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Review Article

Seronegative Autoimmune Hepatitis

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Abstract

Autoimmune hepatitis (AIH) is a relatively rare liver disease with varying worldwide incidence of from 0.7 to 2 per 100,000 people. It is characterized by the presence of auto-antibodies. However, an average of 10% of AIH cases have AIH symptoms and pathology but lack autoimmune serology. For such seronegative AIH (snAIH) cases, there is currently no established diagnostic algorithm for diagnosis. and improper or delayed diagnosis of snAIH can lead to no or inappropriate treatment that results in progression to fulminant hepatitis or cirrhosis. This review aims to review the current literature and to present an update of seronegative autoimmune hepatitis, including its pathophysiology, clinical presentation, methods of diagnosis, and treatment in order to increase awareness and emphasize the necessity for timely management.

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Introduction

Autoimmune hepatitis (AIH) is an insidious inflammatory disease of the liver that affects 1 in 200,000 people annually in the USA. [1–3] AIH has a bias for women at all ages. The diagnosis of AIH is established through scoring systems developed by The International Autoimmune Hepatitis Group (IAIHG) exclude other diseases and histological features that reflect a chronic inflammatory state. [1] The presentation of AIH can overlap with other autoimmune conditions, complicating efforts to make a diagnosis. [4] In addition to liver biopsy data, serological confirmation of the presence autoantibodies has been helpful in screening and confirming the diagnosis of seropositive autoimmune hepatitis (spAIH). Studies have demonstrated a wide range of 7–34% and an average of 10% of AIH patients who do not present with auto-antibodies. [5–9] Because of the lack of auto-antibodies for screening, seronegative autoimmune hepatitis (snAIH) is underdiagnosed. A liver biopsy is required to make the diagnosis, and that further delays initiation of treatment. [1, 9] Furthermore, there is no established algorithm for diagnosing snAIH. [9] The aim of this report is to review the literature on proposed pathogenesis, presentation, diagnosis, and management of snAIH and propose a potential workup for its diagnosis.

Clinical presentation

As in AIH, the presentation of snAIH ranges from asymptomatic to debilitating symptoms including fulminant hepatic failure. [6, 10] snAIH often present with nonspecific symptoms of varying severity, such as fatigue, lethargy, abdominal pain, and arthralgia involving small joints. Acute and severe presentations occur in less than 7% of cases. However, acute presentations may present covertly as well, with normal serum gamma globulin levels, low pretreatment international diagnostic scores, but with histopathology demonstrating centrilobular zone 3 necrosis consistent with snAIH. [9] Fatal presentations were associated with viral-like prodromal symptoms including jaundice, nausea, vomiting, and abdominal pain. [2, 3] In one study, patients initially presented at an outpatient clinic initially, inferring that cases of very severe hepatitis were admitted directly to the hospital and were not included in the study, possibly biasing the clinical and laboratory outcomes. [2] Chronic presentations have been reported to range widely from 1–34% of cases. [11, 12] snAIH has also been association with other autoimmune diseases whose manifestations may also be present. [3, 6]

Pathogenesis

AIH is thought to result from the development of genetic variants in products that regulate B and T cell peripheral immune tolerance, contributing to a breach in B cell tolerance. That results in autoantibodies, and cell-mediated organ damage. [12] The proposed pathophysiology of AIH includes genetically predisposed individuals exposed to an environmental trigger such as drugs and viruses, that elicit T cell-mediated autoimmunity, circulating autoantibodies, and hyperglobulinemia. The exact mechanism involved in the pathogenesis of snAIH is unknown. Given the

Keywords: Seronegative autoimmune hepatitis; Autoantibody negative; Autoimmune hepatitis; Cryptogenic hepatitis.

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine transaminase; ANA, antinuclear antibodies; anti-ASGPR, anti-asialoglycoprotein receptor; anti-dsDNA, anti-double-stranded DNA; anti-LC1, anti-liver cytosol 1; anti-LKM-1, anti-liver and kidney microsomes; anti-LKM-, anti-liver kidney microsomal 3; anti-SMA, anti-smooth muscle antibodies; anti-SLA-LP, anti-soluble liver antigen/liver-pancreas anti-UGT, anti-UDP-glucuronosyltransferase; BAFF, B cell activating factor; IAIHG, International Autoimmune Hepatitis Group; IFN, interferon; MELD, Model for End-stage Liver Disease; pANCA, perinuclear anti-neutrophil cytoplasmic antibody; snAIH, seronegative autoimmune hepatitis; spAIH, seropositive autoimmune hepatitis; TNF, tumor necrosis factor..

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many similarities to AIH, some investigators have proposed that the pathogenetic mechanisms of snAIH are similar to those of AIH. [3] However, the lack of autoantibodies suggests absent or decreased B cell activity with apparent maintenance of increased T cell activity. Although the absence of autoantibodies is the main diagnostic difference, that characteristic may not represent a fundamental difference in pathogenesis, as it may be an epiphenomenon rather than a difference in the core mechanisms of disease. [9] Various theories implicating viral infection have been proposed, but no exogenous viral genomic sequences have been found to be associated with snAIH in pre- and post-inoculation studies in non-human primates. [12] Two main explanations of B cell deviations in seronegative autoimmune diseases have been proposed. Only antibodies that are not detectable with commercially available assay kits are involved, or the titers of typical and atypical antibodies are below the level of detectability because of low production or the presence of blocking antibodies. [9, 13]

Typical and atypical antibodies

Typical autoantibodies used for serological diagnosis of AIH are anti-nuclear antibody (ANA), anti-smooth muscle antibody (SMA, anti-actin), and anti-liver kidney microsomal antibody 1 (LKM-1). Atypical antibodies that may be diagnostically useful in AIH cases when typical antibodies are negative are anti-neutrophil cytoplasmic antibody (pANCA), anti-asialoglycoprotein receptor antibody (ASGPR), anti-liver cytosolic antibody 1 (LC1), anti-soluble liver antigen/liver-pancreas antibody (SLA/LP), anti-liver kidney microsomal antibody 3 (LKM-3, anti-UDP-glucuronosyltransferase (UGT)).

In a study conducted by Wang *et al*., [14] 17 of 167 patients (□10%) with AIH were found to have no autoantibodies which is consistent with snAIH. snAIH patients also had lower serum IgG levels and more advanced histological stages compared with classical AIH patients. This suggests that the lack of positive serologies may be related to differences in overall B cell activity rather than a specific defect in the generation of specific autoantibodies. Strengths of the study lie in its comprehensiveness in describing the presentation, diagnosis, and management of AIH. Other investigations have been limited by a lack of standardized immunofluorescence in laboratories of the studies reviewed. [7, 8] Studies with greater strength in diagnosing snAIH found an absence of even unconventional autoantibodies such as anti-soluble liver antigen/liver-pancreas (SLA-LP). [3, 12, 15] A strength of the review is its sample size, but methodology for excluding studies was not clearly described, leaving open the possibility of publication bias.

Antibodies below the level of detectability

Seronegativity could result from an inability to detect antigen-antibody complexes by current assays because of antigen-antibody complexes, the presence of only antibodies for which commercially tests are not available, or fluctuations in levels in which peaks are missed. [9] Enzyme-linked immunosorbent assays exists for ANA, SMA, LKM-1, LC1, and SLA/LP. As a reflection of detectability, titer cutoffs of >1:40 for both SMA and ANA have been reported. Anti-SLA/LP is highly specific for the diagnosis of AIH and correlates with worse prognosis. [16] Mean titer values for that autoantibody were 1:600 for Type 1 AIH and 1:1,300 for type 2 AIH. pANCA can only be detected by immunofluorescence, which makes it less available compared with other serology assays.

Some reports indicate that plasma cell frequencies may be increased in snAIH cases with increased frequencies of plasma cells producing IgM, IgG, and IgA, suggesting the lack of autoantibodies in snAIH, and seronegativity is a matter of timing. [9, 17– 19] It has been proposed that early intense antigen-antibody complex formation can confound assays. Wang *et al*. [14] found that upon further review of subjects with snAIH during a 5 to 26 months follow-up, four of 17 (23%) became ANA positive. That suggests that autoantibody levels fluctuate, and supports the practice of serial measurements of autoantibodies. [14, 17– 19] The authors recommended testing with non-standard antibodies. [9]

It has been reported that in some cases of snAIH, treatment with corticosteroids resulted in the paradoxical appearance of autoantibodies, suggesting a role for an immune-mediated B cell inhibitory process that responded to steroids. However, treatment varied between the two groups, with use of prednisolone 40 mg daily alone or the combination of prednisolone 30 mg and azathioprine 50 mg. The study also failed to find a correlation between the late appearance of ANA and treatment regimen. [14] Tapering of prednisolone was also conducted in an individualized manner based on the response of the liver function tests.

Serial autoantibody testing after initial diagnosis of spAIH should be interpreted with caution. A study at the Mayo Clinic examined 107 patients with clinical and histological spAIH and tried to correlate the appearance and disappearance of SMA and ANA over time. The study assessed autoantibody expression outcomes with and without conventional corticosteroid regimens. It was found that as much as 76% of spAIH patients diagnosed by liver pathology and the presence of antibodies, lost SMA or ANA autoantibodies over 128 ± 9 months. [20] In some cases, but not others, loss of the antibodies was associated with improved liver enzymes, liver function tests, and histopathology findings. It was also noted that positivity fluctuated with time-on-treatment. Therefore, caution should also be taken with initial negative antibody testing as appearance of antibodies can be delayed. [20] The findings also underscore the importance of ruling out immunosuppression before assigning a diagnosis of snAIH. [12]

B cell and T cell dysfunction in the pathogenesis of snAIH

B cell depleting antibody (anti-CD20) treatment has been shown to be effective in AIH patients who did not respond to conventional therapy. [21] B cell depletion was accompanied by significantly decreased number and cytotoxic activity of T cells (Fig. 1). Three mechanisms have been proposed for the role of B cells in AIH, auto-antibody generation, antigen presentation, and cytokine/chemokine production. [21] The polyfunctional tasks of B cells in hepatitis implies that snAIH likely had B cells that lacked auto-antibody function, but continued B cell presentation.

In spAIH, autoantibody titers, total immunoglobulin levels, and specific antibodies (anti-LKM-1 and anti-LC1) have been shown to correlate with disease activity by direct antibody-mediated damage or activating liver-infiltrating T-lymphocytes. Cytokines such as interleukin (IL)-21 are produced by follicular T-helper cells, and drive B cell activation, plasma cell differentiation, and immunoglobulin production (Fig. 1). [9, 22] Serum levels of IL21 in AIH patients have been shown to be positively correlated with the grade of necroinflammatory activity, total serum bilirubin levels, and increased severity. Murine models demonstrated that blocking IL21 led to suppressed T-helper cells and muted development of AIH. [21] However, the studies did not address the role of serology and B cells in snAIH. There ap-

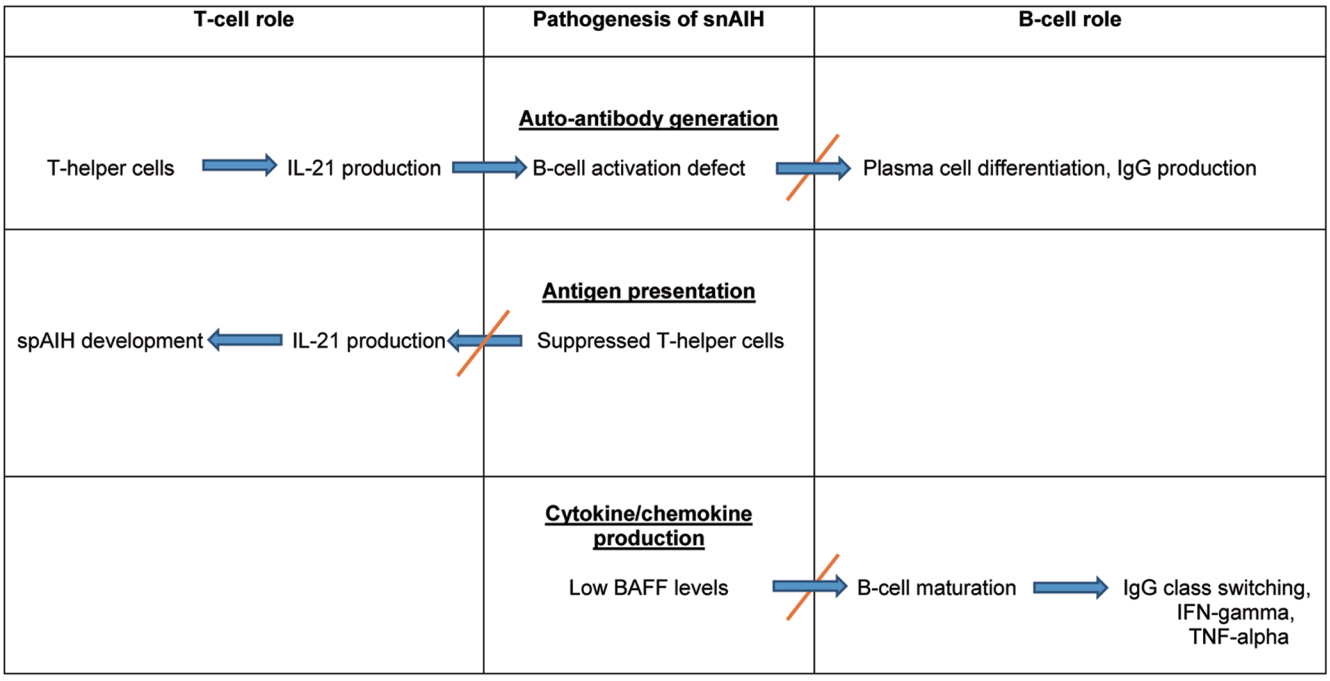

Fig. 1. Proposed pathogenic mechanisms involving T cell and B cell dysfunction in seronegative autoimmune hepatitis. Three proposed mechanisms for snAIH include auto-antibody generation, antigen presentation, and cytokine/chemokine production resulting from B cell and T cell dysregulation. Blocked blue arrows represent progression of normal physiology. Red font indicates the proposed mechanism of pathology. Blocked blue arrows with red strike indicate sequelae of halted or suppressed processes. BAFF, B cell activating factor; IFN, interferon; TNF, tumor necrosis factor.

pears to be a key role for B cells as antigen-presenting cells in spAIH given that B cell depletion with anti-CD20 in a murine model of spAIH both prevented induction and treated disease after onset. [23]

B cells in AIH also regulate the immune response through both cytokine release and recruitment of other immune cells. In a murine model of AIH, increased numbers of polyfunctional B cells were found to secrete interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α). [23] C-X-C Motif Chemokine Ligand 10 (CXCL10), or IFN-γ-inducible protein 10 are produced by B cells and stimulate hepatic inflammation and fibrosis. [24] Treatment of AIH with B cell depletion was associated with a decrease in CXCL10 levels. [23, 25] B cell activating factor (BAFF) level was also correlated with CXCL10 in AIH patients. [26] BAFF is a TNF family member and is critical for B cell maturation and immunoglobulin class-switch recombination. Excessive BAFF can lead to the development of autoreactive B cells and stimulation of the adaptive immune response. [27] BAFF is likely to be less prevalent in snAIH and subsequently inhibits the stimulation of immunoglobulin class switching and B cell maturation. [27] Without B cells, the inflammatory response required for hepatitis diagnosis would not be present. It is likely that B cell dysfunction is a cause of seronegativity.

Diagnosis

The diagnosis of snAIH is given to non-treated cases with consistent spAIH symptoms and pathology, but lacking autoimmune serology and other liver diseases associated with exposure to hepatotoxic medications or alcohol (<25 g of alcohol daily), or viral, hereditary, or metabolic disorders. [9] Because there are no established diagnostic criteria for snAIH, the diagnosis of snAIH may be made on the basis of the combination of a hepatic pattern of serum aminotransferases, lack of positive autoantibodies, lack of elevation of total IgG, typical histological findings, immunogenetic background (e.g., other autoimmune disorders in the patient or family and/or HLA typing). Given the similarity of snAIH and spAIH, the diagnosis of snAIH through the use of established scoring systems for diagnosing AIH and exclusion of other liver pathologies have been used. [9, 15, 28] Supporting clinical findings include family history, concurrent immune diseases, liver enzyme tests, HLA genotype, and liver histopathology (Fig. 2). Ultimately, rapid improvement with corticosteroid therapy supports the diagnosis of AIH, and thus snAIH. [3, 11, 20, 28- 31] Studies have emphasized the fact that snAIH can convert to AIH with time even without treatment. [12, 32] Therefore, the diagnosis of snAIH should not be based on a single sample. Rather, serial monitoring of serology is recommended to identify cases of AIH with delayed or fluctuating serologies.

Laboratory tests

snAIH cases often have elevated serum aminotransferases with normal or moderately elevated alkaline phosphatase. [7] Patients with snAIH are more likely to have albumin less than 35 g/l and an international normalized ratio greater than 1.2. [2] However, laboratory values often fluctuate and are occasionally normal. As previously noted, HLA may play a role in diagnosing snAIH. as reported by Heringlake, *et. al*. [12] snAIH was associated with certain HLA haplotypes including HLA DR4, HLA-B8, HLA-CW7 compared with cryptogenic liver disease. [12] HLA-DR3 was noted to be rare in snAIH and more common in AIH. However, the HLA haplotyping was performed on only 20 of the 43 patients who were presumed to have snAIH in that study. Limitations of the study included possible bias because of differences in diagnostic methods. Fifty-two of the 76 patients had liver biopsies at

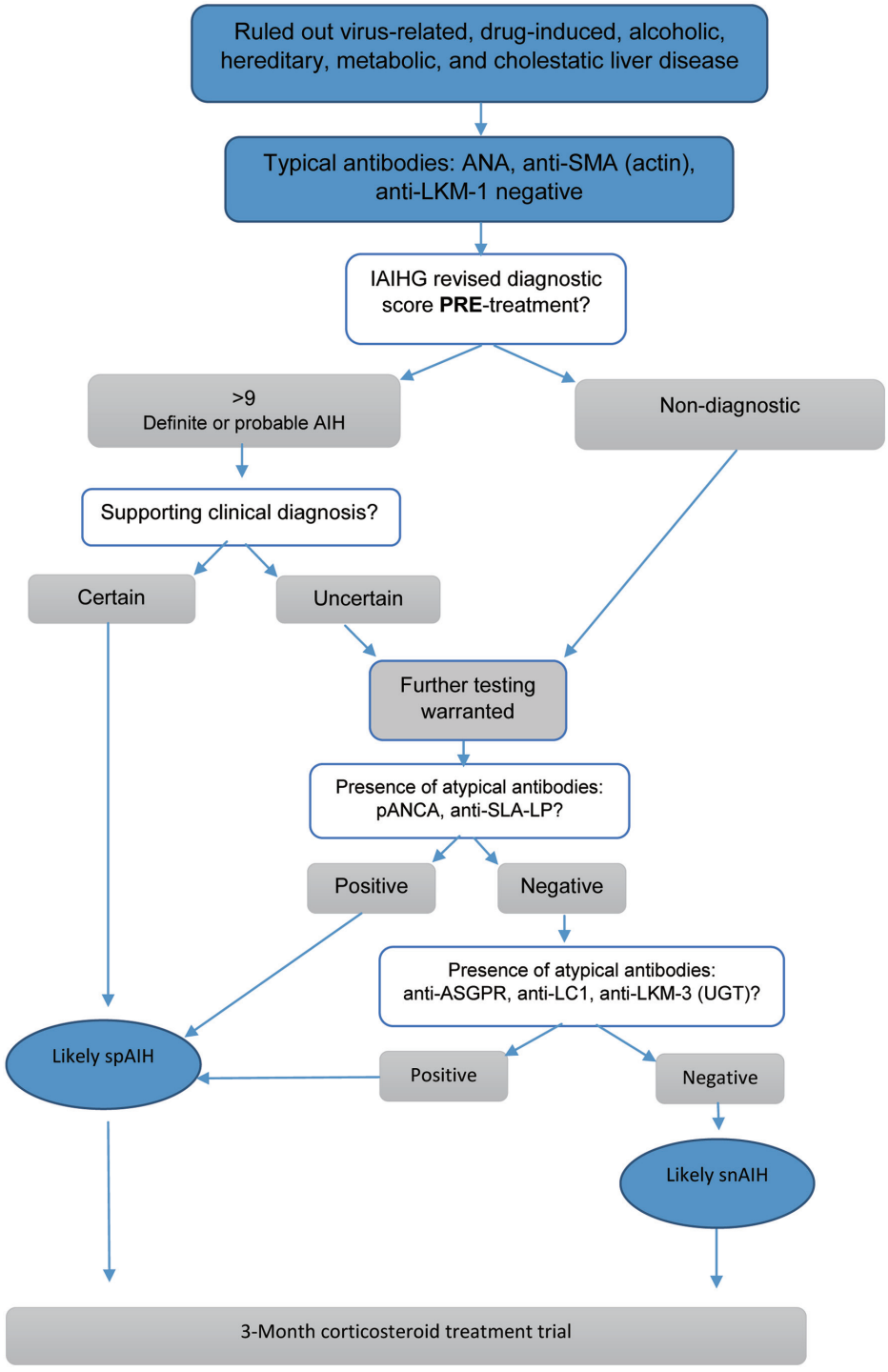

Fig. 2. Algorithm for the diagnostic workup of seronegative autoimmune hepatitis (Adapted from Czaja, 2011). [9] snAIH are non-treated cases with the symptoms and pathology of AIH, but lacking typical autoimmune serology and absence of other etiologies of hepatitis including virus-related, drug-induced, alcoholic, hereditary, metabolic, and cholestatic liver disease. The initial workup includes exclusion of these other etiologies and seronegativity. Next, the IAIHG revision diagnostic score should be implemented, and yielding a score of >9 with supportive clinical diagnosis indicates a definite or probable diagnosis of AIH. If a clinical diagnosis is lacking or if the IAIHG score pretreatment is <9, atypical antibodies should be reviewed. The absence of these atypical autoantibodies makes the diagnosis of snAIH likely, while the presence of atypical antibodies supports a diagnosis of AIH. Both diagnoses of snAIH and AIH should be further confirmed with a 3-month corticosteroid treatment trial. Blue boxes represent diagnosis decision. Clear boxes represent questions of diagnosis. Gray boxes represent answers of diagnostic questions. AIH, autoimmune hepatitis; ANA, antinuclear antibodies; anti-ASGPR, anti-asialoglycoprotein receptor antibody; anti-LC1, anti-liver cytosol antibody; anti-LKM-1, anti-liver and kidney microsomes; anti-LKM-3, anti-liver kidney microsomal 3; anti-SLA-LP, anti-soluble liver antigen/liver-pancreas antibody; anti-SMA, anti-smooth muscle antibodies; anti-UGT, anti-UDP-glucuronosyltransferase; IAIHG, International Autoimmune Hepatitis Group; pANCA, perinuclear anti-neutrophil cytoplasmic antibody; snAIH, seronegative autoimmune hepatitis; spAIH, seropositive autoimmune hepatitis.

an outside hospital. They were evaluated by different pathologists, and not all the snAIH patients received the same therapy. [12] Out of the 37 who received immunosuppressive therapy, 34 responded to the intervention.

As noted above, snAIH requires the absence of elevated conventional autoantibodies including ANA, SMA, and anti-LKM-1. In a study by Wang *et al*., [8] 17 of 167 patients with AIH were be seronegative. Those patients had lower serum IgG levels and more advanced histological stages compared with classical AIH patients. Kaymakoglu *et al*. [15] also classified their subjects as cryptogenic AIH excluding hepatotropic viral etiologies, and also serologic markers associated with AIH type 1 and 2. There was support for a diagnosis of snAIH with response to immunosuppressive therapy. However, anti-SLA and other atypical autoantibodies were not measured, as only in recent years have standardized test systems become available for detecting them.

Liver biopsy

Liver pathology is necessary for the diagnosis of snAIH, not only to show lesions typical for AIH, but also to exclude other disease entities. [15, 31, 33] There are no morphological features that are pathognomonic or specific for snAIH. However, typical features include cirrhosis (19–83%), interface periportal or periseptal hepatitis (75–83%), lymphoplasmacytic necroinflammatory infiltrate, and plasma cell infiltration (17–50%). [6, 7, 33, 34] Advanced histological stage (S3, S4) is more commonly seen in snAIH than classical AIH. [9, 14, 35] Many of the snAIH liver biopsies were reviewed by more than one liver pathologist, reducing the chance of interpretation bias. [7, 8, 14]

Although it has been reported that up to 40% of snAIH cases have low IgG levels in addition to absent autoantibodies, plasma cell infiltration is usually as extensive as in AIH. [31, 36] In acute presentations, liver biopsies in 97% of subjects showed centrilobular and submassive necrosis and moderate to severe plasma cell infiltration. [6, 30, 35, 37] Acute-onset pathology also had less fibrosis than patients with more active disease. [6] The presence of interface hepatitis in the absence of steatosis, well-defined granulomas, hepatic inclusions, marked bile duct injury or loss, and iron excess are consistent with the diagnosis in patients with chronic snAIH. [34, 37] Steatosis is not a common finding in AIH. Therefore, if fatty infiltrate is present, other conditions in addition to snAIH should be considered. [9]

Classification

IAIHG revised comprehensive scoring system

Most studies used the revised comprehensive, as opposed to the simplified, International Autoimmune Hepatitis Group (IAIHG) scoring system, as there is no scoring system that is tailored solely for snAIH. [1, 9, 12, 15, 28, 38] Kaymajoglu *et al*. [28] reported that patients with cryptogenic hepatitis and lacking immune-serologic markers for AIH, had the same features of AIH, including clinical phenotype, disease severity, histological findings, and treatment outcomes. That indicates that the revised scoring system, and not the autoantibody status, was an important diagnostic tool in snAIH. Other studies have also demonstrated that patients with snAIH and scored for AIH had superior responses to corticosteroid therapy. [12, 15, 28]

The timeliness of diagnosing snAIH is critical, as low scores on the simplified criteria have been associated with an increased risk of decompensation to fulminant hepatitis. [39] It is important to note that each scoring system can support, but not override the clinical diagnosis. [30] Sherigar *et al*. [1] described a case of seronegative AIH that was diagnosed with the use of the IAIHG revised comprehensive criteria. Other liver diseases were excluded with viral and hepatitis panels and thorough medical and social history review. ANA, SMA, anti-LKM-1, and anti-LKM-3 autoantibodies and serum IgG levels were within normal limits. The patient was noted to have a slightly elevated pANCA. A liver biopsy demonstrated circumferential interface hepatitis. At first, the simplified diagnostic criteria were used, but revealed a score of 4, which is not diagnostic for AIH. Even if the patient did include testing for SLA/LP antibodies, and these were positive, the score would still not meet the cutoff for diagnosing AIH. The revised criteria were subsequently used to score the clinical features and confirmed a diagnosis of AIH with a score of >17. That indicates the possible utility in reassessment of cryptogenic active hepatitis for snAIH using the revised scoring system. A limitation of the report was a lack of follow-up data.

Treatment

Pharmacologic agents

A positive response to immunosuppressive therapy can strengthen the diagnosis of snAIH. [11, 12, 32, 35, 37] However, a lack of response to therapy does not exclude the diagnosis of snAIH or spAIH. [7, 34] Three-month treatment trials with corticosteroids should be considered in all patients regardless of the serological findings to further confirm diagnosis in patients with a positive response. [7, 9] Given that spAIH and snAIH are similar in most respects apart from their serology, theoretically, the treatment for both should be the same, including steroid use with or without the addition of azathioprine. However, the literature provides variations in dosing and duration of both induction and maintenance periods.

Reported treatment regimens vary greatly, which limits comparison of responses to immunosuppression across studies. Treatment regimens initially developed for AIH, and later studied in snAIH, included two phases, an induction phase consisting of 30 mg of prednisone daily and azathioprine 50 mg daily, or prednisone 60 mg daily; and a maintenance phase including prednisone 10 mg daily and azathioprine 50 mg daily, or prednisone 20 mg daily. [9] If there is uncertainty of the diagnosis, treatment should be administered for 3 months with the higher dose of prednisone, given that azathioprine requires some time to reach effective levels. [9] Although never formally studied, the combination of budesonide and azathioprine may be useful for treatment in patients prone to side effects from prednisone, including patients with hypertension, diabetes, obesity, or osteoporosis.

Gassert *et al*. [11] conducted a retrospective review over a 5-year period and found no significant differences between patients with spAIH and those with snAIH with respect to age, sex, serum ALT levels, and AIH pretreatment diagnostic scores. Twenty of the 30 spAIH patients underwent pretreatment liver biopsies, and eight more had post-treatment liver biopsies. A total of six snAIH patients were included following a liver history confirmation. Studies have shown that clinical presentation alone does not increase probability of diagnosing snAIH and that histological variations help to support the diagnosis of snAIH. [9, 19] Of the snAIH patients, 83.3% had moderate to severe interface hepatitis vs. 85% in the spAIH group), 83.3% had advanced fibrosis vs. 40% in the pAIH group, and 17% had plasma cells vs. 60% in the spAIH group. Treatment was standardized among spAIH

and snAIH patients, with administration of prednisone 20 mg daily. The mean time to remission was recorded when ALT levels normalized, and was similar in in spAIH and snAIH patients (2.6 vs. 2.7 months, respectively). Within 3 months, 88.9% of spAIH patients and 66.7% of snAIH were in remission. One year later, one of the six snAIH patients had an increase in serum aminotransferase levels, indicating possible relapse or misdiagnosis. Strengths of the study include testing of all patients for atypical antibodies, including SLA. There was standardization in the treatment regimen with prednisone 20 mg daily, which was tapered over several months. However, there was some variation in the initiation of azathioprine 50 mg daily beginning sometime within the first 2 months of steroid therapy in patients who had a decline in serum ALT levels. A major limitation includes the small sample size of only 30 patients with spAIH and six with snAIH. That greatly reduced the power of the findings.

Heringlake *et al*. [12] approached treatment in a different way by using responsiveness to treatment to support the diagnosis of snAIH. Among the initial 126 cryptogenic chronic liver disease patients, the IAIHG scoring system identified approximately one-third of the cohort to have snAIH and thought to benefit from treatment with immunosuppressants. Thirty-seven presumed snAIH patients were allocated to receive both prednisolone and azathioprine combination therapy or monotherapy prednisolone 1 mg/kg/day induction therapy followed by a stepwise reduction of steroids. Patients who had at least a probable-AIH score of 10–12 points before treatment and 12–14 points after treatment, had superior responses to corticosteroid therapy. [9, 12, 32, 35, 40] Although there were inconsistencies with the immunosuppressive regimen administered, a total of 34 of the 37 who received any immunosuppressive treatment responded favorably. Two of the nine patients with cryptogenic liver disease receiving immunosuppressive therapy responded positively with a p-value of <0.005. A positive treatment response was thus supportive of a snAIH diagnosis. [12]

Kaymakoglu *et al*. [28] looked into possible regimens for relapsing snAIH and spAIH patients. Initially all patients received a treatment regimen of prednisone 30 mg daily and azathioprine 50-75 mg daily with a gradual reduction of steroids after a month. There were regular assessments with biochemical tests and physical exam at 3–6-month intervals. Response to therapy was assessed using the IAIHG scoring system. Follow-up for response assessment in cryptogenic hepatitis subjects was a median of 48 months, ranging between 36–60 months. Shorter follow-up times leaves room for the possibility of overestimation of responses to treatment. After 2 years, seven cryptogenic cases and one spAIH case were thought to have achieved complete remission, and thus treatment was stopped for these subjects. Out of the seven cryptogenic AIH subjects, six relapsed in 1 to 20 months after stopping treatment (71%). One AIH subject relapsed (100%). Combination therapy was restarted in patients with relapse after drug withdrawal, and they maintained complete remission thereafter. [28] Overall, responses to immunosuppressive therapy were similar in both the snAIH and spAIH groups. The study did not define complete remission, making it difficult to determine long term effects of treatment and to rule out over estimation bias.

Liver transplant

If immunotherapy does not result in improved outcomes, end-stage cases may be treated by liver transplant. AIH accounts for only 2.3% of all liver transplants. Early recognition of snAIH is often delayed as a result of the exhaustive workup required to make the diagnosis of snAIH. [11] The 5-year survival of patients and their grafts in AIH ranged from 73% to 92%, and the actuarial 10-year survival after transplant was >70%. [9] Patients with acute liver failure who had an underlying diagnosis of snAIH were at an increased risk of developing graft hepatitis following transplant. [2] Patients with Model for End-stage Liver Disease (MELD) scores greater than 35 did particularly poorly in transplant outcomes. [3] Improved rates of spontaneous survival were associated with steroid use in patients with initial significant aminotransferase levels greater than 30 times normal, but more prominent if subject had illness of less than 2 weeks duration.

Wigg *et al*. [41] studied 110 consecutive cases of seronegative acute liver failure requiring liver transplants over a course of 12 years. The study endpoint was short-term mortality of less than 2 months rather than all-cause mortality. Thirty-one deaths occurred in the seronegative group, of which 20 (67%) occurred in less than 2 months. Two patients with short-term mortality died from graft ischemia and one died from primary graft non-function. In those with late mortality, chronic rejection occurred in two patients and primary graft non-function following a regraft occurred in one patient. Adjusting for pretransplant risk factors of early mortality, it was found that survival following transplantation for snAIH was 83%, 81%, 73% at 2, 12, and 60 months, respectively. The most common cause of early death was sepsis or multiorgan dysfunction. Factors contributing to risk of early death included high donor body mass index, recipient age of more than 50 years, and non-Caucasian recipient ethnicity. Interestingly, pretransplant renal function was not identified as an important predictive variable for post-transplant outcomes, as seen in non-seronegative causes of acute liver failure. It was inferred that the donor liver quality rather than the recipient comorbidities held more importance in determining early death risk. [41]

Conclusions and recommendations

Seronegative autoimmune hepatitis is a diagnosis that is all too often missed or delayed. It should be considered earlier in cases that present like AIH, but have a negative serology for autoantibodies, and other causes for hepatitis and immunosuppressive treatment are excluded. Additional work up includes the use of IAIHG comprehensive diagnostic scoring criteria, liver histology, and demonstration of a positive treatment response to corticosteroids. These factors help to support a diagnosis of snAIH. However, there is no definitive test currently available for diagnosis. It is important to recognize that repeat antibody testing is important because of the dynamic nature of antibody levels. The absence of auto-antibodies in snAIH with preserved multifunctionality of B cells, suggests B cell dysregulation rather than inactivation. Further research into the pathogenesis of snAIH may provide insight into earlier and more specific diagnostic modalities.

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Conflict of interest

GYW has been an editor-in-chief of *Journal of Clinical and Translational Hepatology* since 2013. SBA has no conflict of interests related to this publication.

Author contributions

Wrote the manuscript and prepared figures (SBA), proposed the idea for the review and performed critical revisions of the manuscript (GYW).

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