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Application of Histopathology, Immunohistochemistry, and Antibody Titers in the Diagnosis and Treatment Response Assessment of Autoimmune Liver Diseases

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Abstract

Objective

To investigate the differences in histopathological characteristics, immunohistochemical marker expression, and serum antibody titers among autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), AIH-PBC overlap syndrome (OS)[39], and drug-induced autoimmune-like hepatitis (DI-AILH), providing basis to enhance the accuracy of differential diagnosis and rational prediction of treatment response.

Methods

A retrospective collection of liver biopsy specimens from 40 patients at the Department of Gastroenterology, Leshan People's Hospital, between January 2022 and August 2025 was conducted, including 11 AIH cases, 11 PBC cases, 10 AIH-PBC OS cases, and 8 DI-AILH cases. A comprehensive approach utilizing HE staining (assessing inflammation grade, fibrosis stage, and characteristic pathological changes), immunohistochemistry (detecting markers such as Mum-1, CD38, CD3, CD68), serum antibody titers (anti-nuclear antibody, ANA), and liver biochemical indices (ALT, ALP, IgG) was employed to quantitatively compare histological and immunohistochemical differences among the four groups. Patients with AIH, PBC, and OS were followed up for 6 months to analyze the correlation between treatment response and antibody titers, biochemical indices, and pathological changes.

Results

Both the AIH and OS groups exhibited significant interface hepatitis, hepatocyte rosette formation, and emperipolesis. The scores for portal plasma cell + lymphocyte infiltration [AIH: (2.00 ± 1.61), (3.91 ± 0.30); OS: (1.90 ± 1.20), (3.70 ± 0.48)] were significantly higher than those in the PBC group [(1.00 ± 0.63), (2.55 ± 1.13)] and the DI-AILH group [(0.38 ± 0.52), (2.38 ± 1.30)] (all $P < 0.05$). Inflammation grade (Scheuer grade) [AIH: (3.64 ± 0.50), OS: (3.00 ± 0.67)] and fibrosis stage (Scheuer stage) [AIH: (3.09 ± 0.83), OS: (2.80 ± 0.79)] were also higher in the AIH and OS groups compared to the other two groups (all $P < 0.05$). Serologically, the proportion of high-titer ANA (≥ 1:640) in the OS group was 80.0% (8/10), significantly higher than in other groups ($P < 0.05$). IgG levels in the AIH group [2520.00 (2190.00, 3565.00)] were significantly higher than in the other three groups ($P < 0.05$), while ALT levels in the DI-AILH group (360.12 ± 420.84) were significantly higher than in the other three groups (all $P < 0.05$). The 6-month follow-up showed no significant difference in the complete biochemical response rate between the AIH and OS groups ($P > 0.05$). Non-responders in the PBC group had significantly higher ALP and ALT levels [ALP: 917.00 ± NA; ALT: 215.00 ± NA] compared to responders [ALP: 195.50 ± 95.68 U/L; ALT: 64.40 ± 47.68 U/L] (both $P < 0.05$). Multivariate logistic regression analysis showed that elevated IgG was an independent diagnostic factor for AIH (OR = 1.02, 95% CI: 1.00-1.04, $P < 0.05$), and high-titer ANA was an independent predictive factor for OS (OR = 3.41, 95% CI: 1.12–10.38, $P < 0.05$). Immunohistochemical

features: OS showed the most prominent CD3 and CD38 expression, reflecting dual "cellular immunity + immune activation" overlay. AIH exhibited active CD68 and Mum-1 expression, suggesting a combined "cellular immunity (macrophages) + humoral immunity" drive. PBC had Mum-1 levels comparable to AIH but lower T-cell and immune activation involvement, leaning towards "humoral immunity + bile duct-specific injury". DI-AILH generally showed lower involvement of all immune markers, reflecting the fundamental difference of its "drug-induced" immune injury mechanism compared to traditional autoimmune liver diseases.

Conclusion

Combining histopathological features (interface hepatitis, rosette formation), immunohistochemical markers (Mum-1, CD3, CD38, CD68), and serological indices (IgG, ANA titer, ALT) can effectively differentiate AIH, OS, PBC, and DI-AILH, improving diagnostic accuracy. Early identification of OS and selection of targeted immunosuppressive regimens can improve treatment response. Pre-treatment ALP and ALT levels in PBC patients can serve as important reference indicators for second-line treatment decisions.

Introduction

Autoimmune liver diseases (AILD) are a group of chronic inflammatory hepatobiliary diseases mediated by aberrant autoimmune responses, primarily including autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and overlap syndromes (OS). Among these, AIH and PBC are clinically most common and may present concurrently or sequentially in the same patient, known as AIH-PBC (OS). Diagnosis currently follows the internationally "Paris Criteria" updated 2011 [3 49]. Furthermore, drug-induced autoimmune-like hepatitis (DI-AILH), a special type of drug-induced liver injury, exhibits high similarity in clinical symptoms, serology, and histology to AIH[21–31], but responds well to corticosteroid therapy with a low relapse rate after discontinuation. According to the 2023 guidelines for the diagnosis and treatment of drug-induced liver injury(DILI) [32], differential diagnosis between DI-AILH and AIH requires combining liver biopsy pathological features with long-term follow-up (≥ 6 months). This study systematically analyzes the differences in clinical characteristics, histopathological morphology, immunohistochemical marker expression, serum antibody titers, and biochemical response among patients with AIH, PBC, AIH-PBC (OS), and DI-AILH, aiming to identify key indicators for differential diagnosis and explore predictive factors for treatment response, providing references for precise clinical diagnosis and treatment.

1. Materials and Methods

1.1 Study Subjects Forty patients who underwent ultrasound-guided liver biopsy at the Department of Gastroenterology, Leshan People's Hospital, from January 2022 to August 2025 were retrospectively enrolled and divided into four groups based on diagnostic criteria: AIH group (11 cases), PBC group (11

cases), AIH-PBC (OS) group (10 cases), and DI-AILH group (8 cases). Baseline clinical data (gender, age, comorbidities of autoimmune diseases), laboratory indices, liver histology data, and treatment outcomes were collected, with a follow-up duration of 6 months and an endpoint of August 2026. Inclusion Criteria: AIH: Meeting the 2021 revised diagnostic scoring system [49] (≥ 15 points, definitive diagnosis) by the International Autoimmune Hepatitis Group (IAIHG) [5]. PBC: Meeting at least 2 of the following: Elevated alkaline phosphatase (ALP) and γ-glutamyl transferase (γ-GT) persisting for ≥ 6 months; Positive anti-mitochondrial antibody (AMA); Histological evidence of lymphocytic interlobular cholangitis. AIH-PBC (OS): Meeting the 2011 "Paris Criteria" [6] (simultaneously satisfying the AIH simplified diagnostic criteria ≥ 6 points and the main PBC diagnostic criteria). DI-AILH: Based on the RUCAM (Roussel Uclaf Causality Assessment Method) scale for drug and liver injury causality [7] (score ≥ 6) and clinically presenting with "autoimmune-like features" (positive anti-nuclear antibody(ANA)or elevated IgG, accompanied by interface hepatitis). Exclusion Criteria: Coexisting viral hepatitis (hepatitis B, C, etc.), metabolic liver diseases (Wilson's disease, hemochromatosis); alcoholic liver disease, non-alcoholic fatty liver disease, or other causes of liver dysfunction; coexisting liver malignancies (hepatocellular carcinoma, cholangiocarcinoma) or alpha-fetoprotein (AFP) > 100 ng/mL; liver biopsy specimen length < 10 mm or containing less than 6 lobules (insufficient reliability for pathological assessment).

1.2 Research Methods

1.2.1 Collection of Clinical and Laboratory Indices Patient gender, age, and comorbidities of rheumatic immune diseases (e.g., rheumatoid arthritis, Sjögren's syndrome) were recorded, along with biochemical response status after 6 months of treatment (complete response: ALT, ALP return to normal range; partial response: ALT, ALP decrease ≥ 50% from baseline but not normal; no response: not meeting partial response criteria). Peripheral venous blood was collected at enrollment. ALT, total bilirubin (TBil), γ-GT, and ALP levels were measured using an automatic biochemical analyzer. Immunoglobulin G (IgG) and immunoglobulin M (IgM) levels were measured by immunoturbidimetry. ANA titers were detected by indirect immunofluorescence (categorized as low titer < 1:320, medium titer 1:320-1:640, high titer ≥ 1:640).

1.2.2 Liver Histology and Immunohistochemistry Assessment Liver tissue specimens were fixed in 4% formaldehyde, embedded in paraffin, and sectioned consecutively at 4 μm for HE staining. Two experienced liver pathologists performed blinded evaluations, using the Scheuer scoring system(Table 1)[8] for inflammation grading (0–4) and fibrosis staging (0–4). A semi-quantitative scoring system [9–10] was used for infiltrating inflammatory cells (lymphocytes, plasma cells, neutrophils, eosinophils): 0 points (no infiltration), 1 point (mild infiltration, involving < 10% of field), 2 points (moderate infiltration, involving 10%-30% of field), 3 points (marked infiltration, involving 30%-50% of field), 4 points (severe infiltration, involving > 50% of field). Immunohistochemistry Detection: The EnVision two-step method was used with primary antibodies: Mum-1 (rabbit anti-human, 1:100), CD38 (mouse anti-human, 1:200), CD3 (rabbit anti-human, 1:150), CD68 (mouse anti-human, 1:200). Positive expression was defined by the

presence of brown-yellow granules in the cytoplasm or cell membrane. The percentage of positive cells was calculated, with ≥ 10% considered positive expression.

Table 1
Criteria for histological evaluation

Histological Characteristics	Scoring System	Scoring Criteria
InflammatoryCell (TypePortal Tract / Lobule)	0/1/2/3/4	0 point: Absent
Lymphocyte	0/1/2/3/4	1 point: ≤5%
Plasma Cell	0/1/2/3/4	2 points: 5%□20%
Neutrophil	0/1/2/3/4	3 points: 20%□50%
Eosinophil	0/1/2/3/4	4 points: >50%
Rosette Formation	Absent/Present	Hepatocyte arrangement around central lumen; mononuclear lymphocytes in hepatocyte cytoplasm
Emperipolesis	Absent/Present	
Inflammation Grade	0/1/2/3/4	Schruer Score
Fibrosis Stage	0/1/2/3/4	Schruer Score
Inflammatory Cell Cluster		
Portal Tract	Absent/Present	Cluster: ≥5 plasma cells aggregated
Lobule	Absent/Present	
Cholestasis	Absent/Present	Yellow material deposition in bile ducts or hepatocytes
Bile Duct Injury	Absent/Present	Inflammatory reactive change of bile duct epithelial cells
Steatosis	0/1/2/3/4	NASH Steatosis Scoring
Hepatocyte Necrosis	0/1/2/3/4	0 point: Absent1 point: Focal necrosis (punctate) 2 points: Focal necrosis (patchy) 3 points:Bridging confluent necrosis 4 points: Bridging massive necrosis
ANATiter Classification		Negative:<320Low:<320Moderate: 320-640High: >640
InflammatoryCell (TypePortal Tract / Lobule)	0/1/2/3/4	0 point: Absent

1.3 Statistical Methods Data analysis was performed using SPSS 27.0 statistical software. Normally distributed measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA), with pairwise comparisons using the LSD-t test. Non-normally distributed data were expressed as median (interquartile range) [M (Q1, Q3)], with comparisons among multiple groups using the Kruskal-Wallis H test and pairwise comparisons using the Nemenyi test. Count data were expressed as number (percentage) [n (%)], with intergroup comparisons using the χ^2 test or Fisher's exact probability method. Univariate and multivariate logistic regression analyses were used to identify independent predictive factors for diseases. Pearson/Spearman correlation analysis was used to assess the association between ANA titer and various indices. A P-value < 0.05 was considered statistically significant.

2. Results

2.1 General Data Among the 40 patients, 6 were male (15.0%) and 34 were female (85.0%), with a male-to-female ratio of 1:5.7. The mean age was (55.77 ± 12.34) years, predominantly middle-aged and elderly patients (≥ 50 years old accounted for 67.5%, 27/40). Five patients (12.5%) had other autoimmune diseases (2 Sjögren's syndrome, 2 rheumatoid arthritis, 1 systemic lupus erythematosus). Age and gender distribution across the four groups: The mean age of the DI-AILH group (48.75 ± 4.46 years) was significantly lower than the other three groups (AIH: 64.55 ± 10.68 years; PBC: 53.09 ± 13.68 years; OS: 54.70 ± 12.71 years) (P < 0.05).

2.2 Histological Features

2.2.1 Inflammation and Fibrosis Degree Both inflammation grade and fibrosis stage were higher in the AIH and OS groups compared to the DI-AILH and PBC groups. Inflammation grade: [AIH: (3.64 ± 0.50), PBC: (2.36 ± 0.67), OS: (3.00 ± 0.67), DI-AILH: (2.25 ± 1.16)]. Fibrosis stage: [AIH: (3.09 ± 0.83), PBC: (2.36 ± 1.29), OS: (2.80 ± 0.79), DI-AILH: (1.38 ± 0.74)]. The differences were statistically significant (all P < 0.05).

2.2.2 Characteristic Pathological Changes Rosette formation: AIH group 8 cases (72.7%), AIH-PBC (OS) group 7 cases (70.0%), significantly more than DI-AILH group 1 case (12.5%) and PBC group 2 cases (18.2%) (all P < 0.05). Portal inflammatory cell cluster infiltration: AIH group 9 cases (81.8%), AIH-PBC (OS) group 8 cases (80.0%), significantly more than PBC group 4 cases (36.4%) and DI-AILH group 3 cases (37.5%) (all P < 0.05). Severe interface hepatitis (Ishak grade 3): AIH group 3 cases (27.3%), AIH-PBC (OS) group 2 cases (20.0%), not present in the PBC and DI-AILH groups (P < 0.05).

2.3 ANA Titer and Disease Correlation Low-titer ANA (< 1:320) was mainly distributed in the AIH group (8/11, 72.7%) and DI-AILH group (7/8, 87.5%). Medium/high-titer ANA [(1:320-1:640), (> 1:640)] was mainly distributed in the PBC group (9/11, 81.8%) and AIH-PBC (OS) group (8/10, 80.0%). The intergroup difference was statistically significant (P < 0.05).

2.4 Biochemical and Immunoglobulin Levels IgG: AIH group [2520.00 (2190.00, 3565.00)] was significantly higher than PBC group [1890.00 (1580.00, 2125.00)], AIH-PBC (OS) group [1705.00 (1612.50, 2180.00)], and DI-AILH group [1760.00 (1210.00, 2202.50)] (all $P < 0.05$). ALT: DI-AILH group (360.12 ± 420.84) was significantly higher than AIH group (295.36 ± 247.30), AIH-PBC (OS) group (116.50 ± 63.97), and PBC group (78.09 ± 64.10) (all $P < 0.05$).

2.5 Treatment Response Analysis Six-month complete biochemical response rate: PBC group 90.9% (10/11), AIH group 72.7% (8/11), AIH-PBC (OS) group 60.0% (6/10). The difference in complete response rate between the AIH and OS groups was not statistically significant ($P > 0.05$). In the PBC group, the single non-responder had significantly higher ALP and ALT levels compared to responders (both $P < 0.05$). In the AIH group, ANA titer showed a borderline negative correlation with treatment response ($r=-0.58$, $P = 0.055$), suggesting a tendency for higher response rates in patients with lower ANA titers.

2.6 Multivariate Analysis Using "whether it is AIH" as the dependent variable (1 = yes, 0 = no), indicators with $P < 0.1$ in univariate analysis (IgG, ANA titer, plasma cell infiltration score, etc.) were included in multivariate logistic regression. Results showed that elevated IgG was an independent diagnostic factor for AIH (OR = 1.01, 95% CI: 1.01 ~ 1.01, $P < 0.05$). Using "whether it is AIH-PBC (OS) " as the dependent variable (1 = yes, 0 = no), indicators with $P < 0.1$ in univariate analysis (ANA titer, IgM, ALP, etc.)[20–21] were included in multivariate logistic regression. Results showed that high-titer ANA (≥ 1:640) was an independent predictive factor for AIH-PBC (OS) (OR = 15.75, 95% CI: 2.30 ~ 107.93, $P < 0.05$).

2.7 Immunohistochemical Expression Comparison of positive rates of immunohistochemical markers among the four groups: (Figure 2–Figure 3) shows the comparison of positive rates for CD38, CD3, CD68, and Mum-1 immunohistochemical staining in AIH, PBC, DI-AILH, and AIH-PBC (OS) patients. AIH-PBC (OS): Showed the most prominent T-cell infiltration and immune activation (CD3, CD38), reflecting dual "cellular immunity + immune activation" overlay. AIH: Exhibited active macrophage infiltration (CD68) and plasma cell function (Mum-1), representing a combined "cellular immunity (macrophages) + humoral immunity" drive. PBC: Had plasma cell function (Mum-1) comparable to AIH, but lower T-cell and immune activation involvement, leaning towards "humoral immunity + bile duct-specific injury". DI-AILH: Generally showed lower involvement of all immune markers, reflecting the fundamental difference of its "drug-induced" immune injury mechanism compared to traditional autoimmune liver diseases.

3. Discussion

"differential diagnosis among AILD (AIH, PBC, AIH-PBC(OS)) and DI-AILH" and "factors influencing 6-month treatment response." It analyzes the specific manifestations and clinical significance of each disease group from three dimensions: pathological features, biochemical indices (e.g., IgG, ALP), and immunohistochemical mechanisms (e.g., CD3, CD38, CD68, Mum-1). Ultimately, it proposes diagnostic optimization suggestions and points out research limitations, providing references for clinical practice.

1. Diagnostic Dilemmas and Multidimensional Evaluation Strategies of Autoimmune Hepatitis (AIH)

Autoimmune hepatitis (AIH), a classic autoimmune liver disease, has a prognosis that is directly determined by treatment response.,A 2024 multicenter retrospective study by the International Autoimmune Hepatitis Group involving 2559 AIH patients showed that failure to achieve complete biochemical response (normalization of transaminases and IgG) at 6 months of treatment was an independent risk factor for liver-related death and liver transplantation [19,20,21,33]; furthermore, patients achieving response within 6 months had a significantly higher 20-year survival rate (91%) compared to non-responders (61%). This conclusion highlights the critical role of early accurate diagnosis and timely treatment in AIH prognosis. However, current AIH diagnosis faces significant bottlenecks—lack of specific biomarkers, reliance on scoring systems [1], low positivity rate and specificity of autoantibodies; some antibody-negative patients may even have normal IgG levels [19,41,42,43], further increasing diagnostic difficulty, leading to delayed immunosuppressive therapy for some patients and ultimately affecting treatment response. This study provides new insights for AIH diagnosis from pathological and immunological perspectives. Pathologically, AIH patients exhibit significant interface hepatitis, Rosette formation, emperipolesis, and clustered plasma cell infiltration at the limiting plate; portal lymphocyte infiltration is also pronounced [38]. Inflammation grade and fibrosis degree are higher than in other disease groups, likely due to cumulative chronic immune responses. Biochemically, elevated immunoglobulin IgG is an important factor influencing AIH diagnosis [19,20,21,37]. Immunohistochemistry further shows high expression of macrophage infiltration marker CD68 and plasma cell differentiation marker Mum-1 [40], suggesting AIH is a disease driven by both "macrophage-mediated cellular immunity and plasma cell-dominated humoral immunity" [16, 17, 18]. Based on this, characteristic pathological manifestations, immunohistochemical markers, and immunoglobulin levels can be integrated clinically to construct a multidimensional assessment system, breaking through traditional diagnostic limitations and improving AIH diagnostic efficiency.

2. Differentiation between DI-AILH and AIH: From Morphological Similarity to Mechanistic Distinction
   DI-AILH [44], as a special type of DILI [50], shares histological similarities with AIH—such as lymphoplasmacytic infiltration in portal stroma and interface hepatitis, and varying degrees of confluent necrosis in liver parenchyma. However, different drugs can induce heterogeneous histological phenotypes, and there are fundamental differences in pathogenesis and clinical management between DI-AILH and AIH. Precise differentiation is crucial to avoid overtreatment or undertreatment. Serological characteristics provide important clues for differentiation. Low-titer autoantibodies are often associated with chronic DILI and idiosyncratic DILI, while higher IgG levels are closely linked to DI-AILH. Hisamochi et al. [45] found in a series study of 62 patients that the serum IgG-to-normal ratio could serve as a predictive indicator for DI-AILH, and serum IgG >1800 mg/dL had high sensitivity for DI-AILH. In this study, the average IgG level in the DI-AILH group was 1760.00 mg/dL, second only to the AIH group, further validating the clinical value of this association. Differences in immune mechanisms are the core basis for differentiation. This study found that the expression levels of various immune markers (CD3, CD38, CD68, Mum-1) in DI-AILH were generally lower than in traditional AILD like AIH, reflecting the fundamental difference between its "drug-induced" immune injury mechanism and autoimmune-driven liver disease—the immune

abnormalities in DI-AILH are more likely a "secondary" reaction triggered directly or indirectly by drugs, rather than a "primary" lesion caused by autoimmune system dysregulation. This mechanistic difference not only clarifies the pathological boundary between the two but also provides theoretical support for formulating differential treatment strategies (e.g., priority to drug withdrawal and observation for DI-AILH, prompt immunosuppressive therapy for AIH).

3. Clinical Challenges and Exploration of Mechanistic Features in PBC and AIH-PBC OS
4. Treatment Response Challenges in PBC PBC, as an AILD centered on bile duct injury, sees approximately 40% of patients showing poor response to first-line UDCA, posing a significant clinical challenge. Previous studies suggest that alkaline phosphatase (ALP) is significantly associated with PBC protein components, and OS. Degree of liver inflammation and fibrosis are also key factors influencing response [18]. This study further confirms that elevated ALP and ALT are independent risk factors for non-response to treatment in PBC. More notably, some patients who do not fully meet diagnostic criteria for OS exhibit AIH-like features, termed "PBC with AIH-like features" in the literature [33, 34]. Failure to promptly add immunosuppressive therapy for such patients may lead to uncontrolled disease progression. Therefore, exploring "whether PBC patients with elevated ALP and ALT should initiate second-line drugs [35, 36] or immunosuppressants earlier" is necessary to improve treatment outcomes. Immunologically, plasma cell function (Mum-1 expression) in PBC is comparable to AIH, but T-cell infiltration (CD3) and immune activation (CD38) involvement are lower, suggesting its pathological mechanism leans more towards "humoral immunity dominance + bile duct-specific injury" [46, 47]. This contrasts sharply with the AIH model of "synergistic drive of cellular + humoral immunity," explaining why PBC treatment primarily focuses on bile duct protection, while AIH requires suppression of systemic immune responses.
5. Recognition and Immune Overlay Mechanism of [14□48] is the most common overlap form in AILD, with a prevalence of about 5%~15% among PBC patients. The diagnosis is mainly based on the Paris criteria [3], and treatment recommends UDCA combined with immunosuppressants. However, due to complex clinical presentations, early recognition is challenging. This study found many commonalities in histopathology between OS and AIH [39]—such as interface hepatitis, Rosette formation, emperipolesis, clustered plasma cell infiltration at the limiting plate, and portal lymphocyte enrichment, with higher inflammation grade and fibrosis degree. High-titer ANA is an independent influencing factor for OS, and whether it can serve as a marker predicting progression to OS warrants further exploration in larger-scale studies. Immunohistochemical features further reveal the pathogenesis of OS: OS shows the most prominent T-cell infiltration (CD3) and immune activation (CD38) levels among the four disease groups, embodying a dual mechanism of "cellular immune activation + immune response overlay"—inheriting the T-cell-mediated immune response from AIH while retaining the bile duct injury-related immune characteristics of PBC. This also leads to more complex conditions and higher treatment difficulty in OS, necessitating combination therapies to balance different dimensions of immune abnormalities.

4.Conclusion

Combining histopathological features (interface hepatitis, rosette formation)[12–13], immunohistochemical markers (Mum-1, CD38, CD3, CD68)[15], and serological indices (IgG, ANA titer, ALP) can significantly improve the differential diagnostic accuracy among AIH, AIH-PBC (OS), PBC, and DI-AILH. Elevated IgG is an independent diagnostic factor for AIH, and high-titer ANA is an independent predictive factor for AIH-PBC (OS), serving as core serum markers for disease classification. Pre-treatment ALP and ALT levels in PBC patients can predict response to ursodeoxycholic acid (UDCA) therapy; high levels indicate a risk of non-response, suggesting the need for early assessment for second-line therapy. Early precise classification and individualized treatment (e.g., combined "corticosteroids + UDCA" regimen for AIH-PBC (OS)) are key to improving the prognosis of patients with AILD.

Study Limitations and Future Perspectives

This study has certain limitations. Firstly, the sample size in each group is limited, and conclusions are based on small-sample statistics, potentially introducing selection bias. Secondly, differences in patient compliance may affect timely assessment of treatment efficacy, leading to potential bias in data interpretation.

Future research could advance in three directions: First, conducting large-scale, multicenter cohort studies to validate findings such as the "AIH multidimensional diagnostic model," "DI-AILH IgG threshold," and "PBC response risk factors." Second, further exploring the predictive value of high-titer ANA for OS and developing more precise disease progression assessment tools. Third, integrating immunohistochemical markers with molecular detection to identify potential specific biomarkers, further enhancing diagnostic and therapeutic precision.

With the deepening of research on AILD, more underlying pathogenic mechanisms will be elucidated, and more efficient assessment methods will be applied in clinical practice, ultimately addressing the core issues of "difficult differentiation and poor response," and providing patients with better diagnostic and therapeutic services.

Declarations

Standard Approval Statement:This study was approved by the Ethics Committee of the Leshan People's Hospital (Approval No. LYLL–2026–KY 075) on February 24, 2026. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study

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Author Contribution

Research conception and design: Su Jing; Paper writing: Su Jing; Data collection and major revisions of the paper: Chen Yuqing, Luo Rui; Paper revision and final review: Liang Shiyi.

References

1. Shen Y, Lu C, Men R et al. Clinical and pathological characteristics of autoimmune hepatitis with acute presentation[J]. Canadian Journal of Gastroenterology & Hepatology, 2018, 2018: 3513206.
2. Degott C, Zafrani ES, Callard P, et al. Histopathological study of primary biliary cirrhosis and the effect of ursodeoxycholic acid treatment on histology progression[J]. Hepatology. 1999;29(4):1007–12. 10.1002/hep.510290444.
3. Boberg KM, Chapman RW, Hirschfield GM, et al. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue[J]. J Hepatol. 2011;54(2):374–85. 10.1016/j.jhep.2010.09.002.
4. Lewis JH, Mortensen ME, Zweig S, et al. Efficacy and safety of high-dose pravastatin in hypercholesterolemic patients with well-compensated chronic liver disease: results of a prospective, randomized, double-blind, placebo-controlled, multicenter trial[J]. Hepatology. 2007;46(5):1453–63. 10.1002/hep.21848.
5. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis[J]. Hepatology. 2008;48(1):169–76. 10.1002/hep.22322.
6. Chazouillères O, Wendum D, Serfaty L, et al. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy[J]. Hepatology. 1998;28(2):296–301. 10.1002/hep.510280203.
7. Danan G, Teschke R. Roussel uclaf causality assessment method for drug-induced liver injury: present and future[J]. Front Pharmacol. 2019;10:853. 10.3389/fphar.2019.00853.
8. Desmet VJ. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis [Hepatology. 1981;1:431–435][J]. Journal of Hepatology, 2003, 38(4): 382–386. 10.1016/s0168-8278(03)00005-9
9. de Boer YS, van Nieuwkerk CM, Witte BI, et al. Assessment of the histopathological key features in autoimmune hepatitis[J]. Histopathology. 2015;66(3):351–62. 10.1111/his.12558.
10. Younes R, Caviglia GP, Govaere O, et al. Long-term outcomes and predictive ability of non-invasive scoring systems in patients with non-alcoholic fatty liver disease[J]. J Hepatol. 2021;75(4):786–94. 10.1016/j.jhep.2021.05.008.

11. Gonzalez RS, Washington K. Primary biliary cholangitis and autoimmune hepatitis[J]. Surg Pathol Clin. 2018;11(2):329–49. 10.1016/j.path.2018.02.010.
12. Gurung A, Assis DN, McCarty TR, et al. Histologic features of autoimmune hepatitis: a critical appraisal[J]. Hum Pathol. 2018;82:51–60. 10.1016/j.humpath.2018.07.014.
13. Balitzer D, Shafizadeh N, Peters MG, et al. Autoimmune hepatitis: review of histologic features included in the simplified criteria proposed by the international autoimmune hepatitis group and proposal for new histologic criteria[J]. Mod Pathol. 2017;30(5):773–83. 10.1038/modpathol.2016.267.
14. Rust C, Beuers U. Overlap syndromes among autoimmune liver diseases[J]. World J Gastroenterol. 2008;14(21):3368–73. 10.3748/wjg.14.3368.
15. Zhao SX, Li WC, Fu N, et al. Emperipolesis mediated by CD8(+) T cells correlates with biliary epithelia cell injury in primary biliary cholangitis[J]. J Cell Mol Med. 2020;24(2):1268–75. 10.1111/jcmm.14752.
16. Tai Y, Wang Q, Korner H, et al. Molecular mechanisms of T cells activation by dendritic cells in autoimmune diseases[J]. Front Pharmacol. 2018;9:642. 10.3389/fphar.2018.00642.
17. Valentine KM, Davini D, Lawrence TJ, et al. CD8 follicular T cells promote B cell antibody class switch in autoimmune disease[J]. J Immunol. 2018;201(1):31–40. 10.4049/jimmunol.1701079.
18. Mix H, Weiler-Normann C, Thimme R, et al. Identification of CD4 T-cell epitopes in soluble liver antigen/liver pancreas autoantigen in autoimmune hepatitis[J]. Gastroenterology. 2008;135(6):2107–18. 10.1053/j.gastro.2008.07.029.
19. Abe K, Takahashi A, Nozawa Y, et al. The utility of IgG, IgM, and CD138 immunohistochemistry in the evaluation of autoimmune liver diseases[J]. Med Mol Morphol. 2014;47(3):162–8. 10.1007/s00795-014-0082-z.
20. Lee BT, Wang Y, Yang A, et al. IgG: IgM ratios of liver plasma cells reveal similar phenotypes of primary biliary cholangitis with and without features of autoimmune hepatitis[J]. Clin Gastroenterol Hepatol. 2021;19(2):397–9. 10.1016/j.cgh.2019.11.024.
21. Lee H, Stapp RT, Ormsby AH, et al. The usefulness of IgG and IgM immunostaining of periportal inflammatory cells (plasma cells and lymphocytes) for the distinction of autoimmune hepatitis and primary biliary cirrhosis and their staining pattern in autoimmune hepatitis-primary biliary cirrhosis overlap syndrome[J]. Am J Clin Pathol. 2010;133(3):430–7. 10.1309/AJCPE93GZSHUNTAI.
22. Zhuge YZ, Wang X. Hepatic sinusoidal obstruction syndrome associated with pyrrolizidine alkaloids[J]. J Practical Hepatol. 2021;24(5):617–20. 10.3969/j.issn.1672-5069.2021.05.003.
23. Li YS, Bao J, Xu Y, et al. Clinicopathological analysis of 16 cases of pyrrolizidine alkaloids-associated hepatic sinusoidal obstruction syndrome[J]. Chin J Hepatol. 2020;28(4):332–7. 10.3760/cma.j.cn501113-20190724-00269.
24. Dignan FL, Wynn RF, Hadzic N, et al. BCSH/BSBMT guideline: diagnosis and management of veno-occlusive disease (sinusoidal obstruction syndrome) following haematopoietic stem cell transplantation[J]. Br J Haematol. 2013;163(4):444–57. 10.1111/bjh.12558.

25. Cooperative Group for Hepatic and Gall Disease, Chinese Society of Gastroenterology, Chinese Medical Association. Expert consensus on diagnosis and treatment of pyrrolidine alkaloids-related sinusoidal obstruction syndrome (2017, Nanjing)[J]. J Clin Hepatol. 2017;33(9):1627–37. 10.3969/j.issn.1001-5256.2017.09.003.
26. Wang Y, Zhuge YZ, Zhang F, et al. Treatment for Gynura segetum caused hepatic vein occlusive disease: a single-center retrospective study[J]. Chin J Digestion. 2016;36(12):811–5. 10.3760/cma.j.issn.0254-1432.2016.12.003.
27. Zhuge Y, Liu Y, Xie W, et al. Expert consensus on the clinical management of pyrrolizidine alkaloid-induced hepatic sinusoidal obstruction syndrome[J]. J Gastroenterol Hepatol. 2019;34(4):634–42. 10.1111/jgh.14612.
28. Yang S, Liu DL, Tan YY, et al. Defibrotide in treatment of hepatic sinusoidal obstruction syndrome caused by Gynura segetum: a case report[J]. Chin J Liver Dis (Electronic Version). 2022;14(2):69–72. 10.3969/j.issn.1674-7380.2022.02.011.
29. Richardson PG, Carreras E, Iacobelli M, et al. The use of defibrotide in blood and marrow transplantation[J]. Blood Adv. 2018;2(12):1495–509. 10.1182/bloodadvances.2017008375.
30. Aziz MT, Kakadiya PP, Kush SM, et al. Defibrotide: an oligonucleotide for sinusoidal obstruction syndrome[J]. Ann Pharmacother. 2018;52(2):166–74. 10.1177/1060028017732586.
31. Li KY, Li YX, Liu ZH, et al. Clinical characteristics analysis of 80 cases with hepatic sinusoidal obstruction syndrome[J]. Henan Med Res. 2018;27(10):1774–7. 10.3969/j.issn.1004-437X.2018.10.017.
32. Andrade RJ, Aithal GP, de Boer YS, et al. Nomenclature, diagnosis and management of drug-induced autoimmune-like hepatitis (DIALH): an expert opinion meeting report[J]. J Hepatol. 2023;79(3):853–66.
33. Anonymous. Classification of chronic viral hepatitis: a need for reassessment[J]. J Hepatol. 1991;13(3):372–4. 10.1016/0168-8278(91)90084-o.
34. Wen M, Men R, Fan X, et al. Worse response to ursodeoxycholic acid in primary biliary cholangitis patients with autoimmune hepatitis features[J]. Dig Dis. 2021;39:366–74.
35. To U, Silveira M. Overlap Syndrome of Autoimmune Hepatitis and Primary Biliary Cholangitis[J]. Clin Liver Disease. 2018;22(3):603–11.
36. European Association for the Study of the Liver. EASL clinical practice guidelines: Autoimmune hepatitis[J]. J Hepatol. 2015;63(4):971–1004.
37. Michelle A, et al. Post-translational modification-specific autoantibodies are associated with complete biochemical remission in autoimmune hepatitis[J]. Hepatology. 2023;78(2):601–14.
38. Yoshida EM, et al. Low-dose interleukin-2 treatment for refractory autoimmune hepatitis: A case series[J]. Hepatology. 2023;77(2):679–83.
39. European Association for the Study of the Liver. EASL clinical practice guidelines on the management of autoimmune hepatitis[J]. J Hepatol. 2025. 10.1016/j.jhep.2025.03.017. [Epub ahead of print].

40. Yang WJ. Pathological diagnosis of autoimmune hepatitis[J]. J Clin Hepatol. 2024;40(6):1082–7.
41. Huang LH, Chen YL, Lin L, et al. Research on the pathogenesis of autoimmune liver disease and intervention of traditional Chinese medicine based on NLRP3[J]. Pharmacology and Clinics of Chinese Materia Medica; 2024. (In press).
42. Cai QY, Yang ZH, Pei F. Application of histopathology, immunohistochemistry and fluorescence in diagnosis and differential diagnosis of autoimmune liver diseases[J]. Chin J Gastroenterol Hepatol. 2024;33(1):1–8.
43. Xiao Q, Tan SZ, Zhao TH, et al. Diagnostic significance of antinuclear antibody and liver histopathology in male patients with autoimmune liver disease[J]. J Clin Hepatol. 2019;35(5):1070–3.
44. Ma LL, Zhu QT, Xu JC, et al. Value of liver function indexes and autoantibody indexes in differential diagnosis of autoimmune liver diseases[J]. Anti-Infection Pharm. 2024;21(1):64–9.
45. Feng J, Huang Y, Huang S. Clinical expression analysis of autoantibodies in autoimmune liver diseases[J]. Labeled Immunoassays Clin Med. 2022;29(9):1523–7.
46. Andrade RJ, Aithal GP, de Boer YS, et al. Nomenclature, diagnosis and management of drug-induced autoimmune-like hepatitis (DIALH): an expert opinion meeting report[J]. J Hepatol. 2023;79(3):853–66.
47. Stine JG, Northup PG. Autoimmune-like drug-induced liver injury: a review and update for the clinician[J]. Expert Opin Drug Metab Toxicol. 2016;12(11):1291–301. 10.1080/17425255.2016.1211110.
48. National Health Commission of the People's Republic of China. Guidelines for the diagnosis and treatment of primary biliary cholangitis (2025 edition)[J]. J Clin Hepatol. 2025;41(8):1504–6.
49. Wang QP, Gan MF. Pathological diagnosis of primary biliary cholangitis[J]. J Clin Hepatol. 2024;40(6):1088–92.
50. Yao SJ, Li YL, Chen MJ, et al. Diagnosis and treatment of overlap syndrome of autoimmune liver diseases[J]. J Clin Hepatol. 2023;39(8):1952–7.

Figures

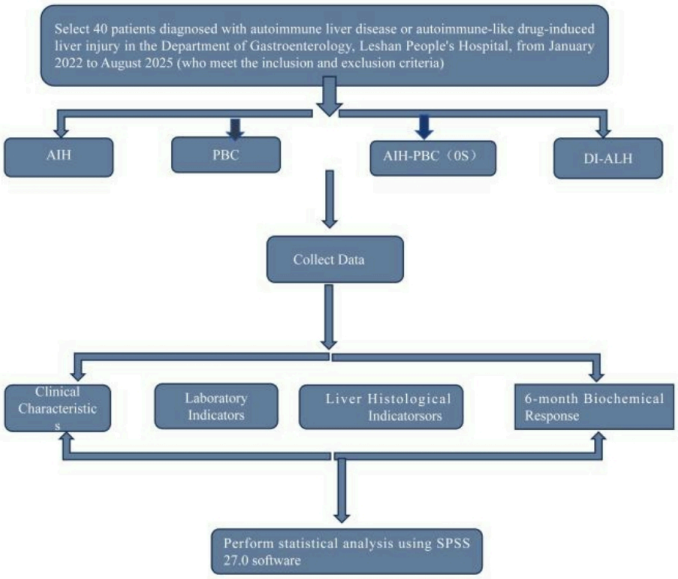


Figure 1

Flow chart of the study design

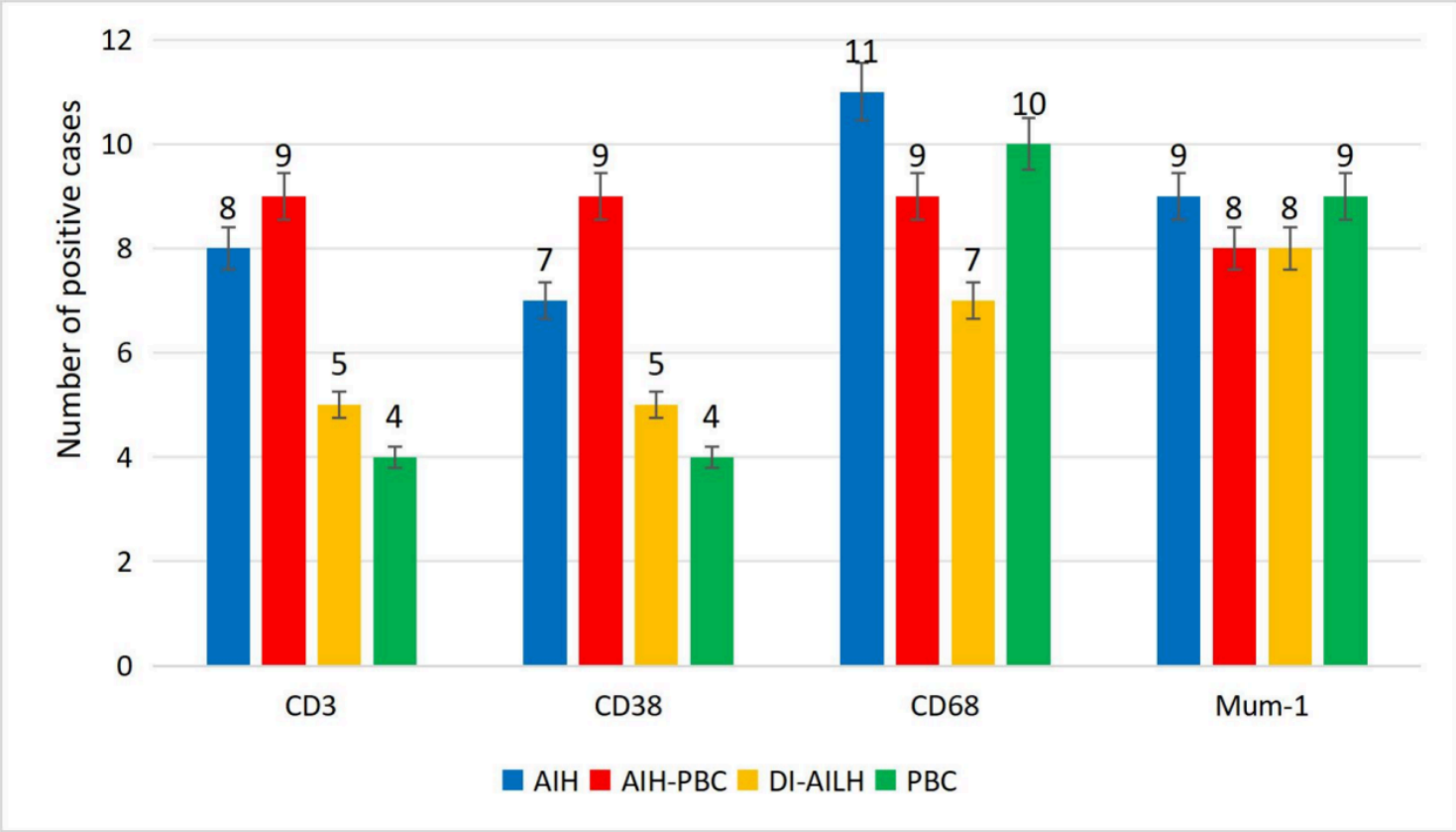


Figure 2

Comparison of the number of CD38, MUM1, CD4 and CD8 positive cells in AIH,PBC,DI-AILH and OS patients

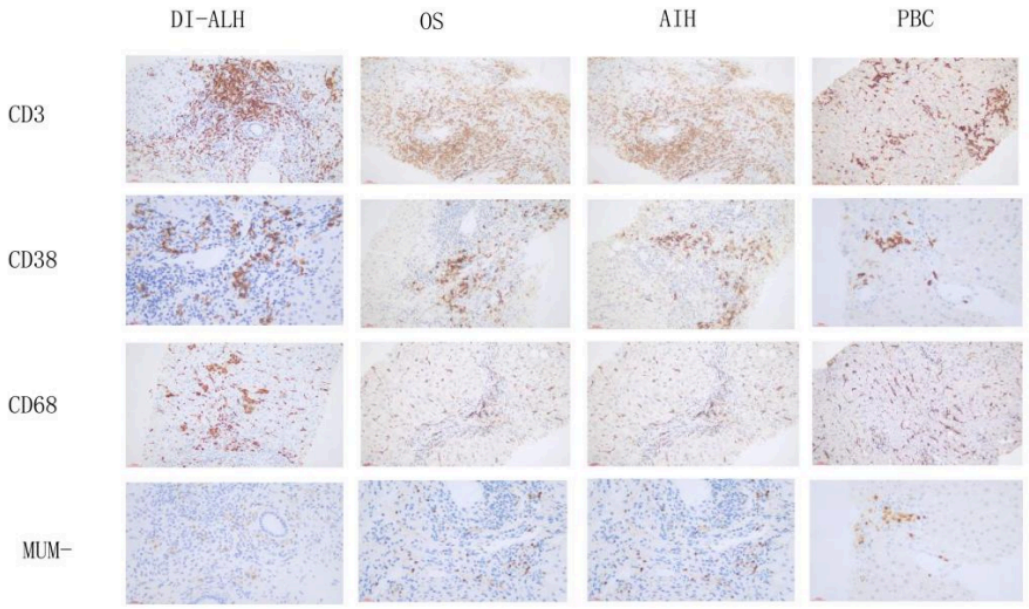


Figure 3

Pathological findings of CD38, MUM1, CD3, and CD68-positive staining in patients with autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), drug-induced autoimmune-like hepatitis (DI-ALH), and AIH-PBC overlap syndrome (OS)