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Diagnostics of Autoimmune Hepatitis Enabled by Non-Invasive Clinical Proteomics

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ABSTRACT

Background: Autoimmune hepatitis (AIH) may be difficult to diagnose and distinguish clinically and biochemically from other chronic liver diseases like metabolic dysfunction-associated steatotic liver disease (MASLD).

Aims: To identify pathways involved in the pathogenesis and identify disease-specific biomarkers of AIH.

Methods: We recruited 19 newly diagnosed patients with AIH, 17 with MASLD, and 19 healthy controls. Liver tissue and plasma were collected, and untargeted mass-spectrometry-based proteomics was performed. For classification of AIH versus MASLD and healthy, machine learning analyses were performed employing logistic regression models on liver and plasma proteome data. Findings were validated using data from the United Kingdom Biobank (UKB).

Results: We identified 7632 liver and 556 plasma proteins with 2521 liver and 227 plasma proteins differing between AIH and healthy, including 56 overlapping. Metabolic dysregulation and systemic immune activation characterised the AIH liver and plasma proteome, respectively. Plasma proteome profiling enabled classification of AIH from MASLD and healthy with an area under the receiver operating characteristic curve of 0.91 (0.09 SD). Validation in the UKB was possible for 8 of 20 diagnostic proteins and showed consistent directional changes. Three proteins (C7, ICAM1, cAST) were significantly different between AIH

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and MASLD/healthy in unadjusted analyses, and 6 of 8 proteins (C7, ICAM1, cAST, IGFBP3, TIMP1, TTR) were significantly different when adjusting for age and sex.

Conclusions: Clinical proteomic analyses of paired liver-plasma samples from patients with AIH enabled high diagnostic potential. Proteomics may constitute a novel non-invasive diagnostic tool for AIH if validated in larger, age- and sex-matched cohorts.

Clinical Trial Number: NCT05335603

1 | Introduction

Autoimmune hepatitis (AIH) is a rare immune-mediated fibroinflammatory liver disease that is clinically challenging to diagnose as no accurate biomarker exists [1–3]. The disease has a heterogeneous presentation with a spectrum ranging from acute liver failure [4] to the more frequent presentations with unspecific symptoms or asymptomatic subtle chronic liver inflammation only discovered through accidental findings of elevated liver enzymes [2]. Consequently, one-third of all patients with AIH, including children, have developed cirrhosis at the time of diagnosis [5]. As treatment with immunosuppressive medication can hamper disease progression, early and accurate diagnosing is essential.

Diagnosis of AIH is based on clinical, biochemical (particularly transaminase elevation) and serological (elevated IgG and presence of autoantibodies) findings, along with mandatory histological assessment [1, 2, 6]. However, no histological feature is pathognomonic to AIH, and the histological picture varies depending on the phenotypic presentation [6, 7]. Chronic AIH is characterised by a predominantly portal-based lymphoplasmacytic inflammation associated with interface hepatitis, and acute AIH typically reveals a lobular-based inflammation with sparse portal/periportal histological features of chronic hepatitis. Due to morphologic overlap with viral and drug-induced hepatitis, these etiologic factors must be excluded clinically.

Two scoring systems from the International AIH Group [8, 9] strive to encompass the various diagnostic parameters to assist AIH diagnostics. The scoring systems are challenged by criteria not specific for AIH (e.g., rosette formation and emperipolesis) and are primarily based on chronic AIH [6]. Hence, they focus on portal/periportal inflammation, which is mainly seen in chronic AIH, as well as elevated IgG levels and detectable autoantibodies, which may be absent in acute severe AIH [4, 10]. Modification of the histological criteria to address these difficulties has been proposed by the International AIH Pathology Group [6]. Nonetheless, they will not suffice to rule out or verify AIH with certainty, and the diverse presentations and lack of specific biomarkers make diagnosis challenging.

Another clinical difficulty lies in the differentiation between AIH and other liver diseases, particularly when conditions share overlapping clinical and biochemical features. This is especially true for diseases that present with elevated liver enzymes and hepatic steatosis on diagnostic imaging, as these findings are common in multiple liver disorders. The most important of these is metabolic dysfunction-associated steatotic liver disease (MASLD), given its high prevalence—affecting approximately 25% of the global population. There are no specific biomarkers for MASLD, making this

diagnosis difficult to exclude in patients without a classical presentation of AIH. This diagnostic ambiguity also poses a problem, as patients with AIH who have metabolic syndrome could be misdiagnosed with MASLD [11, 12].

Proteomics is widely used to generate insight into disease mechanism(s), biomarkers, and future treatment targets [13]. These methods can either focus on a preselected list of proteins (targeted) or analyse the entire detectable protein content of a sample (untargeted) [14], thereby producing a hypothesis-free, unbiased dataset ideal for biomarker discovery and characterisation [15]. Protein profiles of patients with AIH have been assessed using micro-array [16] as well as mass-spectrometry [17–20], revealing alterations in, e.g., immune-related proteins (polymeric immunoglobulin receptor [PIGR]), coagulation (Von Willebrand Factor [VWF]), growth factor-binding proteins (insulin-like growth factor [IGF]-binding protein complex acid-labile subunit [IGFALS]), and transport proteins (transthyretin [TTR], apolipoprotein E [APOE]).

In this study, we applied state-of-the-art untargeted mass-spectrometry-based proteomics on both liver and plasma samples from patients with newly diagnosed, untreated AIH, MASLD and healthy individuals (discovery cohort). First, we investigated the differences between AIH and healthy, with the aim of gaining insight into AIH pathophysiology. Secondly, we used machine learning algorithms to analyse proteomics to identify biomarker panels able to distinguish AIH from not only healthy, but also MASLD. Results were validated in the United Kingdom Biobank (UKB) which is a large research resource containing targeted plasma proteomics of approximately 54,000 individuals [21, 22].

2 | Materials and Methods

2.1 | Discovery Cohort

2.1.1 | Recruitment and Sample Collection

For a detailed description of the methods section and diagnostic criteria, see the File S1 and Table S1. We recruited 19 newly diagnosed patients with AIH, 17 with MASLD and 19 healthy individuals (Figure 1A) from our prospective cohort study FALL ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT05335603) identifier NCT05335603). The study was approved by the Regional Committee on Health Research Ethics (H-19038266) and performed in accordance with the Declaration of Helsinki (1975). All patients with AIH and MASLD and 9 of 19 healthy individuals (47%) underwent a liver biopsy for histological assessment and proteomics analyses (snap frozen liver tissue). Fasting plasma samples were obtained on the day of liver biopsy (Figure 1B). Liver tissue and plasma were stored at –80°C until mass spectrometry analyses. We were unable to

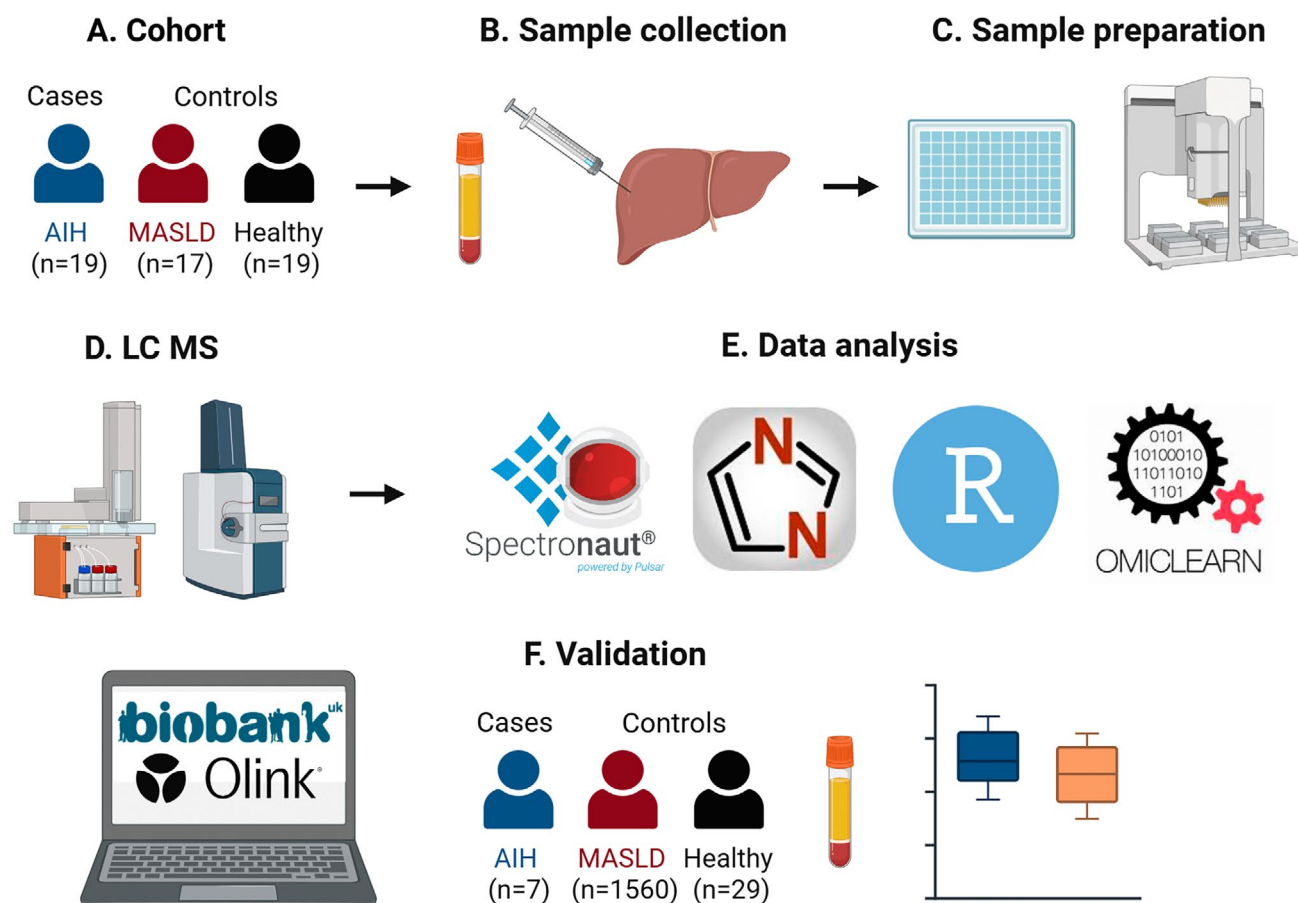


FIGURE 1 | Study design. (A) Newly diagnosed patients with AIH ($n = 19$), MASLD ($n = 17$) and healthy individuals ($n = 19$) were recruited from the Gastro Unit. (B) Snap-frozen liver tissue and plasma were collected at baseline. (C) Samples were prepared using a 96-well format and with automated sample loading. (D) Mass-spectrometry-based tissue and plasma proteomics were performed using PASEF-DIA on Bruker timsTOF instrument. (E) Processing of raw mass spectrometry data was performed by Spectronaut (liver) and DIA-NN (plasma). Bioinformatic analyses were performed with R. For the classification of AIH versus MASLD and healthy, machine learning analyses were performed with the OmicLearn platform. (F) Proteins from our predictive plasma proteome model were validated using the Olink plasma proteomics dataset from United Kingdom Biobank. AIH, autoimmune hepatitis; LC MS, liquid chromatography mass spectrometry; MASLD, metabolic dysfunction-associated steatotic liver disease.

obtain plasma from one patient with AIH (5%) and liver from two patients with MASLD (12%).

2.1.2 | Proteomics Profiling and Machine Learning Modelling

Liver and plasma samples were lysed with lysis buffer, and peptides analysed by EvoSep-Bruker timsTOF instrument (Figure 1C,D). Raw liver and plasma mass spectrometry files were processed using Spectronaut and DIA-NN, respectively (Figure 1E). Intensity values representing label-free quantifications (LFQs, i.e., relative concentrations) were log2-transformed, proteins with less than 70% of valid values in at least one group were removed, and missing values were imputed using the MinProb approach [23].

To address our first aim—understanding disease pathophysiology—we performed differential abundance analysis comparing patients with AIH to healthy controls only. This analysis was performed in R version 4.3.2 with the Limma package (3.58.1) which fits a linear model [24, 25] (Figure 1E).

A protein was considered differentially abundant if a false discovery rate adjusted p value by Benjamini–Hochberg was < 0.05 .

For our second aim—identification of diagnostic biomarkers for AIH—we performed machine learning using the online platform OmicLearn [26] to discover protein panels that were able to distinguish AIH from both MASLD and healthy controls combined (Figure 1E). Logistic regression was applied with a repeated stratified cross-validation approach. Several models were tested: (1) the predictive value of enzymatically measured (standard biochemistry) alanine aminotransferase (ALT); (2) plasma proteome; (3) plasma proteome and ALT combined; (4) liver proteome; (5) liver proteome and ALT combined; (6) plasma and liver proteome; (7) plasma and liver proteome combined with ALT. Model performances were evaluated with area under the receiver operating characteristic curve (ROC AUC), area under the curve of the precision–recall curve (PR AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Results are reported as the mean of all individual splits. For a detailed explanation of the performance metrics, please see Table S2.

In addition to the primary analyses, we performed sensitivity analyses both for the mechanistic and biomarker discovery aims by excluding patients with cirrhosis to assess the potential effect of advanced fibrosis. Furthermore, to investigate whether the observed proteomic alterations were specific to AIH rather than reflecting liver injury in general, we conducted a differential abundance analysis comparing AIH with MASLD. For the mechanistic analysis of AIH versus healthy, we additionally performed differential abundance analyses adjusting for age and sex to evaluate the potential influence of these demographic factors on the proteomic differences observed.

2.2 | Validation in United Kingdom Biobank

To validate our findings from the machine learning modelling, we used the UKB which is a large biomedical research database integrating data such as anthropometric measurements, standard biochemistry, diagnostic imaging and health records from more than 500,000 individuals recruited between 2006 and 2010 and continuously followed [21] (Figure 1F). All study participants gave their informed consent at baseline, and the UKB study protocol was approved by the UK North West Multi-centre Research Ethics Committee. For plasma proteomics, we used the data generated by Olink (Uppsala, Sweden) who have measured 2923 proteins (Olink Explore 3072 Proximity Extension Assay) expressed as Normalised Protein eXpression (NPX) units (an arbitrary and normalised unit on a log₂ scale) from approximately 54,000 UKB participants' baseline visit [22].

We identified individuals with AIH (cases) using ICD-10 diagnoses recorded between 1996 and February 2024. MASLD individuals were identified using ICD-10 diagnoses and diagnostic imaging together with anthropometric measurements, medical treatment history and biochemistry, securing concurrent metabolic dysfunction as recommended by Rinella et al. [27]. Healthy individuals were defined as individuals without any ICD-10 diagnoses, no signs of liver injury and not having metabolic dysfunction. This was to mimic the healthy group from our discovery cohort as much as possible. A detailed definition of diagnoses is listed in Table S3. Healthy and MASLD groups were collapsed into a combined control group.

As our discovery cohort consisted of newly diagnosed, untreated patients with AIH with active liver inflammation, we aimed to select AIH cases in UKB with liver inflammation at the time of proteomic analyses, i.e., at the baseline visit/recruitment. Hence, we included only AIH cases with elevated ALT levels at baseline, using a cut-off of 45 U/L for females and 70 U/L for males, based on clinical reference values in the Capital Region, Denmark.

The Olink Explore 3072 Proximity Extension Assay measures a predefined protein list. As only a subset of the prediction proteins identified during machine learning in our discovery cohort was measured by Olink, we were not able to validate the entire plasma model. Instead, we aimed to see if the predictive proteins also measured by Olink were differentially expressed in UKB AIH cases compared with UKB controls using Wilcoxon tests and visualised with box plots. Also, multiple linear regression

analyses adjusting for age and sex were performed. Analyses were performed in R version 4.3.0, and a $p < 0.05$ was considered statistically significant.

3 | Results

3.1 | Discovery Cohort

3.1.1 | Baseline Characteristics

All patients with AIH had type-1 AIH [2] with varying degrees of liver inflammation and fibrosis, herein five patients (26%) having cirrhosis at the time of diagnosis (Table 1 and Tables S4 and S5). Concurrent MASLD was present in 5 (26%) patients with AIH. In the MASLD group, only one patient (6%) had cirrhosis. Patients with AIH were older and had a larger proportion of female sex than in the MASLD and healthy groups ($p < 0.001$ and 0.007, respectively). As expected, patients with AIH also had higher levels of ALT, aspartate aminotransferase (AST) and alkaline phosphatase (ALP), reflecting ongoing liver inflammation ($p < 0.001$). Two patients with AIH (11%) had started prednisolone at the time of sample collection.

3.1.2 | Liver and Plasma Proteomics in AIH Versus Healthy

3.1.2.1 | One Third of the Liver and Plasma Proteome Are Altered by AIH. We identified 7632 liver proteins and 556 plasma proteins, with 337 present in both plasma and liver. The PCA plots demonstrated a clear separation between AIH and the MASLD and healthy groups in plasma (Figure 2A). The differentiation between AIH and MASLD based on the liver proteome was not as distinct (Figure 2B). PCA plots (Figure S1) suggested that cirrhosis may partially explain some of the observed variance in our dataset, potentially influencing the underlying patterns we identified. Therefore, sensitivity analyses excluding patients with cirrhosis were also performed.

Comparing AIH versus healthy, 2521 liver proteins (33% of total liver protein detection) and 227 plasma proteins (41% of total plasma protein detection) were significantly up- or downregulated; 56 of these proteins were significantly up- or downregulated in both the liver and the plasma (Figure 2C–F, Table 2). A complete list of all differentially expressed proteins, along with their associated log₂ fold changes (FCs) and adjusted p values, is provided in Tables S6 and S7.

3.1.2.2 | Proteins Involved in T Cell Activation and Auto-reactive Antibodies Are Upregulated in AIH. Among the differentially expressed proteins, intercellular adhesion molecule 1 (ICAM1) and immunoglobulin heavy variable 4–34 (IGHV4.34) were upregulated in plasma (FC 2.13 and FC 2.07, adjusted $p < 0.001$, respectively) and liver (FC 1.76, adjusted P value 0.001 and FC 2.60, adjusted p 0.014, respectively). Both proteins remained upregulated in plasma after adjusting for age and sex (adjusted $p < 0.020$). AIH is considered a T-cell mediated disease resulting in B-cell activation and antibody production [28], and ICAM1 is a cell-surface glycoprotein and adhesion receptor playing a crucial role in T-cell activation and migration

TABLE 1 | Baseline characteristics of those participants having plasma proteomics.

	AIH	MASLD	Healthy	<i>p</i>
Number	18	17	19	—
Age (years)	60 [48, 71]	49 [36, 58]	23 [22, 26]	<0.001
Female	16 (89%)	7 (41%)	9 (47%)	0.007
Non-smoker	17 (94%)	13 (81%)	19 (100%)	0.104
Alcohol (units/week)	1 [0, 4]	0 [0, 4]	3 [2, 7]	0.100
BMI (kg/m ²)	27.4 [23.5, 31.7]	30.0 [26.6, 34.7]	22.6 [21.3, 23.3]	<0.001
Fat mass (%)	40.8 (6.9)	37.6 (8.0)	20.7 (9.7)	<0.001
VAT (L)	2.6 (1.5)	3.8 (1.4)	0.5 (0.4)	<0.001
Concurrent MASLD	5 (28%)	—	0 (0%)	0.020
Cirrhosis	5 (28%)	1 (6%)	0 (0%)	0.019
Child-Pugh A	3 (17%)	1 (6%)	0 (0%)	0.148
Child-Pugh B	2 (11%)	0 (0%)	0 (0%)	0.125
Fibroscan				
Median (kPa)	9.4 [6.4, 13.5]	6.0 [5.1, 9.9]	4.1 [3.8, 4.3]	<0.001
CAP (dB/m)	242 (59)	288 (63)	174 (48)	<0.001
Treatment				
Steroid	2 (11%)	0 (%)	0 (0%)	0.125
Laboratory tests				
Haemoglobin (mmol/L)	8.7 (0.9)	8.8 (1.1)	8.7 (0.8)	0.916
Leukocytes (×10 ⁹ /L)	6.6 (1.4)	6.6 (2.3)	5.0 (1.4)	0.006
Thrombocytes (×10 ⁹ /L)	246 (75)	255 (65)	230 (46)	0.477
Creatinine (μM)	65 [56, 69]	75 [63, 85]	73 [63, 88]	0.051
Albumin (g/L)	35 [32, 36]	40 [38, 42]	41 [40, 43]	<0.001
INR	1.1 [1.0, 1.1]	0.9 [0.9, 1.0]	1.0 [1.0, 1.1]	<0.001
ALT (U/L)	179 [75, 358]	71 [57, 91]	20 [17, 26]	<0.001
AST (U/L)	106 [68, 220]	42 [35, 47]	20 [17, 25]	<0.001
ALP (U/L)	112 [80, 140]	81 [68, 115]	61 [50, 82]	<0.001
GGT (U/L)	102 [64, 165]	64 [37, 110]	—	0.385
Bilirubin (μmol/L)	11 [7, 13]	11 [7, 14]	8 [7, 12]	0.442

Note: There was 1 of 19 patients with AIH not having plasma obtained for proteomics analyses. Baseline characteristics of those participants having liver proteomics are listed in Table S4. Continuous data are reported as mean (SD) if normally distributed with homoscedasticity, and *P* values were calculated using one-way ANOVA. Otherwise, continuous data are reported as median [quartile 1, quartile 3], and *p* values were calculated using the Kruskal–Wallis test. Categorical data are reported as number (%), and *p* values were calculated using a chi-squared test.

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; GGT, gamma-glutamyl transferase; INR, international normalised ratio; MASLD, metabolic dysfunction-associated steatotic liver disease; VAT, visceral adipose tissue.

[29]. The protein has previously been found to be elevated in AIH, although it is also increased in other chronic liver diseases [30–33]. IGHV4.34 is part of the variable region in the heavy chain of immunoglobulins, which is involved in antigen recognition. Immunoglobulins are produced by B-cells and expressed on their cell surface or secreted [34]. IGHV4.34-encoded antibodies are known to be autoreactive [35] and associated with autoimmunity, e.g., systemic lupus erythematosus [36].

3.1.2.3 | The Insulin-Like Growth Factor System Is Impaired in AIH. We found a downregulation of the IGF system, which is synthesised by the liver and is involved in maintaining normal insulin sensitivity and mediating growth-promoting functions of growth hormone [37]. The peptide hormones IGF1, IGF2 and their binding proteins (IGFBP) IGFBP3, IGFBP5 and IGFBP6 were downregulated in plasma (FC <0.65, adjusted *p* < 0.001); IGFBP6 was also downregulated in the liver

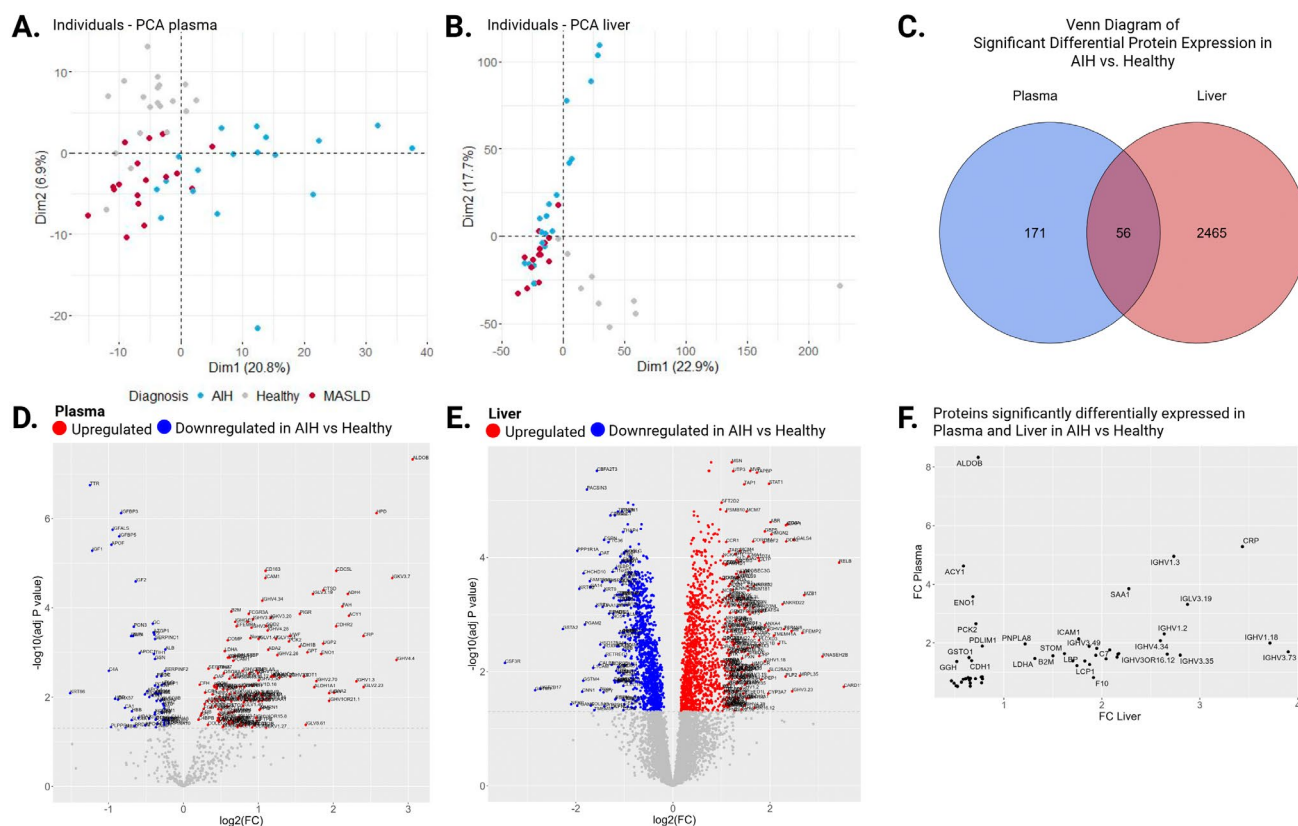


FIGURE 2 | Proteomics results: AIH versus Healthy individuals (including MASLD in PCA). (A) PCA was performed on plasma samples from patients diagnosed with AIH (blue, $n = 18$), MASLD (red, $n = 17$) and healthy individuals (grey, $n = 19$). Samples were colour-coded by diagnosis to reveal separation patterns based on principal components. (B) As (A) for liver samples (AIH, $n = 19$; MASLD, $n = 15$; healthy, $n = 9$). (C) Venn diagram displaying the number of unique and common proteins that were significantly differentially expressed in liver (brown) and plasma samples (blue) when comparing AIH with healthy individuals. (D) Volcano plot generated to show the upregulated (red) and downregulated (blue) proteins in plasma from AIH patients ($n = 18$) compared with healthy individuals ($n = 19$). (E) As (D) for liver samples (AIH, $n = 19$; healthy, $n = 9$). (F) Scatter plot created to correlate the FC of the 56 common proteins (see Venn diagram C) that were significantly differentially expressed in both liver and plasma when comparing AIH with healthy individuals. Each point represents a protein, highlighting the relationship between its expression in both tissues. Adj, adjusted; AIH, autoimmune hepatitis; dim, dimension; FC, fold change; MASLD, metabolic dysfunction-associated steatotic liver disease; PCA, principal component analysis.

(FC 0.66, adjusted $p < 0.001$). In contrast, IGFBP4 and IGFBP7 were upregulated in plasma (FC 1.60 and FC 1.38, respectively, adjusted $p = 0.02$).

IGF2 and IGFBP5 remained downregulated in plasma after adjusting for age and sex (adjusted p value 0.031 and 0.017, respectively).

3.1.2.4 | Common Differentially Expressed Proteins Indicate Plasma Alterations Originating From the Liver. Of the 56 proteins that were differentially expressed in liver and plasma (Figure 2F, Table 2), some were consistently elevated in both tissues, including proteins involved in inflammation and immune response. These included c-reactive protein (CRP), serum amyloid A-1 protein (SAA1), beta-2-microglobulin (B2M), complement component C7 (C7), ICAM1 and, in particular, a variety of immunoglobulin heavy and light chain variants, suggesting a liver-dependent systemic response [32, 38–41]. The proteome of both liver and plasma showed impaired liver function and protein synthesis in AIH, including downregulation of albumin (ALB) [42], IGFALS [37], proteins involved in lipid metabolism (e.g., apolipoprotein F [APOF],

apolipoprotein M [APOM], phosphatidylcholine-sterol acyl-transferase [LCAT], zinc-alpha-2-glycoprotein [AZGP1]) [43–45] and the transport proteins TTR (FC < 0.50 , adjusted $p < 0.001$ in both liver and plasma) and retinol-binding protein (RBP4; FC plasma = 0.62, adjusted $p < 0.001$).

Of the mentioned proteins, APOF, AZGP1 and TTR remained differentially expressed in both liver and plasma after adjusting for age and sex, and CRP, B2M, ICAM1, several IGHV and IGLV proteins, APOM and RBP4 remained differentially expressed in plasma after adjusting for age and sex (adjusted P value < 0.040).

3.1.2.5 | ALT and AST Elevation in AIH Comprise ALT1 and Cytoplasmic AST. We measured two isoforms of both ALT and AST: ALT1 (also named GPT) and ALT2 (also named GPT2) plus cytoplasmic AST (cAST; also named GOT1) and mitochondrial AST (mAST; also named GOT2). In the liver, all four isoforms were detected, but only ALT2 was downregulated in AIH compared with healthy (FC 0.67, adjusted $p = 0.002$). In plasma, we detected both isoforms of AST (cAST and mAST), ALT1 but not ALT2. cAST and ALT1 were upregulated in plasma in AIH compared with

TABLE 2 | Plasma and liver protein alterations in AIH versus healthy.

Gene name	Protein name	Level	Protein description
<i>Immune response and inflammation</i>			
IGHV1.18, 1.2, 1.3, 3.35, 3.49, 3.73, 4.34, 5.51	Immunoglobulin heavy variables	↑ plasma ↑ liver	Part of immunoglobulin heavy chain variable region, involved in antigen recognition
IGKV1.5, 1.8, 1.16, 1.17, 1.27, 1.39, 3.7, 3.20, 1D.8, 1D.16, 1D.33, 3D.11, 3D15, 3D.20	Immunoglobulin kappa variables	↑ plasma	Part of immunoglobulin light chain variable region, involved in antigen recognition
IGLV3.19, 4.69	Immunoglobulin lambda variables	↑ plasma ↑ liver	Part of immunoglobulin light chain variable region, involved in antigen recognition
B2M	Beta-2-microglobulin	↑ plasma ↑ liver	Light chain of the MHC class I complex; stabilises the structure for antigen presentation in immune surveillance
HLA.B	HLA class I histocompatibility antigen, B alpha chain	↑ plasma ↑ liver	Heavy chain of the MHC class I complex; presents peptide antigens to CD8+ T cells for immune surveillance
CD8A	T-cell surface glycoprotein CD8 alpha chain	↑ liver	Coreceptor for MHC class I, enabling T-cell activation, cytotoxic response, and memory formation
TAPBP	Tapasin	↑ liver	Involved in the association of MHC class I with transporter associated with antigen processing (TAP) and in the assembly of MHC class I with peptide
TAP1, TAP2	ABC-type antigen peptide transporter	↑ liver	Antigen transporters, crucial for antigen presentation to MHC class I molecules
C7	Complement component C7	↑ plasma ↑ liver	Part of the complement system, involved in immune defence
ICAM1	Intercellular adhesion molecule 1	↑ plasma ↑ liver	Facilitates leukocyte-endothelial adhesion and T-cell activation
SAA1, CRP	Serum amyloid A-1, C-reactive protein	↑ plasma ↑ liver	Acute-phase protein involved in inflammation and immune response
GBP3, GBP4	Guanylate-binding protein 3 and 4	↑ liver	Interferon-inducible GTPase that plays important roles in innate immunity
PIGR	Polymeric immunoglobulin receptor	↑ plasma	Mediates transcytosis of IgA and IgM across mucosal epithelial cells
STAT1	Signal transducer and activator of transcription	↑ liver	Signal transducer and transcription activator that mediates cellular responses to interferons, cytokines and growth factors
NCF4	Neutrophil cytosol factor 4	↑ liver	Part of the NADPH-oxidase complex, critical for oxidative burst and reactive oxidant generation
SERPINB9	Serpin B9	↑ liver	Inhibits granzyme B protecting cells from granzyme B mediated apoptosis and playing a role in immune regulation and cytotoxic lymphocyte activity
<i>Metabolism and transport</i>			
TTR	Transthyretin	↓ plasma ↓ liver	Transports thyroxine hormone (T4) and retinol, the latter by forming a complex with RBP4

(Continues)

TABLE 2 | (Continued)

Gene name	Protein name	Level	Protein description
RBP4	Retinol-binding protein	↓ plasma	Transports retinol by forming a complex with TTR
IGF1 and 2	Insulin-like growth factor I and II	↓ plasma	Involved in growth, cell proliferation together with involvement in lipid, protein, and glucose metabolism
IGFBP3 and 5	Insulin-like growth factor-binding protein 3 and 5	↓ plasma	Bind to and prolong the half-life of IGFs, which are involved in growth and metabolism
IGFBP4 and 7	Insulin-like growth factor-binding protein 4 and 7	↑ plasma	Bind to and prolong the half-life of IGFs, which are involved in growth and metabolism
IGFALS	Insulin-like growth factor-binding protein complex acid-labile subunit	↓ plasma ↓ liver	Bind to and prolong the half-life of IGF1, which is involved in growth and metabolism (also mentioned under “Growth and cell regulation”)
ALDOB	Fructose-bisphosphate aldolase B	↑ plasma ↓ liver	Involved in glycolysis, gluconeogenesis, and fructose metabolism
PCK1	Phosphoenolpyruvate carboxykinase 1	↓ liver	Cytosolic isoform of PEP CK. Rate limiting enzyme in gluconeogenesis
PCK2	Phosphoenolpyruvate carboxykinase 2	↑ plasma ↓ liver	Mitochondrial isoform of PEP CK. Rate limiting enzyme in gluconeogenesis
GALK1	Galactokinase	↓ liver	Catalyses phosphorylation of galactose
HK3	Hexokinase-3	↑ liver	Catalyses phosphorylation of hexoses and initiating glycolysis by converting glucose to glucose-6-phosphate
APOF, APOM	Apolipoprotein F and M	↓ plasma ↓ liver	Regulates lipid metabolism and lipoprotein transport
APOE, APOA1	Apolipoprotein E and A1	↓ liver	Regulates lipid metabolism and lipoprotein transport
LCAT	Phosphatidylcholine-sterol acyltransferase	↓ plasma ↓ liver	Synthesised mainly in the liver and secreted to plasma where it esterifies cholesterol on HDL and LDL surfaces
AZGP1	Zinc-alpha-2-glycoprotein	↓ plasma ↓ liver	Lipid mobilising factor involved in fat metabolism
PLIN5	Perilipin-5	↓ liver	Lipid droplet-associated protein
NPL	N-acetylneuraminase lyase	↑ liver	Catalyses the cleavage of sialic acids and prevents them from being recycled and returning to the cell surface
GPX2	Glutathione peroxidase 2	↑ liver	Reduces hydroperoxides using glutathione, maintaining cellular redox balance
<i>Coagulation</i>			
F2	Prothrombin	↓ plasma	Precursor to thrombin. Functions in blood homeostasis, inflammation, and wound healing
F9	Coagulation factor 9	↓ plasma	Involved in blood coagulation by converting factor X to its active form
VWF	Von Willebrand factor	↑ plasma	Serves as an adhesion molecule for platelets thereby initiating platelet plug formation. Also an acute-phase protein

(Continues)

TABLE 2 | (Continued)

Gene name	Protein name	Level	Protein description
TBXAS1	Thromboxane-A synthase	↑ liver	Catalyses the conversion of prostaglandin H2 to thromboxane A2, an inhibitor of blood vessel constriction and platelet aggregation
PROC	Vitamin K-dependent protein C	↓ plasma	Regulates blood coagulation by inactivating factors Va and VIIIa
<i>Cell adhesion and structural</i>			
CDHR2	Cadherin-related family member 2	↑ plasma	Involved in cell adhesion and maintaining epithelial integrity
CDH1	Cadherin-1	↑ plasma ↓ liver	Involved in cell adhesion and maintaining epithelial integrity
<i>Growth and cell regulation</i>			
FGL1	Fibrinogen-like protein 1	↑ liver ↑ plasma	Immune suppressive molecule that inhibits antigen-specific T-cell activation Secreted by, and promotes growth of, hepatocytes
IGFALS	Insulin-like growth factor-binding protein complex acid-labile subunit	↓ liver ↓ plasma	Bind to and prolong the half-life of IGF1, which is involved in growth and metabolism (also mentioned under “Immune response and inflammation”)
ADA2	Adenosine deaminase 2	↑ plasma ↑ liver	Involved in extracellular adenosine degradation; regulates cell proliferation and differentiation
HMOX1	Heme oxygenase 1	↑ liver	Catalyses heme degradation, generating biliverdin, carbon monoxide, and iron; protects cells from apoptosis
<i>Others</i>			
FAM151A	Family with sequence similarity 151 member A	↓ liver	Membrane protein with unknown function
FAM169A	Family with sequence similarity 169 member A	↑ liver	Nuclear inner membrane with unknown function Limited known function, possibly involved in cell signalling
ANXA9	Annexin A9	↓ liver	Acetylcholine receptor with low affinity, targeted by pemphigus vulgaris antibodies in keratinocytes

Note: ↑ increased protein levels in AIH compared with healthy. ↓ decreased protein levels in AIH compared with healthy.

healthy (FC 3.05, adjusted *P* value 0.003; FC 3.15, adjusted *P* value <0.001, respectively), whereas mAST was unchanged (FC 0.87, adjusted *p* = 0.320). There was an almost perfect correlation between levels of enzymatically measured (standard biochemistry) plasma ALT and plasma ALT1 together with enzymatically measured AST and cAST in patients with AIH (Pearson *r* 0.96 and 0.98, respectively, *p* < 0.001), and a non-significant correlation between enzymatically measured AST and mAST (Pearson *r* 0.28, *P* value 0.313).

3.1.2.6 | Enrichment Analysis Identifies Metabolic Dysregulation in the Liver and Systemic Immune Activation of Plasma in AIH. To better understand which biological processes, molecular functions and cellular components are altered in AIH compared with healthy controls, an enrichment analysis of the differentially expressed proteins was performed. The liver and plasma proteome uncovered distinct molecular traits. Functional analyses of the biological processes in the liver (Figure S2) showed a marked enhancement in protein

processing, characterised by significant enrichment in ribonucleic acid (RNA) metabolism, cytoplasmic translation and protein assembly. Additionally, energy production pathways were upregulated, indicating increased metabolic activity. In contrast, plasma samples showed enrichment in immune-related processes, including wound healing, blood coagulation, complement activation and both humoral and cellular immune responses (Figure S3).

Molecular function analysis of the liver (Figure S4) also revealed enrichment in activities related to protein synthesis and energy metabolism, as well as cellular structure (cadherin and actin binding). In the plasma, molecular functions (Figure S5) were enriched in enzyme activities and binding activities related to immune responses (e.g., complement and integrin binding).

Analysis of enriched cellular components in the liver (Figure S6) revealed enhancement of structures related to protein synthesis (e.g., ribosome) and transport (e.g., cytoplasmic vesicle lumen),

as well as mitochondrial structures involved in energy metabolism. In plasma (Figure S7), the analysis identified components associated with the extracellular matrix, secretory pathways and various blood components (e.g., blood microparticles and lipoprotein particles). Taken together, the results from the enrichment analyses suggest that while metabolic dysregulation is a dominant feature in the liver during AIH, systemic immune activation is a hallmark of the AIH plasma proteome.

3.1.2.7 | Excluding AIH Patients With Cirrhosis has a Greater Impact on the Plasma Proteome Than the Liver Proteome. Excluding patients with cirrhosis decreased the number of differentially expressed proteins when comparing AIH with healthy individuals: 2311 proteins in the liver were detected (a decrease of 8% compared to analysis including patients with AIH and cirrhosis), 115 proteins in plasma (decrease by 45%), with 34 proteins in common between liver and plasma (decrease by 39%) (Figure S8). A complete list of all these proteins, along with their associated log2 FCs and adjusted *p* values, is provided in Tables S8 and S9.

When excluding patients with cirrhosis, 678 proteins were no longer significantly altered. This is partly due to a decreased statistical power, but it also suggests that the lost proteins may reflect the cirrhotic stage. For instance, proteins synthesised in the liver and involved in coagulation (e.g., coagulation factor 9 (F9)) and the innate immune system (e.g., complement component C8 alpha chain [C8A], complement component C8 beta chain [C8B], complement subcomponent C1r [C1R] and C1s [C1S]) were downregulated in plasma in the total AIH cohort, but unchanged when excluding patients with cirrhosis. This is in line with the impaired liver function, coagulopathy [46] and increased susceptibility to infections in cirrhosis [47, 48]. Many of the immunoglobulin heavy chain proteins (IGHV gene families) and light chain kappa proteins (IGKV gene families) were upregulated in plasma in the total AIH cohort but unchanged when excluding patients with cirrhosis. This corresponds to the well-known hyperglobulinemia observed in cirrhosis [41, 49]. Also, metalloproteinase inhibitor 1 (TIMP1) was upregulated in plasma in the total AIH cohort but unchanged when excluding patients with cirrhosis. The protein is associated with cirrhosis [50] and included in the enhanced liver fibrosis (ELF) test [51]. Interestingly, TIMP1 is downregulated by IGF1 [52], which was also reduced in our study. Regarding the dysregulated IGF system observed in the total AIH cohort, downregulation of IGF1, IGF2, IGFBP3, IGFBP5 and IGFBP7 in plasma remained significant after excluding patients with cirrhosis, indicating that these changes are likely related to underlying AIH-related pathophysiological processes beyond cirrhosis alone. In contrast, IGFBP4 and IGFBP7 were no longer upregulated in plasma after excluding cirrhosis, suggesting that their elevation could be more associated with advanced fibrosis or cirrhosis-related alterations.

3.1.3 | Liver and Plasma Proteomics in AIH Versus MASLD

To better investigate whether some of the observed proteomic changes in AIH reflected general liver inflammation/damage or were more closely linked to AIH-specific pathophysiology, we compared AIH to MASLD. Here, we found 976 liver proteins

(13% of total liver protein detection) and 182 plasma proteins (33% of total plasma protein detection) which were significantly up- or downregulated, with 29 of these proteins significantly up- or downregulated in both the liver and the plasma. A complete list of all differentially expressed proteins, including log2 FCs and adjusted *p* values, is provided in Tables S10 and S11.

Proteins involved in immune processes like ICAM1, many IGLV and IGHV proteins, including IGHV4.34, were upregulated in AIH plasma and ICAM1 also in AIH liver when compared with MASLD. The findings remained significant also after excluding cirrhosis.

The IGF system was also notably dysregulated in AIH compared with MASLD. In plasma, IGF1, IGF2, IGFBP3 and IGFBP5 were downregulated (FC < 0.78, adjusted *p* < 0.050), and IGFBP5 was similarly downregulated in liver (FC 0.72, adjusted *p* = 0.021). Conversely, IGFBP2 was upregulated in both liver and plasma (FC > 2.13, adjusted *p* < 0.050), and IGFBP7 was upregulated in liver (FC 1.56, adjusted *p* = 0.029). After exclusion of patients with cirrhosis, only the upregulation of IGFBP2 in plasma remained significant. This contrasted with the AIH versus healthy comparison, where broader alterations of the IGF system persisted after excluding cirrhosis.

The transport proteins TTR and RBP4 were downregulated in plasma (FC < 0.58, adjusted *p* < 0.002), and TTR was also downregulated in liver in AIH (FC 0.69, adjusted *p* = 0.010). These differences remained significant after excluding cirrhosis.

3.1.4 | Diagnostic Classification of AIH Using Machine Learning

To evaluate the predictive performance of the protein findings in a diagnostic classification of AIH versus MASLD and healthy, we performed machine learning on our plasma and liver proteome data, individually and combined. For comparison, we evaluated the predictive performance of enzymatically measured ALT levels, which are commonly used in AIH diagnosing [8] and for evaluating treatment response [2] (Table 3 and Figure 3). Overall, all models demonstrated excellent discriminative ability with ROC AUCs ranging from 0.89 to 0.94. Model 1, which relied solely on ALT, performed the worst across all metrics, indicating its limited effectiveness in classifying AIH versus MASLD and healthy. The plasma proteome (model 2) demonstrated the highest PPV of 0.88 and specificity of 0.94, indicating that a positive result is highly likely to accurately reflect AIH, thereby minimising the risk of false positives. However, it was more challenging to identify AIH effectively, reflected by a sensitivity of only 0.64. The liver proteome in combination with ALT (model 5) performed as well as the liver and plasma proteome combined (model 6), both achieving the highest ROC AUC (0.94) and PR AUC (0.94), reflecting excellent overall class discrimination.

The 20 proteins selected as features for the plasma (model 2) and liver (model 4) proteome models are listed in Figure 3, which illustrates the importance of the proteins (Figure 3A,C), their variations between AIH and MASLD/healthy individuals (Figure 3B,D), and the ROC curve for the plasma proteome

TABLE 3 | Mean performance metrics of different models classifying AIH versus MASLD and healthy.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
ROC AUC	0.89 (0.09)	0.91 (0.09)	0.93 (0.08)	0.92 (0.08)	0.94 (0.07)	0.94 (0.08)	0.94 (0.08)
PR AUC	0.83 (0.12)	0.87 (0.12)	0.90 (0.11)	0.92 (0.07)	0.94 (0.07)	0.94 (0.07)	0.94 (0.07)
Sensitivity	0.54 (0.22)	0.64 (0.23)	0.71 (0.20)	0.77 (0.15)	0.77 (0.19)	0.78 (0.21)	0.77 (0.21)
Specificity	0.91 (0.10)	0.94 (0.11)	0.92 (0.11)	0.89 (0.13)	0.88 (0.15)	0.86 (0.14)	0.86 (0.14)
PPV	0.77 (0.26)	0.88 (0.18)	0.85 (0.20)	0.86 (0.15)	0.85 (0.17)	0.84 (0.15)	0.84 (0.15)
NPV	0.80	0.84	0.86	0.84	0.83	0.84	0.84
Accuracy	0.79 (0.09)	0.84 (0.10)	0.85 (0.11)	0.84 (0.09)	0.83 (0.12)	0.83 (0.09)	0.82 (0.09)

Note: Mean performances (SD) of different models classifying patients with AIH ($n = 19$ if liver proteome without plasma proteome, $n = 18$ if plasma proteome) against controls, the latter comprising healthy individuals ($n = 19$ if plasma proteome without liver proteome, $n = 9$ if liver proteome) and patients with MASLD ($n = 17$ if plasma proteome without liver proteome, $n = 15$ if liver proteome). A logistic regression approach with repeated stratified cross validation was used via the OmicLearn Platform. Standard deviation of NPV was not available in the output from OmicLearn. Model 1: ALT. Model 2: Plasma proteome. Model 3: Plasma proteome + ALT. Model 4: Liver proteome. Model 5: Liver proteome + ALT. Model 6: Plasma proteome + Liver proteome. Model 7: Plasma proteome + Liver proteome + ALT. Abbreviations: AIH, autoimmune hepatitis; AUC, area under the curve; MASLD, metabolic dysfunction-associated steatotic liver disease; NPV, negative predictive value; PPV, positive predictive value; PR AUC, area under the curve of the precision-recall curve; ROC AUC, area under the receiver operating characteristic curve.

model (model 2, Figure 3E) and liver proteome model (model 4, Figure 3F).

To evaluate whether these proteins provided additional value to ALT alone, the proteins were categorised based on their correlation with ALT: those with strong correlation (adjusted $R^2 > 0.50$), weak correlation ($0.20 \leq \text{adjusted } R^2 \leq 0.50$), and no correlation (adjusted $R^2 < 0.2$) (Figures S9 and S10, Tables S12 and S13). For both models, more than half the proteins did not correlate with ALT, including plasma proteins from the IGF system (IGFBP3, IGFBP5, IGF2), immunoglobulin heavy and lambda variables (IGHV1.2, IGHV3.49, IGHV4.34, immunoglobulin lambda variable 1–47 (IGLV1.47)) and TIMP1, and IGFALS in the liver. Proteins strongly associated with ALT comprised UTP—glucose-1-phosphate uridylyltransferase (UGP2), prothrombin (F2), ICAM1, GOT1 (cAST) and fructose-bisphosphate aldolase B (ALDOB) in the plasma and signal transducer and activator of transcription (STAT1), hexokinase-3 (HK3) and guanylate-binding protein 3 (GBP3) in the liver.

When excluding patients with cirrhosis, all proteins remained differentially expressed except TIMP1 in plasma. This highlights the association of TIMP1 with fibrosis [51] and its limited diagnostic relevance in patients with non-cirrhotic AIH. The set of selected proteins for the plasma and liver models changed after excluding patients with cirrhosis, despite the fact that 39 of 40 proteins initially selected as top predictors remained differentially expressed (Figure S11). In the plasma model, 9 of 20 proteins remained selected, 8 of those 9 proteins being in the top 10 most important prediction proteins out of 20, and TTR being the most important prediction protein in both the total cohort and the non-cirrhotic cohort plasma model. In the liver model, 12 of 20 proteins remained selected. The plasma and liver models still performed well with ROC AUCs of 0.88 and 0.92, respectively. A complete summary of model statistics is provided in Table S14.

3.2 | Validation in United Kingdom Biobank

In the UKB cohort, we identified 39 AIH cases. Among these, 32 had a normal ALT at baseline and were subsequently excluded

from the study. The control group consisted of 1560 individuals with MASLD and 29 healthy controls, who were included for subsequent analyses.

Baseline characteristics of the cohort are listed in Table S15. The AIH group included both males and females (female sex 57.1%) with a median age of 62 years [Q1 59, Q3 65] and a median ALT of 96 U/L [84, 109]. At the time of analysis, 6 of 7 had not yet received an ICD-10 AIH diagnosis, meaning that they had incident AIH. One individual with prevalent AIH had been diagnosed around 10 years prior. The remaining 6 incident AIH cases received their diagnosis between 1 and 13 years after recruitment. Among them, one was diagnosed with cirrhosis 1 month before recruitment but received the AIH diagnosis 1 year after recruitment.

Among the 20 plasma predictive proteins identified through machine learning in our discovery cohort, 8 were measured by Olink: C7, ICAM1, GOT1 (cAST), TIMP1, VWF, F2, IGFBP3 and TTR (Figure 4). In the discovery cohort, C7, ICAM1, GOT1 (cAST), TIMP1 and VWF were found to be significantly up-regulated in AIH compared with controls, with the increases in C7, ICAM1 and GOT1 (cAST) confirmed in the validation cohort ($p < 0.05$). These results align with expectations, as C7 and ICAM1 are known to be associated with immune responses (see Table 2 and as described previously), and GOT1 (cAST) is correlated with ALT. In the validation cohort, TIMP1 and VWF showed only nominal increases in AIH compared with controls. TIMP1 and VWF are associated with cirrhosis [50, 53], and the lower prevalence of cirrhosis in the validation cohort (14%) compared with the discovery cohort (26%) may account for the absence of significant elevation of these proteins. Similar to our findings in the discovery cohort, F2, IGFBP3 and TTR were decreased in AIH compared with controls, though these changes were not statistically significant ($p > 0.05$).

The 12 prediction proteins not measured by Olink and thus not validated were IGHV3.49, IGHV4.34, IGHV1.2, IGHV1.3, IGLV1.47, immunoglobulin heavy constant gamma 1 (IGHG1), calcium-independent phospholipase A2-gamma (PNPLA8), ALDOB, caldesmon (CALD1), UGP2, IGF2 and IGFBP5.

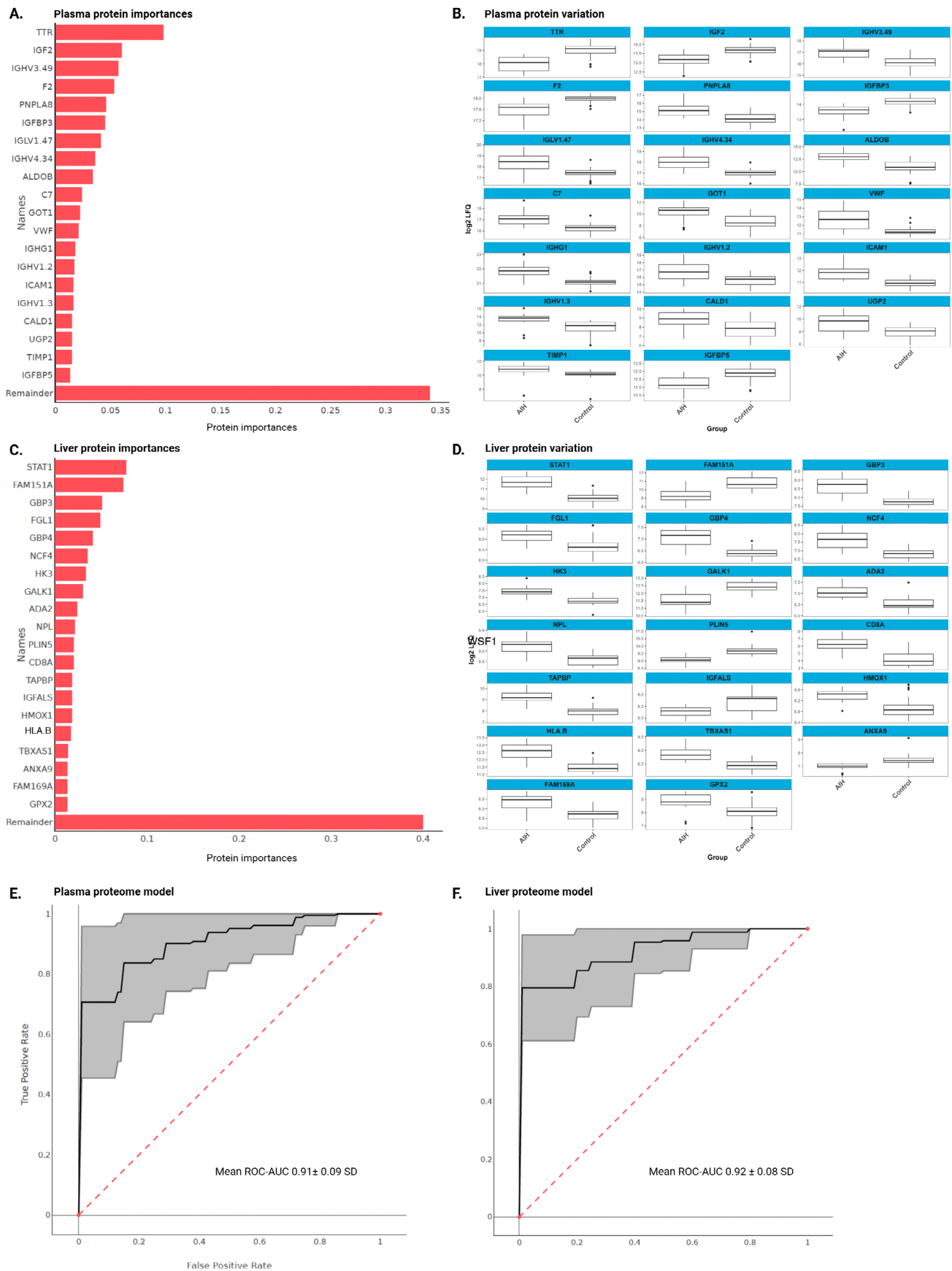


FIGURE 3 | Legend on next page.

FIGURE 3 | Protein importance, variation and ROC curves for plasma and liver proteome models. (A) Top 20 plasma proteins ranked by their importance in the predictive plasma proteome model (model 2). (B) Variation in log2 LFQ of the top 20 plasma proteins (listed in A) between AIH and controls (MASLD + healthy). Greater differences in variation indicate better predictive proteins. (C) As (A) for liver (model 4). (D) As (B) for liver. (E) ROC curve for plasma proteome model. The red stippled line represents the chance level (AUC = 0.50). The black solid line indicates the mean ROC curve, with the grey shaded area showing ± 1 standard deviation around the mean. (F) As (E) for liver (model 4). ADA2, adenosine deaminase 2; AIH, autoimmune hepatitis, ALDOB, fructose-bisphosphate aldolase B; ANXA9, annexin A9; C7, complement component C7; CALD1, caldesmon; CD8A, T-cell surface glycoprotein CD8 alpha chain; F2, prothrombin; FAM151A, protein FAM151A; FAM169A, family with sequence similarity 169 member A; FGL1, fibrinogen-like protein 1; GALK1, galactokinase; GBP3, guanylate-binding protein 3; GBP4, guanylate-binding protein 4; GOT1 cytoplasmic aspartate aminotransferase; GPX2, glutathione peroxidase 2; HK3, hexokinase-3; HLA.B, HLA class I histocompatibility antigen, B alpha chain; HMOX1, heme oxygenase 1; ICAM1, intercellular adhesion molecule 1; IGF2, insulin-like growth factor II; IGFALS, insulin-like growth factor-binding protein complex acid labile; IGFBP3, insulin-like growth factor-binding protein 3; IGFBP5, insulin-like growth factor-binding protein 5; IGHG1, immunoglobulin heavy constant gamma 1; IGHV1.2, immunoglobulin heavy variable 1–2; IGHV1.3, immunoglobulin heavy variable 1–3; IGHV3.49, immunoglobulin heavy variable 3–49; IGHV4.34, immunoglobulin heavy variable 4–34; IGLV1.47, immunoglobulin lambda variable 1–47; LFQ, label-free quantifications; MASLD, metabolic dysfunction-associated steatotic liver disease; NCF4, neutrophil cytosol factor 4; NPL, N-acetylneuraminase lyase; PLIN5, perilipin-5; PNPLA8, calcium-independent phospholipase A2-gamma; ROC, receiver operating characteristic; ROC AUC, area under the receiver operating characteristic curve; STAT1, signal transducer and activator of transcription; TAPBP, tapasin; TBXAS1, thromboxane-A synthase; TIMP1, metalloproteinase inhibitor 1; TTR, transthyretin; UGP2, UTP—glucose-1-phosphate uridylyltransferase; VWF, von Willebrand factor.

Excluding the AIH individual already having an ICD-10 recorded AIH diagnosis at analysis (hence having prevalent AIH with presumably ongoing medical treatment) did not change the results. Excluding individuals with cirrhosis (one AIH individual, six MASLD controls) resulted in C7 no longer being differentially expressed in AIH compared with controls ($p = 0.140$). Adjusting for age and sex in multiple linear regression analyses resulted in significant changes of C7, ICAM1, GOT1 (cAST), TIMP1, IGFBP3 and TTR in AIH compared with healthy individuals (Table S16).

4 | Discussion

This study is the first to describe alterations in the liver and plasma proteome of patients with newly diagnosed, untreated AIH compared with MASLD and healthy individuals. We found profound alterations of the AIH liver and plasma proteome, and that metabolic dysregulation and systemic immune activation were key features. Proteins involved in T-cell activation and auto-reactive antibodies were upregulated, and the IGF system was dysregulated. We also found that, in AIH, ALT and AST (enzymatically measured) consisted of the isoforms ALT1 and cAST. Both the liver and plasma proteome performed well in differentiating AIH from MASLD and healthy, demonstrating promising biomarker panels. Validation in UKB showed consistent directional changes for the proteins that were analysed in the UKB.

IGF1 and IGF2 are peptide hormones synthesised in the liver together with their binding proteins. They have insulin-like functions, contributing to the maintenance of normal insulin sensitivity, but their main roles rely on mediating the growth-promoting functions of growth hormone. Generally, the IGF system is considered hepatoprotective, being involved in organ development, growth and regeneration, together with involvement in lipid, protein and glucose metabolism. Treatment with systemic IGF1 in rats has shown to reduce fibrosis formation in the liver and improve liver function [54, 55]. Impairment of the IGF system in chronic liver diseases like MASLD, cirrhosis

and hepatocellular carcinoma is well established [37] and aligns with many of our findings in AIH, although MASLD has been associated with increased levels of IGFBP3 [56].

In AIH, potential dysregulation of the IGF system remains unexplored. We identified consistent downregulation of IGF1, IGF2, IGFBP3 and IGFALS in AIH compared with both healthy controls and MASLD. Interestingly, these alterations were attenuated after exclusion of patients with cirrhosis in the AIH versus MASLD comparison but remained evident when comparing AIH with healthy controls. One possible explanation is that the IGF system may be more closely associated with fibrotic processes rather than cirrhosis alone. In our cohort, both the AIH and MASLD groups exhibited varying degrees of fibrosis (53% with F1–F3 fibrosis and 65% with F1–F2 fibrosis, respectively; Table S5), but cirrhosis was more prevalent in AIH (26%) than MASLD (6%) (Table 1 and Table S5). Thus, when comparing AIH with healthy controls, who had no fibrosis and no cirrhosis, the IGF system alterations persisted, whereas the fibrosis burden between AIH and MASLD was more similar after excluding cirrhosis, potentially explaining the loss of significance. However, our study was not sufficiently powered to adjust for fibrosis stage in the analyses. The disruptions in the IGF system in patients with AIH, observed in our study, could also be associated with the well-known disturbances of glucose metabolism observed in these patients [57–59]. While these findings highlight potential dysregulation of the IGF system in AIH, functional validation through mechanistic studies or targeted assays in independent patient cohorts would be necessary to confirm these results and elucidate their biological relevance.

ALT and AST are found in different organs within the body, with the highest concentration observed in the liver. They are both considered tissue leakage proteins, normally present in the blood in low concentrations, but with rising levels upon tissue damage. ALT1 has been localised to the cytosol and endoplasmic reticulum in human hepatocytes, whereas ALT2 has been localised to the mitochondria and endoplasmic reticulum of human skeletal muscle cells [60]. Our study confirms previous findings that upon liver inflammation, increased leakage of ALT1 but not ALT2 to the blood is observed, whereas leakage of ALT2 occurs in other

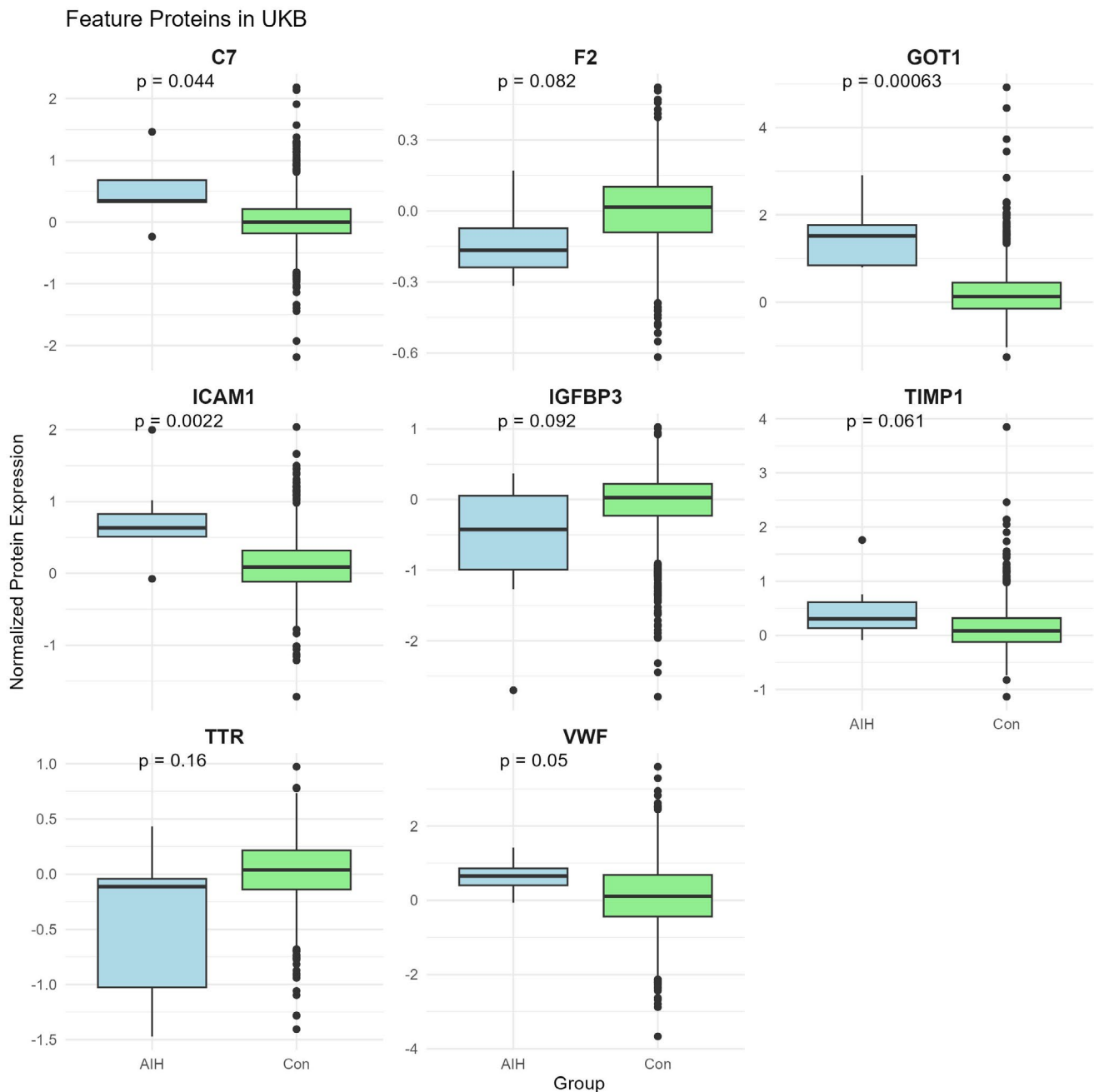


FIGURE 4 | Validation of plasma prediction proteins in UKB. Boxplots of NPX values (i.e., arbitrary concentrations) of those plasma prediction proteins (listed with gene names) identified by machine learning methods in the discovery cohort which were also measured by Olink (UKB) in 7 AIH cases and 1589 controls (1560 MASLD individuals and 29 healthy individuals). The boxes represent the interquartile range and the line within the box the median. *p* were calculated using Wilcoxon rank-sum test. AIH, autoimmune hepatitis; C7, complement component C7; F2, prothrombin; GOT1, cytoplasmic aspartate aminotransferase; ICAM1, intercellular adhesion molecule 1; IGFBP3, insulin-like growth factor-binding protein 3; MASLD, metabolic dysfunction-associated steatotic liver disease; NPX, normalised protein expression; TIMP1, metalloproteinase inhibitor 1; TTR, transthyretin; UKB, United Kingdom Biobank; VWF, von Willebrand factor.

situations such as muscle injury [61, 62]. In rats, leakage of cAST preceded that of mAST during liver ischemia, suggesting mAST was leaked to the blood only after severe damage of hepatocytes [63]. This might explain our finding of increased cAST, but not mAST, in plasma. Our findings of an almost perfect correlation between enzymatically measured ALT and ALT1 together with AST and cAST underscore that mass spectrometry concentrations are accurate, although relative, underlining the validity of proteomics. It also suggests that isoform-specific measurements

could be relevant in distinguishing liver-derived enzyme elevations from those originating in other tissues, offering potential diagnostic value in clinical practice.

Previous proteomic studies in AIH are scarce. Early studies (2004–2012) used mass spectrometry to identify a few selected proteins using gel electrophoresis techniques [16, 64–68]. More recently, serum proteomics have been done in adult patients with AIH cirrhosis (2019) [18] and paediatric patients with AIH

(2023) [17], and saliva proteomics have been performed in adult AIH cohorts (2023) [19, 20]. When aiming for the identification of novel biomarkers, these studies have some limitations, though. First, when evaluating diagnostics, it is a concern that the cohorts were not restricted to newly diagnosed or untreated patients with AIH. Diagnostic biomarkers assessed after treatment are likely to be attenuated after immunosuppressive medication is initiated. Secondly, the studies included healthy individuals as control groups, which may not be ideal in biomarker discovery as findings may not be AIH specific but could indicate, e.g., liver inflammation in general. Regarding liver proteomics, only one study has, to our knowledge, been performed in a small cohort of AIH explant livers (2011) [69].

Minghui et al. [18] conducted an untargeted serum proteomics experiment in 16 patients with AIH cirrhosis and 16 sex-matched healthy controls, identifying 108 differentially expressed proteins in AIH cirrhosis ($p < 0.05$ and an FDR < 0.01). Several of those were also observed in our study, including increased levels of VWF, PIGR, IGHG1 and C7 and decreased levels of TTR, IGFALS, APOE, apolipoprotein A1 (APOA1), inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1), vitamin K-dependent protein C (PROC) and C8B. Using a panel of eight proteins (VWF, TTR, IGFALS, APOE, adiponectin [ADIPO], PIGR, gelsolin [GELS]), Minghui et al. achieved a ROC AUC of 1.00 (95% confidence interval 1.00–1.00) in discriminating AIH cirrhosis from healthy controls. Evaluation by Western blot in a larger cohort (90 patients with AIH cirrhosis, 90 patients with non-cirrhotic AIH, 120 controls) confirmed these alterations, although they were less pronounced in non-cirrhotic AIH, except for PIGR, which showed greater increases in non-cirrhotic AIH compared with AIH cirrhosis. Of their predictive proteins, TTR, VWF and IGFALS were also identified as key predictive proteins in our plasma models. Particularly, TTR was the most important predictor in classifying AIH from healthy and MASLD, irrespective of cirrhosis or not.

TTR has primarily been associated with amyloidosis as misfolded TTR proteins can aggregate into amyloid fibrils [70]. However, downregulation of TTR, as observed in AIH in our study, may also reflect alterations in Vitamin A homeostasis. TTR is primarily produced in the liver and transports thyroxine hormone and Vitamin A (retinol), the latter by forming a complex with RBP4. Vitamin A is involved in the maintenance of growth and vision, systemic metabolic regulation, herein lipid and carbohydrate metabolism, and plays regulatory roles in innate and adaptive immune responses [71, 72]. Dietary Vitamin A is taken up by hepatocytes and primarily stored in lipid droplets in quiescent hepatic stellate cells. Upon activation during liver injury, hepatic stellate cells differentiate into activated myofibroblasts and lose their Vitamin A storage, underscoring the well-known association of Vitamin A deficiency and fibrosis formation in chronic liver disease [73, 74]. Downregulation of TTR is not specific for AIH and has also been observed in MASLD [75] and alcohol-related liver disease [76]. Nevertheless, downregulation of TTR was more pronounced in AIH in our study (FC TTR plasma and liver < 0.70 in AIH versus MASLD). This may be explained by Vitamin A's involvement in immune system regulation [72] in addition to involvement in lipid metabolism and fibrosis [71, 73].

The pattern of more pronounced alterations of the plasma proteome in cirrhosis found by Minghui et al. [18] is consistent with

findings from our study and has also been observed in MASLD [75] and alcohol-related liver disease [76]. Clinically, this aligns with the phenotypical presentation as non-cirrhotic liver damage is often asymptomatic, while cirrhosis frequently manifests with observable alterations like sarcopenia, ascites, gynaecomastia and splenomegaly.

The lower ROC AUC of the plasma model found in our study (0.91) compared to Minghui et al. [18] (1.00) may result from methodological differences and cohort composition. Our repeated stratified cross-validation approach minimises overfitting and better accounts for variability, while their single ROC analysis might overestimate performance. Additionally, Minghui et al. [18] used healthy controls, likely more distinct from AIH patients, whereas our control group included both healthy individuals and MASLD patients, providing a more clinically relevant but challenging classification scenario.

Serum proteomics has also been performed in paediatric patients with AIH by Sirbe et al. [17]. They recruited 16 prevalent patients with AIH and 2 newly diagnosed patients with decompensated AIH cirrhosis and compared their serum proteomic profile with 18 age- and sex-matched healthy individuals using two 92-target panels (inflammation and cardiometabolic) from Olink. They did not observe any significant alterations at an adjusted P level. This may be a consequence of studying the plasma proteome of patients already receiving medical treatment, which aims to normalise the condition, together with the use of targeted proteomics, only studying a pre-selected list of proteins, undoubtedly overlooking potential interesting alterations.

4.1 | Strengths and Limitations

Our study has several notable strengths. First, the inclusion of liver tissue from not only AIH patients but also healthy individuals and patients with MASLD allowed us to identify disease-specific alterations. Secondly, combining liver and plasma proteomics offered a unique opportunity to trace liver-specific alterations in plasma. As plasma reflects processes from across the body—and is influenced by various comorbidities—disease-specific changes in plasma can be difficult to identify and often require large sample sizes [77]. By combining tissue and plasma proteomics, we were able to identify disease-specific changes in plasma. Importantly, our study included newly diagnosed, untreated patients with AIH, which is particularly valuable for biomarker discovery, as diagnostic biomarkers are most relevant prior to treatment initiation. Additionally, by sharing our dataset, including data on all the differentially expressed proteins, we enable other researchers to explore and validate their own hypotheses.

A limitation of our study is the lack of age and sex matching in the discovery cohort (AIH group was older and a higher proportion of females), and we cannot leave out that some observed changes may be influenced by these factors. A systematic review by Johnson et al. [78] identified 32 proteins that change significantly with age, reported in at least five studies. Of the proteins reported in our study, only albumin and cAST were identified as age-specific proteins in the review. Since albumin and cAST are known to vary due to liver disease, we do not believe these

changes are primarily age-related in our study. Also, cAST, which was one of the proteins in our plasma model validated in UKB, remained significantly increased in the validation cohort in UKB after adjusting for age and sex.

Although previous proteomics studies have observed sex differences in serum/plasma protein levels, findings are inconsistent [79, 80]. For example, SAA1 [79] and B2M [80], both of which are involved in inflammation and immune response and elevated in AIH in our discovery cohort, have been reported at higher levels in females than in males. Potential sex-related differences in the human liver proteome remain unexplored.

To address this limitation, we also performed sensitivity analyses adjusting for age and sex in our discovery cohort. Several proteins, including B2M as mentioned above, remained significantly altered after adjustment, suggesting that these findings are not solely driven by demographic differences.

Another limitation of our study is the lack of identification of low abundant peptides, like chemokines, which play roles in immune diseases like AIH [28]. This underrepresentation derives from the sensitivity in untargeted proteomics, where detection tends to favour highly abundant proteins. In biological samples, proteins span a wide range of concentrations and sizes, and low abundant proteins are often missed.

A limitation of our validation in UKB is the use of targeted Olink proteomics, which measures a preselected list of proteins, unlike our untargeted approach. Consequently, many immune-mediated predictive proteins from our plasma model were not included in the Olink panel, limiting our ability to fully validate the plasma model in UKB. Additionally, our sample sizes in both the discovery and validation cohorts were small, highlighting the need for further validation in larger cohorts.

5 | Conclusions

In this study, we conducted the first parallel characterisation of the liver and plasma proteome in newly diagnosed, untreated AIH patients. Using an untargeted approach, we found profound alterations of both the liver and plasma proteome and promising diagnostic biomarker panels with good diagnostic accuracy. Sensitivity analyses adjusting for age and sex confirmed that several key proteomic differences, including immune-related proteins, were not solely driven by demographic differences. Our findings have potential to improve the diagnostic work-up of patients suspected of having AIH. To confirm our findings and refine the panels, we plan to perform liver and plasma proteomics on larger, age- and sex-matched cohorts.

Author Contributions

Anne-Sofie Houlberg Jensen: conceptualization, writing – original draft, writing – review and editing, validation, formal analysis, visualization, investigation, methodology, data curation, software, project administration. **Henriette Ytting:** writing – review and editing, supervision, investigation. **Annelaura Bach Nielsen:** validation, formal analysis, data curation, writing – review and editing, software. **Mikkel Parsberg Werge:** writing – review and editing, investigation. **Elias Badal Rashu:** investigation, writing – review and editing. **Liv Eline**

Hetland: investigation, writing – review and editing. **Mira Thing:** investigation, writing – review and editing. **Puria Nabilou:** investigation, writing – review and editing. **Johan Burisch:** writing – review and editing. **Anders Ellekær Junker:** investigation, writing – review and editing. **Lise Hobolth:** investigation, writing – review and editing. **Christian Mortensen:** investigation, writing – review and editing. **Flemming Tofteng:** writing – review and editing. **Flemming Bendtsen:** writing – review and editing. **Søren Møller:** investigation, writing – review and editing. **Mogens Vyberg:** investigation, writing – review and editing. **Reza Rafiolsadat Serizawa:** investigation, writing – review and editing. **Marie Winther-Sørensen:** writing – review and editing, data curation, software. **Jesper Sloth Kellemann:** writing – review and editing. **Lise Lotte Gluud:** conceptualization, methodology, project administration, supervision, writing – review and editing, resources. **Nicolai Jacob Wewer Albrechtsen:** conceptualization, methodology, supervision, project administration, writing – review and editing, resources, writing – original draft.

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Ethics Statement

Informed consent was obtained from all participants. The cohort study FALL was approved by the Regional Committee on Health Research Ethics (H-19038266). The UKB study protocol was approved by the UK North West Multi-centre Research Ethics Committee.

Conflicts of Interest

H.Y. has been principal investigator in clinical trials run by Gilead and Cymabay and has participated in advisory board and congress travel with IPSEN. A.E.J. reports grants or contracts from Alexion Pharmaceutical A/S. L.H. is sub-investigator in clinical trials run by Boehringer Ingelheim and reports speaker fees from Norgine. F.B. reports grants from Ferring and Tillotts Pharma. R.R.S. is a consultant for Merck. L.L.G. reports grants or contracts from Novo Nordisk, Gilead, Sobi, Pfizer, and Norgine; and consulting fees from Novo Nordisk, Astra Zeneca, Sobi, Pfizer, Boehringer-Ingelheim, and Becton Dickinson. N.J.W.A. has received funding, served on scientific advisory panels, and/or speakers bureaus for Boehringer Ingelheim, MSD/Merck, Novo Nordisk, Janssen, and Mercodia. A.-S.H.J., A.B.N., M.P.W., E.B.R., L.E.H., M.T., P.N., J.B., C.M., F.T., S.M., M.V., M.W.-S., J.S.K., declare no competing interests.

Data Availability Statement

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner repository with the dataset identifier PXD065892. The code utilised in the study is available at GitHub: <https://github.com/nicwin98/>.

References

1. C. L. Mack, D. Adams, D. N. Assis, et al., “Diagnosis and Management of Autoimmune Hepatitis in Adults and Children: 2019 Practice

- Guidance and Guidelines From the American Association for the Study of Liver Diseases,” *Hepatology* 72, no. 2 (2020): 671–722, <https://doi.org/10.1002/HEP.31065>.
2. A. W. Lohse, O. Chazouillères, G. Dalekos, et al., “EASL Clinical Practice Guidelines: Autoimmune Hepatitis,” *Journal of Hepatology* 63, no. 4 (2015): 971–1004, <https://doi.org/10.1016/j.jhep.2015.06.030>.
 3. G. Mieli-Vergani, D. Vergani, U. Baumann, et al., “Diagnosis and Management of Pediatric Autoimmune Liver Disease: ESPGHAN Hepatology Committee Position Statement,” *Journal of Pediatric Gastroenterology and Nutrition* 66, no. 2 (2018): 345–360, <https://doi.org/10.1097/MPG.0000000000001801>.
 4. A. D. Yeoman, R. H. Westbrook, Y. Zen, et al., “Prognosis of Acute Severe Autoimmune Hepatitis (AS-AIH): The Role of Corticosteroids in Modifying Outcome,” *Journal of Hepatology* 61, no. 4 (2014): 876–882, <https://doi.org/10.1016/J.JHEP.2014.06.030>.
 5. L. Grønbaek, H. Vilstrup, and P. Jepsen, “Autoimmune Hepatitis in Denmark: Incidence, Prevalence, Prognosis, and Causes of Death. A Nationwide Registry-Based Cohort Study,” *Journal of Hepatology* 60, no. 3 (2014): 612–617, <https://doi.org/10.1016/J.JHEP.2013.10.020>.
 6. A. W. Lohse, M. Sebode, P. S. Bhatthal, et al., “Consensus Recommendations for Histological Criteria of Autoimmune Hepatitis From the International AIH Pathology Group: Results of a Workshop on AIH Histology Hosted by the European Reference Network on Hepatological Diseases and the European Society of Pathology: Results of a Workshop on AIH Histology Hosted by the European Reference Network on Hepatological Diseases and the European Society of Pathology,” *Liver International* 42, no. 5 (2022): 1058–1069, <https://doi.org/10.1111/LIV.15217>.
 7. C. Covelli, D. Sacchi, S. Sarcognato, et al., “Pathology of Autoimmune Hepatitis,” *Pathologica* 113, no. 3 (2021): 185–193, <https://doi.org/10.32074/1591-951X-241>.
 8. F. Alvarez, P. A. Berg, F. B. Bianchi, et al., “International Autoimmune Hepatitis Group Report: Review of Criteria for Diagnosis of Autoimmune Hepatitis,” *Journal of Hepatology* 31, no. 5 (1999): 929–938, [https://doi.org/10.1016/S0168-8278\(99\)80297-9](https://doi.org/10.1016/S0168-8278(99)80297-9).
 9. E. M. Hennes, M. Zeniya, A. J. Czaja, et al., “Simplified Criteria for the Diagnosis of Autoimmune Hepatitis,” *Hepatology* 48, no. 1 (2008): 169–176, <https://doi.org/10.1002/hep.22322>.
 10. S. Yasui, K. Fujiwara, Y. Yonemitsu, S. Oda, M. Nakano, and O. Yokosuka, “Clinicopathological Features of Severe and Fulminant Forms of Autoimmune Hepatitis,” *Journal of Gastroenterology* 46, no. 3 (2011): 378–390, <https://doi.org/10.1007/S00535-010-0316-3>.
 11. M. I. Jalal, M. Brahmabhatt, K. Green, E. M. Weinberg, C. Lammert, and T. Bittermann, “Autoimmune Hepatitis and Metabolic Syndrome-Associated Disease Development: A U.S. Cohort Study,” *Alimentary Pharmacology & Therapeutics* 56, no. 7 (2022): 1183–1193, <https://doi.org/10.1111/APT.17191>.
 12. G. N. Dalekos, N. K. Gatselis, K. Zachou, and G. K. Koukoulis, “NAFLD and Autoimmune Hepatitis: Do Not Judge a Book by Its Cover,” *European Journal of Internal Medicine* 75 (2020): 1–9, <https://doi.org/10.1016/j.ejim.2020.02.001>.
 13. W. Ding, P. Qiu, Y. H. Liu, and W. Feng, “Current Omics Technologies in Biomarker Discovery,” in *Bioinformatics* (IGI Global Scientific Publishing Platform, 2013), 465–497, <https://doi.org/10.4018/978-1-4666-3604-0.ch027>.
 14. R. M. Miller and L. M. Smith, “Overview and Considerations in Bottom-Up Proteomics,” *Analyst* 148, no. 3 (2023): 475–486, <https://doi.org/10.1039/D2AN01246D>.
 15. R. Aebersold and M. Mann, “Mass-Spectrometric Exploration of Proteome Structure and Function,” *Nature* 537, no. 7620 (2016): 347–355, <https://doi.org/10.1038/NATURE19949>.
 16. C. Zingaretti, M. Arigò, A. Cardaci, et al., “Identification of New Autoantigens by Protein Array Indicates a Role for IL4 Neutralization in Autoimmune Hepatitis,” *Molecular & Cellular Proteomics* 11, no. 12 (2012): 1885–1897, <https://doi.org/10.1074/MCP.M112.018713>.
 17. C. Sirbe, M. Badii, T. O. Crişan, et al., “Detection of Novel Biomarkers in Pediatric Autoimmune Hepatitis by Proteomic Profiling,” *International Journal of Molecular Sciences* 24, no. 8 (2023): 7479, <https://doi.org/10.3390/IJMS24087479>.
 18. Z. Minghui, H. Kunhua, B. Yunwen, et al., “Analysis of Differentially Expressed Proteins Involved in Autoimmune Cirrhosis and Normal Serum by iTRAQ Proteomics,” *Proteomics. Clinical Applications* 13, no. 3 (2019): e1700153, <https://doi.org/10.1002/PRCA.201700153>.
 19. G. Guadalupi, C. Contini, F. Iavarone, et al., “Combined Salivary Proteome Profiling and Machine Learning Analysis Provides Insight Into Molecular Signature for Autoimmune Liver Diseases Classification,” *International Journal of Molecular Sciences* 24, no. 15 (2023): 12207, <https://doi.org/10.3390/IJMS241512207>.
 20. A. Olinas, G. Guadalupi, T. Cabras, et al., “Top-Down Proteomics Detection of Potential Salivary Biomarkers for Autoimmune Liver Diseases Classification,” *International Journal of Molecular Sciences* 24, no. 2 (2023): 959, <https://doi.org/10.3390/IJMS24020959>.
 21. C. Sudlow, J. Gallacher, N. Allen, et al., “UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age,” *PLoS Medicine* 12, no. 3 (2015): e1001779, <https://doi.org/10.1371/journal.pmed.1001779>.
 22. B. B. Sun, J. Chiou, M. Traylor, et al., “Plasma Proteomic Associations With Genetics and Health in the UK Biobank,” *Nature* 622, no. 7982 (2023): 329–338, <https://doi.org/10.1038/s41586-023-06592-6>.
 23. C. Lazar, L. Gatto, M. Ferro, C. Bruley, and T. Burger, “Accounting for the Multiple Natures of Missing Values in Label-Free Quantitative Proteomics Data Sets to Compare Imputation Strategies,” *Journal of Proteome Research* 15, no. 4 (2016): 1116–1125, <https://doi.org/10.1021/acs.jproteome.5b00981>.
 24. M. E. Ritchie, B. Phipson, D. Wu, et al., “Limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies,” *Nucleic Acids Research* 43, no. 7 (2015): e47, <https://doi.org/10.1093/nar/gkv007>.
 25. B. Phipson, S. Lee, I. J. Majewski, W. S. Alexander, and G. K. Smyth, “Robust HYPERPARAMETER Estimation Protects Against Hyper-variable Genes and Improves Power to Detect Differential Expression,” *Annals of Applied Statistics* 10, no. 2 (2016): 946–963, <https://doi.org/10.1214/16-AOAS920>.
 26. F. M. Torun, S. Virreira Winter, S. Doll, et al., “Transparent Exploration of Machine Learning for Biomarker Discovery From Proteomics and Omics Data,” *Journal of Proteome Research* 22, no. 2 (2023): 359–367, <https://doi.org/10.1021/acs.jproteome.2c00473>.
 27. M. E. Rinella, J. V. Lazarus, V. Ratzu, et al., “A Multisociety Delphi Consensus Statement on New Fatty Liver Disease Nomenclature,” *Journal of Hepatology* 79, no. 6 (2023): 1542–1556, <https://doi.org/10.1016/j.jhep.2023.06.003>.
 28. B. Terziroli Beretta-Piccoli, G. Mieli-Vergani, and D. Vergani, “Autoimmune Hepatitis,” *Cellular & Molecular Immunology* 19, no. 2 (2022): 158–176, <https://doi.org/10.1038/S41423-021-00768-8>.
 29. M. E. Anderson and T. J. Siahaan, “Targeting ICAM-1/LFA-1 Interaction for Controlling Autoimmune Diseases: Designing Peptide and Small Molecule Inhibitors,” *Peptides* 24, no. 3 (2003): 487–501, [https://doi.org/10.1016/S0196-9781\(03\)00083-4](https://doi.org/10.1016/S0196-9781(03)00083-4).
 30. G. Zöhlrens, T. Armbrust, U. Pirzer, K. M. zum Büschenfelde, and G. Ramadori, “Intercellular Adhesion Molecule-1 Concentration in Sera of Patients With Acute and Chronic Liver Disease: Relationship to Disease Activity and Cirrhosis,” *Hepatology* 18, no. 4 (1993): 798–802, <https://doi.org/10.1002/HEP.1840180408>.
 31. H. Yokomori, M. Obu, T. Uematsu, T. Okada, H. Yamazaki, and M. Oda, “Acute Onset of Autoimmune Hepatitis With Sinusoidal and

- Central Vein Endotheliitis, and Marked Involvement of Activated Dendritic Cells: A Case Report," *Medicine* 97, no. 52 (2018): e13873, <https://doi.org/10.1097/MD.00000000000013873>.
32. A. W. Thomson, S. Satoh, A. K. Nussler, et al., "Circulating Intercellular Adhesion Molecule-1 (ICAM-1) in Autoimmune Liver Disease and Evidence for the Production of ICAM-1 by Cytokine-Stimulated Human Hepatocytes," *Clinical and Experimental Immunology* 95, no. 1 (1994): 83–90, <https://doi.org/10.1111/J.1365-2249.1994.TB06019.X>.
 33. S. Ito, T. Yukawa, S. Uetake, and M. Yamauchi, "Serum Intercellular Adhesion Molecule-1 in Patients With Nonalcoholic Steatohepatitis: Comparison With Alcoholic Hepatitis," *Alcoholism, Clinical and Experimental Research* 31, no. SUPPL. 1 (2007): S83–S87, <https://doi.org/10.1111/J.1530-0277.2006.00292.X>.
 34. H. W. Schroeder and L. Cavacini, "Structure and Function of Immunoglobulins," *Journal of Allergy and Clinical Immunology* 125, no. 2 Suppl 2 (2010): S41–S52, <https://doi.org/10.1016/J.JACI.2009.09.046>.
 35. A. E. Pugh-Bernard, G. J. Silverman, A. J. Cappione, et al., "Regulation of Inherently Autoreactive VH4-34 B Cells in the Maintenance of Human B Cell Tolerance," *Journal of Clinical Investigation* 108, no. 7 (2001): 1061–1070, <https://doi.org/10.1172/JCI12462>.
 36. R. F. van Vollenhoven, M. M. Bieber, M. J. Powell, et al., "VH4-34 Encoded Antibodies in Systemic Lupus Erythematosus: A Specific Diagnostic Marker That Correlates With Clinical Disease Characteristics," *Journal of Rheumatology* 26, no. 8 (1999): 1727–1733, <https://pubmed.ncbi.nlm.nih.gov/10451069/>.
 37. A. Adamek and A. Kasprzak, "Insulin-Like Growth Factor (IGF) System in Liver Diseases," *International Journal of Molecular Sciences* 19, no. 5 (2018): 1308, <https://doi.org/10.3390/IJMS19051308>.
 38. R. Hällgren, "Serum beta2-Microglobulin in Liver Disease," *Scandinavian Journal of Clinical and Laboratory Investigation* 39, no. 5 (1979): 441–447, <https://doi.org/10.3109/00365517909106129>.
 39. T. Lung, B. Sakem, L. Risch, et al., "The Complement System in Liver Diseases: Evidence-Based Approach and Therapeutic Options," *Journal of Translational Autoimmunity* 2 (2019): 100017, <https://doi.org/10.1016/J.JTAUTO.2019.100017>.
 40. Y. R. Ji, H. J. Kim, K. B. Bae, S. Lee, M. O. Kim, and Z. Y. Ryoo, "Hepatic Serum Amyloid A1 Aggravates T Cell-Mediated Hepatitis by Inducing Chemokines via Toll-Like Receptor 2 in Mice," *Journal of Biological Chemistry* 290, no. 20 (2015): 12804–12811, <https://doi.org/10.1074/JBC.M114.635763>.
 41. T. Feizi, "Immunoglobulins in Chronic Liver Disease," *Gut* 9, no. 2 (1968): 193–198, <https://doi.org/10.1136/GUT.9.2.193>.
 42. R. Spinella, R. Sawhney, and R. Jalan, "Albumin in Chronic Liver Disease: Structure, Functions and Therapeutic Implications," *Hepatology International* 10, no. 1 (2016): 124–132, <https://doi.org/10.1007/S12072-015-9665-6>.
 43. S. S. Nayak, A. Ramani, S. S. Kamath, G. N. Kundaje, and A. R. Aroor, "Serum Apoproteins A and B and the Lecithin: Cholesterol Acyl Transferase Activities in Liver Cirrhosis and Hepatic Coma Patients," *Biochemical Medicine and Metabolic Biology* 40, no. 3 (1988): 299–304, [https://doi.org/10.1016/0885-4505\(88\)90132-6](https://doi.org/10.1016/0885-4505(88)90132-6).
 44. R. Feng, X. Guo, Y. Kou, et al., "Association of Lipid Profile With De-compensation, Liver Dysfunction, and Mortality in Patients With Liver Cirrhosis," *Postgraduate Medicine* 133, no. 6 (2021): 626–638, <https://doi.org/10.1080/00325481.2021.1930560>.
 45. T. Liu, X. Luo, Z. H. Li, J. C. Wu, S. Z. Luo, and M. Y. Xu, "Zinc- α 2-Glycoprotein 1 Attenuates Non-Alcoholic Fatty Liver Disease by Negatively Regulating Tumour Necrosis Factor- α ," *World Journal of Gastroenterology* 25, no. 36 (2019): 5451–5468, <https://doi.org/10.3748/WJG.V25.I36.5451>.
 46. M. Peck-Radosavljevic, "Review Article: Coagulation Disorders in Chronic Liver Disease," *Alimentary Pharmacology & Therapeutics* 26, no. Suppl 1 (2007): 21–28, <https://doi.org/10.1111/J.1365-2036.2007.03509.X>.
 47. B. Simbrunner, L. Hartl, M. Jachs, et al., "Dysregulated Biomarkers of Innate and Adaptive Immunity Predict Infections and Disease Progression in Cirrhosis," *JHEP Reports* 5, no. 5 (2023): 100712, <https://doi.org/10.1016/J.JHEPR.2023.100712>.
 48. C. Homann, K. Varming, K. Högåsen, et al., "Acquired C3 Deficiency in Patients With Alcoholic Cirrhosis Predisposes to Infection and Increased Mortality," *Gut* 40, no. 4 (1997): 544–549, <https://doi.org/10.1136/GUT.40.4.544>.
 49. S. Tanaka, Y. Okamoto, M. Yamazaki, N. Mitani, Y. Nakajima, and H. Fukui, "Significance of Hyperglobulinemia in Severe Chronic Liver Diseases - With Special Reference to the Correlation Between Serum Globulin/IgG Level and ICG Clearance," *Hepato-Gastroenterology* 54, no. 80 (2007): 2301–2305.
 50. T. M. Busk, F. Bendtsen, H. J. Nielsen, V. Jensen, N. Brünnner, and S. Møller, "TIMP-1 in Patients With Cirrhosis: Relation to Liver Dysfunction, Portal Hypertension, and Hemodynamic Changes," *Scandinavian Journal of Gastroenterology* 49, no. 9 (2014): 1103–1110, <https://doi.org/10.3109/00365521.2014.934910>.
 51. W. M. C. Rosenberg, M. Voelker, R. Thiel, et al., "Serum Markers Detect the Presence of Liver Fibrosis: A Cohort Study," *Gastroenterology* 127, no. 6 (2004): 1704–1713, <https://doi.org/10.1053/j.gastro.2004.08.052>.
 52. L. Sobrevalls, C. Rodriguez, J. L. Romero-Trejejo, et al., "Insulin-Like Growth Factor i Gene Transfer to Cirrhotic Liver Induces Fibrolysis and Reduces Fibrogenesis Leading to Cirrhosis Reversion in Rats," *Hepatology* 51, no. 3 (2010): 912–921, <https://doi.org/10.1002/hep.23412>.
 53. B. P. van den Boom, M. Stamouli, J. Timon, et al., "Von Willebrand Factor Is an Independent Predictor of Short-Term Mortality in Acutely Ill Patients With Cirrhosis," *Liver International* 43, no. 12 (2023): 2752–2761, <https://doi.org/10.1111/LIV.15728>.
 54. I. Castilla-Cortazar, M. Garcia, B. Muguerza, et al., "Hepatoprotective Effects of Insulin-Like Growth Factor I in Rats With Carbon Tetrachloride-Induced Cirrhosis," *Gastroenterology* 113, no. 5 (1997): 1682–1691, <https://doi.org/10.1053/gast.1997.v113.pm9352873>.
 55. B. Muguerza, I. Castilla-Cortázar, M. García, J. Quiroga, S. Santidrián, and J. Prieto, "Antifibrogenic Effect in Vivo of Low Doses of Insulin-Like Growth Factor-I in Cirrhotic Rats," *Biochimica et Biophysica Acta* 1536, no. 2–3 (2001): 185–195, [https://doi.org/10.1016/s0925-4439\(01\)00045-x](https://doi.org/10.1016/s0925-4439(01)00045-x).
 56. A. Fusco, A. D'Uonno, A. Forgione, et al., "Nonalcoholic Fatty Liver Disease Is Associated With Increased GHBP and Reduced GH/IGF-I Levels," *Clinical Endocrinology* 77, no. 4 (2012): 531–536, <https://doi.org/10.1111/J.1365-2265.2011.04291.X>.
 57. A. S. H. Jensen, H. Ytting, M. Winther-Sørensen, et al., "Autoimmune Liver Diseases and Diabetes," *European Journal of Gastroenterology & Hepatology* 35, no. 9 (2023): 938–947, <https://doi.org/10.1097/MEG.0000000000002594>.
 58. A. S. H. Jensen, M. Winther-Sørensen, J. Burisch, et al., "Autoimmune Liver Diseases and Diabetes: A Propensity Score Matched Analysis and a Proportional Meta-Analysis," *Liver International* 43, no. 11 (2023): 2479–2491, <https://doi.org/10.1111/liv.15720>.
 59. A. S. H. Jensen, H. Ytting, M. P. Werge, et al., "Patients With Autoimmune Liver Disease Have Glucose Disturbances That Mechanistically Differ From Steatotic Liver Disease," *American Journal of Physiology. Gastrointestinal and Liver Physiology* 326, no. 6 (2024): G736–G746, <https://doi.org/10.1152/ajpgi.00047.2024>.
 60. B. Glinghammar, I. Rafter, A. K. Lindström, et al., "Detection of the Mitochondrial and Catalytically Active Alanine Aminotransferase in Human Tissues and Plasma," *International Journal of Molecular Medicine* 23, no. 5 (2009): 621–631, https://doi.org/10.3892/IJMM_00000173/HTML.

61. H. J. Kim, S. Y. Kim, S. P. Shin, et al., "Immunological Measurement of Aspartate/Alanine Aminotransferase in Predicting Liver Fibrosis and Inflammation," *Korean Journal of Internal Medicine* 35, no. 2 (2019): 320, <https://doi.org/10.3904/KJIM.2018.214>.
62. I. Rafter, T. Gråberg, A. Kotronen, et al., "Isoform-Specific Alanine Aminotransferase Measurement Can Distinguish Hepatic From Extrahepatic Injury in Humans," *International Journal of Molecular Medicine* 30, no. 5 (2012): 1241–1249, <https://doi.org/10.3892/IJMM.2012.1106>.
63. W. Kamiike, M. Fujikawa, M. Koseki, et al., "Different Patterns of Leakage of Cytosolic and Mitochondrial Enzymes," *Clinica Chimica Acta* 185, no. 3 (1989): 265–270, [https://doi.org/10.1016/0009-8981\(89\)90216-7](https://doi.org/10.1016/0009-8981(89)90216-7).
64. F. Tahiri, F. Le Naour, S. Huguët, et al., "Identification of Plasma Membrane Autoantigens in Autoimmune Hepatitis Type 1 Using a Proteomics Tool," *Hepatology* 47, no. 3 (2008): 937–948, <https://doi.org/10.1002/HEP.22149>.
65. S. Huguët, V. Labas, J. C. Duclos-Vallée, et al., "Heterogeneous Nuclear Ribonucleoprotein A2/B1 Identified as an Autoantigen in Autoimmune Hepatitis by Proteome Analysis," *Proteomics* 4, no. 5 (2004): 1341–1345, <https://doi.org/10.1002/PMIC.200300757>.
66. Q. Xia, F. Lu, H. P. Yan, et al., "Autoantibody Profiling of Chinese Patients With Autoimmune Hepatitis Using Immunoproteomic Analysis," *Journal of Proteome Research* 7, no. 5 (2008): 1963–1970, <https://doi.org/10.1021/PR700861S>.
67. F. Lu, Q. Xia, Y. Ma, et al., "Serum Proteomic-Based Analysis for the Identification of a Potential Serological Marker for Autoimmune Hepatitis," *Biochemical and Biophysical Research Communications* 367, no. 2 (2008): 284–290, <https://doi.org/10.1016/J.BBRC.2007.12.075>.
68. S. Huguët, J. Vinh, C. Johanet, et al., "Identification by Proteomic Tool of Atypical Anti-Liver/Kidney Microsome Autoantibodies Targets in de Novo Autoimmune Hepatitis After Liver Transplantation," *Annals of the New York Academy of Sciences* 1109 (2007): 345–357, <https://doi.org/10.1196/ANNALS.1398.041>.
69. C. L. Bowlus, E. H. Seeley, J. Roder, et al., "In Situ Mass Spectrometry of Autoimmune Liver Diseases," *Cellular & Molecular Immunology* 8, no. 3 (2011): 237–242, <https://doi.org/10.1038/CMI.2010.72>.
70. F. L. Ruberg and M. S. Maurer, "Cardiac Amyloidosis due to Transthyretin Protein: A Review," *Journal of the American Medical Association* 331, no. 9 (2024): 778–791, <https://doi.org/10.1001/JAMA.2024.0442>.
71. W. S. Blaner, "Vitamin A Signaling and Homeostasis in Obesity, Diabetes, and Metabolic Disorders," *Pharmacology & Therapeutics* 197 (2019): 153–178, <https://doi.org/10.1016/J.PHARMTHERA.2019.01.006>.
72. Z. Huang, Y. Liu, G. Qi, D. Brand, and S. G. Zheng, "Role of Vitamin A in the Immune System," *Journal of Clinical Medicine* 7, no. 9 (2018): 258, <https://doi.org/10.3390/JCM7090258>.
73. J. Song and J. Z. Gordon, "Low Vitamin A Levels Are Associated With Liver-Related Mortality: A Nationally Representative Cohort Study," *Hepatology Communications* 7, no. 5 (2023): e0124, <https://doi.org/10.1097/HC9.0000000000000124>.
74. A. Licata, M. Zerbo, S. Como, et al., "The Role of Vitamin Deficiency in Liver Disease: To Supplement or Not Supplement?," *Nutrients* 13, no. 11 (2021): 4014, <https://doi.org/10.3390/NU13114014>.
75. L. Niu, P. E. Geyer, N. J. Wewer Albrechtsen, et al., "Plasma Proteome Profiling Discovers Novel Proteins Associated With Non-Alcoholic Fatty Liver Disease," *Molecular Systems Biology* 15, no. 3 (2019): e8793, <https://doi.org/10.15252/msb.20188793>.
76. L. Niu, M. Thiele, P. E. Geyer, et al., "Noninvasive Proteomic Biomarkers for Alcohol-Related Liver Disease," *Nature Medicine* 28, no. 6 (2022): 1277–1287, <https://doi.org/10.1038/S41591-022-01850-Y>.
77. P. E. Geyer, L. M. Holdt, D. Teupser, and M. Mann, "Revisiting Biomarker Discovery by Plasma Proteomics," *Molecular Systems Biology* 13 (2017): 942, <https://doi.org/10.15252/msb.20156297>.
78. A. A. Johnson, M. N. Shokhirev, T. Wyss-Coray, and B. Lehallier, "Systematic Review and Analysis of Human Proteomics Aging Studies Unveils a Novel Proteomic Aging Clock and Identifies Key Processes That Change With Age," *Ageing Research Reviews* 60 (2020): 101070, <https://doi.org/10.1016/J.ARR.2020.101070>.
79. K. Miike, M. Aoki, R. Yamashita, et al., "Proteome Profiling Reveals Gender Differences in the Composition of Human Serum," *Proteomics* 10, no. 14 (2010): 2678–2691, <https://doi.org/10.1002/PMIC.200900496>.
80. C. C. Silliman, M. Dzieciatkowska, E. E. Moore, et al., "Proteomic Analyses of Human Plasma: Venus Versus Mars," *Transfusion (Paris)* 52, no. 2 (2012): 417–424, <https://doi.org/10.1111/J.1537-2995.2011.03316.X>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.