Subclinical but significant liver fibrosis in patients with ANCA-associated vasculitis

S.-W. Lee[1,2], D.Y. Kim[3,4], S.H. Ahn[3,4], Y.-B. Park[1,2], K.-H. Han[3,4], J.Y. Park[3,4]

[1]Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul; [2]Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul; [3]Division of Gastroenterology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul; [4]Yonsei Liver Center, Severance Hospital, Seoul, Republic of Korea.

Sang-Won Lee, MD, PhD
Do Young Kim, MD, PhD
Sang Hoon Ahn, MD, PhD
Yong-Beom Park, MD, PhD
Kwang-Hyub Han, MD, PhD
Jun Yong Park, MD, PhD

Please address correspondence to:
Dr Jun Yong Park,
Yonsei Liver Center,
Severance Hospital,
50-1 Yonsei-ro, Seodaemun-gu,
Seoul 03722, South Korea.
E-mail: DRPJY@yuhs.ac

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ABSTRACT

Objective. *We evaluated the laboratory and radiological data on liver and investigate liver fibrosis induced by hepatic manifestation of antineutrophil cytoplasmic antibody-associated vasculitis (AAV) using the aspartate aminotransferase to platelet ratio index (APRI) and an index of fibrosis (FIB-4) in 136 immunosuppressive drug-naïve patients.*

Methods. *We retrospectively reviewed the medical records of 136 patients with AAV without chronic liver diseases or autoimmune diseases. We collected the laboratory and imaging results. We assessed liver fibrosis by APRI and FIB-4. The critical cut-offs of APRI and FIB-4 for predicting liver fibrosis are 0.5 and 1.45. The optimal cut-off of five factor score (FFS) at diagnosis for FIB-4 ≥1.45 was extrapolated by the area under the receiver operator characteristic curve.*

Results. *The mean age at diagnosis was 54.6 years and 32.4% of patients were male (69 MPA, 38 GPA and 29 EGPA). The percentage of patients having the normal results of liver function tests was ranging from 86.0% to 95.6%. There were no patients who exhibited the significantly abnormal findings on imaging studies. Nonetheless, twenty-nine patients with AAV (21.3%) exhibited subclinical but significant liver fibrosis at diagnosis based on FIB-4. Patients with FFS ≥1 had a significantly higher risk of having subclinical but significant liver fibrosis (FIB-4 ≥1.45) than those with FFS <1 (RR 12.486).*

Conclusion. *AAV may increase the results of liver function tests and it may provoke subclinical but significant liver fibrosis at diagnosis. Furthermore, liver fibrosis should be considered in AAV patients having FFS ≥1.*

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of systemic vasculitis involving small vessels from capillaries to intraparenchymal arterioles and venules. AAV is composed of three variants including microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA) (1). Despite the pivotal role of ANCA to initiate pathogenic autoimmunity, the clinical utility of serially measuring ANCA titres is still controversial (2). AAV usually affects almost all the major organs leading to various symptoms and signs as described in Birmingham vasculitis activity score (BVAS) and BVAS for GPA (3, 4). However, hepatic manifestation of AAV has not been emphasised to date unlike other major organs, and furthermore hepatic manifestation is not included in BVAS (3). So far, only a few studies have reported the abnormal results of liver function tests. A previous study reported that liver enzyme levels were elevated in 2~25% of Italian patients with AAV at diagnosis (5), and another recent study demonstrated that 49% of German patients with AAV had abnormal results of liver function tests and GPA patients exhibited liver involvement of AAV more frequently than other variants during the active status (6). Nevertheless, in the real clinical settings, cases exhibiting the serious abnormal results of liver function tests and imaging studies as the initial manifestation of AAV are not common.

On the other hands, given that liver is one of the organs with plenty of capillaries, like lungs and kidneys, AAV may occur in liver more frequently than is expected. However, hepatic manifestation of AAV might have been underestimated due to less significant symptoms than those in other major organs, or ignored by concerns on invasive liver biopsy (7, 8). Theoretically, it can be assumed that autoimmune-related inflammation of AAV can induce the hepatocellular damages, the

expansion of myofibroblast and the activation of stellate cells (9). A series of these responses, in turn, can enhance the potential of liver fibrosis in patients with AAV similar to autoimmune hepatitis and primary biliary cirrhosis (10). Meanwhile, authors recently demonstrated subclinical but significant liver fibrosis in up to 15% of patients with rheumatic diseases, in whom the results of liver function tests and imaging studies were all normal (11-13). Therefore, it must be valuable to first evaluate liver fibrosis in AAV patients. But, due to the limitation of a retrospective study, liver biopsy or transient elastography cannot be performed in AAV patients, to whom immunosuppressive drugs were not administered. Instead, liver fibrosis can be estimated using non-invasive evaluation-equations calculated by the results of liver function tests at diagnosis of AAV, which may compensate for that limitation.

Among various indices for predicting liver fibrosis, the aspartate aminotransferase to platelet ratio index (APRI) and an index of fibrosis (FIB-4) are widely used (14). First, APRI has been introduced as an available method to assess liver fibrosis in chronic hepatitis C and validated in non-alcoholic fatty liver disease and autoimmune hepatitis. APRI is calculated using AST, the upper limit of AST and platelet count. Because APRI <0.5 may rule out significant fibrosis and cirrhosis, the critical cut-off of APRI for predicting moderate fibrosis (S2) to cirrhosis (S4) is set as 0.5 (15). Second, an index of fibrosis (FIB-4) has been also proposed to assess liver fibrosis in HCV-monoinfected patients. FIB-4 is calculated using age, ALT, AST and platelet count. Because FIB-4 <1.45 had a negative predictive value of 94.7% to exclude severe fibrosis with sensitivity of 0.74, the critical cut-off of FIB-4 for predicting significant liver fibrosis (S2 to S4) is set as 1.45 (16). So far, to our best knowledge, there was no report regarding liver fibrosis assessed by APRI and FIB-4 in AAV patients. Hence, in this study, we evaluated the results of liver function tests and the findings of imaging studies, and investigate liver fibrosis induced by hepatic manifestation of AAV using APRI and FIB-4 in 136 immunosuppressive drug-naïve patients at diagnosis.

Patients and methods

Patients

We retrospectively reviewed the medical records of 160 patients with AAV based on the inclusion criteria as follows: i) patients who were first classified as AAV from October 2000 to September 2017 at Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Severance hospital; ii) patients who fulfilled the American College of Rheumatology 1990 criteria for the classification of GPA and EGPA and then reclassified by the algorithm suggested by the European Medicines Agency in 2007, in which authors added the modified contents of the Chapel Hill Consensus Conferences (CHCC) Nomenclature of Vasculitis proposed in 2012 (1, 17-19); iii) patients who had well-documented medical records to assess clinical manifestations at diagnosis and calculate vasculitis activity score represented by BVAS or BVAS for GPA and prognostic factors identified by five factor score (FFS (2009)) at diagnosis (3, 4, 20); iv) patients who had the results of perinuclear (P)-ANCA and cytoplasmic (C)-ANCA or myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA at diagnosis (21); v) patients who had no history of chronic liver diseases, such as viral hepatitis or alcoholic liver diseases identified in the 10th revised International Classification Diseases (ICD-10); vi) patients who had never received both immunosuppressive drugs for AAV or drugs for chronic liver diseases under the Korean Drug Utilisation Review (DUR) system; vii) patients who had no history of autoimmune diseases including autoimmune hepatitis or administration of immunosuppressive drugs for them, which can affect liver function and structure (22); viii) patients who did not suffer from excessive alcohol intake or serious obesity (body mass index >35) (23); ix) patients who had the results of liver function tests at diagnosis including variables of equations to estimate liver fibrosis; x) patients who might have the results of imaging studies on liver including ultrasonography or computed tomography at diagnosis (not obligatory).

Among 160 patients with AAV, nine patients were excluded due to HBsAg positive (n=4), anti-HCV positive (n=1), alcoholic hepatitis (n=3) and non-alcoholic steatohepatitis (n=1). Of these 151 patients with AAV without chronic liver diseases, 15 patients were further excluded due to concomitant autoimmune diseases including rheumatoid arthritis (n=6), Sjogen syndrome (n=4), systemic sclerosis (n=2), ankylosing spondylitis (n=2) and systemic lupus erythematosus (n=1). Finally, 136 patients with AAV were included in this study and selected for statistical analysis (Fig. 1). This study was approved by the institutional Review Board (IRB) of Severance Hospital (4-2017-0673), and the patient's written informed consent was waived by the approving IRB, as this was a retrospective study.

Clinical and laboratory data and radiological evaluation

We obtained age at diagnosis and gender as demographic data. We searched the initial ANCAs: P-ANCA and C-ANCA were evaluated by immunofluorescence assay. MPO-ANCA and PR3-ANCA had been measured by Enzyme-Linked immunosorbent assay (ELISA) kit for anti-PR3 and anti-MPO (Inova Diagnostics, San Diego, USA) before 2013, and by the novel anchor coated highly sensitive (hs) Phadia ELiA (Thermo Fisher Scientific/Phadia, Freiburg, Germany) using human native antigens, performed on a Phadia250 analyser after 2013. Based on the medical records, we refilled the form of vasculitis activity score for AAV and calculated BVAS (representing both BVAS and BVAS for GPA) as well as FFS (2009) at diagnosis (3, 4, 20).

We collected laboratory results at diagnosis including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), while blood cell count, haemoglobin, platelet count, prothrombin time, fasting glucose, blood urea nitrogen, creatinine, serum albumin, alkaline phosphatase (ALP), aspartate

transaminase (AST), alanine transaminase (ALT), total bilirubin and total cholesterol. In addition, the normal ranges of liver function tests were set as the follows: platelet ≥150,000/mm^3, prothrombin time ≤1.16 international normalised ratio (INR), alkaline phosphatase (ALP) ≤115 IU/L, aspartate aminotransferase (AST) ≤40 IU/L, alanine aminotransferase (ALT) ≤40 IU/L, total bilirubin ≤1.2 mg/dL.

We counted the number of patients having the results of liver function tests within normal ranges.

Ninety-seven patients with AAV underwent ultrasonography and computed tomography scan at diagnosis of AAV with reasons as follows: i) for abnormal results of liver function tests; ii) for abdominal manifestations other than hepatic manifestations and iii) for baseline study (Fig. 1). We reviewed the radiological findings of imaging studies and counted the number of cases with abnormal findings.

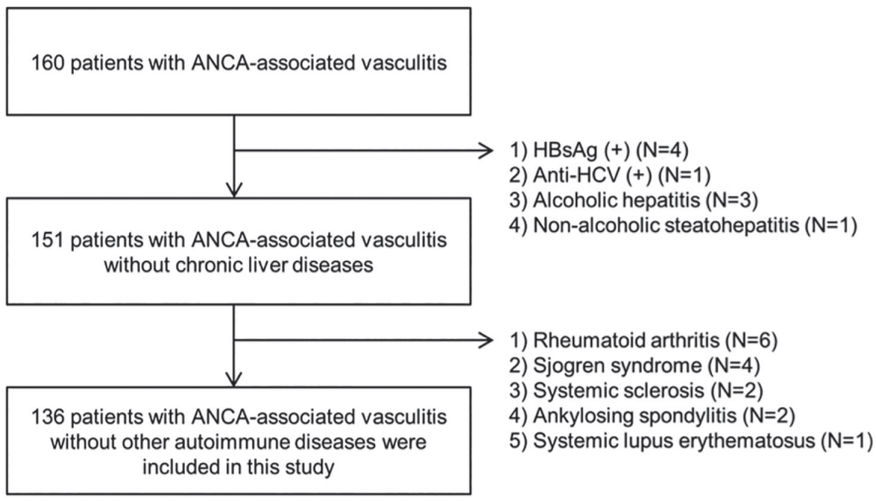

Fig. 1. Selection of the study population. ANCA: antineutrophil cytoplasmic antibody; HBsAg: hepatitis B viral surface antigen; HCV: hepatitis C virus.

Equations to predict significant liver fibrosis

To predict subclinical but significant liver fibrosis in AAV patients, we used two widely used equations as below: i) APRI = AST level (IU/L) / AST upper limit of normal range (IU/L) / platelet count (10^9/L); ii) Fibrosis-4 (FIB-4) = age (years) x AST level (IU/L) / platelet count (10^9/L) / √ALT (IU/L). The critical cut-offs of APRI and FIB-4 to predict liver fibrosis ≥S2 are 0.5 and 1.45 (24).

Statistical analyses

All statistical analyses were conducted using SPSS software (v. 23 for windows; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as number and the percentage. Differences in the results of liver function tests among three AAV variants were investigated by the ANOVA test. Significant differences in BVAS and FFS at diagnosis between the two groups according to the cut-offs of APRI or FIB-4 were evaluated by the Mann-Whitney test. The optimal cut-off of FFS for predicting FIB-4 ≥1.45 was extrapolated by calculating the area under the receiver operator characteristic curve (AUROC) and selecting one with the maximised sum of sensitivity and specificity. In addition, the relative risk (RR) was analysed using contingency tables with the chi square test. *p*-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics of 136 patients with AAV

The baseline characteristics were described in Table I. The mean age at diagnosis was 54.6 years and 44 patients (32.4%) were male. Of 136 patients with AAV, 69 patients were classified as MPA, 38 as GPA and 29 as EGPA. Eighty-two patients (60.3%) had MPO-ANCA (or P-ANCA) and 24 (17.6%) had PR3-ANCA (or C-ANCA). Five patients (3.7%) had both MPO-ANCA (or P-ANCA) and PR3-ANcA (or C-ANCA), and 33 (24.3%) had no ANCA. The mean BVAS at diagnosis was 12.3 and the mean FFS (2009) at diagnosis was 1.2. In terms of the results of liver function tests at diagnosis, the mean platelet count (334, 700.0/mm3), prothrombin time (INR) (1.0), ALT (89.5 IU/L), AST (22.9 IU/L), ALT (22.7 IU/L) and total bilirubin (0.6 mg/dL) were all in the normal ranges.

Radiological features of liver in 97 patients with AAV

All patients with the abnormal results of liver function tests underwent imaging studies. There were no patients who exhibited serious chronic liver diseases on imaging studies. Seventy-eight of 97 patients (80.4%) exhibited normal features of liver structures. Nine patients (9.3%) exhibited fatty liver, 5 patients exhibited hepatic cysts and 4 patients exhibited haemangioma. Liver abscess was incidentally found in one patient who was admitted for high fever and abdominal pain at the same time of diagnosis of AAV.

Subclinical but significant liver fibrosis

Based on the results of liver function tests and imaging studies, we found that the functional abnormalities and structural damages of liver were not apparent at diagnosis of AAV. However, because we did not perform invasive liver biopsy or non-invasive liver stiffness measurement using transient elastography (Fibroscan®) in patients at diagnosis of AAV, we cannot lead to definitive conclusion that hepatic manifestation including liver fibrosis may be rare at the first visit. Instead, we estimated liver fibrosis by APRI and FIB-4. In terms of APRI, the critical cut-off for significantly liver fibrosis (S2-S4) is currently recommended as 0.5 (sensitivity 0.81 and specificity 0.55). When we divided 136 patients with AAV into the two groups according to the critical cut-off of APRI, only three of 136 patients (2.2%) exhibited APRI ≥0.5 (Fig. 2). In terms of FIB-4,

Table I. Baseline characteristics of 136 patients with AAV.

Variables	Values
Demographic data at diagnosis	
Age (year old)	54.6 ± 15.8
Male gender (n, (%))	44 (32.4)
Variants of AAV	
MPA	69 (50.7)
GPA	38 (27.9)
EGPA	29 (21.3)
ANCA at diagnosis (n, (%))	
MPO-ANCA (or P-ANCA)	82 (60.3)
PR3-ANCA (or C-ANCA)	24 (17.6)
MPO-ANCA (or P-ANCA) and PR3-ANCA (or C-ANCA)	5 (3.7)
ANCA negative	33 (24.3)
Vasculitis activity and prognostic factors at diagnosis	
BVAS or BVAS for GPA	12.3 ± 7.7
FFS (2009)	1.2 ± 1.0
Acute reactants at diagnosis	
Erythrocyte sedimentation rate (mm/hr)	62.6 ± 37.2
C-reactive protein (mg/L)	47.4 ± 58.4
Laboratory results at diagnosis	
White blood cell count (/mm^3)	10,394.4 ± 5,077.9
Haemoglobin (g/dL)	11.1 ± 2.3
Platelet count (x1,000/mm^3)	334.7 ± 152.1
Prothrombin time (INR)	1.0 ± 0.2
Fasting glucose (mg/dL)	119.2 ± 46.9
Blood urea nitrogen (mg/dL)	25.7 ± 24.6
Creatinine (mg/dL)	1.9 ± 2.1
Total protein (g/dL)	6.7 ± 0.9
Serum albumin (g/dL)	3.6 ± 0.8
Alkaline phosphatase (IU/L)	89.5 ± 78.5
Aspartate transaminase (IU/L)	22.9 ± 16.7
Alanine transaminase (IU/L)	22.7 ± 29.9
Total bilirubin (mg/dL)	0.6 ± 0.3
Total cholesterol (mg/dL)	167.9 ± 4.0
Proportion of patients with normal results of liver function tests (n, (%))	
Platelet count ≥150,0000/mm^3	130 (95.6)
Prothrombin time (INR) ≤1.16	117 (86.0)
Alkaline phosphatase (IU/L) ≤115 IU/L	117 (86.0)
Aspartate transaminase (IU/L) ≤40 IU/L	126 (92.6)
Alanine transaminase (IU/L) ≤40 IU/L	123 (90.4)
Total bilirubin (mg/dL) ≤1.2 mg/dL	130 (95.6)

Values are expressed as mean and standard deviation or n (%).
AAV: antineutrophil associated vasculitis; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; P-ANCA: perinuclear ANCA; PR3: proteinase 3; C-ANCA: cytoplasmic ANCA; BVAS: Birmingham vasculitis activity score; FFS: five factor score; INR: international normalised ratio.

the critical cut-off for significant liver fibrosis (S2-S4) is currently recommended as 1.45 (sensitivity 0.64 and specificity 0.68). When we divided 136 patients with AAV into the two groups based on the critical cut-off of FIB-4, twenty-nine of 136 patients (21.3%) exhibited FIB-4 ≥1.45 (Fig. 2). In addition, we compared the levels APRI and FIB-4 among MPO-ANCA (or P-ANCA), PR3-ANCA (or C-ANCA), both ANCAs and ANCA negative by One-Way-Anova analysis. We found no statistically significant differences in APRI (p=0.385) and FIB-4 (p=0.075) according to ANCA subgroups.

The association of BVAS or FFS with subclinical but significant liver fibrosis at diagnosis

We do not routinely calculate FIB-4 or perform transient elastography in AAV patients at diagnosis, unless they do not exhibit the significantly abnormal results of liver function tests. Meanwhile, we do routinely assess BVAS and FFS at diagnosis of AAV. Thus, if we find the optimal cut-off of BVAS or FFS for APRI ≥0.5 or FIB-4 ≥1.45 at diagnosis, we could recommend diagnostic tests for liver fibrosis to patients having more than the cut-off.

We compared both BVAS and FFS at diagnosis between patients with APRI ≥0.5 and those with APRI <0.5, but there were no significant differences between the two groups (13.7 *vs*. 12.2 for BVAS, p=0.752 and 1.3 *vs*. 1.2 for FFS, p=0.861) (Fig. 2). We also compared both BVAS and FFS at diagnosis between patients with FIB-4 ≥1.45 and those with FIB-4 <1.45. The mean BVAS at diagnosis did not differ between the two groups (13.6 *vs*. 11.9, p=0.313). However, patients with FIB-4 ≥1.45 had the significantly higher mean FFS at diagnosis than those with FIB-4 <1.45 (1.7 *vs*. 1.1, p=0.002) (Fig. 2).

The optimal cut-off of FFS in predicting FIB-4 ≥1.45

Since the mean FFS at diagnosis was different between patients with FIB-4 ≥1.45 and those with FIB-4 <1.45, we calculated the optimal cut-off of FFS at diagnosis in predicting a potential of subclinical but significant liver fibrosis ≥S2 based on AUROC analysis. We found that 1 of FFS at diagnosis was a strong predictor of FIB-4 ≥1.45 (area 0.667, 95% confidence interval 0.566, 0.769, sensitivity 0.97, specificity 0.31). When we classified 136 patients with AAV into the two groups based on the calculated cut-off of FFS at diagnosis, thirty-four patients (25.0%) were partitioned into the group with FFS at diagnosis ≥1. FIB-4 ≥1.45 was identified more frequently in these patients than in those with FFS <1 (27.5% *vs*. 2.9%, p=0.003). Furthermore, patients with FFS ≥1 had a significantly higher risk of having subclinical but significant liver fibrosis according to FIB-4 ≥1.45 than those with FFS <1 (RR 12.486, 95% CI 1.629, 95.685) (Fig. 3).

Discussion

In this study, we evaluated hepatic manifestation of AAV using the results of liver function tests and the findings of imaging studies. Also we investigated liver fibrosis by APRI and FIB-4

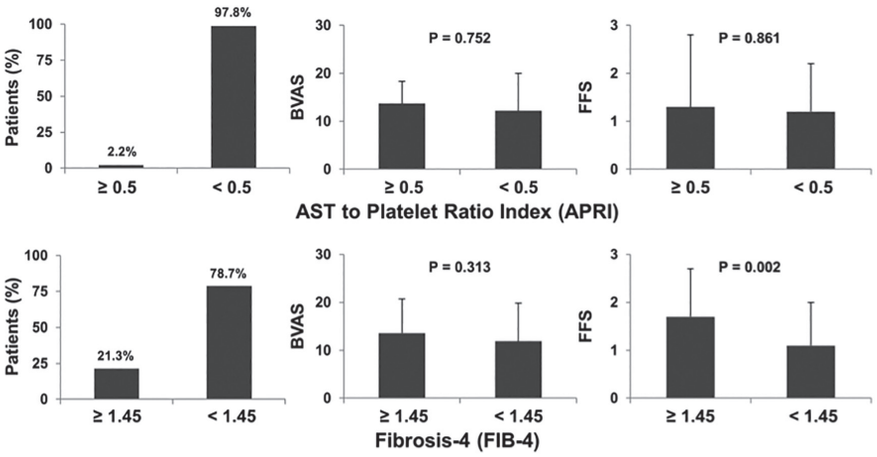

Fig. 2. The association of BVAS or FFS with subclinical but significant liver fibrosis at diagnosis. Among 136 patients with AAV, 3 patients (2.2%) and 29 patients (21.3%) exhibited APRI ≥0.5 and FIB-4 ≥1.45, which suggest significant liver fibrosis. We compared both BVAS and FFS at diagnosis between patients with APRI ≥ 0.5 and those with APRI <0.5, but there were no significant differences between the two groups. We also compared both BVAS and FFS at diagnosis between patients with FIB-4 ≥1.45 and those with FIB-4 <1.45. The mean BVAS at diagnosis did not differ between the two groups. However, patients with FIB-4 ≥1.45 had the significantly higher mean FFS at diagnosis than those with FIB-4 <1.45. BVAS: Birmingham vasculitis activity score; FFS: five factor score; AAV: antineutrophil cytoplasmic antibody-associated vasculitis; APRI: the aspartate aminotransferase to platelet ratio index; FIB-4: an index of fibrosis.

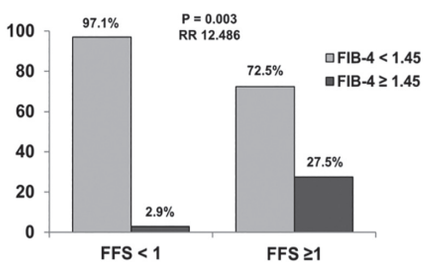

Fig. 3. The optimal cut-off of FFS in predicting FIB-4 ≥1.45. When we classified 136 patients with AAV into the two groups based on the calculated cut-off of FFS at diagnosis, thirty-four patients were partitioned into the group with FFS at diagnosis ≥1. FIB-4 ≥1.45 was identified more frequently in these patients than in those with FFS <1. Furthermore, patients with FFS ≥1 had a significantly higher risk of having subclinical but significant liver fibrosis according to FIB-4 ≥1.45 than those with FFS <1 (RR 12.486). FFS: five factor score; FIB-4: an index of fibrosis; AAV: antineutrophil cytoplasmic antibody-associated vasculitis; RR: relative risk.

in 136 patients with AAV at diagnosis. First, in 136 patients with AAV without chronic liver disease and autoimmune diseases, the mean results of liver function tests at diagnosis were within normal ranges in a considerable number of patients. Furthermore, the percentage of patients having the normal results of liver function tests was ranging from 86.0% to 95.6%. Second, there were no patients who exhibited the significantly abnormal findings on hepatic imaging studies. Third, nonetheless, twenty-nine patients with AAV exhibited subclinical but significant liver fibrosis at diagnosis based on FIB-4. Fourth, patients with FFS ≥1 had a significantly higher risk of having subclinical but significant liver fibrosis (FIB-4 ≥1.45) than those with FFS <1 (RR 12.486). Thus, we conclude that AAV may influence on liver function and furthermore it may provoke subclinical but significant liver fibrosis based on APRI and FIB-4, despite few abnormalities on imaging studies.

A previous study reported that ALT and ALP were remarkably elevated in GPA patients compared to MPA and EGPA patients (6). In order to evaluate the effect of AAV variant on liver function, we compared the mean results of liver function tests among MPA, GPA and EGPA using One-Way-Anova test. However, we could find no significant differences in the mean level of those results among 3 AAV variants at all: PLT (323, 800 for MPA, 378,400 for GPA and 350,900 for EGPA, p=0.378); prothrombin time (INR) (1.0 for MPA, 1.0 for GPA and 1.0 for EGPA, p=0.730; ALP (84.0 for MPA, 129.8 for GPA and 87.6 for EGPA, p=0.108); AST (22.2 for MPA, 22.3 for GPA and 27.8 for EGPA, p=0.480); ALT (20.2 for MPA, 30.5 for GPA and 26.8 for EGPA, p=0.447); total bilirubin (0.5 for MPA, 0.6 for GPA and 0.6 for EGPA, p=0.576). Thus, we conclude that AAV variant may not affect liver function at diagnosis.

In this study, we first applied APRI and FIB-4 to patients with AAV, particularly before immunosuppressive drugs were administered. And we demonstrated 21.3% of AAV patients had a potential of subclinical but significant liver fibrosis at diagnosis. The predictive value of FIB-4 for liver fibrosis has been validated and the critical cut-off of 1.45 for significant liver fibrosis has high specificity of 0.75 (24). On the other hands, in the present study, patients with FIB-4 ≥1.45 exhibited no clinically serious liver structural abnormalities on imaging studies. Therefore, our results may provide a lesson that subclinical but significant liver fibrosis might have been underestimated to date despite the performance of routine laboratory tests or imaging studies.

When we recognise liver fibrosis ≥S2 by FIB-4 ≥1.45, we may consider the prompt performance of transient elastography or liver biopsy. Here, we provide three reasons why we should calculate FIB-4 in all patients with AAV at diagnosis as follows; i) if we recognise FIB-4 ≥1.45, perform transient elastography or liver biopsy, and confirm that liver fibrosis is provoked by AAV (25), we should increase the doses of immunosuppressive drugs or change them to others; ii) if we confirm that liver fibrosis is related to hepatotoxicity of immunosuppressive drugs administered, we should decrease the doses of those drugs or quit them, if possible; iii) if we confirm that liver fibrosis is provoked by incidental liver diseases such as primary sclerosing cholangitis or nodular regenerative hyperplasia which have reactivity to multiple autoantibodies including ANCAs (26), we should refer patients to Hepatologists.

We demonstrated the association between FFS and FIB-4 at diagnosis of AAV. We do not calculate FIB-4 in all patients, but we usually assess FFS in almost all the patients at diagnosis. Therefore, we suggest a clinical and critical pathway for predicting sub-

clinical but significant liver fibrosis by hepatic manifestation of AAV at diagnosis. First, physicians should check whether FFS at diagnosis is more than 1. Second, when FFS is more than 1, physicians should calculate FIB-4. Third, when FIB-4 is more than 1.45, physicians should consider non-invasive liver stiffness measurement using transient elastography. Last, when significant liver fibrosis (≥S2) is detected by transient elastography, physicians should refer patients to Hepatologists for liver biopsy.

Our study has several advantages: first, we first demonstrate that 21.3% of immunosuppressive drug-native patients with AAV had a potential of subclinical but significant liver fibrosis (≥S2) using FIB-4. Second, we provide a clinical and critical pathway for predicting liver fibrosis in AAV patients at diagnosis. Third, we minimise the confounding factors to exclude patients who had concomitant chronic liver diseases and autoimmune diseases and those who were exposed to immunosuppressive drugs.

Meanwhile, our study also has several issues: first, because the aim of this study was to investigate how many patients might exhibit subclinical but significant liver fibrosis, we provided no histological information on liver in patients exhibiting the abnormal results of liver function tests, particularly those having APRI ≥0.5 or FIB-4 ≥1.45. Second, due to the limitation of a retrospective study, we could not collect the result of gamma-glutamyl transferase, which is one of important enzymes to represent liver function. Third, our study was a cross-sectional study, so we could not clarify whether subclinical liver involvement of AAV may be associated with the frequency and severity of adverse events and drug toxicities during the follow-up. We believe that future prospective studies with the histological results and the serial laboratory and imaging data on liver including gamma-glutamyl transferase and FIB-4 in AAV patients with FIB-4 ≥1.45 will overcome these issues of our pilot study.

In conclusion, AAV may increase the results of liver function tests and it may provoke subclinical but significant liver fibrosis at diagnosis. Furthermore, liver fibrosis should be considered in AAV patients having FFS ≥1.

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