Received: 5 March 2019 | Revised: 4 May 2019 | Accepted: 6 May 2019

DOI: 10.1002/jcla.22922

RESEARCH ARTICLE

WILEY

Noninvasive indicators predict advanced liver fibrosis in autoimmune hepatitis patients

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Funding information
This study was funded by the Natural Science Foundation of Anhui Province, Grant/Award Number: 1308085 MH124; and the Natural Science Research of Education Department of Anhui Province, Grant/Award Number: KJ2015ZD31.

Abstract

Background: Liver biopsy is the criterion standard for diagnosing liver fibrosis, but it is not widely used to monitor liver fibrosis because of the invasiveness, risk of complications, and sample errors. Therefore, it is necessary to involve other techniques to monitor liver fibrosis or cirrhosis during clinical practice. The objective was to explore noninvasive indicators to predict advanced liver fibrosis in autoimmune hepatitis (AIH) patients.

Methods: A total of 45 AIH patients and 47 healthy controls were recruited to this retrospective study. Complete blood count and liver function tests were performed for all subjects. AIH patients were divided into "no/minimal fibrosis" group and "advanced fibrosis" group based on liver biopsy.

Results: AIH patients demonstrated significantly higher monocytes, MCV, RDW-CV, RDW-SD, NLR, RDW-CV/PLT, RDW-SD/PLT, TBIL, DBIL, GLB, ALT, AST, GGT, ALP, and GPR and lower WBC, neutrophils, lymphocytes, RBC, HGB, HCT, LMR, TP, ALB, and AAR compared with healthy controls. Patients with advanced fibrosis showed remarkably higher RDW-CV, RDW-SD, RDW-CV/PLT, RDW-SD/PLT, AAR, and FIB-4 and lower RBC, PLT, PCT, and ALB compared with the no/minimal fibrosis group. Logistic regression analysis showed that RDW-SD/PLT was an independent risk factor for advanced fibrosis with an OR (95% CI) of 2.647 (1.383-5.170). Receiver operating characteristic (ROC) analysis revealed that RDW-SD, RDW-CV/PLT, RDW-SD/PLT, FIB-4, and AAR had an area under the ROC curve (AUC) above 0.700 and RDW-SD/PLT had the largest AUC of 0.785 with a cutoff value of 0.239.

Abbreviations: AAR, AST-to-ALT ratio; AGR, ALB-to-GLB ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, AST-to-PLT ratio index; AST, aspartate aminotransferase; AUC, area under the ROC curve; DBIL, direct bilirubin; FIB-4, fibrosis index based on the four factors; GGT, gamma-glutamyltransferase; GPR, GGT-to-PLT ratio; HCT, hematocrit; HGB, hemoglobin; LMR, lymphocyte-to-monocyte ratio; LY, lymphocyte; MCV, mean corpuscular volume; MON, monocyte; NEU, neutrophil; NLR, neutrophil-to-lymphocyte ratio; PCT, plateletcrit; PLR, platelet-to-lymphocyte ratio; PLT, platelet; RBC, red blood cell; RDW-CV, red blood cell distribution width-coefficient of variation; RDW-CV/PLT, RDW-CV-to-PLT ratio; RDW-SD, red blood cell distribution width-standard deviation; RDW-SD/PLT, RDW-SD-to-PLT ratio; ROC, receiver operating characteristic; TBIL, total bilirubin; TP, total protein; WBC, white blood cell.

Liu and Cao contributed equally to this work.

Conclusion: RDW-SD, RDW-CV/PLT, RDW-SD/PLT, FIB-4, and AAR were excellent noninvasive biomarkers and RDW-SD/PLT was an independent risk factor for predicting advanced fibrosis in AIH patients.

KEYWORDS
AST-to-ALT ratio, autoimmune hepatitis, fibrosis index based on the four factors, noninvasive indicators, red blood cell distribution width-to-platelet ratio

1 | INTRODUCTION

Autoimmune hepatitis (AIH) is a generally progressive chronic autoimmune hepatitis whose pathogenesis remains unclear. T cell–mediated events cascade triggered by environmental agents including virus and drugs leading liver necroinflammatory and fibrotic process was a well accepted assumption.[1] The presentation of AIH is heterogeneous and fluctuant. Symptoms of chronic liver disease, such as hepatomegaly, splenomegaly, or jaundice, are common, but sometimes nonspecific symptoms, such as malaise, fatigue, lethargy, itching, and arthralgia, may be the primary complaint.[1] The American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) have published the guidelines for AIH. There are no specific diagnostic parameters for AIH, so the diagnosis of AIH should synthesize characteristic clinical manifestations, biochemical tests, circulating abnormal serum globulin, and autoantibodies. Although autoantibodies are helpful for AIH classification, there is little evidence that autoantibodies play a role in AIH pathogenesis,[1] and titers of autoantibodies have poor correlation with disease activity and treatment response.[1]

The AASLD and EASL guidelines recommend liver biopsy in AIH patients as a prerequisite for diagnosis, prognosis, and treatment decisions.[1,2] Previous studies showed 7% of AIH patients were cirrhotic at the time of diagnosis, and the baseline fibrosis was a risk factor for progression of cirrhosis after treatment.[1] However, because of the invasiveness, risk of complications, and sample errors, the clinical application of liver biopsy is limited. Therefore, many investigators attempted to propose noninvasive diagnostic models based on routine laboratory tests to assess liver fibrosis. Chen B et al[3] used the RDW-to-platelet ratio (RPR) to predict severity of liver fibrosis in patients with chronic hepatitis B (CHB). Kekilli M et al[4] found that the peripheral blood neutrophil-to-lymphocyte ratio (NLR) could forecast advanced fibrosis with high sensitivity and specificity in CHB patients. Yen YH, et al[5] used aspartate aminotransferase-to-platelet ratio index (APRI) and fibrosis index based on the four factors (FIB-4) to evaluate liver fibrosis in chronic hepatitis C patients. However, few studies about the evaluation of liver fibrosis by noninvasive indicators in AIH patients were reported at present.

In this retrospective study, we aimed to investigate the complete blood count, liver function indexes, and other previously published indexes to evaluate the diagnostic value of these indexes for predicting liver fibrosis in AIH patients.

2 | PATIENTS AND METHODS

2.1 | Patients

This study was approved by Medicine Clinical Research Ethics Committee from the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Between December 2008 and June 2018, a total of 45 AIH patients from Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, who underwent liver biopsy were recruited to this retrospective study. All AIH patients were definitely diagnosed according to relevant guideline of the International Autoimmune Hepatitis Group (IAIHG).[6] Patients with viral hepatitis, nonalcoholic fatty liver disease (NAFLD), primary biliary cirrhosis, sclerosing cholangitis, hepatocellular carcinoma (HCC), or other liver disease were excluded. Patients with an inadequate liver biopsy for staging (length <10 mm and/or including <10 portal tracts) or with incomplete clinical/laboratory data were also excluded. Forty-seven age- and gender-matched healthy controls were in good condition for all routine tests, including liver function, blood routine, and abdominal ultrasonography. All subjects were precluded infection or inflammatory diseases within one month before blood collection. Venous blood of AIH patients was collected at the same day with liver biopsy during the first diagnosis as AIH. Venous blood of healthy controls was collected during physical examination.

2.2 | Methods

Demographic and clinical data were collected by reviewing medical records. Hematological complete blood count was tested by Sysmex 5000 hematology analyzer (Sysmex) using 2 mL EDTA-K_2 anticoagulated blood. The complete blood count parameters include white blood cell (WBC), neutrophil (NEU), lymphocyte (LY), monocyte (MON), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), red blood cell distribution width-coefficient of variation (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), platelet (PLT), plateletcrit (PCT), and derived parameters NLR, lymphocyte-to-monocyte ratio (LMR), RDW-CV-to-PLT ratio (RDW-CV/PLT), RDW-SD-to-PLT ratio (RDW-SD/PLT), and platelet-to-lymphocyte ratio (PLR) according to the following formulas: NLR = Neutrophil(10^9/L)/Lymphocyte(10^9/L); LMR = Lymphocyte(10^9/L)/Monocyte(10^9/L); RDW-CV/PLT = RDW-CV(%)/PLT(10^9/L); RDW-SD/PLT = RDW-SD(fL)/PLT(10^9/L); and PLR = PLT(10^9/L)/Lymphocyte(10^9/L). Liver

function parameters were tested by OLYMPUS AU5800 biochemistry analyzer (Beckman Coulter) using 3 mL coagulated peripheral blood after centrifugation at 2564 *g* for 10 minutes. The liver function parameters include total protein (TP), albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and derived parameters AST-to-ALT ratio (AAR), ALB-to-GLB ratio (AGR), APRI, FIB-4, and GGT-to-PLT ratio (GPR) according to the following formulas: AAR = AST(U/L)/ALT(IU/L); AGR = ALB(g/L)/GLB(g/L);

TABLE 1 Demographic data and laboratory parameters of AIH patients and healthy controls

Variables	AIH (n = 45)	Control (n = 47)	*P*
Age	54.29 ± 11.10	54 ± 11.05	0.726[a]
Female/male (n)	37/8	39/8	0.924[c]
WBC ($\times10^9$/L)	5.40 ± 1.75	5.70 ± 0.96	<0.001[a]
Neutrophil ($\times10^9$/L)	2.78 ± 1.35	3.15 ± 0.84	0.028[a]
Lymphocyte ($\times10^9$/L)	1.89 ± 0.64	2.01 ± 0.36	<0.001[a]
Monocyte ($\times10^9$/L)	0.49 ± 0.17	0.41 ± 0.11	0.003[a]
RBC ($\times10^{12}$/L)	4.25 ± 0.56	4.55 ± 0.30	0.002[a]
HGB (g/L)	128.67 ± 16.45	139.32 ± 9.18	0.009[a]
HCT (%)	38.05 ± 4.68	39.95 ± 2.12	<0.00[a]
MCV (fL)	90.30 ± 6.41	87.89 ± 3.27	0.015[a]
RDW-CV (%)	14.30 (13.50-15.40)	12.50 (12.20-13.10)	<0.001[b]
RDW-SD (fL)	45.60 (43.15-52.25)	40.20 (39.20-41.70)	<0.001[b]
PLT ($\times10^9$/L)	172.49 ± 57.36	224.68 ± 48.34	0.299[a]
MPV (fL)	11.30 ± 1.05	10.85 ± 0.96	0.989[a]
PDW (fL)	13.86 ± 2.39	12.93 ± 1.99	0.56[a]
PCT (%)	0.20 ± 0.07	0.24 ± 0.05	0.114[a]
NLR	1.64 ± 1.07	1.62 ± 0.58	0.047[a]
LMR	3.75 (3.09-4.91)	4.65 (3.95-6.33)	<0.001[b]
RDW-CV/PLT	0.08 (0.07-0.11)	0.06 (0.05-0.07)	<0.001[b]
RDW-SD/PLT	0.27 (0.21-0.38)	0.18 (0.15-0.22)	<0.001[b]
PLR	100.15 ± 50.33	114.84 ± 30.94	0.135[a]
TBIL (μmol/L)	21.60 (14.15-36.45)	12.90 (10.30-15.90)	<0.001[b]
DBIL (μmol/L)	7.60 (2.95-15.95)	2.10 (1.70-2.70)	<0.001[b]
TP (g/L)	70.02 ± 8.79	72.88 ± 3.63	0.001[a]
ALB (g/L)	38.00 (35.40-41.25)	43.60(42.10-45.50)	<0.001[b]
GLB (g/L)	32.15 ± 7.31	29.03 ± 3.44	<0.001[a]
ALT (IU/L)	69.00 (35.50-98.50)	18.00 (13.00-20.00)	<0.001[b]
AST (U/L)	48.00 (33.50-98.50)	20.00 (16.00-23.00)	<0.001[b]
GGT (U/L)	115.00 (51-238.50)	18.00 (15.00-24.00)	<0.001[b]
ALP (U/L)	121.00 (85.00-182.00)	78.00 (66.00-87.00)	<0.001[b]
AGR	1.24 ± 0.33	1.53 ± 0.22	0.06[a]
AAR	0.98 ± 0.42	1.25 ± 0.31	0.042[a]

Abbreviations: AAR, AST-to-ALT ratio; AGR, ALB-to-GLB ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; GLB, globulin; HCT, hematocrit; HGB, hemoglobin; LMR, lymphocyte-to-monocyte ratio; MCV, mean corpuscular volume; MPV, mean platelet volume; NLR, neutrophil-to-lymphocyte ratio; PCT, plateletcrit; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; PLT, platelet; RBC, red blood cell; RDW-CV, red blood cell distribution width-coefficient of variation; RDW-CV/PLT, RDW-CV-to-PLT ratio; RDW-SD, red blood cell distribution width-standard deviation; RDW-SD/PLT, RDW-SD-to-PLT ratio; TBIL, total bilirubin; DBIL, direct bilirubin; TP, total protein; WBC, white blood cell.

[a]Student's *t* test.

[b]Mann-Whitney *U* test.

[c]χ^2 test.

TABLE 2 Demographic data and laboratory parameters of AIH patients with no/minimal liver fibrosis and advanced liver fibrosis

Variables	F0-2 (n = 23)	F3-4 (n = 22)	*P*
Age	51.09 ± 10.32	57.64 ± 11.13	0.047[a]
Female/male (n)	20/3	17/5	0.396[c]
WBC ($\times10^9$/L)	5.67 ± 1.71	5.12 ± 1.79	0.301[a]
Neutrophil ($\times10^9$/L)	2.91 ± 1.49	2.66 ± 1.20	0.541[a]
Lymphocyte ($\times10^9$/L)	1.96 ± 0.59	1.81 ± 0.70	0.451[a]
Monocyte ($\times10^9$/L)	0.50 ± 0.17	0.48 ± 0.17	0.752[a]
RBC ($\times10^{12}$/L)	4.42 ± 0.48	4.07 ± 0.60	0.032[a]
HGB (g/L)	131.65 ± 16.98	125.55 ± 15.65	0.217[a]
HCT (%)	39.24 ± 4.51	36.81 ± 4.62	0.082[a]
MCV (fL)	88.85 ± 6.50	91.81 ± 6.09	0.123[a]
RDW-CV (%)	14.06 ± 1.29	15.04 ± 1.57	0.028[a]
RDW-SD (fL)	45.39 ± 4.95	50.16 ± 6.54	0.008[a]
PLT ($\times10^9$/L)	196.70 ± 43.74	147.18 ± 59.81	0.030[a]
MPV (fL)	11.10 ± 1.11	11.51 ± 0.96	0.200[a]
PDW (fL)	13.36 ± 2.30	14.38 ± 2.42	0.167[a]
PCT (%)	0.22 ± 0.05	0.17 ± 0.07	0.008[a]
NLR	1.67 ± 1.29	1.61 ± 0.82	0.849[a]
LMR	4.41 ± 2.47	3.86 ± 1.19	0.344[a]
RDW-CV/PLT	0.07 (0.06-0.09)	0.11 (0.08-0.14)	0.002[b]
RDW-SD/PLT	0.25 ± 0.08	0.42 ± 0.25	0.001[a]
PLR	113.75 ± 60.54	85.93 ± 32.45	0.206[a]
TBIL (μmol/L)	18.30 (12.4-30.3)	25.00 (18.53-45.43)	0.071[b]
DBIL (μmol/L)	4.30 (2.50-12.80)	7.95 (3.30-22.85)	0.122[b]
TP (g/L)	71.67 ± 8.16	68.30 ± 9.28	0.201[a]
ALB (g/L)	39.75 ± 3.90	35.90 ± 4.76	0.005[a]
GLB (g/L)	31.92 ± 6.64	32.40 ± 8.11	0.831[a]
ALT (IU/L)	73.00 (41.00-128.00)	61.50 (30.25-117.50)	0.247[b]
AST (U/L)	48.00 (33.00-99.00)	49.00 (34.25-100.75)	0.919[b]
GGT (U/L)	118 (49-181.00)	111.00 (52.00-253.25)	0.982[b]
ALP (U/L)	127 (105-180)	117.00 (70.50-240.50)	0.251[b]
AGR	1.29 ± 0.28	1.19 ± 0.39	0.323[a]
AAR	0.83 ± 0.32	1.14 ± 0.44	0.011[a]
FIB-4	1.64 (1.05-2.650)	2.76 (2.22-4.90)	0.003[b]
APRI	0.67 (0.43-1.94)	1.31 (0.66-1.83)	0.140[b]
GPR	0.61 (0.28-1.58)	0.76 (0.39-2.40)	0.276[b]

Abbreviations: AAR, AST-to-ALT ratio; AGR, ALB-to-GLB ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, AST-to-PLT ratio index; AST, aspartate aminotransferase; DBIL, direct bilirubin; FIB-4, fibrosis index based on the four factors; GGT, gamma-glutamyltransferase; GLB, globulin; GPR, GGT-to-PLT ratio; HCT, hematocrit; HGB, hemoglobin; LMR, lymphocyte-to-monocyte ratio; MCV, mean corpuscular volume; MPV, mean platelet volume; NLR, neutrophil-to-lymphocyte ratio; PCT, plateletcrit; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; PLT, platelet; RBC, red blood cell; RDW-CV, red blood cell distribution width-coefficient of variation; RDW-CV/PLT, RDW-CV-to-PLT ratio; RDW-SD, red blood cell distribution width-standard deviation; RDW-SD/PLT, RDW-SD-to-PLT ratio; TBIL, total bilirubin; TP, total protein; WBC, white blood cell.

[a]Student's *t* test.

[b]Mann-Whitney *U* test.

[c]χ^2 test.

APRI = [(AST/ULN) × 100]/PLT(10^9/L), where ULN stands for upper limit of normal; FIB-4 = age(YEAR)×AST(U/L)/(PLT(10^9/L)×ALT(IU/L)1/2); and GPR = GGT(U/L)/PLT(10^9/L). All tests were performed strictly according to the manufacturers' protocols and the standard operating procedure (SOP) of the medical laboratory of Shanghai Ninth People's Hospital.

Liver biopsy guided by ultrasound under local anesthesia was performed in all patients using a 16-G disposable needle. A minimum of 1.0cm of liver specimen containing at least 10 portal tracts was required for diagnosis. The specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin and Masson's trichrome. All biopsy specimens were analyzed by an experienced pathologist who was blinded to the clinical data. Liver fibrosis stages were evaluated according to the METAVIR scoring system[7]: F0—no fibrosis; F1—portal fibrosis without septa; F2—portal fibrosis with few septa; F3—numerous septa and without cirrhosis; and F4—cirrhosis. The patients were divided into two groups based on the fibrosis stage: Patients with a grade of F0, F1, or F2 were classified as "no/minimal fibrosis" group, while patients with a grade of F3 or F4 were classified as "advanced fibrosis" group.

2.3 | Statistical analysis

Data were analyzed by IBM SPSS Statistics 20.0 (SPSS Inc). The Kolmogorov-Smirnov test was used to assess the normality and equality of measurement parameters. Data are expressed as mean ± SD for normally distributed variables and as media and range for non-normally distributed variables. Continuous variables were tested by Student's *t* test or Mann-Whitney *U* test, and categorical variables were tested by χ^2 test. The correlation between indexes and liver fibrosis was accessed by binary logistic regression analysis. The "Enter" method was used in univariate logistic regression analysis, and the variables that were statistically significant were entered into multivariate logistic regression analysis. Receiver operating characteristic (ROC) curves were generated to assess the diagnostic performance of each index for liver fibrosis. The cutoff values with both high sensitivity and high specificity were preferred. A two-sided $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Demographic data and laboratory parameters in AIH patients and healthy controls

The data of in vitro laboratory examination of AIH patients and healthy controls were analyzed and are shown in Table 1. The enrolled 45 AIH patients included 37 females (84.44%) and 8 males (15.56%), with a mean age of 54.29 ± 11.10 years, while 47 healthy controls included 39 females (82.98%) and eight males (17.02%), with a mean age of 54 ± 11.05 years. No significant difference in age and gender between AIH patients and healthy controls was found. Among these in vitro laboratory parameters, monocytes, MCV, RDW-CV, RDW-SD, NLR, RDW-CV/PLT, RDW-SD/PLT, TBIL, DBIL, GLB, ALT, AST, GGT, and ALP are significantly higher in AIH patients than in healthy controls, while WBC, neutrophils, lymphocytes, RBC, HGB, HCT, LMR, TP, ALB, and AAR were significantly lower in AIH patients than in healthy controls. Other parameters did not show significant difference between the two groups.

3.2 | Demographic data and laboratory parameters in the no/minimal liver fibrosis and advanced liver fibrosis groups in AIH patients

All 45 AIH patients were divided into no/minimal liver fibrosis group and advanced liver fibrosis group according to liver biopsy. There were 23 patients including 20 females (86.96%) and 3 (13.04%) males with a mean age of 51.09 ± 10.32 years in the no/minimal liver fibrosis group, while there were 17 (77.27%) females and 5 (22.73%) males with a mean age of 57.64 ± 11.13 years in the advanced liver fibrosis group. There was no significant difference in gender between the two groups, but the age of patients in the advanced liver fibrosis group was significantly higher than that in the no/minimal liver fibrosis group. Among these in vitro laboratory parameters, RDW-CV, RDW-SD, RDW-CV/PLT, RDW-SD/PLT, AAR, and FIB-4 in the advanced liver fibrosis group were significantly higher than in the no/minimal liver fibrosis group, while RBC, PLT, PCT, and ALB in the advanced liver fibrosis group were significantly lower than in the no/minimal liver fibrosis group, as shown in Table 2.

3.3 | Analysis of risk factors associated with AIH fibrosis

Binary logistic regression analysis was performed to investigate risk factors associated with advanced liver fibrosis in AIH patients. As shown in Table 3, among these in vitro laboratory parameters, hematological parameters HGB, HCT, RDW-SD, RDW-CV/PLT, RDW-SD/PLT, PLT, and PCT and liver function parameters ALB and FIB-4 were associated with liver fibrosis after univariate logistic regression analysis, but only RDW-SD/PLT was the independent risk factor to predict advanced fibrosis in AIH patients with an OR (95% CI) of 2.647 (1.383-5.170).

3.4 | Diagnostic performance of liver fibrosis risk factors

Receiver operating characteristic curves were adopted to evaluate the performance of indexes in identifying no/minimal fibrosis patients from advanced liver fibrosis patients, as shown in Figure 1. ROC analysis indicated that RDW-SD, RDW-CV/PLT, RDW-SD/PLT, AAR, and FIB-4 showed an excellent diagnostic value, with AUC (95% CI) of 0.716 (0.566-0.867), 0.773 (0.635-0.910), 0.785 (0.650-0.919), 0.709 (0.556-0.863), and 0.757 (0.614-0.900), respectively. The corresponding cutoff values were 44.350, 0.093, 0.239, 0.765, and 2.260, as shown in Table 4.

4 | DISCUSSION

AIH is a chronic progressive inflammatory liver disease with unspecific onset and heterogeneous clinical presentation. Liver fibrosis is one of the common complications during AIH progression, and even

TABLE 3 Univariate and multivariate analyses of the relationships between in vitro laboratory parameters and fibrosis in AIH patients

	Univariate analysis		Multivariate analysis	
Variables	**OR (95% CI)**	***P***	**Adjusted OR (95% CI)**	***P***
WBC ($\times10^9$/L)	0.816 (0.479-1.392)	0.456		
Neutrophil ($\times10^9$/L)	0.587 (0.187-1.850)	0.363		
Lymphocyte ($\times10^9$/L)	0.730 (0.425-1.254)	0.255		
Monocyte ($\times10^9$/L)	0.980 (0.573-1.678)	0.942		
RBC ($\times10^{12}$/L)	0.574 (0.325-1.016)	0.057		
HGB (g/L)	0.549 (0.308-0.979)	0.042		
HCT (%)	0.540 (0.306-0.955)	0.034		
MCV (fL)	1.511 (0.879-2.596)	0.135		
RDW-CV (%)	1.440 (0.824-2.517)	0.201		
RDW-SD (fL)	1.892 (1.057-3.387)	0.032		
PLT ($\times10^9$/L)	0.425 (0.222-0.813)	0.010		
MPV (fL)	1.308 (0.737-2.320)	0.359		
PDW (fL)	1.177 (0.690-2.009)	0.550		
PCT (%)	0.393 (0.203-0.763)	0.006		
NLR	1.018 (0.601-1.725)	0.948		
LMR	0.818 (0.480-1.394)	0.460		
RDW-CV/PLT	2.214 (1.157-4.238)	0.016		
RDW-SD/PLT	2.674 (1.383-5.170)	0.003	2.647 (1.383-5.170)	0.003
PLR	0.576 (0.326-1.017)	0.057		
TBIL (μmol/L)	1.598 (0.916-2.790)	0.099		
DBIL (μmol/L)	1.598 (0.916-2.790)	0.099		
TP (g/L)	0.721 (0.415-1.253)	0.246		
ALB (g/L)	0.527 (0.294-0.945)	0.031		
GLB (g/L)	1.239 (0.711-2.159)	0.449		
ALT (IU/L)	0.703 (0.408-1.212)	0.205		
AST (U/L)	1.055 (0.622-1.788)	0.844		
GGT (U/L)	1.018 (0.601-1.725)	0.948		
ALP (U/L)	0.759 (0.443-1.300)	0.315		
AGR	0.665 (0.380-1.165)	0.154		
AAR	1.700 (0.955-3.026)	0.071		
FIB-4	2.532 (1.329-4.823)	0.005		
APRI	1.476 (0.854-2.554)	0.163		
GPR	1.367 (0.796-2.347)	0.257		

Abbreviations: AAR, AST-to-ALT ratio; AGR, ALB-to-GLB ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, AST-to-PLT ratio index; AST, aspartate aminotransferase; DBIL, direct bilirubin; FIB-4, fibrosis index based on the four factors; GGT, gamma-glutamyltransferase; GLB, globulin; GPR, GGT-to-PLT ratio; HCT, hematocrit; HGB, hemoglobin; LMR, lymphocyte-to-monocyte ratio; MCV, mean corpuscular volume; MPV, mean platelet volume; NLR, neutrophil-to-lymphocyte ratio; PCT, plateletcrit; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; PLT, platelet; RBC, red blood cell; RDW-CV, red blood cell distribution width-coefficient of variation; RDW-CV/PLT, RDW-CV-to-PLT ratio; RDW-SD, red blood cell distribution width-standard deviation; RDW-SD/PLT, RDW-SD-to-PLT ratio; TBIL, total bilirubin; TP, total protein; WBC, white blood cell.

many patients were found with advanced fibrosis at the first time when diagnosed as AIH. Liver fibrosis is also crucial for the prognosis and treatment choice for AIH. Liver biopsy is the criterion standard for diagnosing liver fibrosis, but the clinical application of liver biopsy is limited because of the invasiveness, risk of complications, and sample errors. Therefore, it is necessary to involve other techniques

to monitor liver fibrosis practically and conveniently. Noninvasive indicators, acquired through routine in vitro laboratory test, showed great potential in monitoring liver fibrosis or cirrhosis during clinical practice, especially in chronic viral hepatitis.[8] However, few noninvasive indicators have been reported in the evaluation of liver fibrosis in AIH patients at present.

In this study, we carried out the complete blood count and liver function tests of AIH patients and healthy controls. Complete blood count is a widely used and easily acquired laboratory test in clinical practice. It comprises WBC, RBC, HGB, PLT, and their morphological indexes, such as MCV and RDW. White blood cell count, 5-part differential (neutrophil, monocyte, lymphocyte, eosinophil, and basophil), and derived indexes such as NLR and LMR are well-known markers of infection and inflammation.[9,10] A study from Yang Z found that NLR and LMR were significantly increased in systemic autoimmune rheumatic diseases (SARDs), compared with healthy individuals. Furthermore, NLR and LMR may be useful tools to reflect inflammatory status of SARDs.[11] Another study reported by Huang Y showed that NLR and LMR were significantly increased in patients with Guillain-Barré syndrome (GBS) and closely relevant to clinical pathophysiological status.[12] In this study, we found that WBC, neutrophils, lymphocytes, and LMR are significantly lower, while NLR and monocytes are remarkably higher in AIH patients compared with healthy controls, consistent with the previous study reported by Zeng T, et al[13] The reduction in peripheral neutrophils and lymphocytes in AIH patients may have resulted from the exhaustion or migration from blood to the liver,[14] while increased NLR indicated that AIH patients lost more lymphocytes than neutrophils in their peripheral blood. On the contrary, the amount of monocytes increased in AIH patients, which may be caused by continuous inflammation and mobilization of monocytes from the bone marrow to the peripheral blood.[15] The relationship between NLR and fibrosis was controversial in previous studies on whether NLR could be a new marker for predicting fibrosis in patients with nonalcoholic fatty liver disease.[16] In this study, neither NLR nor LMR showed any significant difference between the no/minimal liver fibrosis group and the advanced liver fibrosis group, indicating that NLR or LMR could not be a predictor of fibrosis in all kinds of liver diseases under different pathogenesis backgrounds.

We also found that MCV, RDW-CV, RDW-SD, RDW-CV/PLT, and RDW-SD/PLT are significantly higher, while RBC, HGB, and HCT are remarkably lower in AIH patients compared with healthy controls.

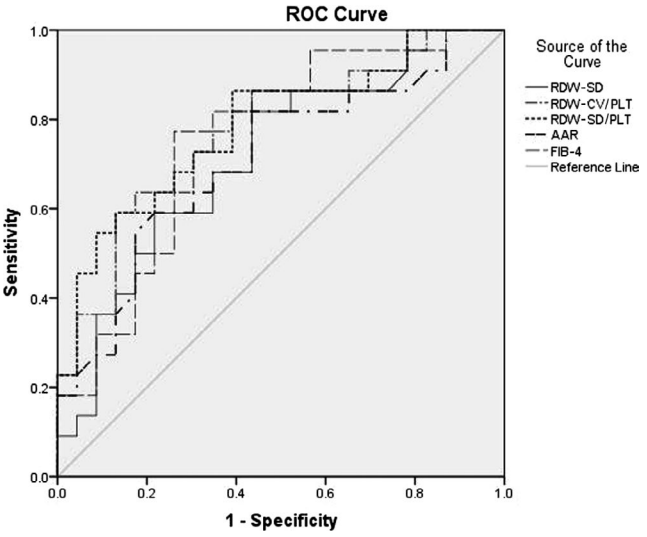

FIGURE 1 Receiver operating characteristic curves of in vitro laboratory parameters for advanced liver fibrosis in AIH patients

Furthermore, RDW-CV, RDW-SD, RDW-CV/PLT, and RDW-SD/PLT are significantly increased, while RBC, PLT, and PCT are remarkably decreased in the advanced liver fibrosis group compared with the no/minimal liver fibrosis group. Anemia (low hemoglobin) has been well known to be associated with an increased risk of mortality, and hemolytic anemia is commonly present in chronic liver disease, particularly cirrhosis.[17,18] MCV reflects the volume of red blood cells, and RDW reflects the size variability of erythrocytes. In some measuring platforms, rather than RDW, coefficient of variation of the red blood cell distribution width (RDW-CV) and standard deviation of the red blood cell distribution width (RDW-SD) were used to describe red blood cell width distribution. AIH patients, especially patients with advanced fibrosis, were more susceptible to hemolytic anemia. Increasing RDW-CV/RDW-SD in AIH patients may be caused by several reasons: Proinflammatory cytokines suppress the maturation of erythrocytes and accelerate the rebirth of large reticulocytes into peripheral blood circulation, resulting in increased anisocytosis; in addition, portal hypertension leads to hypersplenism followed by erythroclasis, RBC distortion, and hemolytic anemia; moreover, AIH patients often suffer from reduplicative hypohepatia and secondary malnutrition, leading to lack of iron and other hematopoietic materials, so that many immature erythrocytes entry into peripheral circulation. The half-life of red blood cells is relatively longer than many

TABLE 4 Diagnostic accuracy of different indexes for prediction of liver fibrosis in AIH patients

Variables	Optimized cutoff	Sensitivity (%)	Specificity (%)	AUC (95% CI)	*P* value
RDW-SD (fL)	44.350	81.81	56.52	0.716 (0.566-0.867)	0.013
RDW-CV/PLT	0.093	63.64	82.61	0.773 (0.635-0.910)	0.002
RDW-SD/PLT	0.239	86.36	60.87	0.785 (0.650-0.919)	0.001
AAR	0.765	81.81	56.52	0.709 (0.556-0.863)	0.016
FIB-4	2.260	77.27	73.92	0.757 (0.614-0.9000)	0.003

Abbreviations: AAR, AST-to-ALT ratio; FIB-4, fibrosis index based on the four factors; PLR, platelet-to-lymphocyte ratio; RDW-CV/PLT, RDW-CV-to-PLT ratio; RDW-SD, red blood cell distribution width-standard deviation; RDW-SD/PLT, RDW-SD-to-PLT ratio.

other indexes, such as bilirubin and albumin; therefore, RDW-CV/RDW-SD represents a relatively stable index. Consistent with many previous studies that reported that low PLT counts were associated with advanced liver fibrosis,[19-22] we found decreased PLT and PCT in the advanced fibrosis group in AIH patients. The decreased PLT may be caused by hypersplenism and the reduced thrombopoietin production as a result of excessive damaged liver cells in advanced fibrosis patients.[23,24]

As expected, higher serum TBIL, DBIL, ALT, AST, ALP, and GGT levels were found in AIH patients compared with healthy controls, consistent with other kinds of hepatitis.[13,25] Protein synthesis is an important function of the liver. Protein synthesis ability declined along with hepatocytes damaging,[26] resulting in decreased serum levels of TP and ALB. While increased GLB was attributed to excessive autoantibodies produced in AIH patients.[27] Interestingly, although slightly elevated TBIL and DBIL and decreased ALT, ALP, and GGT were found in the advanced fibrosis group, which was well known as "biliary enzyme separation",[28] no significant differences were found between the no/minimal liver fibrosis group and the advanced fibrosis group in these parameters including TBIL, DBIL, ALT, AST, ALP, GGT, TP, ALB, and GLB, indicating that these liver enzymes could not predict liver fibrosis independently. We also evaluated the combined parameters such as AAR, APRI, and FIB-4, which were previously used to identify the presence of liver fibrosis and the severity of fibrosis in chronic hepatic C patients.[29-33] In our study, we found that AAR and FIB-4 were superior to APRI in distinguishing advanced liver fibrosis from no/minimal liver fibrosis in AIH patients. These results are consistent with the previous finding focused on AIH patients.[13] However, a study by Abdollahi M[34] concluded that FIB-4 and APRI were superior to AAR at distinguishing severe fibrosis from mild-to-moderate fibrosis in chronic hepatitis C patients. Moreover, a previous study focused on hepatitis B and C found that there was no significant relationship between the degree of liver fibrosis and the AAR score.[35] Therefore, we need different specific biomarkers to identify fibrosis stages in hepatitis with different pathogeny.

In light of the present study, we found that RDW-SD, RDW-CV/PLT, RDW-SD/PLT, AAR, and FIB-4 showed a valuable performance to identify advanced liver fibrosis after a ROC analysis, while only RDW-SD/PLT is an independent risk factor to predict advanced liver fibrosis through a multivariate logistic regression analysis. RDW-CV is calculated from the erythrocyte volume distribution histogram and represents the coefficient of variation of erythrocyte volume around mean corpuscular volume (MCV), while RDW-SD is calculated from the width of erythrocyte volume distribution curve at a level 20% above baseline and is expressed in femtoliters (fL).[36] A study reported by Robbins CS[37] indicated that RDW-CV had higher sensitivity and efficiency than RDW-SD when evaluating anisocytosis in microcytic MCV ranges. However, in normocytic and macrocytic MCV ranges, RDW-SD presented better performance than RDW-CV in evaluating anisocytosis. AIH patients had an elevated MCV (Table 1), and our study showed that RDW-SD was superior to RDW-CV in identifying advanced liver fibrosis. This is concordant with the result of Wang J's study, which showed that RDW-SD rather than RDW-CV was one of the independent predictors of advanced fibrosis in patients with chronic hepatitis B.[38] Consistent with our study, Taefi A[39] showed that the RDW-to-platelet ratio can strongly predict the degree of fibrosis or cirrhosis in patients with chronic hepatitis such as chronic hepatitis B, chronic hepatitis C, alcoholic hepatitis, and primary biliary cirrhosis.

In conclusion, our study demonstrates for the first time that RDW-SD/PLT is an independent risk factor to predict advanced liver fibrosis in AIH patients. RDW-SD/PLT is a noninvasive indicator that could be easily obtained from hematological complete blood count, which is routinely tested for AIH patients in clinical laboratory. Therefore, RDW-SD/PLT could serve as a routinely used reference indicator to monitor liver fibrosis in all AIH patients.

ACKNOWLEDGMENTS

The authors would like to thank the staffs of Clinical Laboratory Department of the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, for performing routine laboratory hematological and biochemistry test. We are also grateful to Professor Hu Xiqi, Department of Pathology, Fudan University, for performing pathological evaluation.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

1. European Association for the Study of the Liver. EASL Clinical practice guidelines: autoimmune hepatitis. *J Hepatol*. 2015;63(6):1543-1544.
2. Manns MP, Czaja AJ, Gorham JD, et al. Diagnosis and management of autoimmune hepatitis. *Hepatology*. 2010;51(6):2193-2213.
3. Chen B, Ye B, Zhang J, Ying L, Chen Y. RDW to platelet ratio: a novel noninvasive index for predicting hepatic fibrosis and cirrhosis in chronic hepatitis B. *PLoS ONE*. 2013;8(7):e68780.
4. Kekilli M, Tanoglu A, Sakin YS, Kurt M, Ocal S, Baqci S. Is the neutrophil to lymphocyte ratio associated with liver fibrosis in patients with chronic hepatitis B? *World J Gastroenterol*. 2015;21(18):5575-5581.
5. Yen Y-H, Kuo F-Y, Kee K-M, et al. APRI and FIB-4 in the evaluation of liver fibrosis in chronic hepatitis C patients stratified by AST level. *PLoS ONE*. 2018;13(6):e0199760.
6. Alvarez F, Berg PA, Bianchi FB, et al. International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol*. 1999;31(5):929-938.
7. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. *Hepatology*. 1996;24(2):289-293.

8. Li Y, Cai Q, Zhang Y, et al. Development of algorithms based on serum markers and transient elastography for detecting significant fibrosis and cirrhosis in chronic hepatitis B patients: significant reduction in liver biopsy. *Hepatol Res*. 2016;46(13):1367-1379.
9. Hu Z-D, Sun YI, Guo J, et al. Red blood cell distribution width and neutrophil/lymphocyte ratio are positively correlated with disease activity in primary Sjögren's syndrome. *Clin Biochem*. 2014;47(18):287-290.
10. Kurtul A, Yarlioglues M, Celik IE, et al. Association of lymphocyte-to-monocyte ratio with the no-reflow phenomenon in patients who underwent a primary percutaneous coronary intervention for ST-elevation myocardial infarction. *Coron Artery Dis*. 2015;26(8):706-712.
11. Yang Z, Zhang Z, Lin F, et al. Comparisons of neutrophil-, monocyte-, eosinophil-, and basophil-lymphocyte ratios among various systemic autoimmune rheumatic diseases. *APMIS*. 2017;125(10):863-871.
12. Huang Y, Ying Z, Quan W, et al. The clinical significance of neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio in Guillain-Barré syndrome. *Int J Neurosci*. 2018;128(8):729-735.
13. Zeng T, Yu J, Tan L, et al. Noninvasive indices for monitoring disease course in Chinese patients with autoimmune hepatitis. *Clin Chim Acta*. 2018;486:135-141.
14. Miao QI, Bian Z, Tang R, et al. Emperipolesis mediated by cd8 t cells is a characteristic histopathologic feature of autoimmune hepatitis. *Clin Rev Allergy Immunol*. 2015;48(2–3):226-235.
15. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol*. 2011;11(11):762-774.
16. Kara M, Dogru T, Genc H, et al. Neutrophil-to-lymphocyte ratio is not a predictor of liver histology in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. 2015;27(10):1144-1148.
17. Duclos B, Bories P, Mirouze D, Navarro M, Michel H. Anemia caused by acanthocytosis in the cirrhotic patient: diagnosis and prognostic significance. 3 cases. *Ann Med interne (Paris)*. 1983;134(7):643-646.
18. McHutchison JG, Manns MP, Longo DL. Definition and management of anemia in patients infected with hepatitis C virus. *Liver Int*. 2006;26(4):389-398.
19. Ekiz F, Yüksel O, Koçak E, et al. Mean platelet volume as a fibrosis marker in patients with chronic hepatitis B. *J Clin Lab Anal*. 2011;25(3):162-165.
20. Hui AY, Chan H-Y, Wong V-S, et al. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. *Am J Gastroenterol*. 2005;100(3):616-623.
21. Zeng M-D, Lu L-G, Mao Y-M, et al. Prediction of significant fibrosis in HBeAg-positive patients with chronic hepatitis B by a noninvasive model. *Hepatology*. 2005;42(6):1437-1445.
22. Pan JJ, Yang CF, Chu CJ, Chang FY, Lee SD. Prediction of liver fibrosis in patients with chronic hepatitis B by serum markers. *Hepatogastroenterology*. 2007;54(77):1503-1506.
23. Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. *J Clin Invest*. 1966;45(5):645-657.
24. Kawasaki T, Takeshita A, Souda K, et al. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis. *Am J Gastroenterol*. 1999;94(7):1918-1922.
25. Ozer J, Ratner M, Shaw M, Bailey W, Schomarker S. The current state of serum biomarkers of hepatotoxicity. *Toxicology*. 2008;24(3)5:194-205.
26. Zhu JY, Han Y. Autoimmune hepatitis: unveiling faces. *J Dig Dis*. 2015;16(9):483-488.
27. Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: current diagnostic and clinical challenges. *World J Gastroenterol*. 2008;14(21):3374-3387.
28. Li WG. Patients with chronic severe hepatitis and serum biochemical indicators of prognosis. *Modern Preventive Medicine*. 2011;38(18):3757-3758.
29. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38(2):518-526.
30. Giannini E, Risso D, Botta F, et al. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. *Arch Intern Med*. 2003;163(2):218-224.
31. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. *Hepatology*. 2007;46(1):32-36.
32. Ohta T, Sakaguchi K, Fujiwara A, et al. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. *Acta Med Okayama*. 2006;60(2):77-84.
33. Cross TJ, Rizzi P, Berry PA, Bruce M, Portmann B, Harrison PM. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol*. 2009;21(7):730-738.
34. Abdollahi M, Pouri A, Ghojazadeh M, Estakhri R, Somi M. Non-invasive serum fibrosis markers: a study in chronic hepatitis. *Bioimpacts*. 2015;5(1):17-23.
35. Eminler AT, Ayyildiz T, Irak K, et al. AST/ALT ratio is not useful in predicting the degree of fibrosis in chronic viral hepatitis patients. *Eur J Gastroenterol Hepatol*. 2015;27(12):1361-1366.
36. Walters JG, Garrity PF. RDW-SD and RDW-CV: their relationship to RBC distribution curves and anisocytosis. *Sysmex J Int*. 1993;3:40-45.
37. Robbins CS. Horse impacts: research findings and their implications. *J Bras Patol Med Lab*. 1993;49(5):324-331.
38. Wang J, Yan X, Yang Y, et al. A novel predictive model using routinely clinical parameters to predict liver fibrosis in patients with chronic hepatitis B. *Oncotarget*. 2017;8(35):59257-59267.
39. Taefi A, Huang CC, Kolli K, Ebrahimi S, Patel M. Red cell distribution width to platelet ratio, a useful indicator of liver fibrosis in chronic hepatitis patients. *Hepatol Int*. 2015;9(3):454-460.

How to cite this article: Liu L, Cao J, Zhong Z, et al. Noninvasive indicators predict advanced liver fibrosis in autoimmune hepatitis patients. *J Clin Lab Anal*. 2019;33:e22922. https://doi.org/10.1002/jcla.22922