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Review Article

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Autoantibody Profiling and Diagnostic Performance of Serum and Liver Tissue Markers in Patients with Autoimmune Hepatitis: A Review

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Abstract

Autoimmune hepatitis has no single diagnostic test. It is identified by assembling autoantibodies, immunoglobulin G, liver histology and the exclusion of viral disease into a score. Each component behaves differently, and the antibody most people order first is the least specific one available.

Objectives: To review the published evidence on serological and histological markers in autoimmune hepatitis: how the individual autoantibodies perform, how the two diagnostic scoring systems compare, what liver biopsy contributes, and where each fails.

Material and Methods: Narrative review of diagnostic accuracy studies, scoring system validations, meta-analyses and society guidance indexed in PubMed and the major hepatology and immunology journals. Studies were eligible if they reported the accuracy of an autoantibody or scoring system for diagnosing autoimmune hepatitis in a defined population, or the contribution of histology to that diagnosis. No new patient data were generated; every estimate quoted is one published by the original investigators.

Results: Pooling 29 studies, antinuclear antibodies gave a sensitivity of 65.0 per cent and specificity of 75.1 per cent, smooth muscle antibodies 59.3 and 92.6 per cent, and antibodies to soluble liver antigen 19.4 and 98.9 per cent, with diagnostic odds ratios of 7.38, 31.55 and 16.87 respectively. In 435 patients assessed by both scoring systems, the revised original system was more sensitive (100 against 95 per cent) while the simplified system was more specific (90 against 73 per cent) and more predictive (92 against 82 per cent), and excluded the diagnosis more reliably in diseases with concurrent immune features (83 against 64 per cent). The simplified criteria at a threshold of six reported sensitivity and specificity of 88 and 97 per cent in the derivation cohort, 90 and 95 per cent in a Chinese validation and 96 and 97 per cent in a German one.

Conclusion: No autoantibody both identifies and confirms the disease. Antinuclear antibodies are the most frequently positive and the least discriminating; antibodies to soluble liver antigen are close to definitive when present but absent in four patients out of five. The two scoring systems fail in opposite directions and are best used for different purposes rather than as alternatives.

Abbreviations: AIH – Autoimmune Hepatitis, ANA – Antinuclear Antibodies, SMA – Smooth Muscle Antibodies, anti-SLA/LP – Antibodies to Soluble Liver Antigen/Liver Pancreas, anti-LKM1 – Anti-Liver Kidney Microsomal Antibody Type 1, anti-LC1 – Anti-Liver Cytosol Antibody Type 1, IgG – Immunoglobulin G, IAIHG – International Autoimmune Hepatitis Group, PBC – Primary Biliary Cholangitis, PSC – Primary Sclerosing Cholangitis, DILI – Drug-Induced Liver Injury, DOR – Diagnostic Odds Ratio.

Keywords: Autoimmune Hepatitis; Autoantibodies; Anti-SLA/LP; Diagnostic Criteria; Liver Histology

Introduction

Autoimmune hepatitis was described as a clinical entity six decades ago [1] and remains one of the few liver diseases with no confirmatory test. Diagnosis rests on a combination of circulating autoantibodies, raised immunoglobulin G, compatible liver histology and the exclusion of viral

and drug-induced causes [2,3].

Because no single element is decisive, the International Autoimmune Hepatitis Group formalised the combination into a score, first in 1993 [4], then as a revised system based on twelve or thirteen components in 1999 [5], and finally as a simplified four-component system in 2008 [6]. Both remain in use, which is itself a signal that neither is wholly satisfactory.

The serological picture is conventionally divided by antibody profile: type 1 defined by antinuclear or smooth muscle antibodies, type 2 by antibodies to liver kidney microsome or liver cytosol, and a proposed third group defined by antibodies to soluble liver antigen [7,8,9]. Whether these represent distinct diseases or serological variants of one is contested [3,10,27,35].

What is less often set out plainly is how differently the individual antibodies behave as tests. This review examines the accuracy of each, compares the two scoring systems where they have been applied to the same patients, and considers what liver histology adds.

Material and Methods

Search strategy: PubMed and the archives of the major hepatology and clinical immunology journals were searched for studies of diagnostic markers in autoimmune hepatitis. Search terms combined autoimmune hepatitis with antinuclear antibody, smooth muscle antibody, anti-SLA/LP, anti-LKM1, anti-LC1, immunoglobulin G, simplified criteria, revised original criteria, liver biopsy and diagnostic accuracy. Reference lists of retrieved validations and guidelines were screened by hand.

Eligibility: Studies were included if they reported the diagnostic accuracy of one or more autoantibodies or of a scoring system in a defined population with suspected autoimmune hepatitis, or the contribution of histological features to diagnosis. Paediatric validations are included where they bear on the same instrument and are identified as such. Studies of autoantibodies in other liver diseases are cited where they define specificity.

Data extraction and handling: Design, sample size, comparator population, assay method, score threshold and reported accuracy estimates with confidence intervals were extracted as published. The comparator population is reported alongside every specificity figure, because specificity against healthy controls and against other chronic liver diseases are different quantities. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 4 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

The three main antibodies behave very differently: A meta-analysis pooling 29 studies quantified all three. Antinuclear antibodies gave a pooled sensitivity of 0.650 (95% CI 0.619-0.680) and specificity of 0.751 (0.737-0.764). Smooth muscle antibodies gave 0.593 (0.564-0.621) and 0.926 (0.917-0.934). Antibodies to soluble liver antigen gave 0.194 (0.168-0.222) and 0.989 (0.985-0.993) (Figure 1) [11].

The pattern is a clean trade-off. The antibody ordered first and positive most often is the least specific: a positive antinuclear antibody raises the probability of autoimmune hepatitis by a likelihood ratio of only 3.03, and one in four patients without the disease will also be positive [11]. Antibodies to soluble liver antigen are the mirror image, present in fewer than one patient in five but very rarely in anyone else, with a negative likelihood ratio of 0.839 that makes a negative result uninformative [11].

Overall discrimination, expressed as the diagnostic odds ratio, ranks smooth muscle antibodies highest at 31.55, ahead of soluble liver antigen at 16.87 and antinuclear antibodies at 7.38 (Figure 2) [11]. The middle antibody, not the most sensitive or the most specific, is the most useful single test.

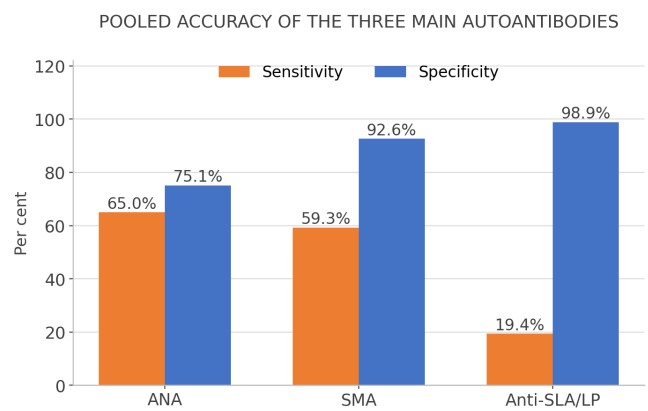


Figure 1: Pooled sensitivity and specificity of the three principal autoantibodies, from 29 studies [11]

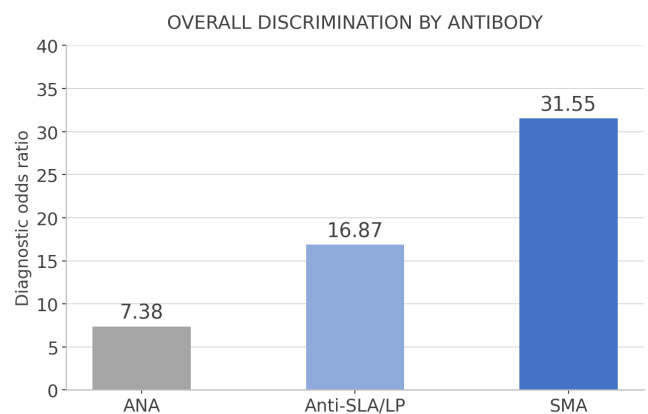


Figure 2: Diagnostic odds ratio for each antibody from the same pooled analysis [11]

The less common antibodies: Antibodies to liver kidney microsome type 1 define type 2 disease, occur predominantly in children and adolescents, and are considered highly specific [7,12]. They frequently coexist with antibodies to liver cytosol type 1, which can also occur alone [8,13]. Specificity has been questioned where chronic viral hepatitis is present, though a controlled comparison found antibodies to liver cytosol in a third of patients positive for liver kidney microsome antibodies with hepatitis C and in almost none of those without, and no soluble liver antigen antibodies in any viral hepatitis patient [13].

Antibodies to soluble liver antigen were initially proposed as defining a third disease type in patients negative for the conventional markers, but they are now understood to occur alongside them and are better regarded as an additional marker of type 1 disease [9,13]. They were incorporated into the 2008 simplified criteria on that basis [6]. Roughly a fifth of patients lack all of the conventional antibodies at presentation, and it is in that group that the less common specificities earn their place [3,14].

Serology does not predict what happens next: The historical division into serological types carried an implicit prognostic claim that has not held up. Large observational cohorts report comparable clinical, biochemical, histological and prognostic features across the serological subgroups [3,15]. Antibodies to soluble liver antigen have been associated with a more severe course and higher relapse rates [16,17], but the association may reflect coexisting antibodies to Ro52 rather than a distinct phenotype [3,16]. Antibody titres are unreliable markers of relapse, and monitoring is better based on aminotransferases and immunoglobulin G [3,27]. Response criteria and endpoints have themselves been inconsistently defined across

this literature, which complicates any comparison of outcome between serological subgroups [38], and the disease carries a measurable burden on health utility irrespective of subtype [37].

Study	n	Focus	Principal reported finding
Alvarez et al. 1999 [5]	—	Criteria	Revised original scoring system defined
Hennes et al. 2008 [6]	—	Criteria	Simplified four-component system; 88% and 97% at score 6
Czaja 2008 [18]	435	Comparison	Original more sensitive, simplified more specific
Yeoman et al. 2009 [19]	—	Validation	Simplified criteria in acute and chronic disease
Qiu et al. 2011 [20]	405	Validation	Simplified criteria 90% sensitive, 95% specific
Zhang et al. 2014 [11]	29 studies	Meta-analysis	Anti-SLA/LP specificity 98.9%, sensitivity 19.4%

Table 1: Principal studies reviewed, with focus and the finding as reported by the original investigators

Two scoring systems that fail in opposite directions: The clearest comparison applied both systems to 435 patients with diverse chronic liver disease, 153 of whom had autoimmune hepatitis by codified clinical criteria. The revised original system was more sensitive, 100 against 95 per cent, and seven patients diagnosed by it were non-diagnostic by the simplified system. The simplified system was more specific, 90 against 73 per cent, and more predictive, 92 against 82 per cent (Figure 3) [18].

The difference is most visible in the awkward cases. The revised original system assigned a diagnosis of autoimmune hepatitis to 20 of 21 patients with cryptogenic chronic hepatitis, against five by the simplified system, and the simplified system more often excluded the diagnosis in diseases with concurrent immune features, 83 against 64 per cent [18]. The practical reading is that the older system is better at capturing atypical disease and the newer one better at ruling out mimics, so they answer different questions.

Validation of the simplified criteria has been broadly consistent. The derivation study reported sensitivity of 88 per cent and specificity of 97 per cent at a score of six or more [6]; a Chinese cohort of 405 patients reported 90 and 95 per cent [20]; a German cohort of 70 cases against 211 controls reported 96 and 97 per cent (Figure 4) [21]. A Greek comparison of the two systems reached the same conclusion, that they are complementary rather than alternatives [36]. At the higher threshold of seven for definite disease, sensitivity falls sharply, to 43 per cent in the German cohort against 100 per cent specificity [21].

TWO SCORING SYSTEMS IN THE SAME 435 PATIENTS

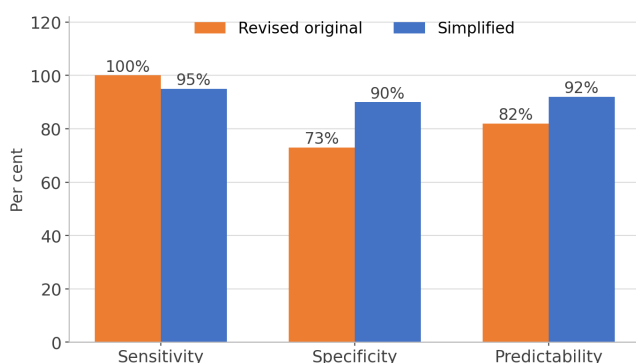


Figure 3: The revised original and simplified scoring systems applied to the same 435 patients [18]

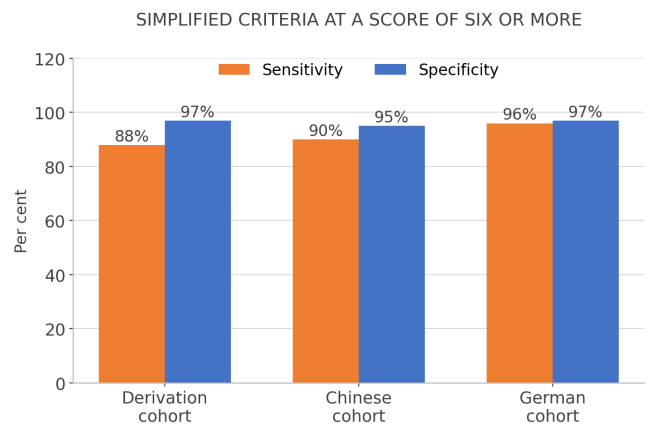


Figure 4: Simplified criteria performance at a score of six or more across three cohorts [6,20,21]

What the biopsy contributes: Histology is one of only four components in the simplified score and remains part of every diagnostic algorithm [2,6]. Its role is partly confirmatory and partly exclusionary, since the differential includes drug-induced injury, steatohepatitis and the cholestatic diseases, several of which carry overlapping serology [18,22]. Paediatric validation has shown that patients falling short of the classical criteria may nonetheless be diagnosed on histology, clinical presentation and treatment response [23,24].

Updated histological criteria have been proposed and evaluated against the 2008 version, with comparable sensitivity and better specificity for probable disease, and a higher area under the curve [25]. That direction of travel, tightening the histological component rather than adding serological ones, reflects where the residual diagnostic uncertainty actually sits.

Marker	Sensitivity	Specificity	Best use
ANA	65.0%	75.1%	Screening; a positive result means little alone [11]
SMA	59.3%	92.6%	Best single discriminator, DOR 31.55 [11]
Anti-SLA/LP	19.4%	98.9%	Near-confirmatory when present [11,16]
Anti-LKM1	—	High	Defines type 2; mainly paediatric [7,12]
Liver histology	—	—	Confirmation and exclusion of mimics [18,25]

Table 2: Reported performance of each marker and where it is most useful

WHAT RAISING THE THRESHOLD COSTS

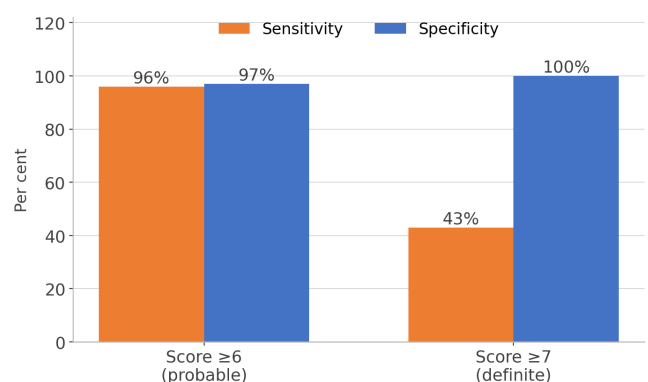


Figure 5: Sensitivity and specificity of the simplified criteria at the probable and definite thresholds in the German cohort [21]

How much each result actually shifts the diagnosis: Likelihood ratios express the practical value of a result better than sensitivity and specificity do, because they say how far a test moves the probability. Antinuclear antibodies carry a positive likelihood ratio of 3.03 and a negative ratio of 0.464; smooth muscle antibodies 11.74 and 0.449; antibodies to soluble liver antigen 11.09 and 0.839 (Figure 6) [11].

Read this way the asymmetry is stark. A negative smooth muscle antibody halves the odds of disease, while a negative anti-SLA/LP barely moves them at all. Two of the three antibodies are useful in only one direction, and which direction differs between them [11,26].

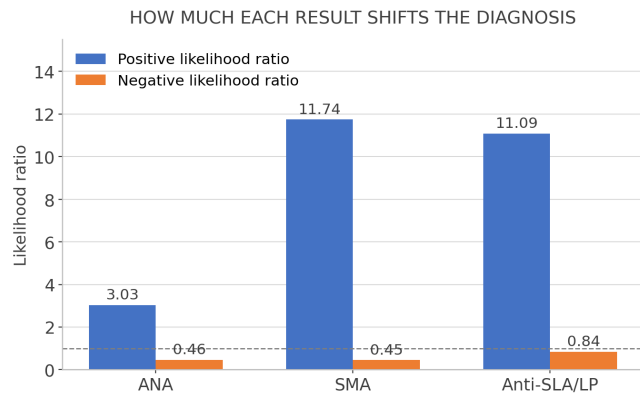


Figure 6: Positive and negative likelihood ratios for each antibody from the pooled analysis [11]

The wider diagnostic setting: Autoimmune hepatitis occurs across a clinical spectrum from asymptomatic disease found incidentally to fulminant failure [26,27], and a substantial minority of patients are asymptomatic at diagnosis yet progress [28]. Current guidance from both the American and European societies retains histology as a requirement and treats serology as supportive rather than decisive [2,29,30].

Two overlapping situations complicate serological interpretation particularly. Chronic viral hepatitis generates autoantibodies that mimic the pattern of autoimmune disease [13,31,32], and features of steatohepatitis are present in a substantial share of patients with genuine autoimmune hepatitis, which confounds both histology and the scoring systems [33]. De novo disease after liver transplantation is a further distinct entity [34]. In each of these the antibody result alone is actively misleading, which is the argument for retaining a multi-component score rather than moving to a single serological test [18,35].

Antibody	Target antigen	Associated type	Note
ANA	Heterogeneous nuclear antigens	Type 1	Common in other liver disease [11,35]
SMA	Actin and other cytoskeletal proteins	Type 1	Best single discriminator [11]
Anti-SLA/LP	UGA serine tRNA-associated protein	Type 1	Often with anti-Ro52 [11,16]
Anti-LKM 1	Cytochrome P450 2D6	Type 2	Mainly paediatric [7,35]
Anti-LC1	Formiminotransferase cyclodeaminase	Type 2	May occur alone [13,31]

Table 3: Target antigens of the diagnostic autoantibodies and the disease type each defines

Discussion

Two conclusions follow, and both are about how these tests are used rather than how good they are. The first is that the antibody most often ordered is the weakest. A positive antinuclear antibody carries a likelihood ratio of 3.03 and is found in a quarter of people without the disease [11], which in a population where autoimmune hepatitis is uncommon leaves the post-test probability low. Reporting it as though it were confirmatory is a recurring source of misdiagnosis.

The second is that antibodies to soluble liver antigen are the opposite kind of test and should be used accordingly. At 98.9 per cent specificity a positive result is close to decisive, but at 19.4 per cent sensitivity a negative one excludes nothing [11]. Ordering it as part of a first-line panel and treating a negative result as evidence against the diagnosis inverts what the test can do.

Choosing between the scoring systems

The two systems are usually presented as though the newer had superseded the older, but the head-to-head comparison shows they fail differently [18]. The revised original system captures atypical presentations that the simplified system misses, and correspondingly over-diagnoses cryptogenic hepatitis. The simplified system excludes mimics more reliably and misses atypical disease. Neither is superior; they are suited to different clinical questions, and the sensible approach is to apply the simplified system first and fall back on the revised original where it returns a negative result in a patient who nonetheless looks like a case.

The threshold matters as much as the instrument. Raising the simplified score from six to seven moved sensitivity from 96 to 43 per cent in one cohort while gaining three percentage points of specificity [21]. A definite-disease threshold is useful for research cohorts and actively unhelpful for clinical decisions.

Study limitations

The limitations belong to the underlying literature. Both scoring systems were validated against a reference standard that itself incorporates the components being tested, so sensitivity of the revised original system is 100 per cent by construction in several cohorts [18,20,21]. Autoantibody assays differ between laboratories in method, substrate and cutoff titre, and pooled accuracy figures conflate them [11]. Specificity depends heavily on the comparator group, and cohorts differ in how many patients with cholestatic or drug-induced disease they include [18,20]. Several validations are single-centre and retrospective [21]. Paediatric and adult populations differ in antibody distribution and in the performance of the same criteria [23,24].

Two gaps deserve naming. No study reviewed here reports outcomes for patients treated on the basis of a positive score who later proved to have another diagnosis, which is the group the specificity figures are about. And no prospective comparison has tested a sequential strategy applying the simplified system first and the revised original second, although that is what the comparative data imply.

Conclusion

Autoimmune hepatitis is diagnosed by assembling components that each perform poorly alone, and the assembly conceals how differently they behave. Antinuclear antibodies are positive most often and discriminate least, with a diagnostic odds ratio of 7.38 against 31.55 for smooth muscle antibodies. Antibodies to soluble liver antigen are near-specific at 98.9 per cent but present in fewer than one patient in five, so they confirm and never exclude. The two scoring systems fail in opposite directions, the older capturing

atypical disease at the cost of over-diagnosis and the newer excluding mimics at the cost of missing atypical cases. Using them sequentially rather than choosing between them, and reading a negative anti-SLA/LP as uninformative rather than reassuring, is what the published accuracy data support.

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Conflict of Interest

No competing interest attaches to any of the three authors.

Ethics Statement

Every study discussed here was already in the public record. As nothing was gathered from patients and nobody joined a study, an ethics submission did not apply.

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