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Review article: autoimmune hepatitis – current management and challenges

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SUMMARY

Background
Autoimmune hepatitis (AIH) is a disease of unknown aetiology characterised by interface hepatitis, hypergammaglobulinaemia, circulating autoantibodies and a favourable response to immunosuppression.

Aim
To review recent advancements in understanding aetiopathogenesis, clinical, serological and histological features, diagnostic criteria and treatment strategies of AIH.

Methods
Published studies on AIH extracted mainly from PubMed during the last 15 years.

Results
Autoimmune hepatitis has a global distribution affecting any age, both sexes and all ethnic groups. Clinical manifestations are variable ranging from no symptoms to severe acute hepatitis and only seldom to fulminant hepatic failure. Autoimmune attack is perpetuated, possibly via molecular mimicry mechanisms, and favoured by the impaired control of regulatory T-cells. A typical laboratory finding is hypergammaglobulinaemia with selective elevation of IgG, although in 15–25% of patients – particularly children, elderly and acute cases – IgG levels are normal. Liver histology and autoantibodies, although not pathognomonic, still remain the hallmark for diagnosis. Immunosuppressive treatment is mandatory and life-saving; however, to meet strict response criteria, the conventional therapy with prednisolone with or without azathioprine is far from ideal.

Conclusions
Autoimmune hepatitis remains a major diagnostic and therapeutic challenge. The clinician, the hepato-pathologist and the laboratory personnel need to become more familiar with different expressions of the disease, interpretation of liver histology and autoimmune serology. According to the strict definition of treatment response issued by the 2010 AASLD guidelines, many patients are nonresponders to conventional treatment. Newer immunosuppressive agents targeting pathogenetic mechanisms can improve patient management, which needs to be tailored on a case-by-case basis.

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INTRODUCTION

Autoimmune hepatitis (AIH) is an unresolving progressive liver disease that affects preferentially females and is characterised by interface hepatitis, hypergammaglobulinaemia, circulating autoantibodies and a favourable response to immunosuppression.[1–3]

Due to the absence of a specific marker of the disease and the large heterogeneity of its clinical, laboratory and histological features, AIH diagnosis may be difficult. Therefore, the International AIH Group (IAIHG) met for the first time some 20 years ago and proposed a cumulative score,[4] which was subsequently revised[5] and simplified.[6]

AIH is a relatively rare disease with prevalence rates from 10 to 17 per 100 000 in Europe, which are similar to those of primary biliary cirrhosis (PBC).[7–9] However, higher prevalence rates have been reported in areas where epidemiological and prospective studies can be carried out with good accuracy (42.9 and 24.5 cases per 100 000 in Alaska natives[10] and New Zealand[11]), suggesting that the disease might be underestimated or unrecognised in other areas. AIH prevalence and clinical expression appear to vary according to ethnicity. Indeed, Black patients seem to carry a more aggressive clinical course,[12] Alaskan natives have a high frequency of acute disease,[10] patients of Hispanic origin are characterised by an aggressive presentation both biochemically and histologically with a very high prevalence of cirrhosis, whereas Asian patients demonstrate a very poor survival.[13]

AETIOPATHOGENESIS

The dominant hypothesis postulates that AIH is a disease developing in a genetically predisposed individual, who is also exposed to environmental factors. Thereafter, the autoimmune attack is perpetuated, possibly via molecular mimicry and is favoured by the impaired control of regulatory T-cells.

Genetics of AIH

AIH is a 'complex trait' disease, which does not follow the typical Mendelian pattern of inheritance. The strongest association is with genes located within the human leucocyte antigen (HLA), particularly those encoding the HLA class II DRB1 alleles.

In Europe and North America, DRB1*0301 and DRB1*0401, encoding for the HLA-DR3 and HLA-DR4 antigens, respectively, confer susceptibility to AIH-type 1 (AIH-1).[14, 15] DRB1*0405 and DRB1*0404 confer susceptibility to AIH in Japan, Argentina and Mexico,[16] whereas DRB1*1301 allele (HLA-DR13) confers susceptibility in patients from Argentina.[17, 18] Susceptibility to AIH-type 2 (AIH-2) is conferred by the possession of DRB1*0701 (HLA-DR7) and DRB1*0301 (HLA-DR3).[19]

Molecular mimicry in AIH

In AIH, the best example of molecular mimicry is represented by the antiliver/kidney microsomal antibody type 1 (anti-LKM1), which targets cytochrome P450IID6 (CYP2D6). CYP2D6 shares sequence homologies with hepatitis C virus (HCV), cytomegalovirus and herpes simplex virus type 1,[20–22] infectious agents that could act as triggering factors and initiate an autoimmune attack in genetically susceptible hosts. In addition, the accessibility of CYP2D6 on the outer surface of hepatocyte plasma membrane suggests that autoantibody-dependent cytotoxicity could be operative in perpetuating the autoimmune attack directed against the hepatocyte.[23]

Impairment of regulatory T-cells in AIH

A potential role is attributed to a malfunction of regulatory T-cells, particularly CD4+CD25+FOXP3+ T-cells. CD4+CD25+ regulatory T-cells suppress auto-reactive clones through cell/cell contact and releasing cytokines with regulatory activity, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta).[24] CD4+CD25+ regulatory T-cells are numerically reduced and functionally impaired, particularly at the time of AIH diagnosis, whereas, during remission, a partial repopulation ensues.[25, 26] However, using a different methodology and experimental approach, Peiseler *et al.* described normally functioning regulatory T-cells in AIH patients.[27]A univocally accepted set of markers for identifying regulatory T-cells is absolutely necessary for future studies in this area.

A potential pathogenetic contribution to the insufficient control of the pro-inflammatory milieu could also derive from the interaction between the receptor of IL-4 (CD124) and circulating autoantibodies against it.[28] These autoantibodies inhibit STAT6 phosphorylation induced by IL-4 binding to CD124, with a cumulative neutralising effect on IL-4, thus favouring protracted and uncontrolled inflammatory reactions.

Animal models of AIH

The knowledge of the target autoantigens of anti-LKM1 and antibodies against liver cytosol type 1 antigen (anti-LC1), namely CYP2D6 and formiminotransferase cyclodeaminase (FTCD), respectively, allowed the development of a CYP2D6 and a CYP2D6 plus FTCD animal model. [29–32] The immunised mice had a peak serum

aminotransferase 4–7 months after the injection, developed periportal, portal and lobular inflammatory infiltrates, produced anti-LKM1 and anti-LC1 and had liver-infiltrating CD4+, CD8+ and B lymphocytes, including cytotoxic-specific T-cells. Peripheral tolerance and development of regulatory T-cells, but neither sexual hormone nor central tolerance, seem to play a pivotal role in the susceptibility to AIH in females.[33] Most importantly, the adoptive transfer of *ex vivo* expanded regulatory T-cells in mice with AIH restored peripheral tolerance to FTCD, and remission of liver inflammation is achieved.[34]

Regarding the CYP2D6 animal model, chronic hepatitis was triggered only by adenovirus expressing CYP2D6, and was characterised by histological features of AIH, high anti-LKM1 titres, hepatic infiltration with CD4+ lymphocytes and extensive hepatic fibrosis.[35, 36]

Another animal model of AIH has been developed using mice unable to produce natural regulatory T-cells after neonatal thymectomy and genetically devoid of the programmed cell death 1 (PD-1)-mediated signalling.[37] It should be stated, however, that most of the recent animal models provide significant progress in the understanding of AIH-2 pathogenesis, but not for the development of AIH-1, which is by far the most frequent type of AIH.

TOWARDS CLINICAL AND SEROLOGICAL PHENOTYPES OF THE DISEASE

Presentation

The clinical course of AIH is characterised by fluctuated periods of decreased or increased activity and therefore its clinical spectrum is variable ranging from no symptoms to severe acute hepatitis and even fulminant hepatic failure (Table 1).[1, 38] Approximately 11–25% of patients present with an acute onset of AIH, which does not differ from acute hepatitis of other causes.[1, 39, 40] Acute presentation of AIH may contain two different clinical entities. One is the acute exacerbation of chronic AIH (acute exacerbation form of undiagnosed or misdiagnosed AIH) and the other is the true acute AIH without chronic histological changes (acute form of AIH).[38–41] In some patients with acute presentation, immunoglobulin G (IgG) levels are normal and antinuclear antibodies (ANA) are not detected and thus, the

Table 1 | Characteristics of autoimmune hepatitis (AIH)

Variable	
Geoepidemiology	Worldwide in any race
Female: Male ratio	4–6:1
Age at presentation	Any age (bimodal distribution usual with peaks around puberty and between 4th and 6th decades, although a considerable number of patients are even older)
Clinical presentation	Broad range from asymptomatic ('en passant' diagnosis) to acute severe or even fulminant hepatic failure Most common clinical phenotype (almost two-thirds of patients) is characterised by one or more of nonspecific symptoms like fatigue, mild pain in the right upper quadrant, lethargy, malaise, anorexia, nausea, pruritus, jaundice and arthralgia involving the small joints Acute presentation of AIH contains two different clinical entities (the acute exacerbation of chronic AIH and the true acute AIH without histological findings of chronic disease) One-third of patients at diagnosis have developed cirrhosis irrespective of the presence of symptoms or not suggesting a delay in diagnosis due to unfamiliar doctors and laboratories
Physical examination	Depends on the clinical stage of the disease ranging from completely normal to signs and symptoms of chronic liver disease and/or portal hypertension (hepatomegaly, splenomegaly, ascites, varices or hepatic encephalopathy)
Presentation in special conditions	During pregnancy or in the early postpartum period After liver transplantation for other diseases (*de novo AIH)* After administration of drugs or herbals (drug-induced AIH; nitrofurantoin and minocycline implicated in 90% of cases)
Specific features	Frequent presence of a wide variety of other autoimmune or immune-mediated diseases (most common: autoimmune thyroiditis, vitiligo, alopecia, rheumatoid arthritis, diabetes mellitus type-1, ulcerative colitis and coeliac disease)
Complications	HCC development in AIH, although is less common than other liver diseases, does exist and is associated with cirrhosis, suggesting surveillance in all cirrhotic AIH patients

HCC, hepatocellular carcinoma.

physician may not consider AIH. Some of these acute cases of AIH may rarely progress to acute liver failure and this should be kept in mind as the identification of AIH as the aetiology of acute AIH and/or acute liver failure is very important because it became clear that delay in diagnosis and initiation of therapy leads to a poorer prognosis, whereas prompt immunosuppression lowers the risk of evolution of the disease and the need for liver transplantation.[38–42]

AIH was originally described in peripubertal females, but it is now well-known that it can occur globally at any age, in both sexes, and in all ethnic groups.[9, 13, 39, 43–45] An overall bimodal age pattern has been reported at presentation with one peak during childhood and teens and another in middle age between the fourth and sixth decades of life, although recent studies have shown that an increasing number of AIH patients are diagnosed also at older ages (above 60–65 years).[46–49] Commonly, the clinical presentation is characterised by one or more of the following nonspecific symptoms of varying severity: fatigue, general ill health, mild pain in the right upper quadrant, lethargy, malaise, anorexia, weight loss, nausea, pruritus, jaundice and arthralgia involving the small joints, sometimes dating back years (Table 1).[1, 9, 13, 39, 43, 44] Amenorrhoea is also common, whereas maculopapular skin rash and unexplained fever are rare features. Physical examination may be normal, but it may also reveal signs and symptoms of chronic liver disease. In advanced stages, the clinical picture of portal hypertension dominates.

A considerable number of patients at diagnosis (range: 12–35%) are asymptomatic and the final diagnosis is established during investigation for unexplained increase in aminotransferases performed for other reasons.[9, 43, 50–52] Almost one-third of patients at diagnosis have already developed cirrhosis, which is associated with lower overall survival irrespective of the presence of symptoms.[1, 9, 43, 50, 51] The latter finding along with the presence of histological evidence of chronic disease on liver biopsy in a proportion of patients with acute AIH imply that they probably have had subclinical disease for a long time.[1, 38, 40, 42] Actually, this is the diagnostic challenge as subclinical disease often precedes the onset of the disease symptoms, whereas long periods of subclinical disease may also occur after presentation.

Presentation of AIH in special conditions

The disease may be first diagnosed during pregnancy or in the early postpartum period (Table 1). Postpartum exacerbations may occur in patients whose condition improved during pregnancy (presumably due to a change from Th1 to Th2 response).[1, 53–56] This possibility should be actively considered in the differential diagnosis if liver dysfunction particularly accompanied by hypergammaglobulinaemia is observed during pregnancy or more frequently after delivery.

AIH may develop after the administration of several drugs (Table 1). Reactive metabolites created through hepatic metabolism have been shown to bind to cellular proteins. These can then be recognised by the immune system as neoantigens.[2, 3, 57, 58] Drug-induced AIH has been well documented for nitrofurantoin and minocycline, which are implicated in 90% of cases of drug-induced AIH worldwide.[3, 58–60] A recent study showed that after comparing patients with drug-induced AIH with those with AIH that the two groups had quite similar clinical and histological patterns, although the former had lower histological activity and do not seem to require long-term immunosuppression.[60, 61] Other drugs and herbals, such as oxyphenisatin, ornidazole, methyldopa, diclofenac, interferon, atorvastatin, highly active antiretroviral treatment and biologic agents, including infliximab, natalizumab and adalimumab, have also been reported occasionally to induce AIH.[1, 58, 62–64]

The onset of AIH has been recorded in susceptible individuals after viral infections like hepatitis A virus, Epstein–Barr virus (EBV), human herpes virus 6 and measles.[18, 22, 65–67] Vento et al[66] have reported the onset of AIH-1 in two out of 7 susceptible adults after EBV infection, whereas, recently, Cabibi D,[67] Nakajima et al[68] and Zellos et al[69] reported three more cases. From the clinical point of view, these observations indicate that AIH should be considered as an alternate 'emerging' diagnosis in cases with previous viral infections followed by unexplained and prolonged hepatitis.

AIH has been reported after liver transplantation for other liver diseases in adults and children (*de novo* AIH).[70] However, it has been suggested that alternative nomenclature such as 'post-transplant immune hepatitis' or 'graft dysfunction mimicking AIH' or 'post-transplant plasma cell hepatitis' may be more appropriate.[71] Nevertheless, the timely recognition of this entity appears to be crucial for avoiding graft rejection and the need for another transplantation.[70]

A specific feature of AIH is the presence of a wide variety of other autoimmune or immune-mediated diseases in the patient or first-degree relatives, commonly autoimmune thyroiditis, vitiligo, rheumatoid arthritis, diabetes mellitus type-1, ulcerative colitis and coeliac disease (Table 1).[1, 9, 43, 51, 72–75] Rarely, AIH can concur with

other frequent non-autoimmune liver disorders, although, in such cases, early and correct diagnosis is very difficult.[76–80] Taken together, the above associations may further explain the delay of a prompt and accurate diagnosis as the first doctor managing the AIH patient could be unfamiliar with the peculiar heterogeneity of AIH.

Complications

The complications of AIH are the same as in any other chronic liver disease. As stated above, one-third of patients have already developed cirrhosis at the time of diagnosis. For this reason, a timely and correct diagnosis can stop the progression to cirrhosis, decompensated disease and the development of hepatocellular carcinoma (HCC). HCC is a known consequence of AIH-related cirrhosis, although its occurrence in AIH is significantly less frequent compared with other causes of liver cirrhosis.[81, 82] However, a recent population-based study showed that the risk of hepatic and extrahepatic malignancy was significantly increased in AIH patients,[83] whereas, studies from UK, USA and Japan identified the presence of cirrhosis in AIH as the *sine qua non* for HCC development, which subsequently occurs at a rate of 1.1% per year affecting men and women in equal proportions.[82, 84–87] Thus, HCC risk remains sufficient to implicate surveillance in all AIH patients with cirrhosis.

Laboratory findings

Bilirubin concentrations and aminotransferases may range from just above the upper normal limits to more than 50 times these levels, with usually normal or only moderately elevated cholestatic enzymes.[1, 4, 5] These findings do not reliably reflect severity of disease at the histological level. Of interest, recent studies have shown that, along with the elevations of aminotransferases, γ-glutamyl transpeptidase (γ-GT) can also be increased invariably in AIH and, furthermore, might be used as independent predictor of treatment outcome.[43, 51] Aminotransferases and γ-GT may even spontaneously normalise (spontaneous biochemical remission), despite histological evidence of continuing activity. The latter is another critical issue that sometimes may result in delay and/or underestimation of diagnosis as the subsequent hit can be obvious after several months or years and may be completely asymptomatic.

In most patients, but not all, the characteristic laboratory feature is a polyclonal hypergammaglobulinaemia with selective elevation of serum IgG.[4–6, 45, 48, 88–90] It should be emphasised that, in everyday clinical practice, this determination is usually missing and may lead to further underestimation of the disease. Elevation of serum IgA suggests steatohepatitis or drug-induced liver injury rather than AIH, whereas an increase in IgM is more characteristic of either PBC or primary sclerosing cholangitis (PSC). IgA deficiency is not uncommon in children with AIH. However, almost 15–25% of patients, particularly children or elderly and also severe acute cases, may have normal IgG at presentation.[48, 51, 88, 90] Therefore, a diagnosis of AIH should never be ruled out only because of normal IgG. In addition, the physician should be aware that low aminotransferases, bilirubin or IgG do not necessarily equate to mild or inactive disease nor exclude AIH diagnosis.[1, 4, 5]

Another parameter that may be of value and contribute to AIH diagnosis is the serum concentration of complement component C_4, which is persistently low in AIH patients.[1, 4, 5, 91]

Classification and detection of autoantibodies

The detection of non-organ and liver-related autoantibodies, although not pathognomonic, still remains the hallmark for AIH diagnosis in the absence of viral, metabolic, genetic and toxic aetiology of chronic or acute hepatitis.[2–6, 57] According to autoantibody pattern, a subclassification into two major types – AIH-1 and AIH-2 – has been proposed. The clinical and serological phenotypes of the disease associated with AIH-1 and AIH-2 are shown on Table 2. This distinction was initially based on circulating autoantibodies alone, but thereafter, other differences have become apparent (Table 2).

AIH-1. AIH-1 is characterised by the presence of ANA and/or smooth muscle autoantibodies (SMA), which may associate in 60–90% of patients with perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), more appropriately termed peripheral antineutrophil nuclear antibodies (p-ANNA).[2–6, 57, 89–92] AIH-1 accounts for about 75–80% of all cases. In most instances, the ANA staining pattern by indirect immunofluorescence (IIF) show a homogenous diffuse pattern, but speckled patterns are not infrequent, and investigation for different staining patterns appears to have no practical clinical implications.[2–6, 57, 90–93] SMA are detected by IIF on rodent liver and kidney, due to staining of vessel walls, and stomach, due to staining of the muscle layer and directed against structures of the cytoskeleton, such as filamentous actin (F-actin), troponin, tubulin, vimentin and tropomyosin.[2–6, 57, 91, 93, 94] In AIH-1, SMA are predominantly directed against F-actin,[95, 96] but reliance only on antiactin specificity of SMA for AIH diagnosis

Table 2 | Clinical and serological phenotypes of autoimmune hepatitis (AIH)

Feature	AIH-1	AIH-2
Autoantibodies	ANA, SMA, anti-F-actin, antialpha-actinin, anti-SLA/LP	anti-LKM1, anti-LC1, rarely anti-LKM3
Age at presentation	Any age	Usually in children and young adulthood
Genetic susceptibility	HLA DR3, DR4 and DR13	HLA DR3 and DR7
Clinical severity	Variable	Usually acute severe
Histopathological feature at presentation	Mild disease to cirrhosis	Commonly advanced
Failure of treatment	Rare	Frequent
Relapse after drug withdrawal	Variable	Frequent
Need for long-term maintenance therapy	Variable	Very common

AIH-1, autoimmune hepatitis-type 1; AIH-2, autoimmune hepatitis-type 2; ANA, antinuclear antibodies; SMA, smooth muscle antibodies; anti-SLA/LP, antibodies against soluble liver antigen/liver pancreas; anti-LKM1, antibodies against liver kidney microsomal type 1; anti-LKM3, antibodies against liver kidney microsomal type 3; anti-LC1, antibodies against liver cytosol type 1; HLA, human leucocyte antigen.

may result in missed diagnosis of AIH in about 20% of patients as F-actin is a probable, but not the only, target of AIH-specific SMA reactivity.[93, 97]

Titres of at least ≥1:40 in adults and ≥1:20 in children are accepted as positive.[5, 6, 88, 93] However, ANA and/or SMA – usually in low titres – may occur in patients with chronic hepatitis B or C, but, in most of these cases, SMA lack F-actin specificity.[2, 3, 57, 98] During immunosuppression, disappearance of ANA and/or SMA is observed in the majority of patients with AIH-1.[99] However, neither autoantibody titres at first diagnosis nor autoantibody behaviour in the time course of the disease is a prognostic marker for AIH-1.[2, 3, 57, 99] Furthermore, pretransplant ANA and SMA levels do not appear to impact recurrence rates or outcomes following liver transplantation for AIH.[100]

Antibodies to soluble liver antigen (SLA) or liver pancreas (LP), which are now known as one and the same autoantibody designated as anti-SLA/LP, are detected in 15–30% of AIH-1 patients.[101, 102] Anti-SLA/LP is associated with a more severe course of the disease, represents the most specific autoantibody identified in AIH-1[102–107] and occurs at similar frequencies in AIH patients from different geographical regions and ethnic groups.[108] Therefore, anti-SLA/LP seems to be, first, a useful surrogate marker for AIH-1 diagnosis, whereas, secondly, it may also result in a reduction in cases of cryptogenic hepatitis or autoantibody-negative AIH.[109] Anti-SLA/LP target a synthase (S)-converting *O*-phosphoseryl-tRNA (Sep) to selenocysteinyl-tRNA (Sec); thus, its terminologically correct label is SepSecS.[110, 111] As a result, molecular-based assays have been developed for its detection.[103–107]

The reason for anti-SLA/LP association with severe liver inflammation, protracted treatment and relapse after corticosteroid withdrawal is unknown, but it has been reported that antibodies to ribonucleoprotein/Sjogren's syndrome A antigen (anti-Ro/SSA), and particularly against Ro52 (anti-Ro52), were present in 98% of AIH patients with anti-SLA/LP reactivity.[112] The dual presence of anti-SLA/LP and anti-Ro52 was not due to cross-reactivity and was later reported in 77% of European and 96% of North American patients with AIH and anti-SLA/LP.[113, 114] Of interest, anti-Ro52 alone, or in conjunction with anti-SLA/LP, is associated with an adverse outcome in AIH as defined by higher frequencies of progression to cirrhosis and hepatic death or need for liver transplantation.[114] These findings suggest that the prognostic associations ascribed previously to anti-SLA/LP may reflect their almost invariable concurrence with anti-Ro52.

AIH-2. AIH-2 is characterised by the detection of specific anti-LKM1 or infrequently anti-LKM type 3 (anti-LKM3) antibodies[2–6, 22, 57, 88–93, 115] and/or anti-LC1.[2, 3, 57, 93, 116] AIH-2 accounts for less than 10–15% of all cases in Europe and North America, although it is commoner in southern Europe.[43, 45, 47, 51, 117] Using the IIF method on fresh sections of rodent liver, kidney and stomach tissues, the characteristic features of anti-LKM1 are the diffuse staining of cytoplasm of the entire liver lobule and the exclusive staining of the P3 portion of proximal renal tubules.[2, 3, 22, 57, 93] Anti-LKM1 can be easily distinguished from antimitochondrial antibodies (AMA), which stain the proximal and distal renal tubules.[2, 3, 22, 57, 93] Anti-LKM1 target mainly several epitopes of CYP2D6 (molecular weight of 50 kDa).[21, 22, 118–120] Depending on the geographical origin, 0–10% of HCV patients develop

anti-LKM1,[2, 3, 57, 93, 98, 121–125] which are directed mainly against the same target-autoantigen recognised by anti-LKM1 in AIH-2, suggesting cross-reactivity leading to hepatic autoimmunity by molecular mimicry.[2, 3, 21, 22, 119–121, 126, 127] A genetic predisposition such as HLA-DR7 positivity appears to account for anti-LKM1 development in Italian patients with chronic hepatitis C.[128] Screening for anti-LKM is recommended by the IAIHG before the initiation of interferon-alpha-based therapies in HCV patients and, if found positive, a careful monitoring appears reasonable because, occasionally, interferon-alpha may unmask, or provoke, autoimmune hepatic reactions and even 'true' AIH.[5, 6, 76, 93, 129–132] Rarely, AIH-2 may be induced by acute HCV infection and persist even after viral clearance.[133]

Anti-LKM3 alone, or in combination with anti-LKM1, are also detected in about 5–10% of AIH-2 patients.[115] Anti-LKM3 were first described in about 13% of patients with chronic hepatitis D,[134] but only occasionally in HCV patients.[123, 135] Family 1 of UDP-glucuronosyltransferases (UGT1) is the main target-autoantigen of anti-LKM3 (molecular weight of 55 kDa), either in AIH-2 or in chronic hepatitis D.[2, 3]

Anti-LC1 are detected in about 30% of AIH-2 patients[2, 3, 57, 93, 116, 136] and in approximately 50% of anti-LKM1-positive cases.[137, 138] Anti-LC1 is organ-specific, but not species-specific, and is characterised by a cytoplasmic staining of the periportal hepatocytes by IIF. The hepatocellular layer around the central veins is not stained.[116, 136] Anti-LC1 proved to be the sole autoantibody in 10% of AIH patients.[116, 117] It recognises FTCD, a liver-specific metabolic enzyme involved in folate metabolism (molecular weight of 58–62 kDa)[139]. LC1 reactivity is mainly directed to conformational epitopes located in the FT region of FTCD.[140] The detection of anti-LC1 by IIF is usually obscured due to the anti-LKM1 pattern found in most of the anti-LC-positive sera. Therefore, additional techniques such as the Ouchterlony double diffusion, ELISA, immunoblot or counter-immunoelectrophoresis are required for anti-LC1 detection.[116, 136–138, 141] For both anti-LKM and anti-LC1, titres by IIF of at least ≥1:40 in adults and ≥1:10 in children are considered positive.[5, 6, 88, 93]

Problems in autoantibody testing. The IAIHG has published detail guidelines on how to test for autoantibodies relevant to AIH, including the preparation of substrates (especially on how to orient and cut the kidney), application of serum samples, optimal dilution, fluorochrome-labelled revealing agents, selection of controls and diagnostically relevant staining patterns.[93] IIF on fresh frozen sections (of 4–8 weeks store) of a multiorgan substrate from rodents, especially rats, is ideally the preferred first-line screening for ANA, SMA, LMK1, LKM3, LC1 and AMA.[2, 3, 57, 93] The use of immobilised HEp2 cells only for ANA, SMA and AMA detection should be avoided, owing to an increase frequency of false-positive results.

Unfortunately, in real life, the development of locally validated sections for IIF does not seem to be very feasible. In addition, equivalent sections of commercial origin are of variable quality because, to lengthen shelf-life, they are treated with fixatives, which result in enhanced background staining, leading potentially to several difficulties in the interpretation of fluorescence patterns.[89, 93] Therefore, some centres, especially in US, use ELISAs or immunoblot, particularly for the detection of ANA, SMA (F-actin), anti-LKM1, anti-LC1, AMA and anti-SLA/LP.[89]

Autoantibody titres may vary during the course of the disease and therefore it is now clear that low autoantibody titres do not exclude AIH diagnosis, nor do high titres (in the absence of other supportive findings) establish the diagnosis.[2, 3, 57, 89, 93, 142] Furthermore, seronegativity on a single testing cannot exclude AIH; repeated tests may be necessary to allow autoantibody detection. A clinically significant level of positivity would start at the arbitrary dilution of 1/40. In contrast, for subjects up to 18 years, any level of autoantibody reactivity in serum is infrequent, so that positivity at dilutions of 1/20 for ANA and SMA and even 1/10 for anti-LKM and anti-LC1 is clinically relevant.[6, 88, 89, 93] Hence, the laboratory should report any level of positivity from 1/10, with the result interpreted within the clinical context and the age of the patient. Of interest, several laboratories ignore these IIF cut-off points that are recommended by the IAIHG and, by using their own cut-offs (1/80 or even 1/160), expand the number of 'negatives', resulting in further underestimation of the disease and delay of diagnosis.

Other autoantibodies in AIH. Various autoantibodies with limited clinical significance have been reported in AIH. These include antibodies to single- and double-stranded DNA,[2, 3, 143] cardiolipin,[144] histones,[145] cyclic citrullinated peptide,[146, 147] asialoglycoprotein receptor (anti-ASGPR),[3, 89, 109, 148] chromatin,[149] centromere,[3, 109, 150] Ro52,[112–114, 151] alpha-actinin,[143, 152–154] *Saccharomyces cerevisiae*,[155, 156] coeliac disease-related

autoantibodies,[74, 109, 150] AMA,[109, 157–162] lactoferrin[163] and p53 protein.[164]

From this repertoire, AMA, antibodies to alpha-actinin and anti-ASGPR deserve a brief discussion. Although AMA remain the serological hallmark for PBC diagnosis,[3, 157] they can occur in otherwise classical AIH. The prevalence rates of AMA in AIH vary from 3.6% to as high 34%[158–161, 165] in Japanese patients.[158] Most studies agree that AMA detection in AIH does not identify a subgroup that requires different treatment or that evolves quickly into a cholestatic syndrome.[160] In parallel, a long-term study from Canada showed that patients with overt AIH who tested AMA-positive and are treated with corticosteroids had no clinical or histological evidence of PBC, despite the continued detection of AMA over a follow-up of up to 27 years.[161] In contrast, a recent small case study reported three AMA-positive patients with AIH in whom specific features of PBC overlapped in time, suggesting the need of longer follow-up to detect late development of PBC in this setting. [162]

Alpha-actinin is a ubiquitous cytoskeletal protein, which belongs to the superfamily of F-actin cross-linking proteins.[153] This multifunctional molecule has recently gained attention as a possible dominant target-autoantigen in autoimmune diseases, especially systemic lupus erythematosus (SLE) and AIH-1. Indeed, accumulating volume of evidence indicates that anti-dsDNA antibodies may contribute to the pathogenesis of SLE-related glomerulonephritis by cross-reacting with alpha-actinin in murine models as well as in humans.[166] Antialpha-actinin antibodies have also been detected in the sera of more than 40% of patients with AIH-1, characterising, in combination with anti-F-actin antibodies, a subset of patients with clinically and histologically severe form of the disease.[143, 152] This double detection of anti-F-actin and antialpha-actinin antibodies was not due to a cross-reaction and it was highly specific only for AIH-1.[143, 152] Furthermore, it has been shown, in a large cohort of AIH-1 patients, that antialpha-actinin antibodies at baseline could predict response to treatment and therefore, they might be used for monitoring treatment outcome.[154] Of interest, anti-F-actin antibodies target an epitope corresponding to the alpha-actinin-binding domain located at positions 350–375 of the C terminus of human F-actin. [153, 167] All these findings make the implication of alpha-actinin in disease pathogenesis very attractive and point out the need for considerable attention and further investigations.[153, 168]

Anti-ASGPR are common in AIH and they can support diagnosis in patients who are seronegative for conventional antibodies.[109, 148] However, they are also frequently detected in PBC,[109, 169] alcoholic cirrhosis[170] and hepatitis B or C, suggesting low specificity although the assay has recently been improved due to the characterisation of the major antigenic epitopes of ASGPR.[148, 171] Anti-ASGPR detection may also reflect the association of these antibodies with histological activity, which in turn may drive autoantibody production, regardless of the underline disease. Nevertheless, routine determination of these antibodies still awaits standardised and easily accessible assays.

Histological findings

A diagnostic liver biopsy should be performed in all patients with suspected AIH, including those with acute liver failure.[1, 5, 6, 38, 89, 90, 168] Indeed, liver histology is mandatory for AIH diagnosis as has been attested by both the revised and simplified diagnostic criteria (Table 3).[5, 6, 76, 78] Certain histological changes are helpful diagnostically. However, truly disease-specific, pathognomonic findings are still missing.[1, 5, 6] Therefore, a different view of the importance of liver histology in

Table 3 | Simplified diagnostic criteria for the diagnosis of autoimmune hepatitis (AIH)[6]

Variable	Cut-off	Points
ANA or SMA	≥1:40	+1
ANA or SMA	≥1:80	+2*
or LKM	≥1:40	
or SLA/LP	positive	
Liver histology (evidence of hepatitis is a necessary condition)	Compatible with AIH	+1
	Typical AIH†	+2
Serum immunoglobulin G levels	>Upper normal limit	+1
	>1.1 upper normal limit	+2
Absence of viral hepatitis	Yes	+2
Sum		≥6: probable AIH ≥7: definite AIH

ANA, antinuclear antibodies; SMA, smooth muscle antibodies; SLA/LP, antibodies against soluble liver antigen/liver pancreas; LKM, liver kidney microsomal antibodies.

* Addition of points achieved for all autoantibodies (maximum, 2 points).

† To be considered typical, each of the features of typical AIH histology (interface hepatitis, emperipolesis and hepatic rosette formation) had to be present. Compatible features are a picture of chronic hepatitis with lymphocytic infiltration without all the features considered typical.

AIH diagnosis has recently been reported.[172] In this report, the authors concluded that most patients did not need a biopsy as patients with atypical (5%) or compatible (95%) liver histology were similar with respect to biochemical features of AIH.[172] Further multicentre studies are needed to validate these recent findings as liver biopsy is not performed only for diagnostic purposes, but also for defining grading and staging.

A typical feature of AIH is the presence of interface hepatitis, also called piecemeal necrosis (Figure 1a, b). The portal inflammation spares the bile ducts and consists of lymphocytes and abundant ('clustered') plasma cells. The inflammation usually extends into the lobules (Figure 1c). A small subset of AIH patients may also show histological small duct injury, but they lack clinical, serological and immunological features of PBC, and they respond as well to corticosteroids as patients with classical AIH.[173]

The intensity of plasmacytosis can be useful in discriminating AIH from most cases of viral hepatitis. In addition, portal plasmacytosis might have prognostic information as its presence, while on immunosuppressive therapy, is associated with relapse upon drug withdrawal or cessation. However, approximately one-third of AIH patients have few or no portal plasma cells and therefore, the absence of portal tract plasma cell infiltration does not preclude diagnosis.[90, 174] Extensive emperipolesis and hepatocellular rosette formation were also regarded as 'typical' for AIH diagnosis (Figure 1b,d).[6] The word 'emperipolesis' has been generated by two Greek words (*en* meaning inside and *peripolos* meaning patrol) describing the close contact of lymphocytes and hepatocytes as well as the focal intracytoplasmic localisation of lymphocytes within hepatocytes (Figure 1d). Of interest, eosinophils can be present in AIH (Figure 1a) even in the absence of drug-induced AIH, making more prob-

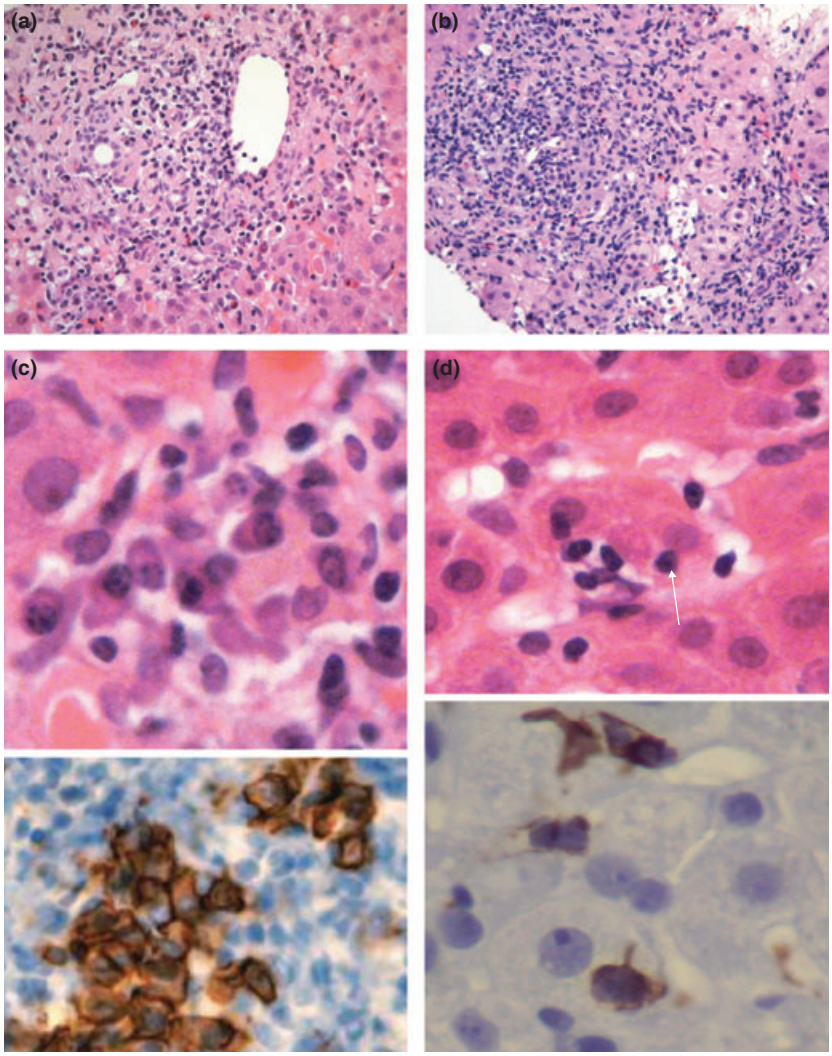

Figure 1 | (a) Portal inflammation consisting of lymphocytes, plasma cells and eosinophils. In addition, interface hepatitis is noted in a case with autoimmune hepatitis-type 1. (b) A case of autoimmune hepatitis with dense lymphocytosis, interface hepatitis and periportal hepatocytic rosettes. (c) Prominent plasma cells in autoimmune hepatitis. They tend to form clusters, better illustrated after CD138 immunostaining (lower left panel). (d) Emperipolesis in routine stain and after CD8+ immunostaining (lower right panel).

lematic the differential diagnosis between these entities. Parenchymal collapse (multiacinar necrosis) in the appropriate clinical and serological background could be helpful to support AIH diagnosis (Figure 2a).[1, 6, 90, 174] Fibrosis is present in all but the mildest or earliest forms of AIH (Figure 2b). In advanced untreated disease, the fibrosis is extensive with cirrhotic changes. Of note, the histological features of necroinflammatory activity and severity of AIH are not in parallel with the biochemical activity of the disease (Figure 2c).[1, 5, 6, 89, 90] For this reason, apart from diagnosis, liver biopsy also provides information on prognosis as almost one-third of patients have cirrhosis or bridging necrosis at presentation, carrying a poorer prognosis than those without.[9, 43, 48, 50]

The findings in patients with acute (Figure 3) to fulminant onset of AIH differ somewhat from those with an insidious presentation.[175, 176] Recently, the US NIH Acute Liver Failure Study Group proposed diagnostic criteria for AIH presenting as acute liver failure.[38] Liver biopsy played an essential role in these criteria and should be performed transjugularly in coagulopathic patients. Two distinctive patterns of massive hepatic necrosis suggestive of an autoimmune pathogenesis were found. One resembling a severe form of the so-called centrilobular variant of AIH with panlobular necrosis and another showing classic AIH with massive hepatic necrosis with sometimes centrilobular involvement. Additional features include the presence of portal lymphoid follicles, a plasma cell-enriched inflammatory infiltrate and central perivenulitis.[38, 42, 177, 178]

Overlap or variant syndromes of AIH

Some patients within the spectrum of AIH present with characteristics of either PBC or PSC. Unfortunately there is no clear-cut consensus regarding their classification and several terms have been used so far, like 'overlap syndrome', 'the hepatic form of PBC', 'autoimmune cholangitis', 'autoimmune sclerosing cholangitis' or 'combined hepatitic/cholestatic syndrome' to describe patients with features of both AIH and PBC or PSC.[179–181] However, as internationally agreed criteria defining 'overlaps' are lacking, their diagnosis is usually difficult and problematic, whereas, due to the lack of standardisation and variations in the populations of the studies, the characteristics of these entities vary between studies.

Recently, an international working party critically reviewed overlap syndromes and found a low sensitivity of the scoring systems for AIH diagnosis in clinically defined overlaps,[182] which is in keeping with results of previous studies.[76, 78] In contrast, the results of another

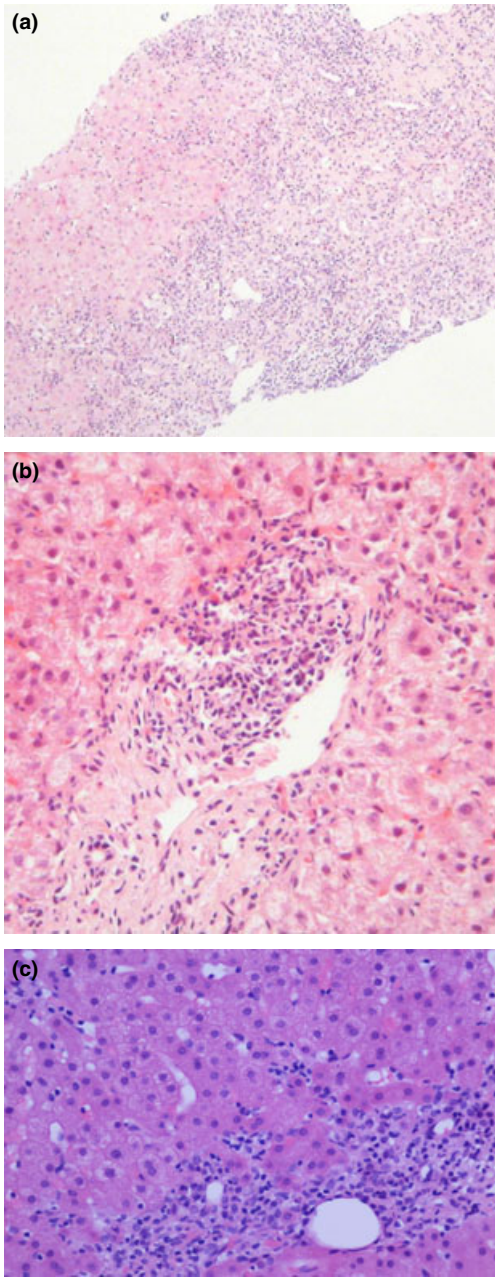

Figure 2 | (a) Extensive periportal parenchymal collapse from a noncirrhotic soluble liver antigen/liver pancreas (SLA/LP)-positive case with autoimmune hepatitis-type 1. In the collapsed area, there is microacinar transformation with regenerative rosettes of variable diameter. (b) Mild interface necroinflammatory activity without significant fibrosis from an untreated patient with autoimmune hepatitis-type 1. (c) No association of biochemical with histological activity; note a portal area with inflammation and ongoing interface necroinflammatory activity from a soluble liver antigen/liver pancreas (SLA/LP)-positive female patient with borderline high transaminases and IgG levels.

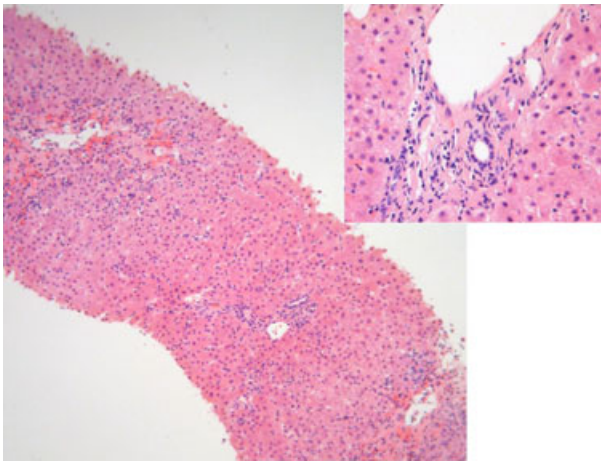

Figure 3 | An acute case of autoimmune hepatitis showing centrilobular injury without significant portal tract involvement (upper right).

study showed that, by the application of the simplified score to 368 PBC patients, the proportion classified as AIH-PBC overlaps was reduced from 12% by the revised IAIHG criteria[5] to 6%,[183] indicating again how the frequencies of 'overlap conditions' are dependent upon the definitions of disease entities.

On the basis of the current, very limited knowledge about the aetiopathogenesis of AIH, PBC and PSC, definition of diagnostic criteria for 'overlap conditions' can only be arbitrary and therefore, the IAIHG position paper did not support the contention of 'overlap syndromes' as distinct diagnostic entities.[182] A recent study,[184] however, concluded that the criteria previously suggested by Chazouilleres et al[185] could identify patients with a clinical diagnosis of AIH-PBC 'overlap syndrome' with high sensitivity (92%) and specificity (97%) and that these criteria performed better than the revised[5] and the simplified[6] score in this regard. Still, these criteria do not represent international consensus.

From the serological standpoint, the concomitant seropositivity for AMA and anti-ds DNA appears to be strictly associated with the clinical and histological diagnosis of AIH-PBC overlap syndrome.[186] In addition, the presence of HLA-DR7 or immunostaining of liver biopsies for IgG or IgM plasma cells has also been proposed as a surrogate marker in the diagnosis of AIH-PBC overlap.[187–189] However, again, no predictable staining pattern for IgG or IgM plasmacytic infiltrates was found in AIH-PBC 'overlap cases', and the IgG specificity of immunostaining for AIH and the IgM sensitivity for PBC were low,[188] although an IgG/IgM ratio of <1 was observed only in PBC, and all 'overlap patients' had a ratio >1.[189]

In the emerging era of IgG4-related diseases,[190] the role of IgG4 response was also investigated in AIH.[191–193] This variant of AIH seems to represent up to a third of AIH patients characterised by high serum IgG4 levels, more intense lymphoplasmacytic periportal infiltrate and marked response to prednisolone therapy compared with IgG4-negative patients.[192, 193] Of interest, IgG4-associated histological lesions were not observed in other chronic liver diseases like HCV or PBC. At present, IgG4 itself does not seem to be directly responsible for the development of liver damage as this subtype does not cause cell-mediated lysis owing to poor binding activity to complement. It is possible that abnormal immunological environments leading to enhanced IgG4 responses, rather than IgG4 itself, underlie the pathogenesis of the liver damage seen in AIH.[192, 193]

Conclusively, the authors are in favour of the IAIHG position, asserting that, in consideration of the low prevalence of such 'overlap syndromes or variants of AIH', patients with autoimmune liver diseases should be categorised as AIH, PBC and PSC, including its small duct variant, respectively, based on the predominating disease and that those with 'overlapping features' should not be considered as being distinct diagnostic entities.[182] The IAIHG scoring systems should not be used to establish such groups of patients. On the other hand, specific therapeutic considerations may be required in patients with PBC or PSC with features of AIH.[194]

DIAGNOSTIC CRITERIA

In case of a compatible liver histology, the diagnosis of AIH is quite easy when other aetiological factors of chronic or acute hepatitis have appropriately been excluded and characteristic circulating autoantibodies and abnormal levels of serum globulins are present (Table 4).[1, 4–6, 89, 150, 168] In principle, however, due to the heterogeneity of the disease and also to the absence of a single diagnostic test such as the detection of HBsAg or AMA in the diagnosis of HBV and PBC, respectively, AIH needs to be considered in the differential diagnosis in any patient with acute or chronic liver disease or unexplained cirrhosis.[109, 168, 195] For these reasons, the establishment of diagnostic criteria of AIH seems mandatory in an attempt to facilitate making the diagnosis in daily clinical practice, particularly in non-expert settings (Table 3), but also to allow enrolment of AIH patients with homogenous patterns into clinical trials.[4–6, 89, 150, 168, 196]

Indeed, in 1999, the IAIHG published the revised diagnostic criteria (Table 4) to standardise the diagnosis for

Table 4 | Descriptive criteria for the diagnosis of AIH from the IAIHG, 1999[5]

Features	Definite AIH	Probable AIH
Liver histology	Interface hepatitis of moderate or severe activity with or without lobular hepatitis or bridging necrosis. No biliary lesions, granulomas or other prominent changes suggestive of a different aetiology.	Same as for definite.
Serum biochemistry	Any serum aminotransferase abnormality, especially if ALP is not markedly elevated. Normal levels of alpha-1-antitrypsin, copper and ceruloplasmin.	As for definite AIH, but patients with abnormal levels of copper or ceruloplasmin may be included provided that Wilson's disease has been excluded by appropriate investigations.
Serum immunoglobulins	Total serum globulin or γ-globulin or IgG concentrations greater than 1.5 times the upper normal limit.	Any elevation of serum globulin or γ-globulin or IgG concentrations above the upper normal limit.
Serum autoantibodies	Seropositivity for ANA, SMA or anti-LKM-1 antibodies at titres greater than 1:80. Lower titres (particularly of anti-LKM-1) may be significant in children. Seronegativity for AMA.	Same as for 'definite', but at titres of 1:40 or greater or presence of other specified autoantibodies.
Viral markers	Seronegativity for markers of current infection with hepatitis A, B and C viruses.	Same as for definite.
Other aetiological factors	Average alcohol consumption less than 25 g/day. No history of recent use of known hepatotoxic drugs.	Alcohol consumption less than 50 g/day and no recent use of known hepatotoxic drugs. Patients who have consumed larger amounts of alcohol or who have recently taken potentially hepatotoxic drugs may be included, if there is clear evidence of continuing liver damage after abstinence from alcohol or withdrawal of the drug.

AIH, autoimmune hepatitis; IAIHG, International Autoimmune Hepatitis Group; ALP, alkaline phosphatase; IgG, immunoglobulin G; ANA, antinuclear antibodies; SMA, smooth muscle antibodies; anti-LKM1, antibodies against liver kidney microsomal type 1; AMA, antimitochondrial antibodies.

clinical trials and population studies.[5] The revised criteria included response to immunosuppressive therapy or relapse after its discontinuation, allowing determination of pre- or posttreatment scores. A pre-treatment score of 15 indicated 'definite' AIH with 95% sensitivity, 97% specificity and 94% diagnostic accuracy. A pre-treatment score ≥10 or posttreatment score ≥12 indicated 'probable' AIH.[5] A pre-treatment score of 10 has 100% sensitivity, 73% specificity and 67% diagnostic accuracy. However, the calculation of this score was relatively complex for everyday clinical use, may be inaccurate when applied in individual patients, especially children, whereas the main aim of clinically useful criteria should be the establishment of a reliable diagnosis as early as possible after clinical presentation and before the initiation of any treatment. Thus, in 2008, the same group published the now widely used simplified diagnostic criteria for AIH based on only four parameters, namely, autoantibodies detection, serum IgG levels, absence of viral hepatitis markers and liver histology (Table 3).[6] A number of studies have shown the utility of these new criteria in different cohorts of patients from different countries spread over four continents with a sensitivity and specificity of more than 90%.[78, 196–201] In this context, the group from Japan reported that the more typical features of disease were present the more useful was the simplified score compared with the revised criteria, whereas, from a large retrospective study including patients with diverse chronic liver disorders (n = 428), it was pointed out the high diagnostic value of the high specificity of the simplified score to exclude AIH. [78] However, as there is no definite gold standard in making AIH diagnosis, precise studies on sensitivity and specificity are not feasible and therefore, clinicians must regard diagnostic scores only as an aid to AIH diagnosis.[202] In patients with a nondiagnostic simplified score, rescoring with the original revised score could be helpful to avoid misdiagnosis.[168, 195, 202]

MANAGEMENT AND OUTCOME

Conventional treatment

In the Seventies, different randomised clinical trials (RCTs) unequivocally demonstrated the survival benefit of immunosuppression in AIH patients.[203–205] These tri-

als not only documented the dramatic positive therapeutic effect of steroid treatment, but also emphasised the dreadful prognosis of symptomatic patients with AIH left without immunosuppressive therapy.[206] Treatment is mandatory and usually effective in patients who have clinical, laboratory or histological features of active liver inflammation.[207, 208] Whether to treat asymptomatic patients with mild disease still is a matter of debate, even if the risk of acute/hyperacute flares with progression of the disease strongly militates in favour of treatment. Patients with inactive or 'burned out cirrhosis' seldom benefit from therapy, and are at increased risk of drug-induced side effects.

From a practical standpoint, AIH treatment can be divided into two phases: (i) induction of remission, and (ii) remission maintenance.[207–210] The standard treatment to achieve remission is monotherapy with high-dose prednisone or prednisolone (usually 1 mg/kg per day), or a reduced initial steroid dose (prednisone or prednisolone 30 mg per day) in combination with 1–2 mg/kg per day of azathioprine, as outlined by the recently published AASLD practice guidelines.[89] Prednisolone is sometimes started in a higher dose than 30 mg/day in combination with azathioprine. Indeed, an individualised dosage of prednisolone (or prednisone) of 1 mg/kg/day plus azathioprine has been proposed as first-line treatment of patients with AIH. The prednisolone is then reduced to 10 mg/day over 2–3 months as aminotransferases are normalised.[90, 207, 209] A previous study showed that noncirrhotic patients who received this dosage had faster normalisation of aminotransferases (77% at 6 months) compared to 39% with standard dose prednisolone in a different randomised trial.[209] Similar findings have been reported from a Greek study as well. Actually, 69.5% of a cohort of treatment-naïve AIH patients, including 35% cirrhotics receiving 0.5–1 mg/kg/day prednisolone plus mycophenolate mofetil (MMF), achieved normalisation of aminotransferases and γ-globulins in less than 3 months.[51] However, the frequency and rapidity of histological resolution, treatment tolerance and long-term outcome, including progression to cirrhosis, relapse after drug withdrawal and treatment failure, require further definition and a firmer evidence base is needed when the abovementioned treatment strategy is administered to patients with AIH.[90, 211]

At variance with the previously dominant view, which considered the disease remission as the reduction of transaminases to less than twice the normal levels,[5] today there is an internationally agreed consensus on the definition of disease remission as complete normalisation of transaminases, along with normal γ-globulins or IgG levels, and possibly of the histological picture.[89, 212]

Long-term treatment with generous steroid dosage may induce predictable side effects such as cosmetic changes ('facies lunaris', dorsal hump formation, 'striae rubrae', weight gain, acne, alopecia, hirsutism) or even more dreadful complications such as osteopenia, brittle diabetes, psychosis, pancreatitis, opportunistic infections, labile hypertension and malignancy.[208, 213] The initial high-dose steroid regimen should therefore be temporally limited and dose tapering actively pursued. The concomitant use of azathioprine, with its steroid-sparing effect, may be very helpful. However, at least 10% of patients may be intolerant to azathioprine and experience nausea, vomiting, arthralgia, fever, skin rash, or may even develop severe side effects such as cholestatic hepatitis, pancreatitis, opportunistic infection, bone marrow suppression and malignancy. In addition, as azathioprine is potentially hepatotoxic, in the severely ill and jaundiced patient, it is advisable to start with high-dose steroids first, and add azathioprine later.[208, 210, 213]

Several proposals of treatment schedules have been recently published[89, 90, 207, 208, 211, 213] and can be used as general guidelines; however, treatment of AIH, particularly dose reduction schedules, should always be adapted to the response of the individual patient, particularly when side effects already developed. Once remission is obtained, its maintenance should be actively pursued, possibly avoiding the reactivation of the disease, defined as an increase in transaminases >3 times the upper normal limit. Azathioprine alone,[209, 214] low-dose steroids,[43] or both[215] are the standard maintenance treatment appropriate to maintain remission with absent or minimal side effects.

Alternative therapies

Overall, 10–20% of patients do not respond to, or are intolerant of, conventional corticosteroid therapy with or without azathioprine use, a nonselective immunosuppressant that acts by inhibition of several enzymes involved in purine synthesis.[216] The measurement of azathioprine metabolites neither provides a foolproof way of avoiding toxicity nor predicts response, and it is time consuming and not widely available.[217, 218]

In addition, recently, Lamers *et al*[215] reviewed the appropriateness of the recommendations for optimal induction and maintenance treatment in AIH, by descriptive analysis of the published RCTs from 1950 to 2009. Surprisingly enough, although the current literature indicates remission rates of 65–80% with conven-

tional therapy,[219] Lamers *et al.* , after the analysis of 11 RCTs with 578 patients including 363 treatment-naïve AIH patients, found much lower percentages of remission on prednisolone treatment (approximately 43%).[215] Therefore, they concluded that treatment of AIH with prednisolone in combination or not with azathioprine is far from ideal, and the search for drugs with a favourable risk–benefit ratio seems mandatory.[215]

In parallel, it has been shown recently that the application of the 2010 response criteria of the AASLD practice guidelines[89] compared with the 2002 criteria[220] flips the previously codified remission rate from 73% to 26%.[212] Of note, patients with complete response defined by normal γ-globulins or IgG and normal transaminases had a very good long-term prognosis virtually free of significant clinical events, whereas patients whose serum aminotransferases were unable to be stably normalised were those with the highest probability of developing long-term complications.[43, 87, 212]

Finally, a very recent large multicentre study from Netherlands showed that relapse of the disease is almost universal when immunosuppression with azathioprine is discontinued in patients with AIH in long-term remission (n = 131), further supporting the concerns for the lack of long-term efficacy of conventional treatment.[221]

Therefore, several strategies have been proposed in recent years, particularly for those not responding to, or intolerant of, conventional treatment, using immunosuppressive drugs derived from the antirejection experience of solid organ transplantation, but also using agents that can redirect thiopurine metabolism towards the biologically active 6-tioguanine (thioguanine) nucleotides (6-TGN) instead of the hepatotoxic metabolites like 6-methylmercaptopurine (6-MMP) as well as new pharmacological, cellular and molecular therapies.[90, 168, 219, 222–224]

Ciclosporin. It is a calcineurin inhibitor and a potent immunosuppressive agent that inhibits IL-2 and T-cell proliferation. Several single-centre studies with ciclosporin for AIH documented improvement in most of the patients treated, especially among the paediatric population.[225, 226] However, a RCT is needed to confirm the efficacy of ciclosporinin AIH, and the long list of side effects (nephrotoxicity, gum hypertrophy, hypertension, hyperlipidaemia, hirsutism, infections and malignancy) has limited its widespread use so far.

Tacrolimus. It is a macrolide antibiotic with immunosuppressive effectiveness 10–200 times greater than ciclosporin. Its mechanism of action is similar to that of ciclosporin. It has been reported to be effective, particularly as salvage therapy and at low doses, in small series of AIH patients who were resistant to standard treatment.[227, 228] As for ciclosporin, its use should be balanced by the relatively frequent side effects (diabetes, neurotoxicity, nephrotoxicity, diarrhoea, pruritus, alopecia).

Mycophenolate mofetil (MMF). It is the pro-drug of mycophenolic acid, which blocks purine synthesis, inhibits DNA synthesis and exerts a selective anti proliferative effect on B and T lymphocytes.[229] MMF has a 5-fold potent inhibitory effect on type II isoform of inosine-5'-monophosphate dehydrogenase, an enzyme of the purine synthesis pathway, that depletes guanosine nucleotide specifically in activated T and B lymphocytes, without affecting type I isoform expressed in other cell types.[229] As a result, MMF tends to be more powerful and better tolerated agent, providing, additionally, selective immunosuppression with minimal side effects, which is the requested standard of therapy in transplantation and autoimmune diseases. [230]

Its use is suggested as an alternative to azathioprine in intolerant patients, usually in association with steroids.[231–240] Richardson *et al.*[231] reported complete biochemical response, with significant decrease in histological activity index on the second biopsy and minimal toxicity in 5/7 patients, while Devlin *et al.*[232] showed a complete response and steroid withdrawal in all 5 patients included in their study. In addition, Chatur *et al*,[233] Inductivo *et al*,[234] Aw *et al*[235] and Wolf *et al*[236] reported 64% (n = 11), 73% (n = 15), 70% (n = 26) and 75% (n = 16) response rates, respectively, while MMF was well tolerated. By contrast, small case-series studies have shown that patients with a previous nonresponse to azathioprine are unlikely to benefit from MMF, although its use resulted in significant decrease in steroid use.[238, 239]

MMF seems to be safe and effective as first-line therapy in inducing and maintaining remission in treatment-naive patients with AIH, with a rapid steroid-sparing effect.[51, 241] Indeed, in the largest prospective series of treatment-naïve AIH patients (n = 59) ever published, it has been shown that MMF at a dose of 1.5–2 g/day in conjunction with personalised dosage of prednisolone (0.5–1 mg/kg/day) resulted initially in 88% response within only 3 months (12% partial responders), even though the definition of complete response used in that study was very strict (normalisa-

tion of transaminases and IgG, disappearance of symptoms and minimal or no inflammation on liver biopsy if performed).[51] Complete remission was achieved in 59.3% of patients (26% and 43% in studies by Muratori et al[43, 212] and Lamers *et al.*[215] using conventional therapy respectively), while prednisolone was withdrawn in 58% in 8 months (22 and 36 months in studies by Johnson et al[214] and Muratori *et al.*[43] using conventional therapy respectively). Severe side effects compelled discontinuation of MMF in only 3% of patients.[51] Of interest, complete normalisation of biochemical indices seems to be achieved after a more prolonged period in AIH patients treated with conventional schedules,[43, 219, 242] as only 11% of these patients enter complete remission in less than 6 months.[242] These findings were independent of the presence or absence of cirrhosis, whereas the response rates in patients who had been treated before with conventional therapy and received MMF as salvage therapy did not significantly differ from those found in treatment-naïve AIH patients.[51] A recent retrospective study reported similar response rates (84%) in 29 AIH patients (including 17 treatment-naïve patients).[241]

Further data from multicentre RCTs are needed on efficacy in improving liver histology and outcome, and information on long-term safety of MMF. These trials seem obligatory and urgent because application of the 2010 AASLD practice guidelines regarding the definition of response will potentially result in increased number of nonresponders to conventional treatment.[89, 168, 212] Due to its teratogenic potential, MMF is contraindicated in pregnancy.

Budesonide. Budesonide is a synthetic corticosteroid with high affinity for the glucocorticoid receptor that undergoes extensive first-pass metabolism. When given in combination with azathioprine (1–2 mg/kg/day), oral budesonide (9 mg/day) appears to be effective in noncirrhotic patients with AIH and seems to have a reduced incidence of corticosteroid-related side effects.[243, 244] Indeed, the European trial compared the combination regimen of budesonide and azathioprine with that of prednisone (40 mg daily, tapered to 10 mg daily) and azathioprine in 203 noncirrhotic AIH patients.[244] The primary end point was to achieve complete remission without the typical steroid-induced side effects. Biochemical remission was achieved more frequently after 6 months in patients treated with budesonide compared with those treated with prednisone (47% vs. 18%), and side effects were fewer (28% vs. 53%).[244]

However, in AIH patients with cirrhosis, the efficacy of budesonide may be reduced and the incidence of corticosteroid-related adverse reactions appears increased. So far, the long-term outcome in patients treated with budesonide regarding the frequency of histological resolution and the durability of the response is unknown, and the low frequency of response (18%) and high occurrence of side effects (53%) in patients treated with conventional therapy are unexplained.[211, 223, 244, 245] Finally, a case of reactivation of AIH during budesonide monotherapy with subsequent response to standard treatment makes the advantages of a more expensive drug as first-line therapy in AIH uncertain.[246]

Allopurinol. Some AIH patients will develop azathioprine-induced hepatotoxicity, which may be difficult to distinguish from AIH nonresponse or relapse without liver biopsy. In this setting, measuring of thiopurine metabolites may provide diagnostic guidance as increased 6-MMP with low or normal 6-TGN concentrations are associated with hepatotoxicity in patients with inflammatory bowel disease,[247] whereas high concentrations of 6-TGN are associated with remission in AIH.[248] The use of allopurinol, which induces preferential azathioprine breakdown leading to higher 6-TGN and lower 6-MMP, might be rationale in AIH patients with intolerance and/or nonresponse due to an unfavourable thiopurine metabolism. So far, very small case-studies have reported that the use of allopurinol (100 mg/day) in combination with low-dose azathioprine (25–50% of the original dose) might be an effective and relatively safe alternative immunosuppression for AIH patients failing standard thiopurine therapy due to preferential 6-MMP metabolism.[224, 249] At present, these results are too preliminary and external validation by RCTs is needed to draw general conclusions.

Treatment of 'difficult to treat patients' and overlap syndromes

Pregnancy and AIH. Although the available studies addressing the question of pregnancy in AIH are relatively few, the conclusive message is uniformly reassuring, indicating that pregnancy in AIH is safe for both mother and child.[53, 250, 251] Steroids are safe as immunosuppressant therapy during pregnancy. Although azathioprine has been designated by the Food and Drug Administration as a category D drug in pregnancy, its use in AIH has not been related to miscarriages or other complications for the mother or the baby.[53, 55, 250, 251] Further support for this notion has been gained by a

recent study of azathioprine use during pregnancy in patients with inflammatory bowel disease.[252] The inflammatory activity of the disease seems to be milder, and is controlled with reduced or even absent immunosuppression[56]; however, postpartum flares are quite frequent, and immunosuppression should be introduced again or increased shortly before the expected date of delivery. A poor disease control in the year prior to pregnancy may be associated with potential complications.[55]

Nonresponsive/noncompliant patient. Response to immunosuppression is considered an ex-post diagnostic criterion;[5] therefore, nonresponse should question the diagnosis first, and then adherence to treatment. Nonresponse is defined as worsening of clinical, laboratory or histological findings in any combination, despite compliance with standard therapy. [89] Many diseases can resemble AIH, including Wilson disease, HCV infection, non-alcoholic fatty liver disease, PBC and PSC. Therefore, reconsideration of diagnosis is needed in all compliant AIH patients with treatment failure by evaluating again the histology and autoimmune serology, whereas investigation for genetic or metabolic diseases of the liver and endoscopic or magnetic resonance cholangiography are also mandatory in this setting.

Compliance can become a problem, especially in paediatric patients entering puberty or adolescents who do not accept the potential development of cosmetic side effects[253]; in addition, patients with anxiety and depression not recognised or treated are more likely to be non-adherent to the therapeutic regimen of AIH and inappropriately considered nonresponders.[254]

Overlap syndromes. These conditions may be difficult to classify and are commonly designated as 'overlap'[179, 182, 255] in an attempt to describe either the sequential presentation of two disorders, or the concomitant presence of two distinct disorders, or a continuum of pathological changes between two disorders without strict boundaries, or as distinct entities on their own. The IAIHG does not endorse such a subclassification, on the ground that the definition of the diagnostic criteria for overlap conditions can only be arbitrary.[182] In addition, due to the low prevalence of 'overlap syndromes', prospective therapeutic trials cannot be expected in the future and a more practical approach is suggested. Therefore, the strategy to treat these patients with a combination of ursodeoxycholic acid and immunosuppression is not evidence-based, and, as a rule, the dominant clinical feature of AIH, PBC or PSC/small duct PSC should be treated first and therapy should be individualised, tailored to each patient and adjusted according to the response.[182, 256] Importantly, however, care must be taken not to expose PBC or PSC patients to the risk of side effects of steroids if this cannot be justified by the beneficial effect.

Potential new therapeutic options according to aetiopathogenesis

Targeting immune cell populations. The emerging role of an impaired regulatory T-cell activity in the pathogenesis of AIH[25, 26, 257] appears to involve not only CD4+CD25+FOXP3+ classical regulatory cells, but also other regulatory cell types, such as NKT cells.[258] Work is in progress to promote expansion and *de novo* generation of regulatory T-cells to reconstitute impaired immune regulation and restoring peripheral tolerance through regulatory T-cell infusion.[259–261] In support of this strategy, a recent paper reported that, in an animal model of AIH, the adoptive transfer of *ex vivo* expanded regulatory T-cells targeted efficiently the inflamed liver, restored peripheral tolerance and induced remission of the disease.[34] Corticosteroid therapy can improve regulatory T-cell function, but in a nonselective fashion.[262]

Antigen-specific regulatory T-cell responses have been recognised in oral toleration studies and in investigational treatments with anti-CD3.[263] An alternative potential approach would be to explore drugs that could restore regulatory T-cell function. Under this context, MMF could be a candidate. Indeed, recent studies have shown that MMF-based immunosuppression increases the percentage and CD25 expression of CD4+Foxp3+ cells, indicating that this therapy – but not corticosteroids – can overturn the repressive effect of calcineurin inhibitors on circulating regulatory T-cells and therefore, may promote T regulatory-mediated suppression of alloreactivity.[264, 265] In parallel, Lee et al[266] have shown, in an experimental model of colitis, that MMF pre-treatment can improve colitis by downregulation of expanded B-cell population through apoptosis and augmentation of regulatory T-cells. Inhibitors of the mammalian target of rapamycin (mTOR), like sirolimus or rapamycin, could be another candidate because it has been shown recently that rapamycin can both promote induction of CD4+ CD25+Foxp3+ regulatory T-cells and inhibit T effector cells function simultaneously.[267]

Autologous haemopoietic stem cell transplantation and mesenchymal stem cell transplantation could be other options for treating patients with severe and/or refractory forms of the disease. Such a therapeutic option

for AIH and other autoimmune diseases has already been reported[268]and supported by findings indicating that bone marrow from patients with AIH have had increased numbers of haemopoietic progenitor cells[269] and plasma cells,[270] whereas bone marrow stromal cells supported normal haemopoiesis.[269] In addition, bone marrow cultures have shown high levels of apoptotic markers, tumour necrosis factor-alpha (TNF-alpha), interferon-gamma, IL-4 and TGF-beta.[271, 272] On the other hand, mesenchymal stem cells that have been isolated from human bone marrow have rescued immune-deficit mice with hepatic failure.[273] Such strategies, even though only rarely can be required, would potentially reduce reliance on whole organ transplantation and avoid the complications of whole organ rejection.[223, 245]

Targeting apoptosis. Programmed cell death is a critical mechanism for preserving immune homeostasis, and medications that can enhance apoptosis of activated lymphocytes and other cellular effectors in autoimmune diseases may short-circuit autoimmunity. Accordingly, rapamycin acting by inhibiting mTOR, a protein that modulates the proliferation and survival of activated lymphocytes, can induce apoptosis of cytotoxic T-cells and antigen-sensitised CD4+ and CD8+ lymphocytes, resulting in a considerable decrease in the production of perforin and granzyme B.[211, 223] Consequently, the apoptosis of hepatocytes targeted by these effectors cells may diminish and the immune-mediated pathway of liver damage is stopped.[274] Of interest, $CD4^+CD25^+$ regulatory T-cells are resistant to the apoptosis induced by rapamycin.[275]

So far, rapamycin has been reported to be effective only as salvage treatment in five patients with *de novo* AIH after liver transplantation who were nonresponders to standard therapies.[276] These preliminary results support the extension of its evaluation, at least in untransplanted patients with refractory AIH.

Monoclonal antibodies. Rituximab is a chimeric monoclonal anti-CD20 antibody that can deplete B lymphocytes by targeting their CD20 cell surface receptor. It has been licensed for use in adults with CD20-positive B-cell lymphoma or rheumatoid arthritis and, recently, for ANCA-associated vasculitis, but it has also been used for off-label indications like refractory non-Hodgkin lymphoma, chronic immune thrombocytopaenic purpura and essential mixed cryoglobulinaemia. Accordingly, rituximab has been used successfully in AIH cases associated with other B-cell-driven diseases.[277] These findings indicate that rituximab may have a role in the treatment of at least refractory AIH. Rare, but serious, side effects have been reported with rituximab administration, including late-onset neutropenia, interstitial pneumonitis, HBV reactivation, intestinal perforation and possible multifocal leucoencephalopathy.[278–280]

Very recently, it has been shown, in a mouse model of fatal AIH, that TNF-alpha is essential in the induction of AIH through upregulation of hepatic CCL20 expression, which allows migration of dysregulated splenic T-cells.[281] As a consequence, the efficacy of anti-TNF-alpha therapy in AIH could have a pathophysiological basis.[282]Weiler-Normann *et al.*[283] reported recently promising results regarding the use of infliximab as a therapeutic option in a case-series of 11 difficult-to-treat patients with AIH. However, TNF-alpha blockade can also be immunogenic, with development of either autoantibodies or true autoimmune diseases, making such therapy a '*two-edged sword*'.[284] Indeed, the induction of AIH is one of the examples of the latter 'therapeutic paradox' during anti-TNF-alpha therapies.[63, 285] This paradox in case of AIH is mainly attributed to the disruption of the regulatory role of TNF-alpha signalling on the immune system. TNF-alpha blockade interferes with the normal cytotoxic T lymphocyte suppression of self-reactive B-cell population leading to autoantibody production, a hallmark of AIH. Furthermore, anti-TNF-alpha therapy disrupts the TNF-alpha-mediated apoptosis of activated T lymphocytes, resulting in unregulated lymphocyte activation.

Conclusively, the use of TNF-alpha blockade seems rationale in the treatment of AIH,[281, 282] but because of the incapability to predict efficiently the 'unforeseen serious complications', like the emergence of severe infections or, in particular, the development and/or deterioration of autoimmunity, safer tools are required to take the risk.[216, 285]

Other biological drugs can also be used to modify the pro-inflammatory intrahepatic cytokine milieu.[286] In particular, Ustekinumab, a human monoclonal antibody that targets the IL-12/IL-23 pathway, and Tocilizumab, a humanised monoclonal antibody targeting soluble IL-6 receptor, are both promising drugs that can effectively skip the balance in favour of regulatory T-cells and therefore control the autoimmune attack.[287]

Liver transplantation

The need for liver transplantation may occur in 10–20% of patients with AIH, mainly for two reasons: (i) severe,

hyperacute AIH resulting in acute or subacute liver failure; (ii) decompensated, end-stage liver cirrhosis/HCC, usually occurring in a patient with longstanding AIH.[288] Five-year survival of liver transplantation for AIH is around 75%. Age significantly affects patient survival after liver transplantation for AIH: in adults, especially above the age of 50 years, there is a significantly increased risk of dying of infectious complications in the early post-operative period.[289] Recurrence of the disease is observed in about 20% of cases and is usually treated with long-term steroids or continuation of azathioprine in the immunosuppression regimen.[290, 291]

CONCLUSIONS

AIH is a relatively rare liver disease of unknown aetiology characterised by interface hepatitis, hypergammaglobulinaemia, circulating autoantibodies and a favourable response to immunosuppression. Due to a large heterogeneity of the genetic, clinical, laboratory, histological and serological features of the disease, AIH might be underestimated or unrecognised. It should be clear that the disease has global distribution affecting any age, both sexes and all ethnic groups.

AIH is developed in genetically predisposed individuals, who are also exposed to diverse triggering factors. Thereafter, the autoimmune attack is perpetuated, possibly via 'molecular mimicry', and is favoured by the impaired control of regulatory T-cells.

Clinical manifestations are variable, ranging from no symptoms to severe acute hepatitis and even fulminant hepatic failure; almost one-third of patients have already cirrhosis at diagnosis, perhaps due to the indolent course of the disease and underestimation of the clinician. Therefore, high clinical suspicion for AIH diagnosis should be raised in every case of unexplained acute or chronic hepatitis. AIH may first be diagnosed during pregnancy or in the early postpartum period, after viral infections or after the administration of several drugs as well as *de novo* after liver transplantation for other reason; a common clinical feature is the presence of a wide spectrum of other autoimmune or immune-mediated diseases in the patient or in first-degree relatives.

Biochemical indices are not characteristic with bilirubin and aminotransferases from just above the upper normal limits to more than 50 times these levels, with normal or only moderately elevated cholestasis; these findings do not reliably reflect severity of the disease at the histological level. Biochemistry may even spontaneously normalise (spontaneous biochemical remission), despite histological evidence of continuing activity; this is a critical issue, which may result in delay and/or underestimation of diagnosis as the subsequent hit can be obvious after several months or years and may be completely asymptomatic. In most patients, the typical laboratory feature is polyclonal hypergammaglobulinaemia with selective elevation of serum IgG; however, almost 15–25% of patients, particularly the children and the elderly, and also those with a severe/acute onset, have normal IgG at presentation; therefore, the diagnosis of AIH should never be ruled out only on the basis of normal IgG.

The detection of non-organ and liver-related autoantibodies, although not pathognomonic, still remains the hallmark of the diagnosis, in the absence of viral, metabolic, genetic and toxic aetiology of chronic or acute hepatitis; the IAIHG has published detailed guidelines on how to test for autoantibodies relevant to AIH; both the laboratory personnel and the clinician need to become more familiar with the disease expressions and the interpretation of liver autoimmune serology to derive maximum benefit for the patient.

Liver histology is mandatory for AIH diagnosis, although no findings are specific for AIH; typical findings include interface hepatitis consisting of lymphocytes and abundant plasma cells; however, one-third of patients have few or no portal or acinar plasma cells and therefore, the absence of portal tract plasma cell infiltration does not preclude diagnosis. Emperipolesis and hepatic rosette formation were also regarded as typical for AIH diagnosis; the histological features in patients with severe-acute to fulminant AIH differs as the lesions predominate in the centrilobular zone, including distinctive patterns of massive hepatic necrosis, presence of lymphoid follicles, a plasma cell-enriched inflammatory infiltrate and central zonal necrosis/perivenulitis.

Because of the low prevalence of 'overlap syndromes', and on the basis of the current, very limited knowledge about the aetiopathogenesis of AIH, PBC and PSC, definition of diagnostic criteria for 'overlap conditions' can only be arbitrary and therefore, patients with autoimmune liver diseases should be categorised as AIH, PBC and PSC, including its small duct variant, respectively, based on the predominating disease; those with 'overlapping features' should not be considered distinct diagnostic entities.

Reliable scores for AIH diagnosis carrying high sensitivity and specificity do exist and the latest simplified score taking into account only four parameters seems easier for everyday use in clinical practice; the absence,

however, of a definite gold standard for AIH diagnosis makes impossible the performance of precise studies on sensitivity and specificity and therefore, clinicians must regard diagnostic scores only as an aid to AIH diagnosis.

Treatment is mandatory and usually life-saving in patients who have clinical, laboratory or histological features of active liver inflammation; treatment can be divided into induction of remission, and remission maintenance either by monotherapy with high-dose corticosteroids or a reduced initial steroid dose in combination with azathioprine. An individualised dosage of prednisolone (or prednisone) of 1 mg/kg/day plus azathioprine has been proposed as first-line treatment of patients with AIH; today, there is an internationally agreed consensus on the definition of disease remission as complete normalisation of transaminases, along with normal IgG levels. Recent systematic review of all published RCTs has shown that treatment of AIH with prednisolone in combination or not with azathioprine is far from ideal; in parallel, a recent large study showed that relapse occurs in virtually all patients with AIH in long-term remission when immunosuppression with azathioprine was discontinued. Therefore, the search for alternative drugs with a favourable risk–benefit ratio seems mandatory. The application of the 2010 AASLD practice guidelines regarding the definition of response is expected to result in increased number of nonresponders to conventional treatment with corticosteroids and azathioprine.

Alternative therapies, such as ciclosporin, tacrolimus, MMF, budesonide, rapamycin, or other new drugs, including biological agents, are very promising and ideally should be tested in the next years, especially for the difficult-to-treat or nonresponder patient. To this endpoint, a network of committed clinical investigators must be generated to evaluate new therapies in multicentre studies.

AUTHORSHIP

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