuppressive Treatment

Absolute	Relative	None
Serum AST > 10-fold ULN; Serum AST ≥ 5-fold ULN and γ-globulin level ≥ 2-fold ULN; Bridging necrosis or multiacinar necrosis on histological examination; Incapacitating symptoms;	Symptoms (fatigue, arthralgia, jaundice); Serum AST and/or γ-globulin less than absolute criteria; Interface hepatitis; Osteopenia, emotional instability, hypertension, diabetes, or cytopenia (white blood cell counts ≤ 2.5x109/L or platelet counts < 50x109/L)	Asymptomatic with normal or near normal serum AST and γ-globulin levels; Inactive cirrhosis or mild portal inflammation (portal hepatitis); Severe cytopenia (white blood cell counts < 2.5x109/L or platelet counts < 50x109/L) or known complete deficiency of TPMT activity precludes treatment with azathioprine; Vertebral compression, psychosis, brittle diabetes, uncontrolled hypertension, known intolerances to prednisone or azathioprine

Uncertain Indications for Treatment

The natural history of autoimmune hepatitis is uncertain in patients who have no or only mild symptoms and in those who have mild laboratory and histological findings. Prospective, randomized, controlled treatment trials have not been performed in these patients, and their indications for treatment remain uncertain and highly individualized (*Tab. 9*) [14]. Asymptomatic individuals with inactive cirrhosis may have an excellent immediate survival without corticosteroid treatment [16]. Other asymptomatic patients who do not have cirrhosis may have inactive disease, and their natural 10-year survival may exceed 80% [16]. There are no guidelines that reliably identify this "safe" population who require no therapy. Spontaneous resolution is possible in some asymptomatic patients with mild disease, but these patients improve less commonly (12% versus 63%, $P < 0.006$) and more slowly than treated patients [14]. Furthermore, untreated asymptomatic patients with mild disease have a lower 10-year survival than treated counterparts (67% versus 98%, $P < 0.01$) [14]. The frequency of spontaneous improvement must be counterbalanced against the frequency of serious drug-related complications when making the treatment decision (12% versus 14%) [14]. Since the mild autoimmune hepatitis can progress and

a rapid and complete response to a normal end point can be anticipated, corticosteroid therapy is favored in asymptomatic mild disease, especially in young individuals who are likely to tolerate the medication satisfactorily [14].

No Indications for Treatment

Corticosteroid therapy is effective only in patients who have clinical, laboratory or histological features of active liver inflammation. Patients with inactive or "burned out cirrhosis" cannot benefit from therapy, and they have an increased risk of drug-induced side effects because their associated hypoalbuminemia, hyperbilirubinemia, and portosystemic shunting can affect protein-binding and disposition of free prednisolone [16,211].

Patients with brittle diabetes, vertebral compression, psychosis, or severe osteoporosis must be critically assessed for a treatment benefit before administering corticosteroids, and azathioprine should be avoided in patients with severe pretreatment cytopenia (white blood cell counts below 2.5x109/L or platelet counts below 50x109/L) or known complete deficiency of thiopurine methyltransferase activity *(Tab. 9)* [10,36].

Treatment Regimens

Corticosteroid therapy is effective for all forms of AIH [10]. Prednisone (or prednisolone) alone or a lower dose in combination with azathioprine ameliorates symptoms and improves the laboratory and histologic manifestations of liver inflammation in most patients [39,40]. It also improves or prevents hepatic fibrosis and increases the 20-year life expectancy to 80% [39]. Outcomes for current therapy can be improved by early recognition and treatment of the disease, continuation of therapy until complete resolution of inflammatory activity, institution of ancillary regimens to prevent complications of the medication, and early identification and treatment of problematic patients [9]. The major determinant of prognosis is the response to corticosteroid therapy, and treatment that is delayed or deferred can result in rapid progression to cirrhosis and liver failure.

Early Recognition and Treatment

Awareness of the diverse manifestations of AIH and the histologic spectrum of the disease at presentation is the key to early diagnosis and therapy. Phenotypic differences among different ethnic groups must be accommodated [11], and the application of the comprehensive and

simplified diagnostic scoring systems of the International AIH Group can be useful in supporting the clinical diagnosis [13].

The treatment of AIH is immunosuppression [24,26,31]. Standard treatment is either prednisone or prednisolone alone or a combination of prednisone and azathioprine (*Tab. 9*). Both are effective in inducing remission in 80% of the cases [41]. Prednisone monotherapy is preferred in children and young adults, while combination therapy is preferred in adult and old patients, in order to give lower doses of steroids, and in this way to minimize adverse effects of long-term treatment with steroids *(Tab. 10)* [19,36].

Treatment Regimens in Adults

Two treatment regimens are equally effective in severe AIH [31]. Prednisone alone (60 mg daily) or a lower dose of prednisone (30 mg daily) in conjunction with azathioprine (50 mg is usually used in the United States or 1-2 mg/kg body weight, which is widely used daily in Europe) (*Tab. 10*) [19,36]. Prednisone may be tapered down to an individual level sufficient to maintain a remission from 20 mg daily onward, reduction should be done by 5 mg every week until 10 mg/day are achieved and even further reduction by 2.5 mg/week have been considered up to 5 mg daily [36]. The maintenance regimen is then continued until resolution of the disease, treatment failure, or drug-intolerance *(Fig. 11)* [11,39].

The combination regimen of prednisone and azathioprine is associated with a lower occurrence of corticosteroid-related side effects than a higher dose prednisone regimen (10% versus 44%), and it is the preferred treatment [48]. Advanced cirrhosis can significantly impair the conversion of prednisone to prednisolone, but this impairment is insufficient to alter treatment response or mandate the administration of prednisolone [36].

Prednisone is appropriate as the sole medication in individuals with severe cytopenia [31], those undergoing a short treatment trial (duration of therapy < 6 months), individuals who are pregnant or contemplating pregnancy, patients with some active malignancies, and individuals with known complete thiopurine methyltransferase deficiency *(Tab. 10)* [40,45,57]. The combination regimen is appropriate in patients who will be treated continuously for at least 6 months or who are at increased risk for drug-related complications, including postmenopausal women

and individuals with emotional instability, osteoporosis, brittle diabetes, labile hypertension, or obesity *(Tab. 10)* [10,39].

Table 10

Immunosuppressive Treatment Regimens for Adults in Autoimmune Hepatitis

	Monotherapy	**Combination Therapy**		
	Prednisone only* ***(mg/day)***	***Prednisone**** ***(mg/day)***	***Azathioprine***	
			USA (mg/day)	***EU(mg/kg/day)***
Week 1	60	30	50	1-2
Week 2	40	20	50	1-2
Week 3	30	15	50	1-2
Week 4	30	15	50	1-2
Maintenance until endpoint	20 and below	10	50	1-2
Reasons for Preference	Cytopenia Thiopurine methyltransferase deficiency Pregnancy Malignancy Short course (≤ 6 months)	Postmenopausal state Osteoporosis Brittle diabetes Obesity Acne Emotional lability Hypertension		

**Prednisolone can be used in place of prednisone in equivalent doses.*

Patients receiving prednisone should undergo eye examinations for cataracts and glaucoma periodically during treatment, and those receiving azathioprine in any dose should be monitored at 6 month intervals for leukopenia and thrombocytopenia. Adjunctive therapies should be based on an awareness of possible complications of the medication, and they should be introduced as appropriate to the individual's perceived risk. *Such therapies should include a regular weight loss exercise program, vitamin D and calcium supplementation. The administration of bone active agents such as bisphosphonates may be appropriate for individual patients [9, 10, 40].* Patients on long-term corticosteroid treatment should be monitored for bone disease by baseline and annual bone mineral densitometry of the lumbar spine and hip.

Like other patients suffering from chronic liver disease patients with AIH should be protected against hepatitis B virus (HBV) and

hepatitis A virus (HAV). Vaccination should be done as early as possible even before immunosuppression is started because of lower response rates [36].

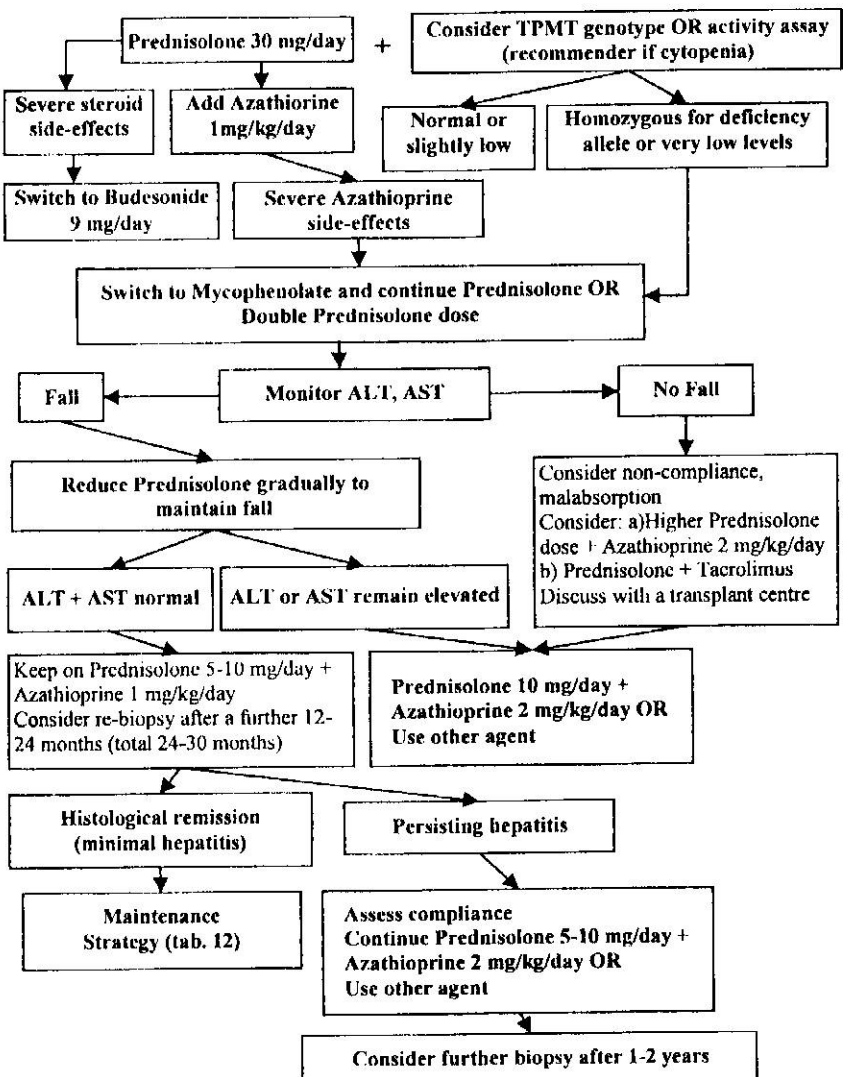

Figure 11. **Suggested induction strategy for autoimmune hepatitis (AIH).**

Treatment Regimes in ChildrenTreatment regimens have been less rigorously established in children than in adults and to some extent, they reflect the preferences of individual centers [9,41]. There have been no randomized, controlled, treatment trials in children with autoimmune hepatitis, but several reports of 17 or more children have documented the efficacy of regimens similar to those used in adults *(Tab. 10)*. Despite the severe disease at presentation, the response to treatment with corticosteroids with or without azathioprine is generally excellent in children. Normalization of liver tests is noted after 6-9 months of therapy in 75%-90% [36].

Treatment-Related Side Effects

The nature and frequency of the side effects associated with each treatment regimen must be explained to the patient prior to the institution of therapy *(Tab. 11)*.

Corticosteroid-Related Side Effects

Cosmetic changes, including facial rounding, dorsal hump formation, striae, weight gain, acne, alopecia and facial hirsutism, occur in 80% of patients after 2 years of corticosteroid treatment regardless of the regimen *(Tab. 11)* [48]. Severe side effects include osteopenia with vertebral compression, brittle diabetes, psychosis, pancreatitis, opportunistic infection, labile hypertension, and malignancy [9, 48]. Severe complications are uncommon, but if they occur, it is usually after protracted therapy (more than 18 months) with prednisone alone (20 mg daily). Corticosteroid-related side effects are the most common causes for premature drug withdrawal in autoimmune hepatitis [10, 11]. Treatment is discontinued in 13% of patients because of complications, and 47% of these have intolerable cosmetic changes or obesity [9, 10]. Twenty-seven percent have osteoporosis with vertebral compression, and 20% have brittle diabetes [9, 10].

Azathioprine-Related Side Effects

Complications of azathioprine therapy in autoimmune hepatitis include cholestatic hepatitis, pancreatitis, nausea, emesis, rash, opportunistic infection, bone marrow suppression and malignancy (*Tab. 11*). Five percent of patients treated with azathioprine develop early adverse reactions (nausea, vomiting, arthralgias, fever, skin rash or pancreatitis), which warrants its discontinuation. The overall frequency of azathioprine-related side effects in patients with autoimmune hepatitis is 10%, and the

side effects typically improve after the dose of azathioprine is reduced or the therapy is discontinued [9, 10, 20, 26].

Table 11

Frequency and Nature of Side Effects Associated with Treatment in Adults with Autoimmune Hepatitis (Adapted from Czaja AJ. 2008)

Prednisone-Related Side Effects		Azathioprine-Related Side Effects	
Type	**Frequency**	**Type**	**Frequency**
Cosmetic (usually mild) Facial rounding Weight gain Dorsal hump striae Hirsutism; Alopecia	80% (after 2 years)	Hematologic (mild) Cytopenia	46% (especially with cirrhosis)
Somatic (usually mild) Emotional instability Glucose intolerance Cataracts		Hematologic (severe) Leucopenia Thrombocytopenia	6% (treatment ending)
Somatic (severe) Osteopenia Vertebral compression Diabetes (brittle) Psychosis Hypertension (labile)	13% (treatment ending)	Somatic (usually mild) Nausea Emesis Rash Fever Arthralgias	5%
Inflammatory/neoplastic Pancreatitis Opportunistic infection Malignancy	Rare	Neoplastic Nonhepatic cell types	3% (after 10 years)
		Hematologic/enteric Bone marrow failure Villous atrophy Malabsorption	Rare (treatment ending)
		Teratogenic during pregnancy	Rare (theoretical)

An important but rare complication of azathioprine treatment is a diarrheal syndrome associated with malabsorption and small intestinal villus atrophy that improves after azathioprine withdrawal. The sinusoidal obstruction syndrome ("veno-occlusive disease") described after renal transplantation has not been reported in azathioprine-treated autoimmune hepatitis, nor has the nodular regenerative hyperplasia described in azathioprine-treated patients with inflammatory bowel disease.

The principal side effect of azathioprine is cytopenia, and the most dire consequence is bone marrow failure (*Tab. 11*) [26, 36]. The frequency

of cytopenia in azathioprine-treated patients with autoimmune hepatitis is 46%, and the occurrence of severe hematological abnormalities is 6% [26,40]. These toxicities are not predictable by either genotyping or phenotyping for thiopurine methyltransferase activity, and the most common cause of cytopenia in these patients is hypersplenism associated with underlying cirrhosis [20]. Patients undergoing azathioprine therapy should have blood leukocyte and platelet counts assessed at 6-month intervals. In patients with severe TPMT deficiency and in those intolerant of azathioprine, the prednisolone-only regime or a lower dose of prednisolone combined with mycophenolate may be used *(Fig. 11)* [19].

Chronic immune suppression in autoimmune hepatitis has been associated with an increased risk of malignancy. The incidence of extrahepatic neoplasm in treated autoimmune hepatitis is 1 per 194 patient-years, and the probability of tumor occurrence is 3% after 10 years [57]. Tumors do not have a predominant cell type, and they are not related to age, sex, treatment regimen or cumulative duration of treatment. The low but increased risk of malignancy associated with chronic low dose azathioprine therapy (1.4-fold greater than normal) must be counterbalanced against the beneficial actions of the drug as a corticosteroid-sparing agent [57,59].

Monitoring and additional management

Patients should be asked about and/or tested for immunity to hepatitis A and hepatitis B infection and susceptible patients should be offered vaccination as soon as possible.

Patients on combination therapy should have baseline and weekly on-treatment monitoring of liver tests, blood sugar and blood count for 4 weeks and then 1-3 monthly thereafter, depending on the responses.

All should receive calcium (1-1.5 g daily) and vitamin D (400 U daily) supplementation. DEXA bone density scans should be performed at commencement of prednisolone-containing treatments and repeated at 1-2-yearly intervals while prednisolone treatment is continued. Patients with osteopenia or osteoporosis should receive bisphosphonates. Prophylaxis with biphosphonates is recommended in corticosteroid-treated patients aged >65 years and in those with a history of fragility fracture.

Screening for glaucoma and cataracts should also be considered after 12 months of prednisolone treatment.

Treatment Endpoints and Courses of Action

Conventional therapy in adults is continued until remission, treatment failure, incomplete response, or drug toxicity (*Tab. 12)* [9,11]. There is no prescribed minimum or maximum duration of treatment. The length of therapy can be based on a fixed minimum duration that is usually associated with a complete response or on a variable duration that is individualized to the desired result and tolerance.

Remission

All adult patients should be given the opportunity to enter a sustained remission that is free of medication *(Tab.12)* [10,14,36]. 90% of adults have improvements in the serum AST, bilirubin, and γ-globulin levels within 2 weeks. Adults rarely achieve resolution of their laboratory and liver tissue abnormalities in less than 12 months, and the probability of remission during therapy diminishes after 2 years. Histological improvement lags behind clinical and laboratory improvement by 3-8 months [9, 10, 63].

Resolution of the laboratory indices (normal serum AST or ALT, γ-globulin, and IgG levels) and tissue manifestations of active liver inflammation (normal liver tissue examination) is the ideal treatment endpoint and the goal of initial therapy (*Tab. 12*) [10]. The average duration of treatment is 18-24 months. Normal laboratory indices before termination of treatment reduces the relative risk of relapse after drug withdrawal by 3-fold to 11-fold compared to patients who do not achieve these results, and 87% of patients who achieve long-term remission have normal laboratory indices prior to the termination of therapy.

The frequency that corticosteroid treatment can achieve a full resolution of the laboratory tests and liver tissue abnormalities is unclear, and whereas pursuit of an ideal treatment endpoint is desirable, it must be tempered by the realization that not all patients can achieve this result or tolerate the required treatment [39]. Daily maintenance doses of medication should remain fixed in adults until the goal of therapy is achieved. Titrations in dose are associated with delayed or incomplete histological improvement, and it can prolong the durations of therapy. Alternate day schedules of prednisone can induce symptomatic and laboratory improvement, but not histological resolution.

Liver biopsy assessment prior to termination of treatment is the only method by which to ensure a full resolution of the disease and an optimal endpoint of therapy. Interface hepatitis is found in 55% of

patients with normal serum AST and γ-globulin levels during therapy, and these individuals typically relapse after cessation of treatment [11]. Their recognition by liver biopsy examination prior to drug withdrawal can justify an extension of treatment. Therefore, a liver biopsy is recommended before termination of immunosuppressive treatment in AIH. Termination of therapy should be considered after at least 2-year treatment, when liver function tests and immunoglobulin levels have been repeatedly normal [36].

Termination of therapy after induction of remission requires a gradual, well-monitored dose reduction over a 6-week period of close surveillance (*Tab. 12*). Patients who are on a protracted course of steroid therapy need to be assessed for adrenal insufficiency. The activity of the disease during and after drug withdrawal is assessed by the appearance of symptoms (fatigue, arthralgias, and anorexia) and the behavior of the laboratory indices of liver inflammation (serum AST and γ-globulin concentrations). Laboratory tests are performed at 3-week intervals during drug withdrawal and for 3 months after termination of therapy. Thereafter, they are repeated at 3 months and then every 6 months for 1 year, and then annually life-long (*Fig. 12*) [10,14].

Table 12

Endpoints of Initial Immunosuppressive Treatment and Courses of Action in Autoimmune Hepatitis

Treatment End point	Criteria	Courses of Action
Remission	Disappearance of symptoms; Normal serum aminotransferases, bilirubin and γ-globulin levels; Normal hepatic tissue or inactive cirrhosis	Gradual withdrawal of prednisone over 6 week period; Serum AST or ALT, total bilirubin, and γ-globulin levels determined at 3 week intervals during and for 3 months after drug withdrawal; Repeat laboratory assessments there after every 6 months for at least 1 year and then every year life long

Treatment failure	Worsening clinical, laboratory, and histological features despite compliance with therapy; Development of jaundice, ascites or hepatic encephalopathy	Prednisone, 60 mg daily, or prednisone, 30 mg daily and azathioprine, 150 mg daily, for at least 1 month; Dose reduction of prednisone by 10 mg and azathioprine by 50 mg for each month of improvement until standard treatment doses are achieved
Incomplete response	Some or no improvement in clinical, laboratory, and histological features despite compliance with therapy after 2 3 years No worsening of condition	Reduction in doses of prednisone by 2.5 mg/month until the lowest level possible (≤10 mg daily) to prevent worsening of serum AST or ALT abnormalities ; Indefinite azathioprine therapy (2 mg/kg daily) as an alternative treatment if corticosteroid intolerance
Drug toxicity	Development of intolerable cosmetic changes, symptomatic osteopenia, emotional instability, poorly controlled hypertension, brittle diabetes or progressive cytopenia	Reduction in dose or discontinuation of offending drug; Maintenance on tolerated drug in adjusted dose

Treatment Failure

Treatment failure connotes clinical, laboratory, and histological worsening despite compliance with conventional treatment schedules; it occurs in at least 9% of patients and may be observed within 3-6 weeks. *(Tab. 12)* [11,14]. Patients who will later fail treatment, die of liver failure or require liver transplantation can be identified early by applying the model of end-stage liver disease (MELD). Early recognition of individuals who are likely to fail corticosteroid therapy may improve their outcome by prompting treatment modifications, including timely liver transplantation.

Treatment failure justifies the discontinuation of conventional treatments, and institution of high dose therapy with prednisone alone (60 mg daily) or prednisone (30 mg daily) in conjunction with azathioprine (150 mg daily) (*Tab. 12*) [10]. Doses at this level are maintained for at least 1 month. Thereafter, the doses of prednisone and azathioprine are reduced each month after improvement in the serum AST level until conventional maintenance doses of medication (original schedule) are reached. 70% of patients improve their clinical and laboratory findings within 2 years, and survival is preserved.

Histological remission is achieved in only 20%, and most patients remain on therapy and at risk for drug-related side effects and/or disease progression [39,49]. The development of hepatic encephalopathy, ascites, and/or variceal hemorrhage during therapy for treatment failure is an indication for liver transplantation [62].

Incomplete Response

Protracted therapy that has improved the clinical, laboratory, and histological indices but not induced complete resolution constitutes an incomplete response *(Tab. 12)* [36]. Thirteen percent of patients fail to enter remission after 36 months of treatment, and they are classified as incomplete responders. In these instances, alternative strategies must be considered *(Fig. 12)*. Long term low dose corticosteroid therapy involves a gradual decrease in the prednisone dose by 2.5 mg per month until the lowest level (≤10 mg daily) is achieved, and the serum AST or ALT level remains stable. Long-term azathioprine (2 mg/kg daily) can also be used to stabilize the serum AST and ALT levels in corticosteroid intolerant individuals who require continuous treatment [10].

Drug Toxicity

Drug toxicity justifies premature discontinuation or alteration of conventional therapy in 13% of patients *(Tab. 12)* [36]. In these instances, therapy with the tolerated agent (prednisone or azathioprine) can be maintained in adjusted dose to prevent worsening in the clinical and laboratory features.

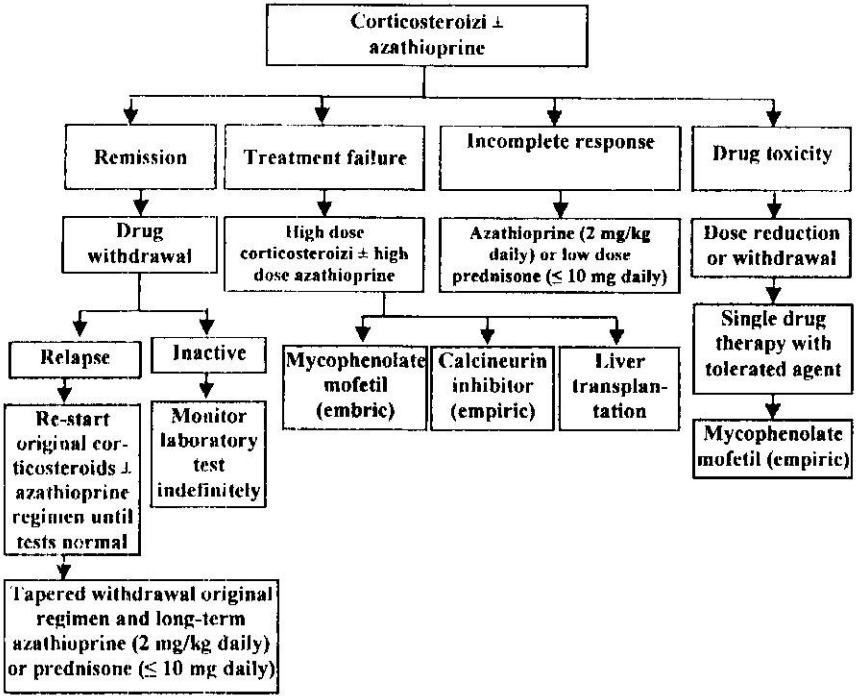

Figure 12. **Management of Autoimmune Hepatitis.** Flow chart of therapy for AIH. Patients are given only corticosteroids or a lower dose of corticosteroids in combination with azathioprine. The outcomes of initial therapy dictate changes in the treatment strategy.

Treatment Endpoints for Children

The treatment endpoints for children are similar to those of adults. Almost all children demonstrate improvement in liver tests within the first 2-4 weeks of treatment with either prednisone or prednisone and azathioprine [9, 10, 36]. Some 80%-90% achieve laboratory remission in 6-12 months. In most treatment protocols, high-dose prednisone (1-2 mg/kg daily) is administered for up to 2 weeks, at which time a gradual decrease in dose is undertaken to reach a maintenance level (usually 0.1-0.2 mg/kg daily or 5 mg daily) in 6-8 weeks [36]. Clinical and laboratory parameters are usually sufficient to determine the adequacy of response. Flares in disease activity, as assessed by an increase in se-

rum AST or ALT level, are treated with a temporary increase in corticosteroid dose.

The goal of treatment in children is to have minimal or no serum AST or ALT abnormality on the lowest dose of medication possible. Long-term, low-dose therapy is anticipated and emotional, cosmetic, and growth-related side effects temper treatment in an individualized fashion. Long-term monotherapy with azathioprine is generally well tolerated, and it is a strategy by which to suppress inflammatory activity and discontinue corticosteroids [36].

Routine monitoring of conventional liver tests and blood counts and amylase are performed at 4 to 6 week intervals. After 2-3 years of treatment, drug withdrawal is considered in children if liver function tests and IgG are repeatedly normal, and autoantibodies negative or $\leq$1:20, for at least 1 year on low-dose corticosteroids. At that time, a liver biopsy examination should be performed and therapy withdrawn only if there is no histological evidence of inflammation. Relapse after drug withdrawal occurs in 60%-80% of children, and parents and patients must be informed that the probability of retreatment is high [36].

Relapse After Drug Withdrawal

Relapse connotes recrudescence of disease activity after induction of remission and termination of therapy [10,39,59]. It is characterized by an increase in the serum AST level to more than three-fold the ULN and/or increase in the serum γ-globulin level to more than 2 g/dL. Laboratory changes of this degree are invariably associated with the reappearance of interface hepatitis in the liver tissue, and they preclude the need for a liver biopsy examination to document relapse.

Progression to cirrhosis (38% versus 4%) and death from liver failure or requirement for liver transplantation (20% versus 0%,) are more common in the patients who relapse multiply than in those who sustain remission after their first treatment [36].

The preferred management of relapse is to reinstitute therapy with prednisone and azathioprine until clinical and laboratory resolution is again achieved and then to eliminate the prednisone while increasing the dose of azathioprine [9,10]. The dose of azathioprine is increased to 2 mg/kg daily as the dose of prednisone is gradually withdrawn. Azathioprine is then continued indefinitely as a chronic maintenance therapy.

87% of adult patients managed by the indefinite azathioprine maintenance strategy remain in remission during a median observation

interval of 67 months [26]. Follow-up liver biopsy assessments show inactive or minimal histological disease in 94%; corticosteroid-related side effects improve or disappear in most patients; and the drug is generally well tolerated. The most common side effect is withdrawal arthralgia, which is encountered in 63% of patients. Myelosuppression occurs in 7%; lymphopenia occurs in 57%; and diverse malignancies of uncertain relationship to the therapy develop in 8%. The major advantage of the azathioprine regimen is the avoidance of corticosteroids and its possible side effects.

An alternative strategy is to administer prednisone in the lowest dose possible to maintain the serum AST level within normal limits or at least below three-fold the ULN. Suppression of the serum AST level to less than three-fold the ULN decreases the likelihood of interface hepatitis on histological examination, and a dose of prednisone less than 10 mg daily is generally well tolerated long-term. 87% of patients can be managed long-term on 10 mg of prednisone daily or less (median dose, 7.5 mg daily) [10]. Observation intervals for up to 149 months have indicated satisfactory outcomes that have justified continued application of the strategy. Side effects associated with the earlier conventional treatments improve or disappear in 85% of patients maintained on low dose prednisone; new side effects do not develop; and survival is unaffected when compared with patients receiving standard dose therapy after relapse.

The major advantages of the low dose prednisone schedule are avoidance of long-term azathioprine therapy in fertile young adults and elimination of the theoretical risks of oncogenicity and teratogenicity [26, 57, 60]. Furthermore the topical steroid budesonide is now being evaluated as an alternative to prednisone or prednisolone in order to achieve or maintain remission with less steroid specific side effects.

Relapse in children is characterized by any manifestation of recrudescent hepatic inflammation after drug withdrawal. Its frequency in children is the same or higher than that observed in adults. Relapse is often associated with nonadherence to treatment. The occurrence of relapse in children justifies reinstitution of the original treatment regimen. Indefinite low-dose therapy can then be instituted after suppression of disease activity using prednisone in combination with azathioprine or 6-mercaptopurine. Maintenance therapy with azathioprine alone is a management option for children who have relapsed [36].

ALTERNATIVE DRUG THERAPIES FOR SUBOPTIMAL RESPONSES

Treatment failure should be managed with high dose prednisone (60 mg daily) or prednisone (30 mg daily) in combination with azathioprine (150 mg daily) before considering other drugs such as cyclosporine, tacrolimus, or mycophenolate mofetil *(Fig. 11, 12)* [36]. When standard treatment fails or drug intolerance occurs, alternative therapies such as cyclosporin, tacrolimus, cyclophosphamide, mycophenolate mofetil, rapamycin, UDCA, and budesonide can be considered. The efficacy of most of these options has not yet been definitively decided and is only reported in small case studies.

Budesonide

Budesonide may offer an alternative to prednisone. Budesonide is a synthetic steroid with high first-pass metabolism in the liver, in principle with limited systemic side effects compared to conventional steroids. In comparison to prednisone the absolute bioavailability of budesonide is less than 6-fold lower but it has an almost 90% first-pass metabolism in the liver, a higher affinity to the glucocorticoid receptor, acts as an anti-inflammatory and immunosuppressive drug and leads to inactive metabolites (6-OH-budesonide, 16-OH-prednisolone) [19, 22].

In a recent multicentre randomised controlled trial in patients with AIH without cirrhosis, budesonide 9 mg/day plus azathioprine 1-2 mg/kg/day for 6 months was more effective in achieving normalisation of serum transaminases and produced fewer steroid-related side effects than prednisolone plus azathioprine [22]. Given the short trial duration and the fact that no follow-up histology data were presented, routine use of this regime in treatment-naïve patients is not currently recommended. However, its use should be considered in non-cirrhotic patients who are intolerant of prednisolone [19]. Longer-term follow-up is needed to better assess the efficacy and safety of budesonide. Based on moderate-quality evidence, the 2011 BSG guidelines strongly recommend use of budesonide for prednisolone-intolerant patients [19].

Deflazacort

This alternative corticosteroid has also been studied for immunosuppression in AIH because of its feature of fewer side effects than conventional glucocorticoids. In a pilot study 15 patients with AIH type I were treated with deflazacort, who had been previously treated with

prednisone with or without azathioprine until they reached a biochemical remission. Remission was sustained for 2 years of follow-up. However, the long-term role of second-generation corticosteroids to sustain remission in AIH patients with reduced treatment-related side effects requires further controlled studies [22].

Cyclosporine A (CyA)

In patients with AIH not responding to standard therapy, CyA has been shown to be of clinical benefit although relapses occurred if the dose of CyA was reduced. Cyclosporine A (CyA) is a lipophylic cyclic peptide of 11 residues produced by *Tolypocladium inflatum* that acts on calcium-dependent signaling and inhibits T cell function via the interleukin 2 gene [19,22]. Cyclosporine has been used successfully to avoid high steroid doses in both adult and pediatric patients.

In a study in children by Alvarez et al, cyclosporine induced biochemical remission of the hepatic inflammatory process in children with autoimmune hepatitis while causing few and well-tolerated adverse effects [3]. In this study, cyclosporine was administered for 6 months alone, followed by combined low doses of prednisone and azathioprine for 1 month, then cyclosporine was discontinued.

In other series CyA has been used predominantly as a salvage strategy or in the context of relapsing or non-responsive AIH. Results in these situations have been favourable, although no long-term reports exist to evaluate safety. Therefore, in considering initiation of CyA, its toxicity profile including the long-term risks of hypertension, renal insufficiency, hyperlipidaemia, hirsutism, infection and malignancy must be balanced against its potential benefits [19].

Tacrolimus

Tacrolimus is a macrolide antibiotic with 10-200 times greater immunosuppressive potency than CyA [22]. Its mechanism of action is similar to that of CyA, although it binds to FK binding protein (an alternative immunophilin). The application of tacrolimus in 21 patients treated for 1 year led to an improvement of aminotransferase and bilirubin levels with a minor increase in serum creatinine levels. Recently, tacrolimus was successful in seven of nine patients with acute AIH who did not respond to corticosteroids. Although tacrolimus represents a promising immunosuppressive candidate drug, larger randomized trials are required to assess its role in the therapy of AIH [19].

Mycophenolate mofetil

Mycophenolate has attracted attention as a transplant immunosuppressant with an important role in the steroid-free immunosuppressive therapy of patients transplanted for chronic hepatitis C infection. Mycophenolate is a noncompetitive inhibitor of inosine monophosphate dehydrogenase, which blocks the rate-limiting enzymatic step in *de novo* purine synthesis. Mycophenolate has a selective action on lymphocyte activation, with marked reduction of both T and B lymphocyte proliferation [22].

MMF has been used predominantly in patients with refractory AIH or with azathioprine intolerance. Most studies have used 2 g/day in divided doses, initially with corticosteroids but with the aim of reducing these. In one study the dose of prednisolone was decreased from a median of 20 mg/day to 2 mg/day after 9 months, with histological improvement noted in all patients. In larger series (29-36 patients) up to one-third of patients discontinued the drug due to poor tolerance or side effects. Only half of patients attained biochemical remission and follow-up histology data were not reported. Patients in whom azathioprine had been ineffective seemed to have a poorer response to MMF than those who had been azathioprine-intolerant.

However, further data are needed, especially on efficacy in inducing histological remission, before MMF can be recommended as a first-line treatment for AIH. There are very few data on the long-term safety of MMF. Development of histological changes were noted in a patient with AIH, including cytoplasmic features of adaptation and nuclear alterations within hepatocytes, and there have been sporadic reports of cerebral lymphoma in patients who have received MMF for other autoimmune diseases. MMF is contraindicated in pregnancy [19].

Other agents with acceptdotal evidence of efficacy

Ursodeoxycholic acid is a hydrophilic bile acid with putative immunomodulatory capabilities. It is presumed to alter HLA class I antigen expression on cellular surfaces and to suppress immunoglobulin production [19]. Its role in AIH therapy or in combination with immunosuppressive therapy is still unclear. In a Japanese study eight patients received ursodeoxycholic acid (UDCA) 600 mg/day for 2 years in whom a significant biochemical improvement was demonstrated. In four patients a biopsy after 12 months showed improvement in inflammation. However, in another study 37 patients refractory to corticosteroid

therapy were randomised to UDCA or placebo for 6 months, together with their standard treatment regimen. No significant benefit from UDCA was found.

Maintenance therapy with cyclophosphamide for up to 12 years without relapse or serious side effects has been successfully achieved in three patients. In case reports, methotrexate, infliximab and rituximab given to patients with resistant AIH resulted in sustained biochemical remission and histological improvement [19].

Transplantation for Autoimmune Hepatitis

Indications and Outcomes

About 10-20% of patients with AIH will require liver transplantation during their lifetime [19]. AIH is the indication for liver transplantation (LT) in approximately 2%-3% of pediatric and 4%-6% of adult recipients in the United States and Europe [36,44].There are two distinct indications. The first is severe acute AIH resulting in acute or subacute liver failure. The second and more common indication for liver transplantation is decompensated chronic liver disease and/or hepatocellular carcinoma, often in a patient with longstanding AIH. The same indications for liver transplantation apply as for other actiologies of cirrhosis in so far as the development of ascites, variceal bleeding, hepatic encephalopathy, spontaneous bacterial peritonitis or hepatorenal syndrome impact significantly on survival. These include a MELD score of >15 or a Child-Pugh score of >10 [32]. Need for LT may result from a failure to diagnose and treat AIH as an etiology of cirrhosis, inadequate response or intolerance to immunosuppressive therapy or noncompliance with treatment.

Untreated patients have a 10-year survival of <30%, and treatment failure requiring LT is often associated with the HLA genotype DRB1*0301. Liver transplantation for AIH is very successful with 5-year and 10-year patient survivals of approximately 75% [46]. A combination of prednisone and a calcineurin inhibitor (tacrolimus more frequently than cyclosporine) is the most common immunosuppression regimen after LT.

Recurrence and *de novo* AIH after liver transplantation

The potential of AIH to recur after liver transplantation is beyond serious debate. Recurrence of AIH, originally described in 1984, is seen in about 20% of recipients [19]. Recurrent AIH in transplant allografts

occurs in approximately 30% of adult and pediatric patients (range 12%-46%) with an average time to recurrence of 4.6 years [36]. The incidence increases with time after LT and accelerates after discontinuation of steroids.

Diagnosis of recurrent AIH is limited by the absence of a specific marker. Autoantibodies such as ANA or ASMA tend to disappear after transplantation but may reappear, albeit at lower titres than before transplantation. The features of recurrent AIH in the liver graft are similar to those of AIH before transplantation. Diagnostic criteria for recurrence include:

- elevation of serum AST or ALT levels;
- persistence of autoantibodies;
- hypergammaglobulinemia and/or elevation of IgG level;
- compatible histopathological findings;
- exclusion of alternative etiologies;
- responsiveness to steroids.

Histopathological abnormalities compatible with recurrent AIH may precede laboratory or clinical evidence of recurrence. Reported risk factors for recurrence included inadequate dosing of immunosuppression (especially discontinuation of prednisone), type 1 AIH and a recipient positive for either HLA-DRB1*03 or DRB1*04 [35].

The most frequent overlapping diagnosis is acute cellular rejection, and both AIH and acute rejection entities share common biochemical and histological features and both respond to corticosteroids. Indeed, patients with AIH hepatitis have an increased risk of acute cellular rejection. In addition, recurrent AIH may precipitate chronic rejection. Recurrent AIH is usually managed by either maintenance of corticosteroids long term or by continuation of azathioprine in the immunosuppression regimen.

SPECIFIC CLINICAL PROBLEMS

Management of AIH in pregnancy

As AIH can often affect women of childbearing age, an important aspect of treatment is one of management of pregnancy where both patients and medical professionals have concerns regarding safety and the use of immunosuppressants. AIH is not contraindication to pregnancy.

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Most experiences indicate that pregnancy and the medication are well tolerated by the mother and the neonate [45]. The major risk is prematurity, and infant mortality relates directly to the degree of prematurity. Fetal loss is higher than in normal mothers, but no greater than in mothers with other chronic illnesses [36]. Fetal mortality has been reported as high as 19% with deliveries usually before the 20th week. Perinatal mortality is 4%; maternal mortality is 3%; the frequency of serious maternal complications is 9%; and the occurrence of an adverse outcome of any type is 26% [45]. Outcomes in autoimmune hepatitis are similar to those in the general population where the frequencies of fetal loss, caesarian section, and still births are 21%, 17%, and 5%, respectively [20].

Preconceptional counseling is advised and termination of immunosuppressive therapy should be attempted where possible. Azathioprine has a category D pregnancy rating by the FDA. It has been associated with congenital malformations in pregnant mice, and low levels of the 6-thioguanine nucleotides are detectable in the newborns of mothers treated for Crohn's disease. Teratogenicity associated with azathioprine therapy therefore is a theoretical consideration, but increased birth defects have not been reported in mothers receiving this treatment, nor have there been apparent adverse consequences of breast feeding by treated mothers [58]. Nevertheless, these human experiences have been anecdotal, and there has not been a comprehensive human study establishing the safety of azathioprine in pregnant women. These findings, however, do justify caution when using azathioprine during pregnancy [20,36]. Patients must be counseled regarding the uncertain risk of azathioprine in pregnancy, and azathioprine should be discontinued, if possible, in patients during pregnancy [36]. Mycophenelate should be avoided in pregnancy, as it is potentially teratogenic [19].

Autoimmune hepatitis can improve during pregnancy, and this improvement may allow reductions in immunosuppressive therapy during pregnancy. Intuitively, little or no treatment during pregnancy is a desirable protective measure for the mother and fetus.

Exacerbations of disease commonly follow delivery as blood estrogen levels fall. The frequency of exacerbation after delivery has been variously reported between 12%-86% [20,45]. Its occurrence must be anticipated, and conventional therapy must be resumed pre-emptively 2 weeks before anticipated delivery and maintained throughout the

postpartum period for at least 3 months after delivery. Contraception should be advised in women with advanced liver disease and features of portal hypertension because they are at risk for variceal hemorrhage during pregnancy [45].

Patients with Cirrhosis

Individuals with cirrhosis at presentation have a higher frequency of drug related complications than those without cirrhosis (25% versus 8%) [36,48]. They also have a high frequency of cytopenia that may compromise their tolerance for azathioprine. Patients with cirrhosis must be closely monitored during therapy, and those individuals with cytopenia should be assessed for thiopurine methyltransferase activity prior to the administration of azathioprine.

Patients with Low Thiopurine Methyltransferase Activity

Patients with near-zero erythrocyte concentrations of thiopurine methyltransferase activity are at risk for myelosuppression during azathioprine treatment [36]. Only 0.3%-0.5% of the population has a severe enzyme deficiency, and not all patients with a deficiency of this degree experience bone marrow failure. Individuals with abnormally decreased but not extreme reductions in thiopurine methyltransferase activity (heterozygous state) tolerate azathioprine satisfactorily at the low dose of 50 mg and the level of enzyme activity may actually increase with continued administration of the drug [11]. The rarity of severe azathioprine-induced myelosuppression, the low dose of azathioprine used in conventional treatment (50 mg-150 mg daily), and the inability to reliably predict risk by phenotypic and genotypic assessments have not supported routine screening for thiopurine methyltransferase activity in AIH. Pretreatment cytopenia, cytopenia developing during therapy, or the administration of higher than conventional doses of azathioprine (>150 mg daily) justifies determination of enzyme activity [14].

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