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Review Article

NAFLD and autoimmune hepatitis: Do not judge a book by its cover

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease (almost 25% of the general population). Autoimmune hepatitis (AIH) is a relatively rare liver disease of unknown aetiology characterized by female predominance and large heterogeneity regarding epidemiology, clinical manifestations, genetics, serology and liver pathology. The potential NAFLD/AIH coincidence or an AIH diagnosis alone instead of NAFLD represent a challenge for clinicians, both in making a correct and timely diagnosis but also in the management of these diseases. The diagnosis of both diseases can be challenging as: (a) reliable laboratory tests to confidently diagnose or exclude NAFLD or AIH are missing; (b) physicians and pathologists are much more familiar with a very common disease like NAFLD so, they do not consider an alternative or additional diagnosis; (c) most NAFLD studies do not investigate the patients for all autoantibodies involved in AIH diagnosis, apply the diagnostic scoring systems for AIH or address the possibility of AIH features on liver histology and (d) the recent European and American practice guidelines for NAFLD do not mention clearly the importance of IgG determination and liver autoimmune serology according to the AIH guidelines. Patients with NAFLD/AIH coincidence have significantly more frequently hypertension, diabetes, obesity, older age, lower transaminases, bilirubin and simplified score for AIH diagnosis but no female predominance compared to AIH patients only. The true outcome of NAFLD/AIH patients is practically unknown while their management is quite problematic because official clinical practice guidelines for this condition are missing.

1. Epidemiology and overview of NAFLD characteristics

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome and is defined by the accumulation of fat in > 5% of hepatocytes at the histological level or by proton magnetic resonance spectroscopy (^{1}H-MRS) in association with insulin resistance in patients who are not alcohol consumers (< 20–30 g/day or < 21 standard drinks/week for men and < 10–20 g/day or < 14 standard drinks/week for women) [1,2]. This disease, largely unknown few decades ago, affects almost a quarter of adults worldwide ranging from 13.5% in Africa to approximately one third in the Middle East and South America [1–3] and therefore, it is now considered the commonest chronic liver disease. The clinicopathological spectrum of the disease includes the non-alcoholic fatty liver (NAFL) or simple steatosis, the non-alcoholic steatohepatitis (NASH) with an estimated prevalence of 1.5–6.5% in the general population, and cirrhosis along with its complications. Fortunately, the vast majority of patients (approximately 90–95%) suffer from NAFL having stable disease for many years without impaired survival while only about 5–10% have or will develop NASH which can progress in one third of them to severe fibrosis, cirrhosis and hepatocellular carcinoma (HCC) (Fig. 1). However, the speed of progression of patients with NASH to the advanced stages as well as the risk factors which are associated with progression of the disease are not clear yet [1–4]. Nevertheless, modelling of NAFLD epidemic has

Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; ULN, upper limit of normal; NAFLD, non-alcoholic fatty liver disease; AST, aspartate aminotransferase; IgG, immunoglobulin G; ANA, antinuclear antibodies; SMA, smooth muscle antibodies; AIH, autoimmune hepatitis; ^{1}H-MRS, proton magnetic resonance spectroscopy; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; T2DM, type-2 diabetes mellitus; AUD, alcohol use disorder; AASLD, American association for the study of liver diseases; AUDIT, alcohol use disorders inventory test; NFS, NAFLD fibrosis score; FIB-4, fibrosis-4 index; NOSA, non-organ specific autoantibodies; IAIHG, International AIH Group; EASL, European association for the study of the liver; IgA, immunoglobulin A; IgM, immunoglobulin M; IIF, indirect immunofluorescence; anti-SLA/LP, antibodies against soluble liver antigen or liver pancreas; CYP2D6, cytochrome P450 2D6

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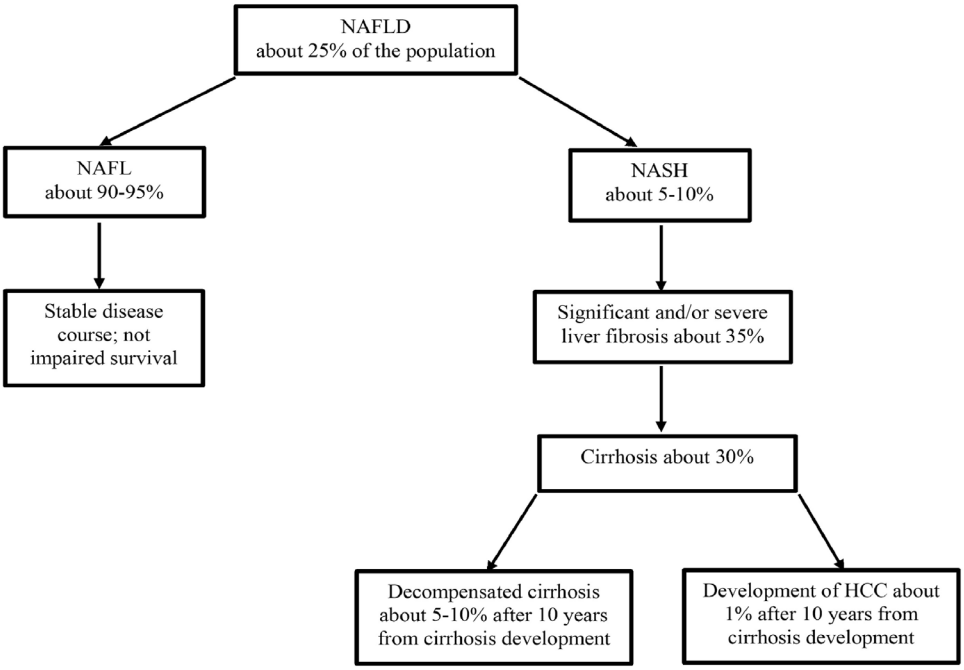

Fig. 1. Proposed natural history of NAFLD. Although the prevalence of NAFLD is high in the general population, most patients suffer from NAFL (simple steatosis) which is associated with stable disease. Only about 5-10% of NAFLD patients have NASH and about 30% of them will develop cirrhosis with an additional risk of decompensation of cirrhosis and development of hepatocellular carcinoma. NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma.

shown that the incidence of decompensated liver cirrhosis, HCC development and liver-related deaths will increase by 168, 137 and 178%, respectively by the year 2030 indicating an underlying nightmare for the health systems all over the world [5].

Risk factors for NAFLD development include all the components of the metabolic syndrome which is defined by at least three of the following five: waist circumference ≥94 cm for men or ≥80 cm for women, history of arterial hypertension or arterial pressure ≥130/85 mmHg, history of established type-2 diabetes mellitus (T2DM) or fasting glucose ≥100 mg/dL, serum triglycerides >150 mg/dl and HDL cholesterol <40 mg/dL for men or 50 mg/dL for women) but also ageing, male sex, ethnicity (e.g. Hispanics), the polycystic ovary syndrome, the obstructive sleep apnoea syndrome and the unhealthy lifestyle such as increased fructose and sugar consumption along with sedentary behaviour [1–4,6–11]. In this context, it has become clear that patients with T2DM bear a very high risk for NAFLD occurrence as the prevalence of NAFLD in this group of patients is as high as of 75% by using transient elastography in combination with the controlled attenuation parameter method [12]. Moreover, this prevalence seems to increase in parallel with obesity in T2DM patients even with normal aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels reaching about 90% in these patients with body mass index (BMI) ≥40 screened by ^{1}H-MRS [13]. However, a subgroup of patients with NAFLD (about 5–10% in the United States and 20% in Europe and Asia) who are lean according to their BMI, is increasingly recognised [14–18]. Although this NAFLD group appears to be more metabolically abnormal than lean individuals without NAFLD, the precise pathogenetic background and pathways which promote and lead to the development of NAFLD in lean subjects have not been clarified yet [14,19]. Lean patients with NAFLD usually have less comorbidities related to the metabolic syndrome and therefore, it is believed that would follow a relatively benign disease course [15,17]. However, although the long-term prognosis of lean NAFLD is unclear, recent data challenge this concept. Indeed, a recent study on 646 patients with biopsy-proven NAFLD (19% with lean NAFLD) showed that although these patients had lower stages of fibrosis, they were at higher risk for the development of severe liver disease compared to overweight or obese patients with NAFLD irrespective of several confounders [16]. Nevertheless, the same study showed that lean patients had no increased risk for overall mortality during a median follow-up of approximately 20 years [16].

The diagnosis of NAFLD can be challenging as reliable laboratory tests to confidently diagnose or exclude it are missing. As a result, any unexplained increase of liver enzymes could be due to NAFLD. In clinical practice, NAFLD diagnosis requires the presence of hepatic steatosis by imaging studies or histology and the absence of several secondary causes of fat accumulation in the liver paying particular attention to the daily alcohol consumption (Table 1) [1,2,20]. According to the recent practice guidance for the diagnosis and treatment of alcohol-related liver diseases from the American Association for the Study of Liver Diseases (AASLD), alcohol drinking above the limits indicated previously are almost definitive of alcohol use disorder (AUD) and potentially supportive of an underlying alcohol-related liver disease and not NAFLD [1–3,21]. In this regard, the presence of AST/ALT ratio >1 in non-cirrhotic patients, disproportionate increase of gamma-glutamyl transpeptidase and/or macrocytosis (high mean corpuscular volume) might be of clinical value in an attempt to discriminate between AUD and NAFLD. However, it is nowadays well known that many patients with AUD may also have metabolic risk factors that probably contribute to the progression of liver disease suggesting an overlap of alcohol-related liver disease and NAFLD. Indeed, the coincidence of drinking habits above safe limits and metabolic risk factors was recently recognized suggesting a serious net result in liver disease severity usually by a more synergistic rather than additive effect of these risk factors [22–25]. Confirmatory to these considerations is the result of a recent study from the United States which showed a remarkable overlap between alcohol-related steatohepatitis and NASH [26]. Nevertheless, as the diagnosis of alcohol-related liver diseases depends largely on the

Table 1
Secondary causes of fat accumulation in the liver.

Alcohol-related fatty liver disease*
Steatogenic drugs (e.g. amiodarone, methotrexate, corticosteroids, valproate)
Chronic hepatitis C (especially genotype 3)
Wilson's disease
Hypopituitarism, hypothyroidism
Starvation, parenteral nutrition
Hypo or abetalipoproteinemia
Inborn errors of metabolism

* Alcohol Use Disorders Inventory Test (AUDIT) and its shorter form AUDIT-C are strongly recommended in an attempt to identify alcohol misuse.

information taken by the patients and/or their families, physicians should be aware to perform a non-judgmental interview in an attempt to minimize underreporting, denial of AUD and uncover alcohol misuse. In these circumstances the use of structured, validated screening tools like the Alcohol Use Disorders Inventory Test (AUDIT) and its shorter form AUDIT-C are still strongly recommended as they are very helpful in identifying alcohol misuse [27–29]. The same in depth investigation should be performed in order to exclude the long-term use of any herbal or non-prescribed supplements, over-the-counter medications or "health foods", weight loss and body building supplements or herbal remedies used as "joint pain remedies" that potentially could induce drug-induced fatty liver disease [1,2]. In other words, physicians should be very careful in excluding AUD and alcohol-related liver disease or steatogenic medications, acting actually as the inspector *Hercules Poirot* and *Miss Marple* of the English crime novelist Agatha Christie before they made a firm diagnosis of NAFLD.

Ultrasound of the liver is still the preferred diagnostic procedure for NAFLD, as it is non-invasive, cost-effective and provides additional information although, ^{1}H-MRS is the only method that can assess quantitatively the liver fat but as it is expensive and not readily accessible outside of research activities, it is not recommended in the everyday clinical setting [1,2,20]. In busy office practices, the use of pocket-sized ultrasound devices by well-trained physicians could be served as point-of-care screening for NAFLD but more validated data on this issue is needed [30]. Other non-invasive methods and scores such as NAFLD fibrosis score (NFS), fibrosis-4 index (FIB-4), vibration controlled transient elastography and MR elastography can be used to identify those NAFLD patients who are at low or high risk for advanced fibrosis [1,2]. On the contrary, diagnosis of NASH still requires a liver biopsy showing all three of the following lesions: steatosis, hepatocyte ballooning and lobular inflammation. Liver histopathology gives us several other important information such as the fibrosis stage of the disease which has proved to be the most important prognostic factor in NAFLD cases as it is independently associated with the liver-related outcomes and mortality [31]. In addition, although liver biopsy is expensive, requires experienced pathologist for interpretation, bears the problem of sampling error and has some morbidity and very rare mortality risk, it should be emphasized that it is invaluable in suspected NAFLD cases if competing aetiologies for liver steatosis and the presence of other coexisting chronic liver disease have not been excluded. Under this context, statistically as in the general population, 20-30% of any liver disease is likely to be affected by NAFLD especially by NAFL and to a lesser extent by NASH including autoimmune liver diseases [32–36].

2. Epidemiology and overview of autoimmune hepatitis characteristics

Autoimmune hepatitis (AIH) is a relatively rare liver disease (20–25 cases/ 100,000 population in Europe) of undefined aetiology characterized by female predominance (male to female ratio: 1 to 3–4) and significant heterogeneity at the epidemiological, clinical, genetic, serological and histological levels (Table 2) [37–44,45–47]. Although in the past there were several preconceptions such as that is a very rare disease and exclusively a disease of children and young females, it is now clear that the distribution of AIH is worldwide, covering all ages (about 30% above 60 years), both sexes (approximately 30% males) and all ethnic groups while its prevalence is increasing in both females and males [38,39,42,44,47–49]. Approximately 20–25% of patients present with an episode of acute hepatitis either as an acute exacerbation of previously undiagnosed and/or misdiagnosed AIH or as an original (genuine) acute onset of AIH without chronic lesions at the histological level. The rest patients present with diverse non-specific symptoms most of which are dating back for years or are completely asymptomatic (Table 2). Another characteristic of AIH is the common association with other autoimmune diseases in the index patient or the first degree relatives. Therefore, AIH patients can be considered for screening both at baseline and during follow-up for concurrent extrahepatic autoimmune diseases, in particular Hashimoto's thyroiditis, in attempt to manage the patient as a whole [38–41,50–52]. Because of this variability of the clinical spectrum of the disease, a delay of diagnosis is common and therefore, physical findings can vary from completely normal to the presence of advanced liver disease (almost one third of adults and half of children have already established cirrhosis at initial diagnosis) [38–42,44,48,49].

Its diagnosis is based on several clinico-pathological features namely, hypergammaglobulinaemia with characteristic elevation of immunoglobulin G (IgG) in most cases, presence of non-organ specific autoantibodies (NOSA), interface hepatitis on liver histology, absence of viral hepatitis markers, and a favourable response to immunosuppression [37–41,43,53]. However, the outcome of AIH, if undiagnosed and untreated, is not favourable leading frequently to cirrhosis, hepatocellular failure and death. The presence of NOSA and interface hepatitis (also called piecemeal necrosis) with characteristic dense plasma cell-rich lymphoplasmacytic infiltrates on liver biopsy are the two important hallmarks for AIH diagnosis [37–41,43,50,53,54]. At the histological level, two other findings such as, the presence of emperipolesis and hepatocellular rosetting are also regarded as typical for AIH diagnosis [37–41,43,54]. However, in the genuine cases with acute severe or even fulminant AIH the histological lesions are different and quite identical to that observed in drug induced liver injury making the diagnosis much more complex and difficult [38–43,55,56]. Of note, neither NOSA nor liver histology bear any truly disease-characteristic finding for the diagnosis of AIH while IgG increase is not found in all patients making also sometimes a timely and correct diagnosis quite problematic. Furthermore, in asymptomatic cases or those who have an insidious onset of the disease bilirubin, AST and ALT values vary from just above the upper limit of normal (ULN) to very high levels adding more challenge and difficulty in the roadmap of a prompt and timely diagnosis of AIH. In other words, similar to NAFLD, any increase of liver enzymes could be due to AIH because there is a lack of reliable laboratory tests to confidently diagnose or exclude AIH [57].

Nevertheless, there are important tools for the diagnosis and study of AIH as attested by the diagnostic scores established by the International AIH Group (IAIHG). The first score was reported in 1993 and subsequently revised in 1999 including descriptive criteria for AIH diagnosis either as "definite" or "probable" based on a numeric scoring system [58,59]. In 2008, the revised score was simplified further in order to be much more friendly in everyday practice (Table 3) [37]. Indeed, contrary to the previous scores, the latter simplified score was specifically designed in an attempt to help at the bedside and not mainly for scientific purposes [60,61]. However, because of poor validation of the abovementioned scores in children, different scores have been proposed recently for the pediatric population [62]. It should also be emphasized that as there is no single gold standard for AIH diagnosis, these diagnostic scores can help to pay attention to a frequently under-recognized and underdiagnosed disease but because of the huge heterogeneity of AIH, making an AIH diagnosis will remain an art despite the development of the improved scoring systems [57–65].

3. NAFLD and AIH: to be, or not to be, that is the question for diagnosis

The potential coincidence of NAFLD and AIH or an AIH diagnosis alone instead of NAFLD represent a challenge for clinicians, both in making a prompt and timely diagnosis but also in the management of these diseases. As stated above, any liver disease including AIH, can be affected by NAFLD in almost 25% of patients because NAFLD has become the commonest liver disease affecting about 25% of the general population [32–36]. Although this is in absolute contrast with the traditional medical school dictum that only a single diagnosis should be investigated for a symptom or for abnormal laboratory values, it seems

Table 2
Main clinical, demographic and laboratory characteristics of patients with autoimmune hepatitis (AIH).

Characteristic	
Sex ratio	Female: Male (3-4:1)
Age at initial presentation (diagnosis)	Any age of all ethnic groups (usually bimodal distribution in puberty and between 4th and 6th decade; one third of patients are older than 60 years)
Clinical manifestations	From asymptomatic to acute severe or even fulminant hepatitis; about 20–25% of patients have acute onset disease because of either an acute exacerbation of previously undiagnosed and/or misdiagnosed AIH or a genuine acute AIH without chronic lesions at the histological level The remaining patients have either an insidious onset (one or more of the following unspecific symptoms: malaise, fatigue, amenorrhea, general ill health, lethargy, anorexia, right upper quadrant pain, weight loss, nausea, jaundice and arthralgias usually involving the small joints) or are completely asymptomatic At diagnosis approximately one third of adult patients and half of children have already cirrhosis suggesting a considerable delay of diagnosis
Classification	AIH type-1: 90% of cases; ANA, SMA or anti-SLA/LP positivity (the latter antibody often with the presence of antibodies against Ro52 antigen; characterises the need for permanent immunosuppression) AIH type-2: about 10% of cases; anti-LKM1, anti-LC1 and rarely anti-LKM3 reactivity; onset in childhood and young adulthood; usually more acute and advanced; frequent treatment failures and frequent relapses after drug withdrawal
Physical examination	It varies from entirely normal to signs of advanced chronic liver disease
Specific characteristics	AIH can occur during pregnancy or more common after postpartum; in established AIH, the disease lessens during pregnancy but postpartum exacerbations are common; maternal and pregnancy outcomes are not different to the general population AIH can develop after liver transplantation for other conditions (*de novo AIH)* AIH can develop after use of drugs, supplements, herbals or biologics including TNF-alpha blockade agents and interferons (very difficult to differentiate from drug induced liver injury) AIH can develop after viral infections (it should be strongly taken into account in cases with previous documented viral infections followed by unidentified and prolonged elevation of aminotransferases)
Aminotransferases and bilirubin	May be slightly or extremely elevated (> 50x ULN)
Cholestatic enzymes	Normal or slightly elevated
IgG	Elevated in ~85% even in the absence of cirrhosis; the presence of "normal" IgG levels does not preclude an AIH diagnosis
Autoantibodies	ANA 70–80%; SMA 70–80%; anti-LKM1 3–5%; anti-SLA/LP 15–30%; pANCA up to 90% often atypical
Extrahepatic autoimmune diseases	Grave's disease, Hashimoto thyroiditis, Vitiligo, Alopecia, Rheumatoid arthritis, Diabetes mellitus type 1, Systemic lupus erythematosus, Psoriasis, Celiac disease, Sjögren's syndrome, Panniculitis, Mononeuritis, Urticaria pigmentosa, Sweet's syndrome, Idiopathic thrombocytopenic purpura, Polymyositis, Haemolytic anaemia, Uveitis, Autoimmune polyendocrinopathy syndrome-type 1

Abbreviations are same as in the text. anti-LKM1, antibodies against liver kidney miscrosomal antigens type 1; anti-LC1, antibodies against liver cytosol antigens type 1; anti-LKM3, antibodies against liver kidney miscrosomal antigens type 3; TNF-alpha, tumor necrosis factor alpha

Table 3
Simplified criteria for autoimmune hepatitis diagnosis (adapted from [1]).

Characteristic	Finding	Score
ANA or SMA pos	≥ 1:40	+1
ANA or SMA pos	≥ 1:80	+2*
or anti-LKM pos	≥ 1:40	
or anti-SLA/LP pos	positive	
Liver histology (presence of hepatitis is necessary)	Typical AIH**	+2
	Compatible with AIH**	+1
	Atypical**	0
Serum IgG levels	> Upper normal limit	+1
	> 1.1 Upper normal limit	+2
*Absence of viral hepatitis****	Yes	+2
	No	0
Sum		≥ 6: probable AIH ≥ 7: definite AIH

Abbreviations are same as in the text.

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

** Presence of typical lesions means the detection of each of the following: interface hepatitis, lymphocytic/lymphoplasmacytic infiltrates in portal tracts and extending into the lobule, emperipolesis (active penetration by one cell into and through a larger cell), and hepatic rosette formation; Compatible liver histology: chronic hepatitis with lymphocytic infiltration without all the features considered typical; Atypical: histological lesions supporting another diagnosis.

*** In chronic cases absence of hepatitis B and C viral markers; in acute cases absence of serological markers of acute hepatitis A, B, C, D and E is needed. ANA or SMA detection is referred to the use of indirect immunofluorescence assay on rodent tissues sections not ELISA.

real in the case of NAFLD and autoimmune liver diseases. Why this happens? Although it is difficult to address this question in a straightforward way, there are many potential answers. First, physicians and pathologists are much more familiar with a very common disease like NAFLD so, in patients with typical signs of metabolic syndrome, negative history of alcohol abuse or hepatotoxic drugs and herbals consumption along with a negative hepatitis viral testing, the presence of increased liver enzymes are interpreted as an expression of NAFLD without considering an alternative or additional diagnosis. The net result is a delayed diagnosis of AIH in those suffering from NAFLD/AIH variant compared to patients with only AIH as amazingly showed by De Luca–Johnson report [33]. This delay has a serious clinical impact as significantly more patients from the NAFLD/AIH group had cirrhosis at baseline liver biopsy compared to patients with AIH alone strongly suggesting the need of careful expert evaluation of liver biopsies even if the diagnosis of NAFL/NASH or AIH appears obvious on the basis of medical history and laboratory results [33].

Second, the situation is much more complicated as there is a lack of reliable laboratory tests to diagnose or exclude NAFLD or AIH while the presence of NOSA has been detected in about 12–48% of NAFLD patients [66,67]. In this context, it should be noted that apart from ANA and SMA, most –if not all- of NAFLD/NASH studies do not investigate the patients for all autoantibodies involved in AIH diagnosis [37–41,53], apply the diagnostic scoring systems of the IAIHG [37,59] or address the possibility of features of AIH at the histological level [37–41,43,55,57]. In this regard, however, it is worthy to underscore that the IAIHG scoring systems have been developed rather to diagnose AIH and increase the discrimination from other liver diseases than to look for common features making therefore, their performance on the diagnosis of NAFLD/AIH coexistence quite problematic [60,63–65,68].

Third, the recent European Association for the Study of the Liver (EASL) and AASLD practice guidelines for the diagnosis and management of NAFLD [1,2] do not mention clearly the importance of IgG determination and liver autoimmune serology according to the AIH guidelines [37–39] in the diagnostic flow-chart to evaluate and follow disease severity in case of suspected NAFLD and metabolic risk factors

[57]. On the other hand, immunoglobulin A (IgA) but not IgG or immunoglobulin M (IgM), is elevated in almost half of NAFLD patients –in particular in those with NASH- and seems to be associated with advanced fibrosis [69–71]. A recent study in almost 5000 adult patients with NAFLD confirmed the abovementioned findings showing also decreased or normal IgG and IgM levels [72]. However, contrary to adults, IgA was neither elevated (only in 4% of patients), nor a biomarker of disease severity in children with NAFLD [73]. It should be emphasised that detail guidelines from the IAIHG on how to test for autoantibodies relevant to AIH have already been published including guidelines for the substrates particularly regarding the orientation and cutting of kidney sections, application of samples, optimal dilution, revealing reagents labelled by fluorochrome, selection of controls and the diagnostic staining patterns [37–41,53,74,75]. Indeed, indirect immunofluorescence (IIF) on fresh frozen sections of about 4-8 weeks store of a multi-organ substrate from rodents, is the ideal preferred first-line screening for the detection of autoantibodies related to AIH but also for other autoimmune liver diseases like primary biliary cholangitis [38–41,50,53,57,59,62,74–76]. In addition, investigation using molecular based assays and not IIF (ELISA or immunoblot) for antibodies to soluble liver antigen or liver pancreas (anti-SLA/LP), which are detected in about 20–30% of AIH patients and seem to characterise patients in whom life-long immunosuppression is required, should be included in first-line screening [37–41,53,74,75,77]. The use of immobilized HEp2 cells as substrate detecting only antinuclear antibodies (ANA), smooth muscle antibodies (SMA) and potentially antimitochondrial antibodies should be avoided in case of suspected AIH because of a high frequency of false-positive results.

However, under real-life circumstances the development of validated sections for IIF at the local level for everyday use is not very feasible making the use of equivalent sections of commercial origin inevitable but clinicians and laboratory personnel should be aware that these slides are of variable quality as, to lengthen shelf-life, they are treated with fixatives, which can result in enhanced background, making the interpretation of IIF patterns potentially difficult. Another difficulty in achieving a correct diagnosis in suspected AIH cases either alone or in combination with NAFLD is regarding the NOSA titres which may vary during the AIH course. So, the physicians should be aware that low titres do not exclude the diagnosis of AIH, nor do high titres in the absence of other supportive findings establish the diagnosis. Moreover, negative results on a single NOSA testing cannot exclude AIH and repeated determinations may be required. Finally, an additional problem is that several laboratories ignore the IIF cut-offs that are recommended by the IAIHG and EASL for children and adults [37–39,62] and by using their own higher cut-offs expand the number of “negatives” leading to a further underestimation of AIH and delay of its diagnosis. Taken together, the final net result could be a rapid progression of AIH if undiagnosed or misdiagnosed and untreated explaining also probably why almost one third of the adults and half of children up to 18 years of age have already established cirrhosis at diagnosis [38–42,44,48,49].

In summary, the diagnosis of patients with AIH either alone or in combination with NAFLD may be quite difficult and complicated because of the wide range of disease manifestations and severity while it should be kept in mind that although NAFLD is by far the most frequent chronic liver disease this could not be always the case.

4. Putative connection between NAFLD and AIH and characteristics of NAFLD/AIH variant

Autoimmune liver diseases may coexist or develop in any kind of chronic liver disease as for example we have already reported in cases with chronic hepatitis B, C or other viral infections and sometimes irrespective of the administration of the old treatments with interferon-a [78–85]. In this context, the hypothetical connection between NAFLD and liver autoimmunity is much less obvious. In a nice Japanese study in 54 consecutive patients with histologically proven NASH, it was found that almost half of patients were positive for liver autoimmune serology testing but most importantly, these positive patients had also features of AIH or primary biliary cholangitis as attested by the significant more severe portal inflammation compared to patients with NASH alone [67]. In addition, these patients were significantly older, much more obese and predominantly women. The investigators explored further their findings in experimental animal models of NASH by using female mice from three different models of NASH. Actually, they evaluated in a complementary way the monosodium glutamate-induced, the choline-deficient L-amino acid defined and the Tsumura Suzuki, obese diabetes mice and they found that almost one third of mice, regardless of the model used, manifest autoimmune features at the histological level [67]. Of note, in parallel with the above findings, obesity has been considered as a risk factor for the occurrence of several autoimmune diseases such as, multiple sclerosis, psoriasis and rheumatoid arthritis [85]. Moreover, obesity seems to affect negatively the course of psoriasis, rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease while it impairs the treatment response of these diseases [85].

Recently a German group tried to shed more light on the putative connection between NAFLD and AIH [86]. Actually, they evaluated how the metabolic liver injury after a high-fat diet could affect AIH pathogenesis in the well-established cytochrome P450 2D6 (CYP2D6) animal model of AIH. The authors were able to prove quite convincingly that pre-existing NAFLD in the CYP2D6 mouse model of AIH resulted in significant strengthening of AIH severity by increasing the frequency of liver autoantigen-specific T cells [86]. So far, it is not clear how this occurs but several key mediators of NAFLD pathogenesis such as, adipokines, cytokines and oxidative stress could be appropriate candidates for inducing the immune tolerance breakdown in susceptible individuals with NAFLD [87–89].

Nevertheless, whatever is the connection between NAFLD and AIH little is known about the characteristics of patients with coincidence of NAFLD and AIH. To date, there is only a large retrospective report from Japan including 1151 subjects with AIH [35]. This report apart from an overall prevalence of NAFLD of about 17%, gave important information on laboratory determinants, liver histology, therapy and complications along with information regarding the laboratory findings before and after treatment. In fact, it was shown that AIH patients with NAFLD had the following characteristics compared to AIH alone (Table 4a). First, they had low female to male ratio and they were older; second, they had lower increase of liver function tests; third, they had progressive

Table 4a
Characteristics of NAFLD/AIH patients compared to AIH patients alone (adapted from [46]).

Characteristic	NAFLD/AIH variant (n = 196)	AIH alone (n = 955)	P value
Females (%)	82.7	88.4	0.033
Age (median, range, years)	64 (55–70)	61 (51–69)	0.004
Cirrhosis at diagnosis (%)	7.8	6.1	NS
AST (median, range, U/L)	112 (57–253)	239 (97–556)	<0.001
ALT (median, range, U/L)	126 (59–279)	285 (109–643)	<0.001
ALP (median, range, U/L)	351 (275–486)	439 (321–611)	<0.001
γ-GT (median, range, U/L)	124 (74–244)	161 (87–271	0.016
Bilirubin (median, range, mg/dL)	0.9 (0.7–1.6)	1.2 (0.8–3.1)	<0.001
IgG (median, range, mg/dL)	2108 (1755–2782)	2178 (1764–2794)	NS
ANA pos (%)	91.8	89	NS
SMA pos (%)	33.9	43.2	NS
Extrahepatic autoimmunity (%)	22.2	26	NS

Abbreviations are same as in text; NS, not statistically significant; γ-GT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase.

fibrosis, mild plasma cell infiltration and/or mild lobular hepatitis but not difference on the prevalence of cirrhosis at diagnosis and fourth, the frequency of prednisolone administration was lower while they had higher frequency of ursodeoxycholic acid administration [35]. However, there was no data on the outcome, the BMI of patients as well as how many had simple steatosis (NAFL) or NASH.

In the western countries and United States, there is only a small retrospective study in 73 consecutive AIH patients which showed that 16% of patients (n = 12) had both NASH and AIH at the histological level while additional 10 patients (14%) had simple steatosis [33]. Of interest, statistically significant proportion of patients with NASH/AIH coincidence had cirrhosis at the initial liver biopsy compared to those with AIH only (50% vs. 18%; p = 0.03). Although these findings are of outmost importance it should be noted that are based on only 22 NAFLD/AIH patients and therefore, general conclusions cannot be drawn safely.

In this context, the IAIHG designed a large multicentre retrospective study which is underway to further clarify this topic. So far, 583 patients with well-established AIH have been included paying particular attention to the characteristics of NAFL and NASH on liver biopsies. An interim analysis showed that NAFLD was present in 126/583 (21.6%) of patients including 107/583 (18.4%) with NAFL/AIH variant (64/107 patients with steatosis only and 43/107 patients with steatosis and lobular inflammation) and 19/583 (3.3%) with histologically proven NASH [36]. These rates of prevalence are within the expected frequencies in the general population and therefore unsurprising. As also expected, patients with NAFLD/AIH variant suffered significantly more frequently from hypertension, T2DM and obesity compared with those with AIH alone while they were less likely to be anti-SLA/LP positive (Table 4b) [36]. Similar to the Japanese study [35], patients with NAFLD/AIH variant were significantly older, had higher triglycerides levels, no female predominance and lower AST, ALT and bilirubin levels (Table 4b). In addition, they had significantly higher BMI and lower simplified score for AIH diagnosis (Table 4b) but no differences regarding the IgG levels, ANA and SMA detection or the presence of cirrhosis according to the initial index liver biopsy.

5. Management and outcome of NAFLD/AIH variant

Unfortunately, very little is known about the outcome of this very important subgroup of AIH. Indeed, the recent large Japanese study did not investigate the prognosis and outcome of these patients. The only parameter that was included in that study was the evaluation of the development of hepatic and extrahepatic malignancies that did not differ between the two groups (with and without NAFLD) [35]. To the contrary, the small study from the United States showed that patients with NASH/AIH coincidence but not with NAFL are more likely to present adverse clinical outcomes and decreased survival [33]. Actually, it was shown that the 12 patients with NASH/AIH had a significantly increased relative risk of 2.5 for unfavourable liver related outcomes and 7.6 for liver related mortality compared to the 51 patients with AIH only. This result could be due to a diagnostic delay which usually accompanies these cases, the pre-existing advanced fibrosis stage (half of NASH/AIH patients had cirrhosis at initial evaluation) and the complexity of individualised management of AIH patients with concurrent NASH as they may not have received the appropriate dose of immunosuppressive agents to achieve long-term remission because of the presence of the metabolic syndrome. On the other hand, the first analysis of the recent large study from the IAIHG [36] showed that response to treatment, progression to cirrhosis during follow-up and liver related deaths or liver transplantation were identical between the two groups. However, the low number of AIH patients with concurrent NASH (19/583; 3.3%) could be responsible for these favourable outcomes [36] which are in parallel with the findings observed in two recent studies from Canada and Greece after the investigation of the potential impact of concurrent NAFLD in 236 and 482 patients with primary biliary cirrhosis, respectively [90,91]. Of note, decompensation of already established cirrhosis during follow-up seemed to be significantly more frequent in patients with NAFLD/AIH variant compared to those with AIH alone (54% vs. 32%, respectively; $p = 0.001$) which may suggest the need of closer follow-up and surveillance in NAFLD/AIH patients with established cirrhosis at diagnosis [36].

Clear practice guidelines for managing patients with the NAFLD/AIH variant and in particular of the NASH/AIH subgroup are missing. However, when managing patients with NAFLD/AIH it is important to consider both diseases in an attempt to have a holistic management of the index patient (Fig. 2). According to the international guidelines for AIH management [38,39,92], an induction therapy is needed initially followed by maintenance treatment with a steroid sparing drug (e.g. azathioprine, 6-mercaptopurine or mycophenolate mofetil). Induction therapy with high dose prednisolone (usually 1 mg/kg/day) will affect negatively diabetes and all the factors of the metabolic syndrome in these patients. Alternatively, a more rapid tapering schedule of steroids, lower doses of corticosteroids during induction or the use of budesonide (a steroid that is rapidly metabolized with low systemic exposure) at initial induction treatment in those who have no established cirrhosis along with an early addition of the steroid sparing agents could be a rationale option. Indeed, a recent multicentre retrospective analysis of 451 AIH patients from 5 European countries showed that lower predniso(lo)ne doses (<0.5 mg/kg/day) resulted in similar rates of transaminases normalization compared to high doses (≥0.5 mg/kg/day) [93] while oral budesonide at a dose of 9 mg/day in association with azathioprine, proved to induce and maintain remission in AIH patients without cirrhosis, bearing in parallel a low frequency of corticosteroids-specific side effects [94]. In our experience, corticosteroids in the short term, even at the usual high doses recommended by the respective authorities in AIH, seem not to bear a significant adverse effect in NAFLD/AIH patients taking into account however, that the patients have been trained and agreed to the treatment adherence in an attempt to manage efficiently both diseases. In other words, in NAFLD/AIH patients who are under immunosuppression strict and intense adherence to the management of all of the components of the metabolic syndrome is strongly required along with lifestyle modifications such as energy restriction, avoidance of fructose-containing foods and drinks, complete avoidance of alcohol or daily alcohol intake strictly below the safe limits, adherence to Mediterranean diet and more physical activity

Table 4b
Characteristics of patients with NAFLD/AIH variant compared to AIH patients alone (adapted from [47]).

Characteristic	NAFLD/AIH variant (n = 126)	AIH alone (n = 457)	P value
Females (%)	71	76	NS
Age (mean, SD, years)	50.2 ± 15.9	44.8 ± 18.7	0.004
AIH simplified score	6.7 ± 1.4	7 ± 1.3	0.02
Cirrhosis at diagnosis (%)	22.7	19.5	NS
Obesity (%)	48	24	<0.001
Arterial hypertension (%)	42	25	<0.001
Diabetes mellitus (%)	32	17	<0.001
BMI (kg/m^2)	30.2 ± 7.1	27 ± 6.9	<0.001
Triglycerides (mean, SD, mg/dL)	137.8 ± 71.6	121.7 ± 67.4	<0.05
AST (mean, SD, U/L)	364.9 ± 574.9	539.9 ± 629.3	<0.05
ALT (mean, SD, U/L)	396.6 ± 536.4	599.5 ± 642	<0.05
ALP (mean, SD, U/L)	148 ± 114.3	188.2 ± 159.8	<0.05
Bilirubin (mean, SD, mg/dL)	3.7 ± 6.4	5.3 ± 7.2	<0.05
anti-SLA/LP pos (%)	6	17	0.005

Abbreviations are same as in text; SD, standard deviation; NS, not statistically significant; ALP, alkaline phosphatase.

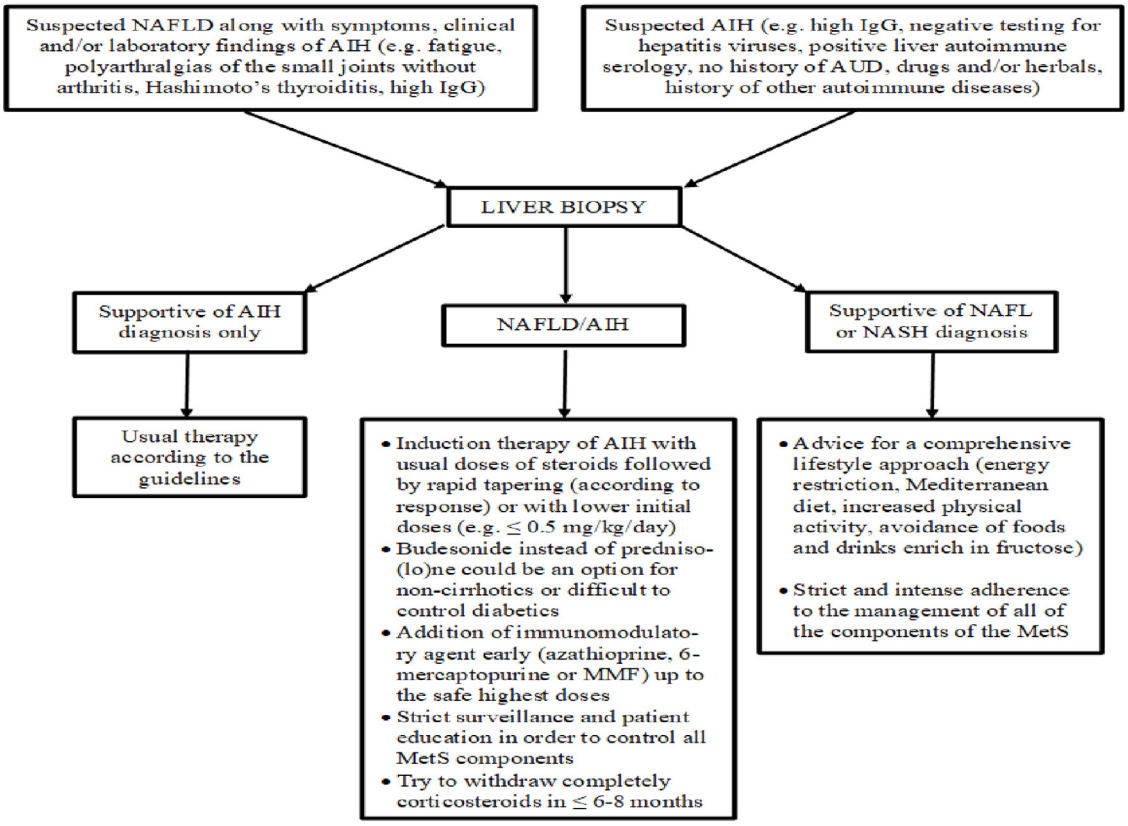

Fig. 2. Proposed diagnostic and treatment algorithm for NAFLD/AIH patients. NAFLD, non-alcoholic fatty liver disease; AIH, autoimmune hepatitis; AUD, alcohol use disorder; NASH, non-alcoholic steatohepatitis; MetS, metabolic syndrome; MMF, mycophenolate mofetil.

(e.g. 150 − 200 min of brisk walking/week in 3 − 5 sessions). However, corticosteroids treatment in the long term (e.g. above or equal of 10 mg/day more than 6–8 months in cases with inability to taper out corticosteroids completely) can be quite detrimental and therefore, closer blood glucose surveillance and modifications of T2DM and arterial hypertension medications seem of major importance.

6. Summary and Conclusions

There is no doubt that NAFLD is by far the most common chronic liver disease in the world (about 25% of the general population). This assumption means that statistically approximately 25% of any particular chronic liver disease could be affected by NAFLD. Primary care internists, general practitioners and hepatologists as well as, clinicians and hepatopathologists need to be aware of this probability including AIH in order to avoid misdiagnosis of patients with coincident NAFLD and AIH as suffering only from NAFLD. AIH diagnosis is no longer a diagnosis of exclusion, but should be based on the medical history, the laboratory findings, the liver autoimmune serology testing, the histological lesions and sometimes in case of uncertainty, in parallel with an evaluation of a course of immunosuppressive treatment [37–41]. Nonetheless, the final diagnosis of AIH in patients with obvious underlying metabolic syndrome remains a challenge both for physicians and pathologists because there is not a single laboratory test to confidently diagnose or exclude either NAFLD or AIH. However, the presence of hypergammaglobulinaemia, strong history of other autoimmune disease –in particular autoimmune thyroiditis- in the index patient or first degree relatives and symptoms of polyarthralgias could support a careful evaluation for AIH presence in patients with metabolic syndrome (Fig. 2).

On the other hand, the impact on outcome and response to treatment of NAFLD in well-established AIH cases is largely unknown although there is evidence that obesity worsens other autoimmune diseases while pre-existing NAFLD potentiate the AIH severity in the experimental animal model of AIH (CYP2D6 mouse model) [85,86]. In a small retrospective study from the United States has been shown that patients with NASH/AIH variant but not patients with NAFL/AIH coincidence are more likely to present with cirrhosis at diagnosis and more likely to have adverse clinical outcomes with decreased survival [33]. These findings were not confirmed in two very large studies from Japan and the IAIHG [35,36]. The latter two studies showed however, that patients with NAFLD/AIH variant are older, have higher BMI, no female predominance, and lower AST, ALT, ALP and bilirubin levels compared to those with AIH alone. Official clinical practice guidelines for the management of NAFLD/AIH variant are missing and therefore, there is an unmet need for future research in pathogenesis and more effective treatments. Until these studies come to final and firm conclusions, a rational treatment approach is suggested schematically in Fig. 2 based on the parallel management of these two conditions which bear high liver related morbidity and mortality.

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Declaration of Competing Interest

The authors declare they have no conflict of interest.

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