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Autoimmune hepatitis with confluent necrosis indicates severe liver injury but responds well to standard immunosuppressive therapy

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Summary. We aimed to study the effects of different extensive confluent necrosis on complete biochemical response, side effects of immunosuppressants, and outcomes in patients with autoimmune hepatitis (AIH). Patients with liver biopsy, receiving standard immunosuppressive therapy (IST), and regular follow-up were retrospectively recruited. Demographic and clinicopathological characteristics between Ishak confluent necrosis scores ≤4 (the non-severe AIH group) and ≥5 (the severe AIH group) were compared. The Kaplan-Meier Survival analysis, Cox regression analysis, and log-rank test were performed. Bilateral $p<0.05$ was considered statistical significance. One hundred and forty-two patients were enrolled, the median age was 56.0, and 83.8% were female. There were no significant differences in aminotransferases and immunological markers between the two groups. Patients in the severe AIH group had significantly worse liver synthetic function, a higher proportion of cirrhosis, and histologically a higher degree of portal inflammation, interface hepatitis, fibrosis stage, and a higher histological activity index score (all $p<0.05$). Patients in the severe AIH group had a lower response than the other group after four weeks (57.1% *vs*. 86.3%, $p=0.002$). However, differences in complete biochemical response (CBR) were insignificant. Eight patients experienced end-point events. Kaplan-Meier survival analysis showed no significant difference between the two groups ($p=0.343$). For adverse effects of IST, patients in the severe group tended toward a higher incidence of corticosteroid adverse effects without statistical significance. Our study indicated that patients with histologically severe confluent necrosis (Ishak score ≥5) had significantly worse liver synthetic function and a higher degree of liver fibrosis before IST. Compared with their counterparts, this subgroup of patients showed delayed biochemical response but eventually comparable CBRs, side effects, and long-term outcomes.

Key words: Autoimmune hepatitis, Inflammatory necrosis, Biochemical response, End-point events, Side effect

Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease of unknown etiology (Rahim et al., 2019; Komori, 2021). It is now considered to be a disease affecting both males and females (Muratori et al., 2023). The annual incidence is 1.00 per 100,000 in

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Abbreviations. AIH, autoimmune hepatitis; AI-ALF, autoimmune acute liver failure; ALT, alanine transaminase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALB, albumin; ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; APRI, aspartate aminotransferase to platelet ratio index; anti-SLA/LP, anti-soluble liver antigen/liver-pancreas; AZA, azathioprine; BMI, body-mass index; BUN, blood urea nitrogen; CAH, chronic active hepatitis; CBR, complete biochemical response; CK7, cytokeratin-7; CK19, cytokeratin-19; Cr, creatine; CsA, cyclosporine; EBV, Epstein-Barr virus; GGT, γ-glutamyltransferase; HAI, histologic activity index; HCC, hepatocellular carcinoma; H&E, hematoxylin-eosin; IAIHG, international autoimmune hepatitis group; ICD, international classification of disease; IgG, immunoglobulin G; INR, international normalized ratio; IST, immunosuppressive therapy; MELD, model for end-stage liver disease; PASD, periodic acid Schiff diastase; PLT, platelet; PT, prothrombin time; SHB, subacute hepatitis with bridging; SHMN, subacute hepatitis with multilobular necrosis; SMA, smooth muscle antibody; TBil, total bilirubin

North America, and 1.37 per 100,000 in Europe (Lv et al., 2019). Wang (2018) reported that the estimated incidence rate of AIH ranges from 0.67 to 2.0 per 100,000 in the Asia-Pacific area and this incidence is expected to increase due to awareness of the characteristics of this disease by hepatologists (Wang et al., 2018). Similarly, the incidence of AIH has significantly increased by 50% in Spain, Denmark, Sweden, and the Netherlands (Gronbaek et al., 2014; van Gerven et al., 2014; Lamba et al., 2021) over decades. For example, Lamba (2021), reported that the incidence of AIH increased from 1.37 per 100,000 in 2008-2010 to 2.39 per 100,000 in 2014-2016 in New Zealand ($p<0.05$) (Lamba et al., 2021).

AIH is characterized by elevated transaminases and immunoglobulin G (IgG) (Liberal et al., 2016; Mieli-Vergani et al., 2018) and the appearance of autoantibody/ies. AIH presents a variety of clinical phenotypes and the most common one is chronic hepatitis. Sometimes, it also can present acute-onset, acute severe hepatitis, or autoimmune acute liver failure (AI-ALF) (Ramachandran et al., 2014). The histological features of AIH are typically manifested as interface hepatitis and lymphoplasmacytic infiltration (Sebode et al., 2018; Webb et al., 2018). Due to rosette and emperipolesis being common in other liver diseases, a panel of liver histologists recommended that they are not pathological characteristics of AIH (Lohse et al., 2022). Interestingly, some AIH patients (12.2-88%) could also present extensive necrosis: submassive and/or massive confluent necrosis (Abe et al., 2012). Ramachandran (2014) found that extensive bridging or multilobular necrosis with portal inflammation were histological features of acute severe AIH (Ramachandran et al., 2014). Stravitz (2011) reported that 42% of autoimmune acute liver failure patients had massive necrosis (Stravitz et al., 2011).

Histological manifestations of severe liver injury are usually associated with severe AIH or autoimmune acute liver failure. However, in the course of our practice, we identified a subtype of AIH patients who had significant confluent necrosis (Ishak score ≥ 5) but without clinically overt severe liver injury. Decades ago, Schalm (1977) compared the clinical characteristics, biochemical responses in 32 chronic active hepatitis (CAH), 36 subacute hepatitis with bridging (SHB) or 30 multilobular necrosis (SHMN), and 30 cirrhosis patients. They found that histological lesions were not associated with clinical or etiological features and the biochemical response was not significantly different between groups (Schalm et al., 1977). Currently, chronic active hepatitis is renamed AIH. Different grading and staging score systems were put forward and showed excellent performance.

Recently, in comparison with AIH patients without severe confluent necrosis (Ishak score ≤ 4), there are scarce reports on whether those with multibridging and/or multilobular necrosis have similar treatment responses, immunosuppressive therapy side effects (IST), and prognosis. Thus, we hypothesized that AIH patients with severe confluent necrosis could achieve similar biochemical remission and prognosis as their non-severe counterparts if timely treatment is instituted. Therefore, we aimed to study the effects of extensive confluent necrosis on treatment response, IST side effects, and prognosis in AIH patients.

Materials and methods

Study population

This is a retrospective longitudinal study of AIH patients diagnosed between January 2002 and April 2022 at Beijing Friendship Hospital, Capital Medical University. According to the International Classification of diseases-10 (ICD-10), patients with a discharge diagnosis of AIH or AIH-associated cirrhosis were retrieved.

Inclusion and exclusion criterion

The inclusion criteria were as follows: (i) ≥18 years old; (ii) the revised International Autoimmune Hepatitis Group (IAIHG) diagnostic score ≥10 points or the simplified International Autoimmune Hepatitis Group diagnostic score ≥6 points (Alvarez et al., 1999; Hennes et al., 2008); (iii) liver biopsy availability; and (iv) standard treatment using glucocorticoids and (or) immunosuppressants.

Exclusion criteria were as follows: (i) concomitant liver diseases: hepatotropic viral hepatitis (A, B, C, D, and E) and non-hepatotropic viral hepatitis (cytomegalovirus and Epstein-Barr virus (EBV) infection), drug-induced liver injury, primary biliary cholangitis, primary sclerosing cholangitis, alcoholic liver disease, genetic and metabolic liver diseases; (ii) bone marrow or liver transplantation; and (iii) follow-up <six months.

Baseline clinical data collection

Clinical and laboratory data included gender, age, body mass index (BMI), past medical history, clinical symptoms and signs, complete blood count, liver biochemistry parameters, prothrombin time (PT), the international normalized ratio (INR), and immunological markers. An antinuclear antibody titer (ANA) 1:80 and smooth muscle antibody (SMA) 1:40 are considered positive. Titers of antibodies to soluble liver antigen/liver-pancreas (anti-SLA/LP) ≥25 by ELISA are regarded as positive. Compensated cirrhosis was diagnosed if one of the following four criteria was met: (1) histologically confirmed cirrhosis; (2) gastro-esophageal varices on endoscopy; (3) evidence of cirrhosis or portal hypertension on imaging profiles; and (4) meeting two or more of these four criteria: a. platelet (PLT)$<100\times10^9$/L with the exclusion of other etiologies; b. albumin (ALB)<35 g/L with exclusion of malnutrition

or kidney diseases; c. INR>1.3; and d. aspartate aminotransferase-to-platelet ratio index (APRI)>2. Decompensated cirrhosis is as follows: (1) cirrhosis, (2) any complication of portal hypertension including ascites, gastroesophageal variceal hemorrhage, hepatic encephalopathy, and hepatorenal syndrome (Xu et al., 2020).

Liver histology

All biopsy tissues were obtained by needle biopsy (16- or 18-gauge). Specimens were formalin-fixed and paraffin-embedded. All slides were stained with Hematoxylin-Eosin (H&E), trichrome, reticulin, periodic acid Schiff diastase (PASD), rhodamine, Prussian blue, cytokeratin-7 (CK7), cytokeratin-19 (CK19), CD38. Liver biopsies were reviewed by a clinical liver pathologist (Zhao XY). The grade of inflammation and stage of fibrosis were assessed according to the Ishak modified histological activity index (HAI) (Ishak et al., 1995). A confluent necrosis score ≥5 (multiple bridging necrosis or multiacinar necrosis) was regarded as severe confluent necrosis (severe AIH group). An Ishak score ≤4 was regarded as non-severe confluent necrosis (non-severe AIH group) (Brunt, 2000). The HAI score comprised the scores for portal inflammation, periportal/interface hepatitis, focal lobular necrosis, and confluent necrosis.

Treatment

All patients were treated with either monotherapy (glucocorticoids) or combination therapy (glucocorticoids plus immunosuppressants) as initial therapy. The initial dose of prednisone/prednisolone was 0.5 mg/kg/d with or without azathioprine: 50 mg/d. Then the medications were adjusted to the maintenance dose according to the treatment response.

Follow-up and outcomes

Follow-up data such as laboratory findings, radiology, and treatment regimens were retrieved from the electronic medical records system. According to the systematic review of response criteria and end-points in AIH by the IAIHG (Pape et al., 2022), complete biochemical response (CBR) was defined as normalization of serum transaminases and IgG below the upper limit normal (ULN) within six months after initiation of treatment (Mack et al., 2020). Response at four weeks was defined as a >50% decrease in serum transaminases after initiation of treatment.

Response after three months was defined as the normalization of serum transaminases and IgG below the ULN. Side effects associated with IST (low-trauma fracture, metabolic syndromes, gastric ulcer, and infection) were also recorded (Sebode et al., 2018). Outcomes were defined as hepatocellular carcinoma (HCC), liver transplantation, liver-related death, and concomitant with other malignant tumors.

Statistical analysis

Statistical analysis was performed by SPSS (version 25.0). Continuous variables were expressed as median (interquartile range) or means ± standard deviations. Categorical data were presented as frequencies and percentages. The comparison of continuous variables between two groups were assessed by the student's t-test or Mann-Whitney U test. Categorical data between two groups were compared by the chi-squared test. Bivariate logistic regression analysis was used to identify

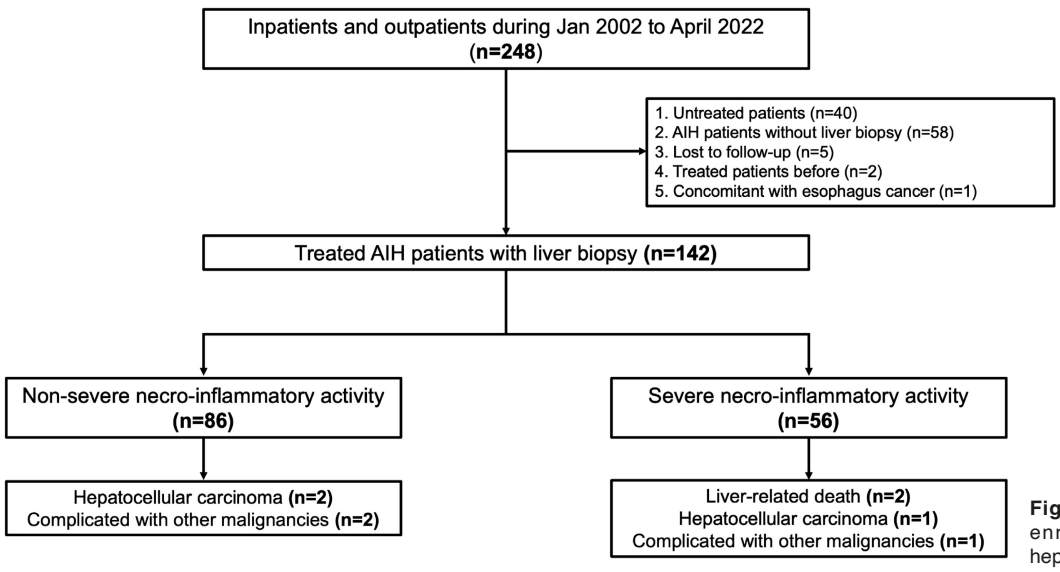

Fig. 1. The flow chart of patient enrollment. AIH, autoimmune hepatitis.

predictive factors of treatment response and side effects. Cox and Kaplan-Meier survival analyses were calculated to investigate prognostic factors of final outcomes. A two-tailed $p<0.05$ was considered statistically significant.

Results

Comparison of clinical characteristics between the severe AIH and non-severe AIH groups at baseline

A total of 248 AIH patients were screened; 40 patients did not accept the standard treatment and 58 had no liver biopsy, thus finally 142 patients were enrolled in this study (Fig. 1). Demographic, clinical, and laboratory characteristics are summarized in Table 1. The median age was 56.0 years (46.0, 63.0) and 119 (83.8%) patients were female; 56 patients had severe confluent necrosis and 86 cases had non-severe confluent necrosis. Age (median: 57.0 *vs*. 54.0 years, $p=0.604$) and gender distribution (female: 82.1% *vs*. 84.5%, $p=0.710$) were comparable between the two groups. At baseline, the severe AIH group had higher serum levels of liver biochemical parameters, such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT) but did not reach statistical significance. ALB was significantly lower (median: 32.6 *vs*. 33.7, p=0.001) while total bilirubin (TBil), INR, and the Child-Pugh score were significantly higher in the severe AIH group [(median: 1.2 *vs*. 1.1, $p<0.001$) and (median: 8.0 vs. 6.0, $p<0.001$), respectively]. The positive rate of ANA and anti-SMA presented no statistical significance (87.5% vs. 78.9%, $p=0.225$) (3.0% *vs*. 6.4%, $p=0.876$,

Table 1. Comparison of demographic and biochemical characteristics of AIH patients between the severe and non-severe disease groups.

Variables	Non-severe AIH group (n=86)	Severe AIH group (n=56)	*p*
Gender (Female, %)	84.5	82.1	0.710
Age (years)	54.0 (51.0,60.0)	57.0 (46.0,65.0)	0.604
ALT (U/L)	108.0 (64.0,209.9)	174.0 (68.0,231.0)	0.094
AST (U/L)	96.0 (87.8,197.0)	209.0 (115.0,289.1)	0.981
ALP (U/L)	136.0 (100.0,140.0)	187.0 (133.0,235.0)	0.259
GGT (U/L)	86.0 (41.0,166.0)	325.0 (126.0,384.0)	0.339
ALB (g/L)	33.7 (29.8,37.3)	32.6 (27.9,34.4)	**0.001**
TBil (μmol/L)	21.1 (16.9,40.4)	29.8 (23.1,173.3)	**0.005**
Cr (μmol/L)	58.7 (49.2,62.0)	44.9 (41.8,58.7)	**0.026**
INR	1.1 (1.0,1.1)	1.2 (1.1,1.3)	**<0.001**
IgG (mg/dL)	1880.0 (1650.0,2740.0)	2470.0 (2100.0,3160.0)	0.061
ANA (>1:80, %)	78.9	87.5	0.225
ANA (>1:160, %)	44.4	41.7	0.764
ANA (>1:320, %)	10.4	13.9	0.573
SMA (%)	6.4	3.0	0.876
SLA/LP (%)	5.0	13.5	0.271
ANCA (%)	15.2	25.0	0.374
PLT (10^9/L)	150.0 (114.0,219.0)	144.0 (96.0,222.0)	0.143
WBC (10^9/L)	4.5 (3.9,5.5)	4.4 (3.4,5.6)	0.785
Liver stiffness (Kpa)	12.0 (8.9,20.2)	24.8 (15.6,42.8)	**0.012**
MELD score	5.3 (2.1,8.1)	5.4 (3.0,9.1)	0.391
Child-Pugh score	6.0 (5.0,7.0)	8.0 (7.0,9.0)	**<0.001**
Child-Pugh class			**<0.001**
A (%)	57.1	25.9	
B (%)	37.1	59.3	
C (%)	5.7	14.8	
Liver cirrhosis (%)	44.0	73.2	**0.001**
Decompensated liver cirrhosis (%)	29.0	47.7	0.104
Interval from onset to liver biopsy (m)	9.0 (3.0,20.0)	6.0 (3.0,20.0)	0.826
Simplified AIH score	7.0 (6.0,8.0)	7.0 (7.0,8.0)	**0.010**
Revised AIH score	16.0 (16.0,18.0)	16.0 (13.019.0)	0.360
Treatment			0.702
GC+AZA (%)	82.6	80.0	
GC (%)	17.4	20.0	
AZA (%)	0.0	0.0	
Endpoint events (%)	4.8	7.1	0.564
Follow-up (month)	47.0 (27.5, 55.0)	34.0 (16.0, 53.0)	0.199

One patient with severe inflammatory necrosis accepted GC and CsA therapy. AIH, autoimmune hepatitis; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; ANA, antinuclear antibody; ANCA, Antineutrophil cytoplasmic antibody; anti-SLA/LP, anti-soluble liver antigen/liver-pancreas; AZA, azathioprine; Cr, creatine; CsA, cyclosporine; GGT, γ-glutamyltransferase; GC, glucocorticoid; IgG, immunoglobulin G; INR, international normalized ratio; MELD, model for end-stage liver disease; SMA, smooth muscle antibody; TBil, total bilirubin. PLT, platelet; WBC, white blood cell.

respectively). The differences in titer of ANA>1:160 and >1:320 between the two groups were both insignificant (41.7% *vs*. 44.4%, *p*=0.764; 13.9% *vs*. 10.4%, *p*=0.573). In the severe AIH group, 13.5% and 25.0% of patients were anti-SLA/LP and antineutrophil cytoplasmic antibody (ANCA) positive, respectively; compared with 5.0% and 15.2% in the non-severe AIH group (*p*=0.876, *p*=0.271). Serum IgG levels showed no significant difference between the two groups (median: 2470.0 vs. 1880.0, *p*=0.061). Liver stiffness in the severe AIH group was significantly higher than in the non-severe AIH group (median: 24.8 *vs*. 12.0, *p*=0.012). The presence of clinical cirrhosis was significantly higher in the severe AIH group (73.2% *vs*. 44.0%, *p*=0.001).

Table 2. Comparison of liver histological features of AIH patients between the severe and non-severe disease groups.

Variables	Non-severe AIH group (n=86)	Severe AIH group (n=56)	*p*
Portal inflammation	2.0 (2.0,3.0)	3.0 (2.0,3.0)	**<0.001**
Periportal/interface hepatitis	2.0 (2.0,2.0)	3.0 (2.0,3.0)	**0.001**
Spotty necrosis	2.0 (2.0,3.0)	2.0 (2.0,3.0)	0.218
HAI score	8.0 (7.0,10.0)	12.0 (12.0,14.0)	**<0.001**
Fibrosis	2.0 (2.0,3.0)	4.0 (3.0,5.0)	**<0.001**
0 (%)	8.3	1.8	
1 (%)	10.7	3.6	
2 (%)	40.5	10.9	
3 (%)	19.0	16.4	
4 (%)	16.7	54.8	
5 (%)	3.6	29.1	
6 (%)	1.2	7.3	

AIH, autoimmune hepatitis; HAI, histological activity index.

Comparison of histological features between the severe AIH and non-severe AIH groups at baseline

Baseline and typical histological features are depicted in Table 2 and Figures 2, 3. We found that the degree of portal inflammation (median: 3.0 *vs*. 2.0, *p*<0.001) and interface hepatitis (median: 3.0 *vs*. 2.0, *p*=0.001) and, thus, the HAI score (median: 12.0 *vs*. 8.0, *p*<0.001) were significantly higher in the severe AIH group in comparison with the non-severe AIH group. The median stage of fibrosis was significantly higher in the severe AIH group (4.0 *vs*. 2.0, *p*<0.001). However, lobular spotty necrosis was comparable between the two groups (*p*=0.218).

Table 3. Comparison of biochemical responses of AIH patients between the severe and non-severe disease groups.

Variables	Non-severe AIH group (n=86)	Severe AIH group (n=56)	*p*
Response after four weeks (%)	86.3	57.1	**0.002**
Response after three-month treatment (%)	31.9	29.8	0.811
CBR after six-month treatment (%)	58.8	44.4	0.134
Response after one-year treatment (%)	71.2	63.8	0.406
Response after two-year treatment (%)	78.3	80.0	0.841

AIH, autoimmune hepatitis; CBR, complete biochemical response.

Table 4. Factors associated with complete biochemical response in AIH patients.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	*p*	OR	95%CI	*p*
Gender	2.43	0.89-6.63	0.082	4.25	1.03-17.66	**0.046**
Age	1.03	0.99-1.06	0.128			
ALT (U/L)	1.00	1.00-1.00	0.607			
AST (U/L)	1.00	1.00-1.00	0.916			
TBiL (μmol/L)	1.00	0.99-1.00	0.298			
ALB (g/L)	1.12	1.03-1.22	**0.007**			
GLB (g/L)	0.94	0.91-10.98	**0.006**			
Cr (μmol/L)	0.98	0.95-1.01	0.203			
INR	0.15	0.01-1.99	0.151			
IgG (mg/dL)	1.00	1.00-1.00	**0.003**	0.99	0.99-1.00	**0.022**
ANA	1.32	0.47-3.75	0.599			
MELD	0.97	0.88-1.07	0.567			
Platelets (10^9/L)	1.01	1.00-1.01	0.148			
Child-Pugh score	0.74	0.57-0.97	**0.027**			
Periportal/interface hepatitis	0.87	0.51-1.48	0.614			
Confluent necrosis	0.83	0.69-0.99	**0.038**			
Fibrosis	0.75	0.58-0.98	**0.034**			
HAI score	0.86	0.75-0.98	**0.028**			
Cirrhosis	0.30	0.14-0.67	**0.003**	0.20	0.08-0.54	**0.001**

Cirrhosis includes compensated cirrhosis and decompensated cirrhosis. AIH, autoimmune hepatitis; ALT, alanine transaminase; AST, aspartate aminotransferase; ALB, albumin; ANA, anti-nuclear antibody; CI, confidence interval; Cr, creatine; γ-glutamyltransferase; GLB, globulin; HAI, histological activity index; OR odds ratio; IgG, immunoglobulin G; INR international normalized ratio; MELD, model for end-stage liver disease; PLT, platelet; TBil, total bilirubin.

Comparison of biochemical responses between the severe AIH and non-severe AIH groups

The majority of patients received glucocorticoid monotherapy or glucocorticoid and azathioprine combination therapy with the exception of one patient who received glucocorticoid and cyclosporine therapy. The median follow-up time [34.0 (16.0, 53.0) *vs*. 47.0 (27.5, 55.0) months] showed no statistical difference between the two groups. Five patients (3.4%) were lost to follow-up. According to the new criteria proposed by the IAIHG, patients in the severe AIH group responded less frequently to the treatment compared with the non-severe AIH group (57.1% *vs*. 86.3%, p=0.002) (Fig.

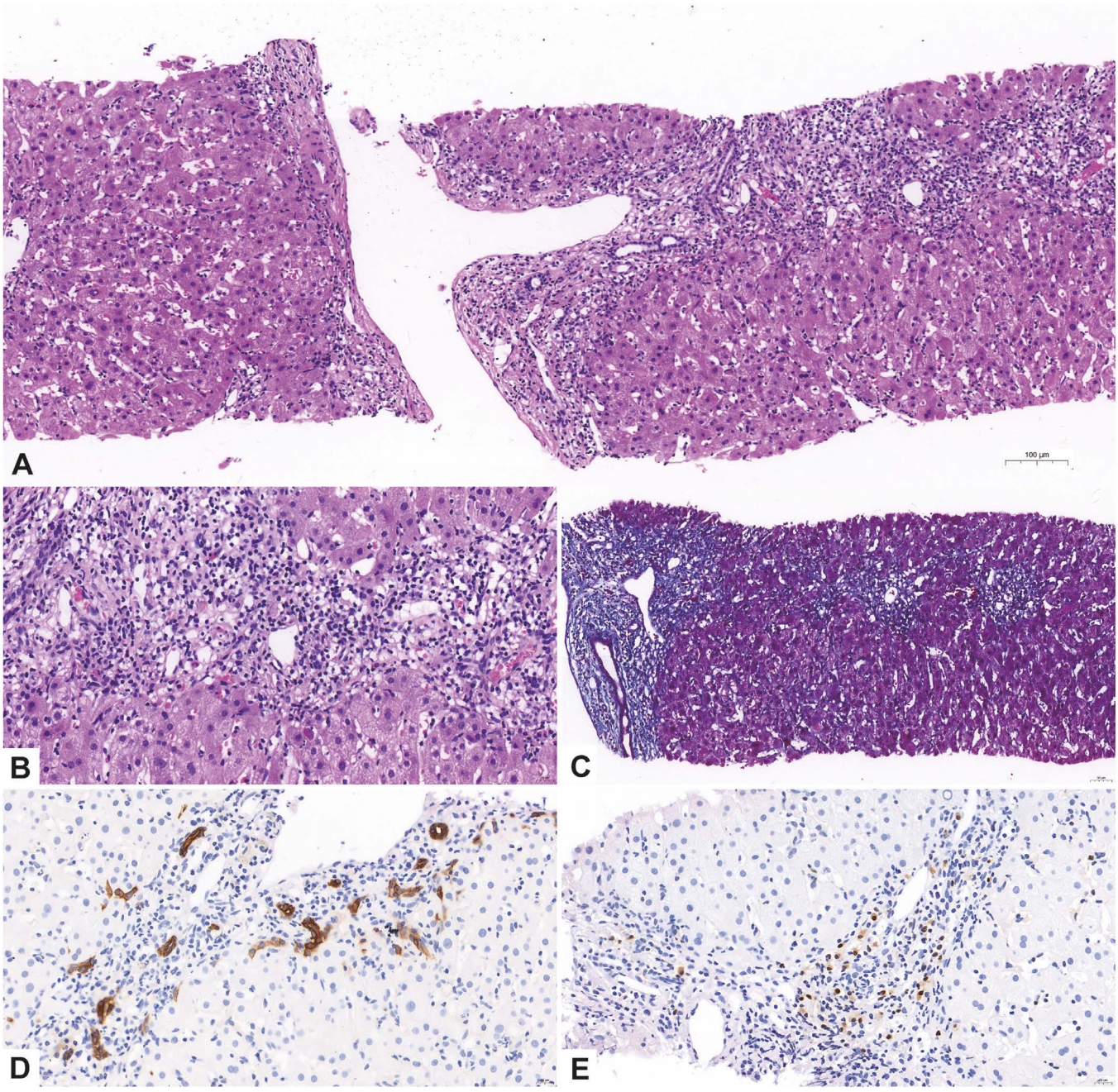

Fig. 2. Representative histological images of the non-severe AIH group. Representative histological images of the non-severe AIH group. **A.** A typical case in the non-severe AIH group, which was characterized by interface hepatitis, plasma cell infiltration with mild perivenular necrosis (H&E). **B.** High magnification of interface hepatitis (H&E). **C.** Mild fibrosis (Masson). **D.** Mild ductular reactions with preserved interlobular bile ducts (CK19). **E.** Lymphocytic and plasmocytic infiltrations (Mum-1). A, × 10; B, D, E, × 40; C, × 20.

5A). Likewise, fewer patients achieved CBR in the severe AIH group, however, without statistical significance (44.4% *vs*. 58.8%, p=0.134). We further calculated the proportion of biochemical response at 12 months. Not surprisingly, the proportion was similar between the two groups (63.8% *vs*. 71.2%, p=0.406) (Table 3).

We further compared the extent and rapidity of the improvement of liver biochemical parameters such as ALT, TB, ALB, and IgG at 6 and 12 months after treatment between the two groups. There was no statistical significance between the two groups (Fig. 4). We found that cirrhosis (OR:0.22, 95%CI 0.07-0.68, p=0.004) was significantly associated with biochemical response at both four weeks and six months, confluent necrosis was significant by univariate logistic regression analysis but insignificant after multivariate analysis.

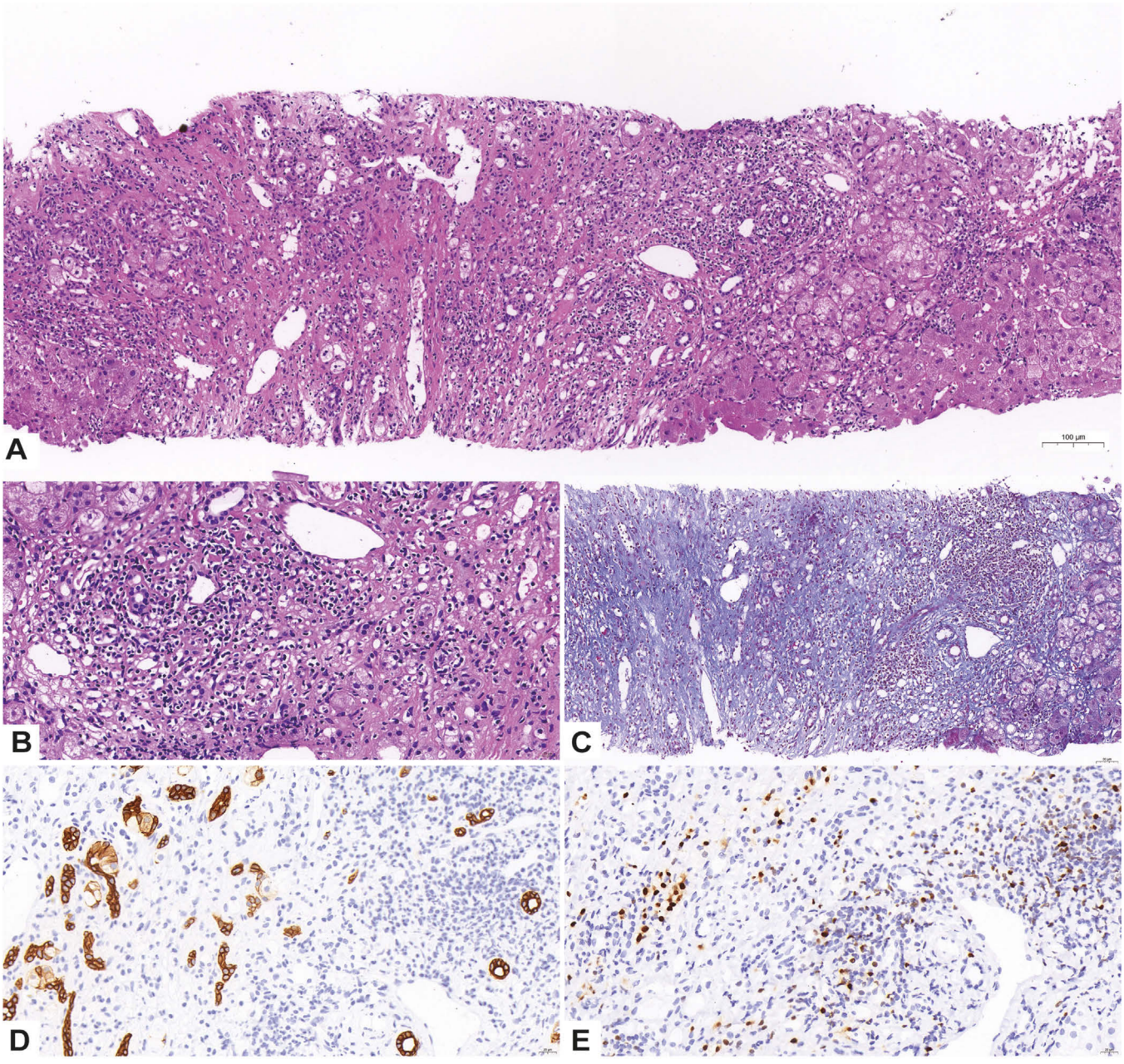

Fig. 3. Representative histological image of the severe AIH group. **A.** Severe AIH histologically presented as multilobular necrosis (Ishak score=6) with a regenerative nodule (H&E). **B.** Inflammatory cells infiltrated into the portal tract (H&E). **C.** Confluent necrosis highlighted by pale blue staining (Masson). **D.** Severe portal inflammation with mild ductular reactions (CK19). **E.** Severe lymphocytic and plasmocytic infiltrations (Mum-1). A, × 10; B, D, E, × 40; C, × 20.

Besides, being female (OR:4.25, 95%CI 1.03-17.66, p=0.046) and elevated IgG (OR:0.99, 95%CI 0.99-1.00, p=0.022) were independent factors associated with CBR (Table 4).

Comparison of clinical outcomes between the severe AIH and non-severe AIH groups

In total, eight patients experienced end-point events, four (7.1%) from the severe AIH group and four (4.8%) from the non-severe AIH group. Two patients progressed to HCC, one patient died, and one patient in the severe AIH group was diagnosed with other malignant tumors. In the non-severe AIH group, two patients developed HCC and two had malignant tumors. Kaplan-Meier cumulative survival of the two patient groups is shown in Figure 5B and there was no statistical significance in terms of end-point events between the two groups (p=0.343).

Table 5. Comparison of IST-associated side effects in AIH patients between the severe and non-severe disease groups.

	Non-severe AIH group (n=86)	Severe AIH group (n=56)	*p*
Incidence of side effects (%)	9.3	16.4	0.209
Specific side effects			0.201
Low-trauma fracture (%)	5.8	5.5	
Metabolic syndromes (%)	3.5	7.3	
Gastric ulcer (%)	0.0	3.6	
Infection (%)	0.0	1.8	

Autoimmune hepatitis, AIH.

Comparison of side effects between the severe AIH and non-severe AIH groups

In comparison, patients in the severe AIH group tended to have more IST-associated side effects (16.4% *vs*. 9.3%) (Table 5). Patients in the severe AIH group presented metabolic disorders more frequently than in the non-severe AIH group and had a comparable ratio of low-trauma fracture (7.3% *vs*. 3.5%, 5.5% *vs*. 5.8%). Gastric ulcer and infection occurred in 3.6% and 1.8% of patients in the severe group in contrast to no patients in the non-severe group.

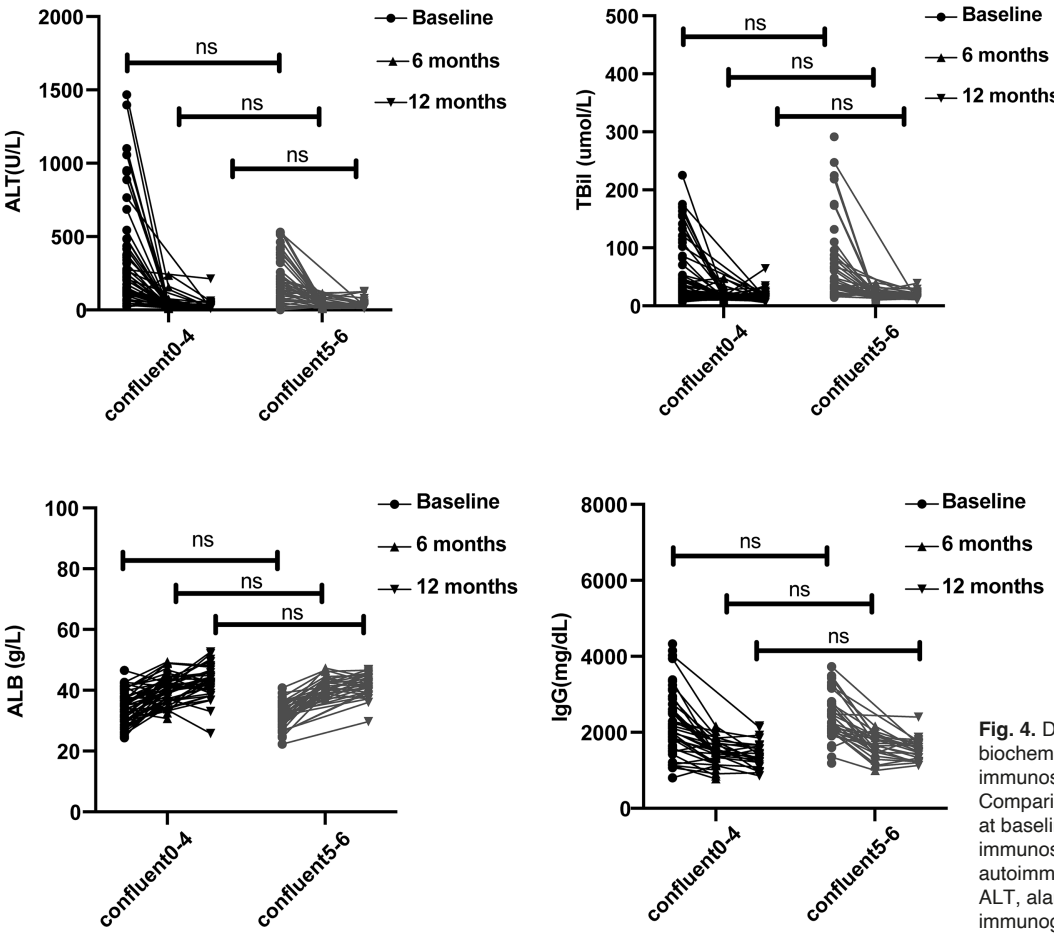

Fig. 4. Dynamic changes in liver biochemical markers after immunosuppressive therapy. Comparison of ALT, TBil, ALB, and IgG at baseline and after 6 and 12 months of immunosuppressive therapy. AIH, autoimmune hepatitis; ALB, albumin; ALT, alanine transaminase; IgG, immunoglobulin G; TBil, total bilirubin.

Discussion

From 2002 to 2021, the prevalence of AIH patients increased from 3.03 ‰ to 33.6 ‰ at our Liver Research Center and from 4.69 to 37.15 per 100,000 person years at this hospital. The proportion of AIH out of all liver diseases increased from 7.86 to 54.97 per 100,000 person years (about seven times) in our center, or from 0.10 to 1.16 per 100,000 person years at the hospital (about 10 times). This epidemiological data was in accordance with reports by Gronbaek and Lamba (Gronbaek et al., 2014; Lamba et al., 2021). It prompted us to analyze the clinical and pathological characteristics of AIH.

Histologically, AIH is commonly characterized by interface hepatitis and plasma cell-rich infiltration. However, during our practice, we occasionally encountered AIH presenting with significant confluent hepatic necrosis such as multiple bridging or multilobular necrosis (Ishak confluent score ≥ 5), not accompanied by clinically overt liver failure. In our cohort, 56 out of 142 AIH patients had severe necrosis indicating more severe liver disease. However, in this subgroup, only nine (16.1%) patients had TBil >171 μmol/L, five (8.9%) patients had INR >1.5, and no patient had hepatic encephalopathy. The results suggested that there is a stage during the course of AIH when histological necro-inflammatory activity outweighs its clinical severity. This prompted us to look further into whether AIH with higher histological severity could respond just as well to IST as those without severe necrosis. More importantly, whether these patients had similar long-term outcomes and IST side effects.

Our study indicated that patients with severe inflammatory necrosis did have significantly worse liver synthetic function and a higher degree of liver fibrosis. Twenty out of 56 patients (35.7%) had an Ishak fibrosis score ≥ 5, which was comparable with the existing data reporting that cirrhosis is present in 28%-33% of AIH patients at presentation (Feld et al., 2005; Czaja and Carpenter, 2006; Liberal and Grant, 2016). Furthermore, Cooksley et al. (1986) reported that AIH patients with bridging necrosis were more likely to develop cirrhosis (Cooksley et al., 1986), which concurred with our study (73.2% *vs*. 44.0%, p=0.001). Manns (2001) reported that in patients with bridging or multilobular necrosis, the incidence of cirrhosis within five years was up to 82% without treatment (Manns and Strassburg, 2001). These data suggest that the degree of histological activity correlated well with clinical severity.

In our cohort, 73.1% of patients responded at four weeks. Patients in the severe AIH group responded less than in the non-severe group (57.1% *vs*. 86.3%, p=0.002). However, the percentage of CBR between these two groups was not significantly different (44.4% *vs*. 58.3%, p=0.143), which was less frequent than in the other two cohorts (four weeks: 83.0%, 92.5%; six months: 61.7%, 77.2%, respectively) (Li et al., 2022; Medas et al., 2022). The multivariate logistic regression analysis also proved that this histological feature did not relate to CBR, despite the association of confluent necrosis between response at four weeks and CBR on univariate logistic regression analysis. Moreover, after adopting standard IST, we also found that the biochemical response rate at 12 months between the two groups was not significantly different: 67.5% in the whole cohort, 63.8% in the severe AIH group, and 71.2% in the non-severe AIH group, which was lower than in studies reported by other groups (80~93%). Sonthalia (2017) reported that severe AIH tended to have a lower biochemical response rate (50% *vs*. 70%, p=0.068) without significance (Sonthalia et al., 2017), which was similar to the results of our study. Only eight patients had end-point events. There was no significant difference between the severe and non-severe AIH groups. Adopting standard IST effectively prevented disease progression. Our data favorably support the treatment of AIH patients with severe inflammatory necrosis, which is parallel to real-world data, suggesting that timely IST is effective and safe in patients with severe AIH (Zachou et al., 2019).

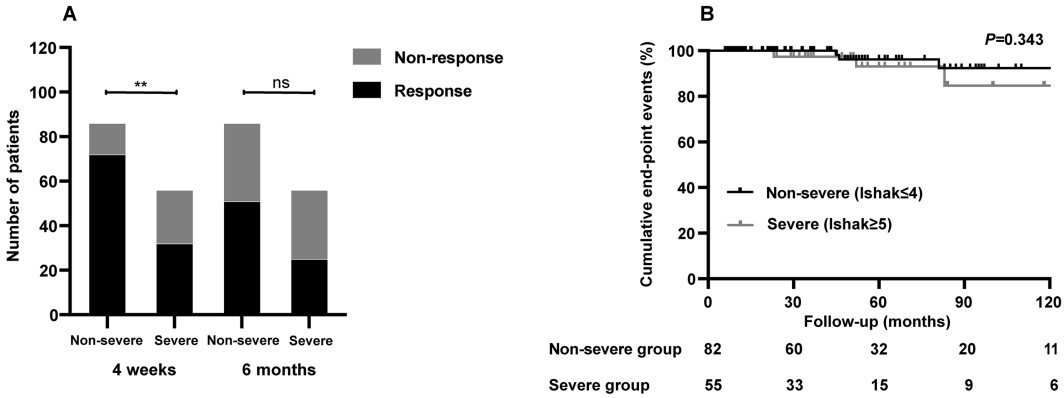

Fig. 5. Treatment response and prognosis of AIH patients between the two groups. **A.** Patients in the non-severe AIH group responded more frequently than in the severe AIH group after four-week treatment (p=0.002). However, the complete biochemical remission rate (CBR) between the two groups showed no significance (p=0.134). **B.** The Kaplan-Meier cumulative survival rate showed no significant difference between the two groups (p=0.343). AIH, autoimmune hepatitis. Non-severe, Ishak ≤4; Severe, Ishak ≥5.

Patients subjected to IST for a long time can have various side effects, 10%-20% of patients may abstain due to side effects (Vergani et al., 2021). AIH patients with severe liver disease or liver failure had a significantly higher incidence of side effects, such as life-threatening infections (Ichai et al., 2007). This may deter the decision to embark on IST. In our study, the overall difference did not reach statistical significance, despite the incidence of side effects being higher in the severe AIH group (16.4% *vs*. 9.3%, p=0.209). This suggests that the severity of histological inflammatory necrosis itself should not prevent the decision to start standard IS therapy even though the side effects are of concern.

Our study has several limitations. This is a single-center, retrospective study where the follow-up data was not sufficient for a full evaluation of the biochemical markers before and after treatment. However, less than 4% of patients were lost to follow-up. To our knowledge, there are relatively few studies focusing on the effects of multiple bridging necrosis and/or multilobular necrosis on the biochemical response and prognosis of AIH patients.

In conclusion, there is a stage during the natural history of AIH that shows a significant extent of necrosis without clinically overt liver failure. Our data favor the active treatment of these patients with standard immunosuppressive therapy for a better biochemical response with comparable long-term outcomes and manageable side effects.

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Interests statement. The authors declare that they have no competing interests.

Ethics Approval. This study has been approved by the Medical Ethics Committee of Beijing Friendship Hospital, Capital Medical University (Approval No.: 2021-P2-303-01). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Author Contribution Statement. Xinyan Zhao and Jidong Jia performed the study concept and design; Xiaoyi Sun, Yu Su, Qianyi Wang, and Jingqi Zhang provided data acquisition; Xiaoyi Sun and Yu Su performed the writing, provided statistical analysis, and interpretation of data; Xinyan Zhao, Jidong Jia, Aileen Wee, and Jimin Liu performed the critical review and revision of the paper; All authors read and approved the final paper.

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Data Availability Statement. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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