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REVIEW

Noninvasive biomarkers for the diagnosis and management of autoimmune hepatitis

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Abstract

Autoimmune hepatitis (AIH) is a rare disease of unclear etiology characterized by loss of self-tolerance that can lead to liver injury, cirrhosis, and acute liver failure. First-line treatment consists of systemic corticosteroids, or budesonide, and azathioprine, to which most patients are initially responsive, although predictors of response are lacking. Relapses are very common, correlate with histological activity despite normal serum transaminases, and increase hepatic fibrosis. Furthermore, current regimens lead to adverse effects and reduced quality of life, whereas medication titration is imprecise. Biomarkers that can predict the clinical course of disease, identify patients at elevated risk for relapse, and improve monitoring and medication dosing beyond current practice would have high clinical value. Herein, we review novel candidate biomarkers in adult and pediatric AIH based on prespecified criteria, including gene expression profiles, proteins, metabolites, and immune cell phenotypes in different stages of AIH. We also discuss biomarkers relevant to AIH from other immune diseases. We conclude with proposed future directions in which biomarker implementation into clinical practice could lead to advances in personalized therapeutic management of AIH.

INTRODUCTION

Autoimmune hepatitis (AIH) is a rare disease of unclear etiology thought to be due to a lack of self-tolerance ultimately leading to liver injury and, in some cases, cirrhosis or acute liver failure.[1–3] The diagnosis requires exclusion of other etiologies and is multilayered, consisting of specific histological abnormalities with elevated liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), immunoglobulin

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ADA, adenosine deaminase; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, antinuclear antibody; ASGPR, anti-asialoglycoprotein receptor; AST, aspartate aminotransferase; BAFF, tumor necrosis factor family B-cell activating factor; CCL, chemokine ligand; CCL11, eotaxin-1; CCL26, chemokine eotaxin-3; DI-AIH, drug-induced AIH; DILI, drug-induced liver injury; FOXP3, forkhead box P3; hnRNP, heterogeneous nuclear ribonucleoprotein; IBD, inflammatory bowel disease; IFN-γ, interferon-gamma; IgG, immunoglobulin G; IST, immunosuppressive therapy; LKM-1, liver kidney microsome type 1; LTR, liver transplant recipients; MIF, macrophage migration inhibitor factor; NK, natural killer; NF-κß, nuclear factor kappa-beta; PBC, primary biliary cholangitis; PD, programmed cell death; RA, rheumatoid arthritis; RNA, ribonucleic acid; RORγt, retinoid-related orphan receptor gamma t; SLE, Systemic Lupus Erythematosus; SLA, soluble liver antigen; SMA, smooth muscle antibodies; sPD1, soluble PD1; SPRi, Surface Plasmon Resonance Imaging; T1D, type 1 diabetes; T-bet, t-box TF expressed in T cells; TE, transient elastography; TF, transcription factor; TGF-ß, transforming growth factor-beta.

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G (IgG) levels, and one or more associated autoantibodies including antinuclear antibody (ANA), smooth muscle antibodies (SMA), and rarely antibodies to liver kidney microsome type 1 (anti-LKM1) and soluble liver antigen (SLA).[3,4] First-line treatment of AIH consists of corticosteroids with azathioprine, to which a majority of patients respond by achieving remission.[4–6] Unfortunately, immunosuppressive therapy (IST) has wide-ranging side effects and is associated with long-term morbidities (infection, malignancy) that reduce patient quality of life and outcomes.[7,8] Because of this, the recent American Association for the Study of Liver Diseases (AASLD) practice guidelines recommend considering IST withdrawal in patients who have liver enzymes and IgG levels within normal limits for at least 2 years.[4,9] This guideline is made with known hesitancy though, as relapse during or after IST withdrawal is common in AIH (>80% in some studies)[8] and thus withdrawal needs to be conducted in a stepwise fashion with close monitoring. Histologically active AIH on liver biopsy, despite normal serum transaminases, predicts relapse following attempted drug withdrawal.[10] Although liver biopsies are specific for active disease on initial presentation, they have risks and are impractical to perform serially, particularly for IST optimization decisions (augmentation, reduction, withdrawal).[11]

Therefore, there is an unmet need for noninvasive blood-based biomarkers that could help predict patients at high risk for relapse or serve as an early marker for relapse. A biomarker has been defined according to the US Food and Drug Administration as a characteristic measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.[12] Although biomarkers can come from a wide heterogeneity of molecular or physiologic characteristics, broad categories of biomarkers include those that measure susceptibility/risk, diagnosis, monitoring, prognostic, predictive, pharmacodynamic response, and safety.

Some of the various desirable traits of biomarkers in the context of AIH include those that are (1) noninvasive, or readily available from a peripheral source such as blood; (2) easily measured, cost-effective, and reproducible across a spectrum of patients with AIH (e.g., pediatric and adult patients, varying levels of disease activity); (3) biologically plausible and sufficiently sensitive to serve as a surrogate of liver histologic inflammatory activity; (4) able to prognosticate at diagnosis, predict biochemical remission following IST, or identify those at higher risk of relapse; (5) identify relapse prior to standard clinical signs and symptoms; and (6) provide insight into relevant immune-based signaling pathways and phenotypes in order to guide personalized therapeutic decisions and promote clinical trials of targeted immunomodulating agents. Although no single biomarker could fulfill each of these diverse characteristics, a determined, systematic approach to biomarker development in AIH is critically important to improve our understanding of the disease, promote the introduction of new targeted therapies, and improve clinical outcomes and quality of life for patients with AIH. The recent 2019 AASLD AIH guidelines cited prognostic and therapeutic biomarkers as a significant unmet need in AIH.[4]

This paper is a review of the available literature on potential candidate biomarkers in adult and pediatric AIH. These biomarkers, selected based on prespecified criteria (see *Methods*), include potential indicators of subclinical disease activity, and predictors of clinical relapse, and remission (Table 1). We also delve into relevant biomarker advances that could be borrowed or gleaned from other immune disease states, such as liver transplantation, drug-induced liver injury, and other nonhepatic autoimmune disorders. We conclude with next steps for novel candidate biomarkers, and how their development and implementation could lead to advances in care and personalization of AIH management.

METHODS

For the purpose of this review of the biomarkers of immune activation and quiescence in different stages of AIH, publications cited in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) were selected in 6/2021–12/2021 using the search words *autoimmune hepatitis* as well as *serum biomarker*, *cytokine*, *chemokine*, *antibodies*, *receptors*, or *regulatory T cells* (see Figure S1). Citations were chosen based upon their relevance to the aim of this article. Articles excluded included articles with animal data, papers not in English without an English abstract, and review articles. Thirty-six articles were identified and 28 were chosen to be discussed in further detail (Table 1). The decision to exclude articles was made based upon several factors: expert opinion that these biomarkers were not clinically promising as well as limited quality of data including small cohorts with low power. The eight articles that were omitted are briefly discussed in the review and further details on those biomarkers can be found in Table S1.

CURRENTLY AVAILABLE BIOMARKERS

Current traditional serum biomarkers of liver injury and treatment response in AIH that are used in practice include aminotransferases (AST and ALT), IgG, and, less frequently, 6-thioguanine (6-TG). Other clinically available candidate markers such as vitamin D and ferritin will be discussed below. Hartl et al. investigated potential predictors in relapse and found that although ALT, IgG, and overall gamma-globulin levels were all normal in patients prior to IST withdrawal, there were variations

TABLE 1 Biomarkers in AIH

Author	Source	# of participants	Biomarker	Increased risk (+)/ protective (-)	Studied in histological disease:		
					Activity	Remission	Relapse
Initial Presentation							
Bayer et al.,[51] 1998	Serum	18 (AIH) 10 (NASH) 16 (control)	TGF-ß1	+	X	X	
Landi et al.,[83] 2013	Serum	40 (AIH) 50 (PBC) 58 (PSC) 54 (HCV) 50 (control)	TNF-α	-	X		
Chaouali et al.,[143] 2020	Serum	50 (AIH) 150 (control)	TNF-α	+	X	X	
Bovensiepen et al.,[47] 2019	PBMCs	49 (AIH) 43 (control)	TNF-producing $CD4^+$ T cells	+		X	
Behfarjam et al.,[80] 2017	RNA	18 (AIH) 18 (control)	T-bet and IFN-ɣ mRNA	+	X		
Migita et al.,[44] 2007	Serum	55 (AIH) 14 (acute hepatitis) 33 (HCV) 33 (control)	BAFF	+	X	X	
Nishikawa et al.,[43] 2016	Serum	80 (AIH)	BAFF	+	X		
Efe et al.,[55] 2014	Serum	68 (AIH) 34 (control)	25(OH)D	-	X	X	X
Ebadi et al.,[56] 2019	Serum	209 (AIH)	25(OH)D	-	X		
Taubert et al.,[57] 2017	Serum	109 (AIH)	Ferritin	-	X	X	
Torgutalp et al.,[50] 2017	Serum	52 (AIH) 28 (control)	ADA	+	X	X	X
Remission							
Longhi et al.,[37] 2004	PBMCs	41 (AIH) 18 (control)	$CD4^+CD25^+$ Treg cells	-	X	X	
Longhi et al.,[38] 2006	PBMCs	25 (AIH) 15 (control)	$CD4^+CD25^+$ Treg cells	-		X	
Ferri et al.,[39] 2010	PBMCs	47 (AIH) 28 (control)	$CD4^+CD25^{hi}$ Treg cells	-	X	X	
Peiseler et al.,[64] 2012	PBMCs	77 (AIH) 42 (control) 8 (NASH)	$CD4^+CD25^+FOXP3^+$ Treg cells	+	X	X	

TABLE 1 (Continued)

Author	Source	# of participants	Biomarker	Increased risk (+)/ protective (-)	Studied in histological disease:		
					Activity	Remission	Relapse
Grant et al.,[40] 2014	PBMCs	41 (AIH) 25 (control)	$CD39^+$ Treg cells	-	X		
Liberal et al.,[59] 2015	PBMCs	43 (AIH) 22 (control)	$CD4^+CD25^+CD127^-$ Treg cells	-	X	X	
Liang et al.,[49] 2018	PBMCs	32 (AIH) 20 (control)	Treg	-	X	X	
Chen et al.,[60] 2019	PBMCs	20 (AIH) 20 (viral) 20 (control)	$Foxp3^+$ Treg cells	-	X		
Gatselis et al.,[65] 2017	Serum	224 (AIH) 249 (PBC) 36 (PSC) 146 (viral hep) 140 (NASH) 114 (control)	DNAse	-	X		
Drug Withdrawal							
Matsumoto et al.,[67] 2014	PBMCs	52 (AIH) 24 (DILI) 30 (viral hep) 11 (PSC) 62 (control)	anti-PD-1 Ab's	+	X	X	X
Mitra et al.,[66] 2015*	RNA	46 (AILD) 15 (control)	FOXP3 TF/RORγt TF ratio	-	X	X	
Behfarjam et al.,[81] 2019	RNA	24 (AIH) 24 (control)	RORγt TF, IL-22 mRNA	+	X		
Derben et al.,[71] 2021	Plasma	60 (AIH)	cytokeratin-18 death marker m65	+	X	X	
Relapse							
Treichel et al.,[74] 1994	Serum	79 (AIH) 122 (PBC) 385 (viral hep) 328 (other)	anti-ASGPR Abs	+	X	X	
Hausdorf et al.,[75] 2009	Serum	45 (AIH) 43 (PBC) 13 (EtOH) 35 (HBV) 53 (HCV) 118 (control)	anti-ASGPR Abs	+	X	X	X

(Continues)

TABLE 1 (Continued)

Author	Source	# of participants	Biomarker	Increased risk (+)/ protective (-)	Studied in histological disease:		
					Activity	Remission	Relapse
Assis et al.,[76] 2013*	Serum	52 (AIH) 309 (PBC) 71 (control)	MIF, CD74	+	X		X
Assis et al.,[78] 2016	Serum, DNA	52 (AIH) 30 (control)	MIF	+	X		

Note: Symbols: *: included patients with PBC.

Abbreviations: Ab: antibody, AIH: autoimmune hepatitis, ADA: adenosine deaminase, ASGPR: asialoglycoprotein receptor, BAFF: tumor necrosis actor family B-cell activating factor, CD: complementary determining, FOXP3: forkhead box P3, HCV: hepatitis C virus, IFN-γ: interferon-gamma, MIF: macrophage migration inhibitor factor, mRNA: messenger ribonucleic acid, PBC: primary biliary cholangitis, PBMCs: peripheral blood mononuclear cells, PD: programmed cell death, PSC: primary sclerosing cholangitis, RNA: ribonucleic acid, RORγt: retinoid-related orphan receptor gamma t, T-bet: t-box TF expressed in T cells, TF: transcription factor, TGF-ß1: transforming growth factor-beta, Treg: regulatory T, 25(OH)D: vitamin D.

within the normal range that distinguished patients that ultimately relapsed.[13] Therefore, they advocated for a goal of ALT less than half the upper limit of normal (ULN) and an IgG level less than 1200 mg/dL. When this was sustained for 2 years, they found 46% of patients experienced relapse after cessation of treatment,[13] which is significantly lower than the current rate of >80%.[8] This is corroborated by Montano-Loza et al. who validated that interface hepatitis disappeared when serum AST levels improved to less than two fold the ULN and that high levels of IgG at IST withdrawal correlated significantly with the risk for relapse.[14]

In contrast, even with biochemical remission, several studies have shown that about 20–50% of patients with AIH still had histological evidence of active disease on liver biopsy which puts them at inevitable risk for relapse after IST withdrawal.[10,15,16] Lüth et al. revealed that although elevated ALT and IgG levels have a 97% positive predictive value for relapse, they have only a 33% negative predictive value for remission, suggesting that these markers are not a sufficient surrogate or replacement for liver biopsy.[17]

In response to these findings, the AASLD changed their guidelines in 2010 to require not only normalization of bilirubin and gamma-globulin levels but also normal serum aminotransferases for at least 2 years prior to consideration of withdrawal of IST.[3] One center showed that with the application of the 2010 criteria to their cohort, the number of their patients that met criteria for remission went from 73% with the 2002 criteria to 26% with the 2010 criteria.[18] Despite evidence that more stringent cutoffs of ALT, IgG, and gamma-globulin levels may be adequate surrogates for histological remission, the evidence is not compelling enough. Therefore, the AASLD continues to recognize liver biopsy as the gold standard to establish the state of histologic remission and exclude inflammatory activity prior to drug withdrawal. Liver biopsy prior to IST withdrawal remains mandatory in children[19] however recent studies in adults suggest that liver biopsy may be optional.[4,20] Furthermore, in patients with AIH and cirrhosis, biochemical remission was recently shown to be an inadequate reflection of histological remission.[21]

Finally, there are limited data on the utility of 6-TG as a surrogate marker for remission in patients on azathioprine. Dhaliwal et al. conducted a study with 70 patients that showed that higher 6-TG levels significantly associated with AIH histological remission,[22] but this was contradicted in other studies.[23–25] The data on the use of 6-TG levels in pediatric AIH is also limited.[26,27] It is important to note, though, a recent study by Candels et al. showed that measuring thiopurine metabolite levels helped maintain remission and even allowed for reduction in dosage of both thiopurines and corticosteroids,[28] providing some promise in its clinical utility. There also may be some practical use of 6-TG to identify azathioprine nonresponders or as a marker of

treatment adherence, but this requires further studies for widespread clinical use.

PATHOPHYSIOLOGY OF AIH

To begin to identify novel candidate biomarkers for exploration, it is important to understand the complex pathophysiology of AIH (Figure 1). Although not completely elucidated, T cell dysregulation plays a primary role in AIH liver injury.[29] This is supported by the finding that a majority of the pathogenic immune cells in active AIH are T cells, predominately $CD4^+$.[30] To a lesser extent, there is involvement of other immune cells including B cells, plasma cells, natural killer (NK) cells, and macrophages.[30] Naïve $CD4^+$ T cells can differentiate into multiple $CD4^+$ T cell subsets based upon the cytokine milieu and the activation of specific transcription factors (TF). For instance, Th1 cells are promoted by IL-12 and interferon-gamma (IFN-γ) [master TF: t-box TF expressed in T cells (T-bet)],[31] Th2 by IL-4 (master TF: GATA-3), Th17 cells are dependent upon the presence of transforming growth factor-beta (TGF-ß) and IL-6 [master TF: retinoid-related orphan receptor gamma t (RORɣt)],[32,33] whereas regulatory cells (Treg) are induced by IL-2 and TGF-ß [master TF: forkhead box P3 (FOXP3)].[33–35]

Each of the Th cell subsets are, in turn, associated with unique inflammatory and anti-inflammatory properties that ultimately play a role in AIH pathogenesis and disease activity. Th1 cells release primarily proinflammatory cytokines including interleukin IL-2 and IFN-ɣ, Th2 cells produce IL-4, IL-10, and IL-13,[29] whereas Th17 cells are responsible for producing IL-17, IL-22, and TNF-α which are major contributors to inflammation.[32] Tregs secrete anti-inflammatory cytokines such as IL-10,[36] and, through multiple mechanisms, inhibit proinflammatory function of effector T cells. Multiple studies have shown that impaired function or reduced numbers of Treg are associated with AIH.[37–41]

A subset of T cells, the follicular helper T cell (Tfh), is responsible for providing help during B-cell maturation and germinal center generation.[42] There is a relative paucity of data about the role of B cells in the pathophysiology of AIH, although hypergammaglobulinemia (namely, elevated IgG) as well as defining antibodies are integral to the diagnosis of AIH. However, recent data has emerged regarding the role of TNF family B-cell activating factor (BAFF), a cytokine essential for the development and maturation of B cells,

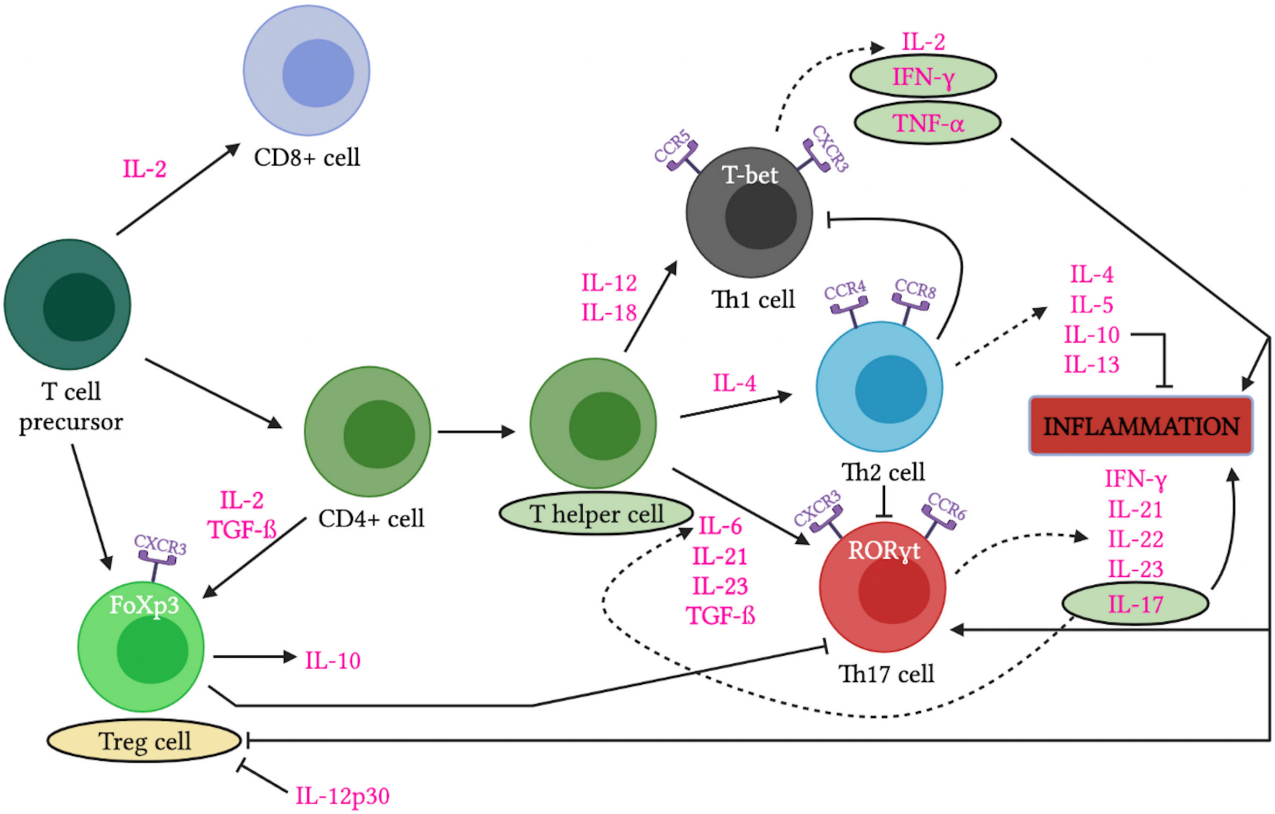

FIGURE 1 The pathophysiology and candidate biomarkers of autoimmune hepatitis. Depiction of the pathways of inflammation that play a role in that pathophysiology of autoimmune hepatitis. Promising markers of disease activity are circled in green and promising markers of quiescence are circled in yellow. CCR/CXCR, chemokine receptor; FoXp3, forkhead box P3; IFN-γ, interferon-gamma; TGF-ß, transforming growth factor-beta; Th, helper T; Treg, regulatory T.

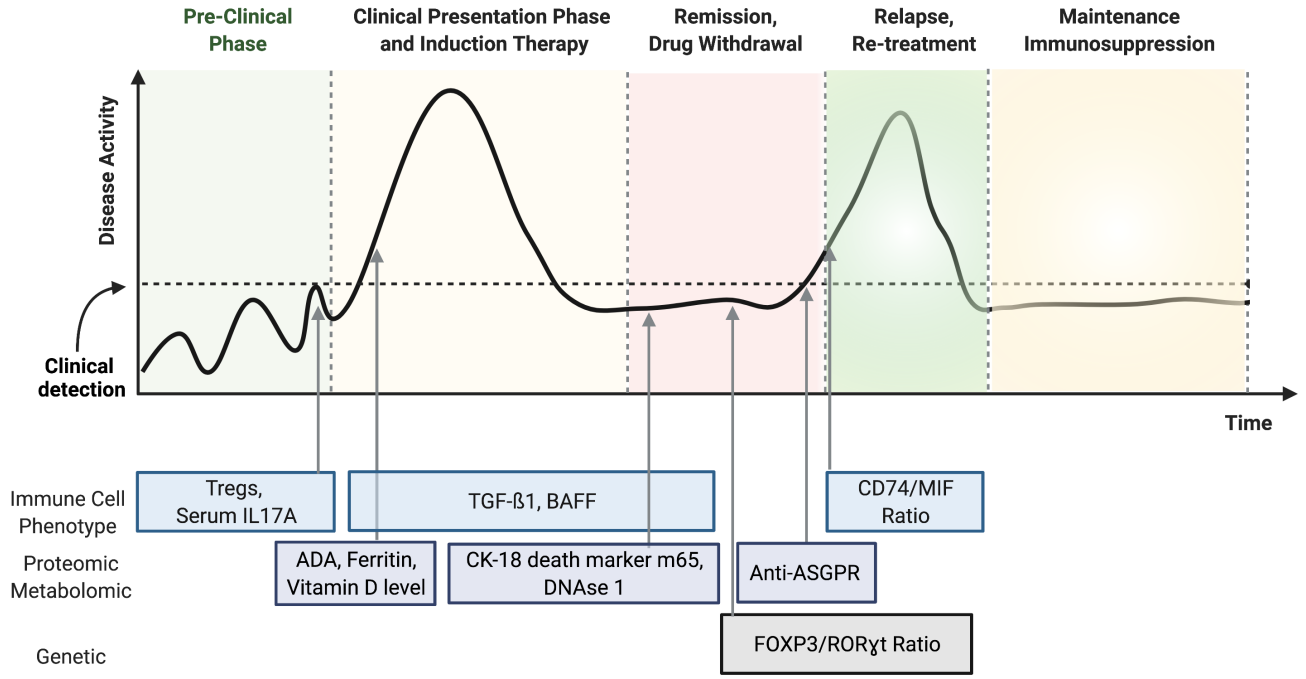

FIGURE 2 Natural history of autoimmune hepatitis and candidate biomarkers. Depiction of the natural history of autoimmune hepatitis with associated potential biomarkers of activity and quiescence at each stage of disease. ADA, adenosine deaminase; ASGPR, asialoglycoprotein receptor; BAFF, tumor necrosis factor family B-cell activating factor; CD, complementary determining; CK, cytokeratin; DNAse, deoxyribonuclease; FOXP3, forkhead box P3; MIF, macrophage migration inhibitor factor; RORγt, retinoid-related orphan receptor gamma t; TGF-ß, transforming growth factor-beta; Tregs, regulatory T.

which is further discussed below as a candidate biomarker.[43–45]

BIOMARKERS OF IMMUNE ACTIVATION AND QUIESCENCE IN DIFFERENT STAGES OF AIH

We will focus the discussion on the leading candidate biomarkers at different phases of disease states of AIH (Table 1). We selected these biomarkers based upon the number of published studies as well as the number of patients and samples with key clinical stages (activity, remission, relapse) tested in each study. We also took into account the ease of application of each biomarker to the clinical setting (Figure 2).

PRECLINICAL PHASE

There are no known preclinical biomarkers capable of identifying signs of AIH before clinical detection or liver damage, but there are certain genetic HLA and non-HLA based polymorphisms that may be associated with AIH such as DRB1*03:01, SH2B3, and CARD10.[4,46] These genetic polymorphisms are not ideal biomarkers, as defined above, as they are fixed values that have little utility in monitoring over time. We do not recommend screening the general population but recognize they may be helpful for risk stratification in patients with other autoimmune features that may be at higher risk for AIH.

CLINICAL PHASE

Initial presentation

This phase is the initial stage of active disease. These biomarkers may have clinical utility in detecting inflammation in the liver prior to increases in serological markers of liver injury (i.e., aminotransferases), in grading disease severity, and/or in earlier prognostication of the disease course.

As discussed above, AIH is primarily driven by a T cell-mediated immune process. The frequency of T cells secreting IL-17 and TNF-α is significantly increased in patients with AIH.[47,48] Liang et al. found that serum levels of IL-17A correlated with liver injury and that levels of circulating Th1 and Th17 cells significantly decreased after IST,[49] which make them a potentially trackable immune marker of disease activity. This study also showed fewer Treg cells in active disease, which will be subsequently discussed as a biomarker of remission.

A recent study found correlations between serum adenosine deaminase (ADA) levels and the severity of interface hepatitis on index liver biopsy.[50] A cutoff of

24.5 U/L identified patients with severe interface hepatitis, making ADA a candidate biomarker for grading severity of disease at initial presentation.

Similarly, serum TGF-ß1 is elevated in active AIH, correlates with active histological disease even in the setting of normal aminotransferases, and normalizes upon biochemical remission. Therefore, it may be a proxy for liver biopsy in predicting histologic activity and remission.[51] BAFF is also elevated in active biochemical and histological AIH disease and reduces with corticosteroid treatment.[43–45]

The vitamin D receptor and vitamin D resistance have been studied for their role in autoimmunity and inflammation.[52–54] Efe et al. reported that serum levels of vitamin 25(OH)-D were associated with liver fibrosis and interface hepatitis in AIH, and nonresponders to IST had significantly lower baseline serum 25(OH)D levels compared to responders.[55] A recent large study confirmed these findings and showed that patients with AIH and severe vitamin D deficiency (<25 nmol/L) were more likely to have treatment nonresponse and liver-related mortality,[56] making it a potential prognosticator at presentation.

Ferritin has also been investigated as a predictive biomarker of treatment response in patients with AIH.[57] Taubert et al. reported that a baseline ferritin level (>2.09 × ULN) and lower immunoglobulin levels (<1.89 × ULN) were associated with complete biochemical remission. Although counterintuitive as ferritin is an acute phase reactant, this study revealed that the hyperferritinemia was quickly reversible with therapy and seemed dysregulated from hepcidin. This was thought to be secondary to human hepatocyte growth factor, which can have potentially favorable immunomodulatory effects.[57] The relevance of iron homeostasis to inflammation and immune tolerance has also been previously reported in patients with liver transplant in which hepcidin and ferritin were differentially elevated in patients with operational tolerance.[58] It is important to note that both these markers are advantageous as they are readily available in clinical practice.

Remission

Remission can be categorized into biochemical (normalization of AST, ALT, and IgG) and histological (lack of inflammation such as interface hepatitis on biopsy) remission.[4] As discussed above, biochemical remission is likely not a sufficient surrogate for histological remission. Therefore, identification of noninvasive biomarkers of histological remission is an unmet need to prevent the requirement of liver biopsy.

Treg cells have been the focus of many studies in AIH as they control effector responses and are deficient in active disease.[37,38,39,59,60] Studies have reported that patients with AIH have fewer peripheral Treg cells in active disease compared to patients in remission and healthy controls.[37,60] Other studies have revealed that, in active AIH, peripheral Tregs are not only decreased in number but also have impaired suppressive function. In particular, peripheral Tregs from patients with AIH have decreased IL-10 secretion and they fail to properly regulate $CD8^+$ T cell function and suppress IFN-ɣ and IL-17 production.[39,40,59,61] Intriguingly, immune-modulating agents such as erythropoietin[62] and IL-2[63] have been shown to increase Treg number and/or function in patients with AIH.

Other studies however reported no difference in Treg cells in active AIH versus remission.[41,64] Peiseler et al. showed that the suppressor function of peripheral Treg cells was not impaired in patients with AIH compared to controls and Treg cells were actually elevated in the liver in active AIH.[64] The authors suggested that the cytokine microenvironment itself may suppress Treg function in the liver.[64] Another theory is that Tregs may actually contribute to the inflammation due to their plasticity and ability to convert into effector cells.[64] Interestingly, Taubert et al. showed a disproportional decrease in intrahepatic Tregs following IST, with the caveat that effector T cells may transiently also express CD25 and FOXP3, making them difficult to distinguish from Tregs.[41] A potential explanation for these contrasting results may reside in the different approaches and markers that have been used to measure Tregs. Through a comprehensive phenotypic analyses of various Treg subsets, McEachern et al. have been able to show that $CD4^+CD25^+CD127^{Low}$ Treg expressing HLA-DR are potentially the ones most impaired in patients with AIH.[62] Further studies are needed to test this hypothesis and whether the amount of circulating Treg correlates with histological remission.

Another marker, serum DNAse1, is an enzyme related to apoptotic cell degradation, may also represent a protective biomarker and favorable profile for remission in AIH. Gatselis et al. revealed that patients with AIH who experienced sustained remission had higher baseline DNAse1 levels compared to partial responders, nonresponders, and subsequent relapsers.[65] The role of DNAse1 in the breakdown of self-DNA and the relationship of self-DNA to inflammation suggests that regulators of autoantigen breakdown may help determine risk of disease activity versus remission.

Drug withdrawal prediction

This is arguably the most significant disease phase for the development of biomarkers in terms of clinical application. AASLD guidelines recommend consideration of IST cessation if possible, despite a known risk of relapse.[4] Therefore, it would be significant to develop biomarkers that could risk stratify patients before IST tapering. This would help with the clinical decision-making for timing

of withdrawal of IST, frequency of monitoring after IST withdrawal, and a more educated risk/benefit discussion with the patient about attempt of withdrawal of IST. These markers should also be more sensitive than currently available serologic tests and allow for closer monitoring during the drug withdrawal phase to detect early signs of immune activation. This could promote a practice of reinitiation of IST prior to increases in aminotransferases and liver injury.

In a further evaluation of T cell phenotyping as biomarkers in AIH, a study by Mitra et al. evaluated the ratio of TF gene expression of Treg (FOXP3) over Th17 (RORγt) cells.[66] The FOXP3:RORγt ratio was <1 in active AIH, high in quiescent disease ($p < 0.001$), and was not significantly different between AIH and primary biliary cholangitis,[66] suggesting a common TF signature. There was also a correlation between the FOXP3: RORγt ratio and the histopathological activity score,[66] indicating a potential surrogate for liver biopsy and patients with more favorable phenotypes for IST withdrawal.

Another potential biomarker is anti-programmed cell death (PD)-1 antibody, an inhibitory T cell receptor that plays a role in regulating T cell activation. Blockade of PD-1 and its ligand PD-L1 are established strategies in cancer immunotherapy, although activation of T cells can lead to detrimental autoimmune consequences.[67–69] Matsumoto et al. showed that serum anti-PD-1 Abs were higher in patients with acute AIH compared to remission and correlated with liver function tests. Interestingly, the presence of anti-PD-1 Abs may be a predictor of poor treatment response and relapse[67] and so may indicate a patient is at higher risk, requiring more frequent serological monitoring during IST withdrawal.

The anti-SLA antibody has also been associated with a two-fold increase in relapse after IST withdrawal,[70] making it a potential prognostic marker for withdrawal consideration. An additional protein is cytokeratin-18 death marker m65, found to have an 86% negative predictive value for detection of incomplete histological remission in a recent multicenter study,[71] which could sway clinicians away from IST withdrawal or obtain a liver biopsy first.

Early relapse detection

Finally, biomarkers during the relapse phase are significant, as relapse is frequent in the disease process of AIH both with and without IST withdrawal.[8] A helpful biomarker in this phase would be a measure of those with resistant relapses that may need higher doses of IST to achieve remission again. This is clinically significant as it would allow the clinician to be more aggressive with IST to minimize liver injury and fibrosis development.

Although ANA and SMA are part of the diagnostic criteria for type 1 AIH, they are neither disease-specific nor correlate with AIH activity in adults.[9] On the contrary, in pediatric populations, LKM-1 as well as SMA may correlate with disease activity, discussed in a later section.[72] Anti-asialoglycoprotein receptor (ASGPR) titers are high in active disease and decrease in response IST.[73–75] Importantly, they appeared to increase prior to elevation of liver enzymes,[75] indicating the potential to predict early diagnosis and relapse of AIH.

Macrophage migration inhibitor factor (MIF) is a cytokine originally studied in Th1-mediated autoimmune disease, including rheumatoid arthritis, systemic sclerosis, and inflammatory bowel disease.[76,77] MIF levels are elevated in patients with AIH despite corticosteroid therapy, and a -173C single nucleotide polymorphism in the *MIF* promoter correlates with steroid resistance in AIH and other disorders.[76,78,79] In addition, the ratio between the soluble, neutralizing MIF receptor (CD74) and MIF negatively correlated with ALT in relapsing patients,[76] showing promise as an immune disease activity biomarker and marker of aggressive disease and steroid resistance.

UNDERSTUDIED BIOMARKERS

We have highlighted the most promising biomarkers according to our literature review, revealing the most studied assays. In addition, it is important to note that there are other potential biomarkers that are understudied but still warrant mention. Gene expression profiles of immune activation, such as IFN-γ, T-bet, and IL-22 transcripts, have higher expression in patients with AIH compared to healthy controls.[80,81] There are various cytokines and chemokines that have also been associated with immune activation in AIH including IL-6, -8, -21, and -23,[48,82–85] as well as CCL2, CXCL9, and CXCL10[86]; whereas others are associated with immune quiescence including IL-2, -4, and -10,[49,60,87] as well as CCL22, CCL13, and eotaxin-1 (CCL11).[83] As noted, several of these biomarker changes are not necessarily unique to AIH and seen in other auto/alloimmune disorders. For instance, Efe et al. reports a potential of angiotensin-converting enzyme to be a serum biomarker for fibrosis in AIH,[88] but will require further investigation and validation before it can be used in clinical practice as it may not be a sensitive marker because it is known to be elevated in other conditions, such as sarcoidosis.

Radiographic biomarkers for AIH are limited, but there is some evidence surrounding transient elastography (TE). TE is an established tool to assess liver fibrosis in various liver diseases, but the data in AIH is limited. A study by Hartl et al. revealed that TE had a high utility in separating severe from nonsevere fibrosis

after 6 months of IST.[89] Of note, they found that it was not a reliable tool before the start of IST.[89] This group also found that remission could potentially be monitored by fibrosis regression via TE,[90] which would be a valuable addition to the monitoring of serum biomarkers during the remission and potential withdrawal of IST. There has also been investigation into alternative techniques of elastography including 'ElastPQ' that which utilizes point shear wave speed measurement,[91] but requires further investigation for clinical use. Both serum and radiographic markers of histologic fibrosis in AIH warrants further investigation.

SPECIFIC BIOMARKERS OF AUTOIMMUNE HEPATITIS IN THE PEDIATRIC POPULATION

There is a paucity of literature with respect to accurate diagnostic and prognostic biomarkers in children with AIH. The bulk of the studies focus on autoantibodies, specifically on the role of anti-LKM1 in defining type 2 AIH, a subtype predominantly found in children. Anti-LKM1 antibodies are present in 1–3% of adults with AIH and in 9–38% of children with AIH, with highest incidences in preteen European and Canadian children.[4] Compared to type 1 AIH, the presentation of type 2 AIH is more acute and severe, with higher rates of relapse and lower remission following IST withdrawal.[92] Historically, autoantibody levels are not established biomarkers of AIH disease activity or treatment outcomes.[4] This dogma was challenged by Couto et al. who identified that the persistence of anti-SMA and anti-actin antibodies correlated with biochemical and histological disease activity in adults and children.[93] After liver transplant, antibodies associated with the diagnosis of de novo AIH include ANA, anti-SMA/anti-actin, and donor-specific anti-HLA antibodies.[94]

As in adults, the PD-1 pathway is associated with pediatric AIH activity. At diagnosis, children with AIH had significantly higher levels of soluble PD-1 compared to other liver diseases and this positively correlated with liver fibrosis stage and the Child Pugh score.[95] Furthermore, soluble PD-1 levels were significantly higher in pediatric patients with AIH with active disease versus remission.[96]

CANDIDATE BIOMARKERS: LESSONS LEARNED FROM OTHER IMMUNE DISEASE STATES

Liver transplantation

The clinical, biochemical, and histological presentations of AIH can mimic allograft rejection and its plasma-cell rich variants in liver transplant recipients (LTR).[97,98] In addition, AIH can recur after LT, with similar presentations and responses to therapy. Thus, biomarkers identifying active versus inactive native AIH could parallel similar profiling in transplant graft immune activation versus quiescence/tolerance, particularly those that are not antigen-specific.

Several LT candidate markers are on the horizon and akin to those developed in nonhepatic organ recipients.[11,99–117] Studies using serial samples have demonstrated increasing levels of microRNAs, donor-specific antibodies, and blood CXCL10 gene expression prior to rejection, mainly during full IST withdrawal in LT tolerance studies.[102,118–120] Additional tolerance assays include blood immunophenotypic assays (Tregs and Vδ1/Vδ2 cell ratios), cytokine gene profiles (NK cells, γδ T cell, Th17 cells, CD8 receptor genes), and genomic microarrays.[103,121]

For the larger LT population not undergoing IST withdrawal, recent studies have reported specific blood gene transcripts that can distinguish rejection from normal graft function and nonrejection causes of graft injury.[122,123] These signatures are detectable in the weeks prior to rejection and resolve with corticosteroid therapy. This is promising as these assays could allow for IST titration guidance during minimization before any biochemical evidence of rejection. Proteoforms may be a more specific marker of immune activation.[103] In summary, these LT biomarker discovery assays should be investigated in AIH given the similar presentations, IST used and weaning considerations.

Drug-induced liver injury (DILI)

Another growing area of research in biomarker discovery is in DILI. Three major areas of biomarker utility are (1) diagnosis - confirmation of a specific drug that may be implicated in DILI i.e., APAP-CYS in predicting APAP-induced DILI[124]; (2) prediction - personalized approach to manage risk of developing DILI after exposure to a certain drug i.e., gene variant HLA B*5701 and abacavir toxicity[124,125]; and (3) prognosis - toxicity in early DILI or chance of mortality i.e., miR122 was used to determine likelihood of delayed injury in APAP-overdose.[124,125]

A smaller subset of patients with DILI display autoimmune features resembling idiopathic AIH.[126,127] Thus, biomarker assessments would be helpful in differentiating between DILI, drug-induced AIH (DI-AIH), and idiopathic AIH.[126] Qu et al. showed significant increases in intrahepatic Tregs in DI-AIH versus AIH.[128] Lammert et al. revealed an IgM predominance in patients with DI-AIH, whereas IgG and IgM autoantibodies characterized idiopathic AIH. Candidate IgGs directed against chromatin, myosin, antimitochondrial antigen, nucleosome antigen, and CENP-B showed high accuracy in distinguishing idiopathic and DI-AIH.[127]

As previously mentioned, immune checkpoint inhibitors targeting PD-1 and CTLA4 can lead to hepatic injury resembling AIH.[129–132] Biomarkers distinguishing checkpoint-associated hepatitis from idiopathic AIH may aid in predicting predisposition to liver injury and treatment response. Zen et al. showed decreased hepatic $CD4^+$ and $CD20^+$ lymphocytes as well as CD20/CD3 and CD4/CD8 ratios in checkpoint inhibitor-hepatitis versus AIH. These results may point to the lack of $CD4^+$ T cell interaction with B cells leading to decreased IgG formation in checkpoint-associated hepatitis.[129] Hutchinson et al. showed a correlation between effector memory $CD4^+$ T cell expansion due to latent CMV with the development of hepatitis following checkpoint inhibitor therapy.[133] This could identify those patients who may be susceptible to checkpoint-associated hepatitis to modify treatment approaches.

Other nonhepatic autoimmune diseases

There is a parallel interest in developing biomarkers across all autoimmune diseases, many of which share characteristics with AIH regarding complex criteria, risk of exacerbations, and lack of therapies to restore tolerance. Lessons and principles from these disciplines may also be helpful in guiding biomarker development in AIH.

Patients with inflammatory bowel disease (IBD) have a growing array of drugs and pathways for therapy, although biomarker development has not been robust or effectively integrated into clinical trials.[134] A recent systematic review of fibrostenosing Crohn's Disease identified categories of promising biomarkers including serum, genetic, and histologic markers, although they are limited by a lack of standardized disease category definitions and the absence of validation.[135]

Connective tissue diseases have undergone significant biomarker development in recent years.[136] This includes interest in biomarkers in the preclinical phase with loss of tolerance (i.e., IFNγ, autoantibodies) and cytokines that are relevant close to the time of diagnosis (i.e., B-lymphocyte stimulator in lupus), as well as immune cell and cytokine-based biomarkers of disease onset and progression. An interesting approach, which could be applied to AIH, came from the Biomarkers of Lupus Disease Study in which patients with active non-organ-threatening SLE were given a monitored withdrawal of IST.[137] The study reported the impact of type 1 IFN signatures on the lupus cytokine pathways and the relationship of IL17RA and B-lymphocyte stimulator levels to different IST regimens. This study also found different gene expression of T cells and IFN, as well as higher frequencies of activated neutrophils, monocytes, and B cells among early versus late flare patients.[138]

In-depth characterization of T cell–based biomarkers has been proposed in type 1 diabetes (T1D).[139] Although the lack of a specific autoantigen in AIH may preclude some of the techniques currently available in T1D, e.g., epitopes from insulin, preproinsulin, and GAD65, antigen-agnostic biomarker approaches could be emulated in AIH. This includes measuring key $CD4^+$ T cell subpopulations by flow cytometry and Treg signatures by nanostring expression assays, where a gene transcript signature of stimulated Tregs can distinguish between new and longstanding T1D, T2D, and controls.[140] Importantly, the development of novel T cell biomarkers in T1D parallels the development of targeted therapies such as anti-CD20 and anti-CD3 drugs. A consensus-based process of T cell–based biomarker development in T1D, ranging from discovery to fit-for-purpose testing and regulatory qualification, could be adopted in AIH.[139]

Lastly, candidate biomarkers may be relevant both for AIH and related autoimmune disorders, such as the shared positivity of relevant autoantibodies. Antinuclear autoantibodies against the heterogeneous nuclear ribonucleoprotein (hnRNP) A2 and B1, two splice variants of a protein involved in mRNA processing, were evaluated making use of Surface Plasmon Resonance Imaging (SPRi), a novel technique designed to evaluate stability of immune complexes. Results showed that the peptide 55–70 of the B1 subunit was highly specific for AIH versus SLE and RA.[141]

FUTURE DIRECTIONS: BENCH TO BEDSIDE AND BEDSIDE BACK TO BENCH, AND THE ROLE OF BIOMARKERS IN CLINICAL TRIALS

The successful introduction of novel AIH biomarkers into clinical care is challenging and requires a thoughtful, longitudinal approach to biomarker development. In this regard, a Roadmap for Discovery and Validation of Candidate Biomarkers can be a useful guide (Table 2). Initial discovery of novel biomarkers should focus on biological plausibility and link histological immune activity with easily measurable peripheral signals such as gene signatures, metabolites, or circulating immune cells. A machine learning approach that incorporates results from multiple different assays may provide distinct signatures that predict diagnosis or remission. Typically, this will involve a small cohort of well-characterized patients with similar disease phenotypes. The relationship of the marker to AIH disease status (active disease, remission, relapse, on/off treatment) should be defined and closely correlated with standard clinical indicators of disease activity (e.g., ALT and IgG, histological findings). Initial testing at this stage can make use of stored biospecimens and retrospective clinical analyses. Confirmation testing of a candidate biomarker utilizing retrospective or prospective cohorts should focus on optimization of the assay or method, development

TABLE 2 Roadmap for discovery and validation of candidate biomarkers in AIH

	Discovery	Confirmation	Validation	Regulatory approval
Key Properties	Biologically plausible Noninvasive or from peripheral blood Correlation with clinical markers (ALT, IgG, histology) and disease state	Assay optimization, standard operating procedure (SOP)	Acceptable interassay variability	Utility in point-of-care testing Cost-efficient and reproducible at routine clinical labs
Testing Setting	Single site/lab	Two/Three site/lab Transferability of assays	Multicenter, international sites/labs	Regulatory Agencies (EMA, FDA) Biomarker qualification process
Clinical Dataset for Correlation	Retrospective	Retrospective, Prospective	Prospective	Prospective Patient Registry
Outcome	Sensitivity and Specificity	Reliability	Sensitivity and Specificity	Correlation with relevant clinical outcomes
Theoretical Application to AIH	Metabolite of hepatic T cell activity measured in peripheral blood in adults with AIH	Metabolite accurately indicates histological activity despite normal ALT while on IST	Metabolite predicts relapse in adults and children with AIH	Metabolite is a biomarker of disease response to IST and can serve as a measurable datapoint in clinical trials

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; EMA, European Medicines Agency; FDA, Food and Drug Administration; IgG, immunoglobulin G; IST, immunosuppressive treatment.

of standard operating procedures, and testing at additional laboratories and clinical sites. Validation of candidate biomarkers should then focus on determining the assay's reliability across multiple centers using prospective cohorts and may be tested across a range of relevant populations, such as adults and children with AIH. In this phase, critical questions about reliability should be addressed including interassay variability from a larger group of laboratories, with further refinement of protocols as a result.

Regulatory approval of a biomarker may be considered as a central final goal of biomarker development in the context of AIH, and one that has been similarly considered in related fields of IBD and type 1 DM.[134,139] This phase of evaluation would encompass additional questions of utility in point-of-care assays that would be performed widely across commercial laboratories, in cost-efficiency considerations, and in use of large, prospective patient registries. From the regulatory perspective of the FDA or EMA, biomarker qualification would entail a careful process in coordination with the regulatory agencies and a resulting designation of a biomarker that can be utilized both clinically and as a surrogate endpoint for clinical trials. A theoretical example is an immune cell metabolite or gene signature that highly correlates with histological disease activity and has added accuracy (e.g., C-statistic) beyond standard laboratory data for determining histological remission in patients with AIH. Such a biomarker could be useful in the context of clinical care and in the context of clinical trials that are designed and powered around remission as an endpoint.

Another example of biomarker utility for clinical use is in the context of development of novel therapies that go beyond standard corticosteroids. This remains an important unmet need in AIH, especially given the meaningful advances in pathway-based targeted therapies in other autoimmune disorders which have moved beyond corticosteroids over the past decades. Indeed, biomarker development may be considered an essential ingredient to achieve this necessary advance in AIH. Peripheral blood biomarkers that could specify which patients have Th17 or Treg predominant abnormalities could help identify subsets of cohorts best suited to a particular targeted immunomodulatory therapy. Furthermore, there is a more fundamental need to improve the structure of clinical trials in AIH, with better patient selection and identification of trial endpoints with the goal of promoting enrollment. Biomarkers that can serve as predictors of response to therapy over a 1- or 2-year period, or biomarkers that can predict the likelihood of relapse after induction therapy, could aid in this process by permitting a more scientifically driven approach to study design and power analyses, as well as shortening the duration of trials themselves.

Finally, the patient experience must remain at the center of all aspects of care and therapeutic innovations

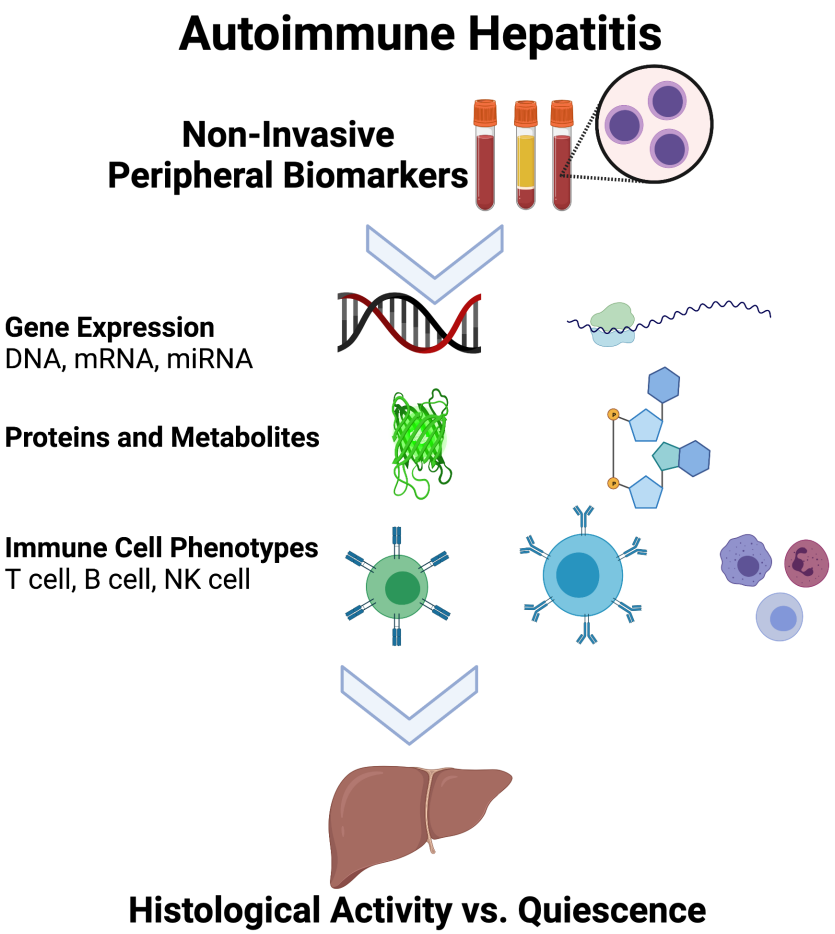

FIGURE 3 Noninvasive peripheral biomarkers in autoimmune hepatitis. Depiction of potential noninvasive peripheral biomarkers in autoimmune hepatitis that may act as surrogates for histological activity vs quiescence. miRNA, microRNA; mRNA, messenger ribonucleic acid; NK, natural killer.

in AIH. In a patient-centered approach context, novel biomarkers that can avoid unnecessary invasive interventions (e.g., substitute for a liver biopsy when considering withdrawal of IST), inform therapeutic decisions (e.g., identify patients who can receive less initial immunosuppression based on immune-sensitivity to agents), and improve quality of life (e.g., identify patients at very low risk of relapse not needing indefinite immunosuppression) would add significant value to the management of AIH.

This patient-centered experience can also be achieved by taking a bedside back to bench approach. Discovery studies where well-characterized populations of patients with AIH with specific clinical disease states (i.e., active disease, remission both on and off IST) are analyzed for various potential biomarkers would likely be of great promise. This approach would also be beneficial as novel treatments are being developed in AIH. For example, as novel therapies targeting B cells are currently being tested in clinical trials,[142] this may prompt further translational studies of B-cell related markers to help clarify the role of B cells in AIH pathogenesis.

CONCLUSIONS

This review highlights promising and emerging candidate biomarkers that may help focus the development of clinically significant biomarkers moving forward, particularly in relation to the different time periods of disease presentation, activity and management (Figure 2). As shown, these include ADA,[50] cytokeratin-18 death marker m65,[71] TGF-ß1,[51] BAFF,[43,44] Anti-ASGPR,[75]. FOXP3/RORγt ratio,[66] DNAse 1,[65] ferritin,[58] CD74:MIF ratio[76] and the vitamin D receptor.[55] Tregs also hold significant promise as protective markers or therapeutic targets in AIH similar to other disease states including organ rejection and DILI.[121]

To summarize, biomarkers have significant promise in improving personalized management of AIH (Figure 3). These markers will play various roles, including early identification of AIH, response to therapy, risk of relapse, and safer IST withdrawal. There is a substantial need for larger validation studies and prospective clinical trials, whereby the most promising candidate biomarkers are used in trials and clinical decision-making.

AUTHOR CONTRIBUTIONS

Claire Harrington: conceptualization, investigation, writing (original draft, review & editing). **Swathi Krishnan**: writing (original draft, review and editing). **Cara Mack**: writing (original draft, review and editing). **Paolo Cravedi**: writing (review and editing). **David N. Assis**: conceptualization, writing (original draft, review and editing). **Josh Levitsky**: conceptualization, writing (original draft, review and editing).

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SUPPORTING INFORMATION

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