Liver Stiffness Assessed by Real-Time Two-dimensional Shear Wave Elastography Predicts Hypersplenism in Patients with Wilson's Disease: A Prospective Study

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Abstract

Background To explore the value of liver stiffness assessed by two-dimensional real-time shear wave elastography (2D-SWE in predicting the occurrence of hypersplenism in patients diagnosed with Wilson's disease (WD).

Methods A total of 90 WD patients were enrolled in this prospective study between May 2018 and December 2018. Clinical data and ultrasound imaging including 2D-SWE liver stiffness of WD patients as baseline data were collected. Patients were followed up for 24 months, or patients developed hypersplenism after enrollment. Risk factors for hypersplenism were determined using cox regression and receiver operating characteristic curve.

Results Twenty-night (32.2%) patients were found developed hypersplenism. The age, the diameter of portal vein, and the liver stiffness were independent risk factors associated with hypersplenism in WD. The cutoff value of liver stiffness for predicting hypersplenism was 10.45 kPa, with sensitivity and specificity of 75.9% and 73.8%, respectively. When patients were divided into two groups according to liver stiffness ≥10.45 kPa or <10.45 kPa, the incidence of hypersplenism were 57.9% vs. 13.5% (P<0.001), and the median time between the enrollment and the development of hypersplenism was 15 months vs. 22 months (P<0.001) for the two groups, respectively.

Conclusion The liver stiffness measured by 2D-SWE was a reliable predictor of hypersplenism in WD patients. Dynamic monitoring WD patients using 2D-SWE is crucial for the early diagnosis of hypersplenism.

Background

Wilson's disease (WD) is a rare autosomal recessive genetic disease [1]. In the patients diagnosed with WD, 75% have liver involvement [2], and 34% eventually develop cirrhosis and hypersplenism [3]. WD patients have been reported 4.4 times more likely to develop hypersplenism than those patients affected with hepatitis B [4]. In the patients with liver disease, the development of hypersplenism is determined when a leukocyte count < 3500/μl and/or a platelet count < 7.5×104/μl [5]. Once hypersplenism occurs, WD patients should be treated with splenectomy [6]. However, the patients may suffer complications such as portal vein thrombosis (PVT) [7] and overwhelming post-splenectomy infection (OPSI) after splenectomy, and the mortality rate related to OPSI can be as high as 50% [8]. Therefore, it is of clinical importance to develop techniques to predict, whether an individual WD patient will develop hypersplenism, and early intervention with splenic artery embolization may be used to decelerate the progression of hypersplenism [9].

The assessment of splenic blood flow using four-dimensional (4D) flow magnetic resonance imaging (MRI) has been reported to predict hypersplenism in previous study [10]. Patients with high compliance is

required in the 4D flow MRI evaluation of splenic blood flow. However, some of the WD patients could not tolerate MRI examination because of neurological and mental symptoms developed during disease progression [1]. It has been reported that liver stiffness assessed by real-time two-dimensional shear wave elastography (2D-SWE) can be applied to predict various hepatic adverse events such as liver cancer caused by hepatitis B and liver failure caused by liver cancer [11, 12], as well as to evaluate whether hepatic patients have complicated portal hypertension and hypersplenism [13–15]. However, so far, no attention has been paid to the value of 2D-SWE to predict hypersplenism in WD patients mainly due to the rarity of this disease.

Therefore, the aim of the study was to conduct a prospective longitudinal cohort study to investigate whether liver stiffness assessment using 2D-SWE can be applied to predict hypersplenism in WD patients.

Materials And Methods

Patients

Consecutive WD patients who were admitted to the Encephalopathy Center of the First Affiliated Hospital, Anhui University of Chinese Medicine between May 2018 and December 2018 were enrolled. Inclusion criteria consisted of a diagnosis of WD with liver involvement according to the Leipzig standard [16] but no hypersplenism at the time of enrollment. Hypersplenism was defined as a leukocyte count < 3500/μl and/or a platelet count < 7.5×10^4/μl [5]. Patients were excluded from the study for the following reasons: combined with hepatitis B, severe liver inflammation indicated by ALT elevation greater than five times upper normal limits [17], failed in liver 2D-SWE assessment, incomplete clinical data at enrollment or during follow-up, not undergo reexaminations as required, and lost to follow-up. This prospective study was approved by the Institutional Review Board of Ethics Committee of the First Affiliated Hospital, Anhui University of Chinese Medicine (2018AH-08), and informed consent was obtained from all participants.

Collection of clinical and imaging data at baseline

The starting point of the study for each patient was defined as the day when the patient underwent liver 2D-SWE. At baseline, clinical data including gender, age, body mass index (BMI), presence of Kayser-Fleischer (K-F) ring, fibrosis index based on four factors (FIB-4) and aminotransferase/platelet count ratio index (APRI) were collected. Laboratory result valid when it was acquired within one week prior to the enrollment. The diameter of the portal vein and the spleen thickness measured by ultrasound, as well as the liver stiffness assessed by 2D-SWE were all documented.

Liver stiffness assessed by 2D-SWE

Liver 2D-SWE measurement was performed upon enrollment for each patient. The Mindray-Resona 7 ultrasonic instrument with the SC5-1U probe (Mindray, China) was used, and the 2D-SWE mode was adopted. The 2D-SWE measurement was performed by one experienced radiologist (8-year experience of

abdominal ultrasound) who had performed more than 300 liver ultrasound examinations in the last two years and at least 50 liver 2D-SWE examinations in the last six months.

On the morning of liver 2D-SWE examination, the patients were in a fasting state. During the examination, they were adopted the supine or left lateral position, with the right hand lifted up to fully expose the intercostal space. Liver 2D-SWE images were obtained from the right liver lobe through the right intercostal space. The image depth was controlled at 8–10 cm, and the size of the liver 2D-SWE sampling frame was adjusted to 4.0 cm × 3.0 cm, which was placed at 1.0 cm below the liver capsule to keep away from the great vessels in the liver. During 2D-SWE examination, the patients were required to hold their breath for 5–6 seconds in the natural breathing state. The elasticity value was measured when the motion stability index (M-STB) was five green asterisks and when the reliability map (RLB) had changed from purple to green [17]. During the measurement, the diameter of the circular region of interest (ROI) was adjusted to 2.0 cm, the data were displayed in kPa (Fig. 1), and the average elasticity in the ROI obtained from each measurement was taken as the recorded value of the current measurement. The measurement was repeated five times continuously, and the median of the recorded values from the five measurements was taken as the final liver elasticity value of the patient. To obtain more accurate and consistent measurement data, we implemented quality control for the images and measurements. If the interquartile range (IQR)/median (Med) was higher than 30% after repeated measurement for five consecutive times, the measurement was considered as failed.

Treatment and follow-up strategy

All the WD patients received de-coppering therapy during follow-up period. The patients regularly took dimercaptosuccinic acid (DMSA) orally at a dose of 10mg/kg/d, bid.

After enrollment, the patients were routinely followed up every month. At each visit, investigation of leukocyte count and platelet count were performed to determine whether the patient developed hypersplenism [5]. The patients were prospectively followed-up for at least 24 months, or the patients developed hypersplenism.

Statistical analysis

Measurement data conforming to normal distribution were expressed as mean ± SD, and intergroup comparison was performed using a *t*-test. Measurement data not conforming to normal distribution were expressed as median (IQR), and the Mann-Whitney U test was adopted for inter-group comparison. Enumeration data were expressed as n (%), and inter-group comparison was performed using an χ^2 test or Fisher's exact test.

The cumulative incidence of hypersplenism was determined based on the hazard ratios derived from Kaplan-Meier survival curves. Cox regression analyses were applied to evaluate the risk factors for hypersplenism in patients with WD. The receiver operating characteristic curve (ROC) and area under the curve (AUC) were used to calculate the predicting hypersplenism efficacy of liver stiffness in WD patients.

In addition, the threshold, as well as its sensitivity and specificity, were determined by calculating the maximum Youden index. Based on the obtained threshold, a Log-rank test was applied to compare and analyze whether there were significant differences when evaluating the risk of hypersplenism between the two groups of patients with Wilson's disease divided by the 2D-SWE threshold. SPSS 25.0 was used for all data analyses in this study, and the differences were considered to indicate statistical significance when $P < 0.05$.

Results

General information of the 90 WD patients at baseline

Ninety WD patients were included in this study (Fig. 2). Among them, 49 (54.4%) were males and 41 (45.6%) were females, with an average age of 29.8 ± 10.6 years (range, 14–63 years). Baseline general information of the 90 patients were summarized in Table 1.

Table 1
Baseline of general information of patients with WD

	Total number of patients	Hypersplenism group	Non- hypersplenism group
Number n (%)	90(100)	29(32.2)	61(67.8)
Male n (%)	49(54.4)	15(51.7)	34(55.7)
Age (years)	29.77 ± 10.62	27.34 ± 7.53	30.92 ± 11.70
BMI (kg/m^2)	21.46 ± 1.65	21.08 ± 1.78	21.64 ± 1.57
K-F ring	65(72.2)	20(69.0)	45(73.8)
FIB-4	1.47 ± 0.20	1.48 ± 0.20	1.47 ± 0.20
APRI	0.74 ± 0.10	0.73 ± 0.10	0.74 ± 0.10
Diameter of portal vein (mm)	11.67 ± 1.80	12.41 ± 2.03	11.31 ± 1.58
Spleen thickness (mm)	51.88 ± 9.02	53.38 ± 5.14	51.16 ± 10.33
Liver stiffness (kPa)	10.10 ± 2.90	12.38 ± 2.77	9.00 ± 2.28

* BMI, body mass index; K-F ring, Kayser-Fleischer ring; FIB-4, fibrosis index based on four factors; APRI, aminotransferase/platelet count ratio index.

Clinical outcome

During follow-up, a total of 29 (32.2%) patients were found developed hypersplenism. The median time between the enrollment and the occurrence of hypersplenism for the whole cohort was 18 months (range,

6–24 months). The cumulative incidence of hypersplenism at 6, 12 and 18 months was 4.4%, 21.1%, and 31.1%, respectively (Fig. 3).

Risk factors associated with hypersplenism in the WD patients

Cox regression analyses revealed that the age, the diameter of portal vein, and the liver stiffness of the patient at enrollment were independent predictive factors associated with hypersplenism (Table 2). Cox regression value for the liver stiffness was 0.325, which suggested that every 1.0 KPa increased in the liver stiffness, the risk of hypersplenism was increased by 32.5%.

Table 2
Cox regression analysis of risk factors for hypersplenism in WD patients

Factor	B	*P*	RR value	95% CI	
				Lower limit	Upper limit
Age	-0.060	0.007	0.942	0.901	0.984
Diameter of the portal vein	0.244	0.012	1.277	1.055	1.545
Liver stiffness	0.325	0.000	1.385	1.231	1.557
*RR, relative risk; CI, credibility interval					

The value of the liver stiffness measured using 2D-SWE in predicting hypersplenism

ROC analyses reported that the AUCs were 0.433 (95%CI: 0.314–0.553) for the age, 0.662 (95%CI: 0.541–0.782) for the diameter of spleen vein, and 0.817 (95%CI: 0.727–0.908) for the liver stiffness in predicting hypersplenism in the WD patients, respectively (Fig. 4). The liver stiffness showed a superior predictive ability than that of the diameter of portal vein and the age of patient ($P < 0.05$).

The optional cutoff value of the liver stiffness in predicting hypersplenism in the WD patients was 10.45 KPa, with sensitivity and specificity of 75.9% and 73.8%, respectively.

To determine a possible association between the liver stiffness and the time point of hypersplenism development, ninety WD patients were sub-divided into high-risk group (with liver stiffness ≥ 10.45 KPa, n = 38) and low-risk group (with liver stiffness < 10.45 KPa, n = 52) based on the cutoff value of 10.45 KPa, respectively. The cumulative incidence of hypersplenism in both groups was shown in Fig. 5. The incidence of hypersplenism in the high-risk group was significantly higher than that of the low-risk group (57.9% vs. 13.5%, $P < 0.001$). The median time between the enrollment and the development of

hypersplenism for the high-risk group was 15 months (range, 6–24 months) and that for the low-risk group was 22 months (range, 6–24 months) ($P < 0.001$) (Fig. 6) (Table 3).

Table 3
Incidence of hypersplenism in the subgroups with different liver stiffness

	Liver stiffness measured by 2D-SWE	Incidence of hypersplenism	The median time of hypersplenism (month)	χ^2	*P*
High-risk group	≥ 10.45 kPa	57.9%(22/38)	15	22.296	<0.001
Low-risk group	<10.45 kPa	13.5%(7/52)	22		

Discussion

In this study, we explored the value of the liver stiffness assessed by 2D-SWE in predicting hypersplenism in the WD patients. Our study demonstrates that the assessment of liver stiffness using 2D-SWE is an effective approach for prospective prediction of hypersplenism in the WD patients. Furthermore, WD patients with a liver stiffness ≥ 10.45 kPa have a significantly higher risk of hypersplenism that might develop in a relatively short term. These findings enable prompt stratification of WD patients with high or low risk of hypersplenism.

In our study, the age of the patient, the diameter of portal vein, and the liver stiffness were independent risk factors for hypersplenism in the WD patients. Furthermore, the liver stiffness showed a higher AUC value of 0.817 when compared with that of the age and the diameter of portal vein. In one study in which 4D flow MRI was used to predict the occurrence of hypersplenism in 20 patients, Keller et al found that the AUC value for blood flow of splenic artery was 0.792 [18], which was slightly lower than that of liver stiffness reported in our study. MR imaging requires contrast agent injection, carries a high cost, and excludes patients with incompatible implanted devices. Most importantly, patients affected with WD typically develop neurological and psychiatric symptoms during disease progression and cannot tolerate MRI examination, making MRI a nonideal modality for assessment of WD patients. Our study suggests that liver stiffness measured using 2D-SWE can be used to assess the risk of hypersplenism in WD patients with high effectiveness and feasibility.

WD is a copper metabolic dysfunction disease, excess copper deposition in the liver tissues causes hepatocyte fatty degeneration, chronic inflammation, liver fibrosis and liver cirrhosis [19]. The pathological process of WD is consistent with other chronic liver diseases. The 2D-SWE is a two dimensions ultrasound-based technology for measuring tissue stiffness. It enables a real-time analysis of

liver stiffness and can discover the underlying pathological processes of liver diseases. Recently, several studies demonstrate that liver stiffness assessed by 2D-SWE provide physicians with an alternative tool in terms of prediction of disease progression [14]. In a meta-analysis in which 746 patients with liver diseases were included, the study suggested that 2D-SWE could be used to forecast portal hypertension with the cutoffs of 15.2–24.6 kPa, with sensitivities raging from 78%-90% and specificities varying from 83%-89% [20]. Our study found that the cut-off value of liver stiffness was 10.45 KPa in predicting hypersplenism in WD patients, with a sensitivity of 75.9% and specificity of 73.8%. The discrepancy in the cutoff value can be partially explained by the difference of the average age of patients enrolled in the two studies. The average age of the 746 patients in the meta-analysis was 53–71 years old, which were older than that of our study (29.8 years old). Of note, so far, no studies have used 2D-SWE to predict hypersplenism in the patients with WD patients. It is possible that liver diseases, such as virus hepatitis, may usually complicated with portal hypertension, while, the probability of concurrent hypersplenism is not as high as WD [3, 4].

When compared with patients with liver stiffness < 10.45 KPa, those with liver stiffness ≥ 10.45 kPa had increased risk of hypersplenism (RR = 1.385). It was worth noting that, in additional to a relatively higher incidence of hypersplenism (57.9% vs. 13.5%), the median time period between baseline and the time points of hypersplenism development in the high-risk patients with liver stiffness ≥ 10.45 kPa was significantly shorter (20 months vs. 14 months, P < 0.001). These results implied that high risk patients may occur hypersplenism at a relatively short term. Our study highlighted that liver stiffness assessed by 2D-SWE can be applied to monitor liver progression of WD patients by stratifying risk of hypersplenism.

There were several limitations in the study. In view of the rare occurrence of WD, the results of this study are only based on one hospital, with a lack of multicenter liver elasticity data. Moreover, the study investigated the laboratory indicators but only FIB-4 and APRI were included for analyses. FIB-4 and APRI are commonly used for monitoring liver fibrosis in chronic hepatitis B and C as well as WD [21]. Whether the prediction efficacy of 2D-SWE will be affected when more laboratory indicators are added in the research needs further investigation.

Conclusion

In conclusion, WD patients with liver stiffness ≥ 10.45 kPa have relatively higher risk of hypersplenism. Monitoring liver stiffness with 2D-SWE is essential for the WD patients to early diagnose hypersplenism, guide clinical treatment and avoid postoperative complications of hypersplenism.

Abbreviations

2D-SWE
Two-dimensional real-time shear wave elastography; WD:Wilson’s disease; PVT:Portal vein thrombosis; OPSI:Overwhelming post-splenectomy infection; 4D:Four-dimensional; MRI:Magnetic resonance imaging; BMI:Body mass index; K-F ring:Kayser-Fleischer ring; FIB-4:Fibrosis index based on four factors;

APRI:Aminotransferase/platelet count ratio index; M-STB:Motion stability index; RLB:Reliability map; ROI:Region of interest; IQR:Interquartile range; Med:Median; DMSA:dimercaptosuccinic acid; ROC:Receiver operating characteristic curve; AUC Area under the curve;

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital, Anhui University of Chinese Medicine, and obtained ethics approval in written (2018AH-08). Patients of this study have provided informed consent for publication of the case.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and analyzed during our study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JW, MH, QZ and LS. The first draft of the manuscript was written by JW and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

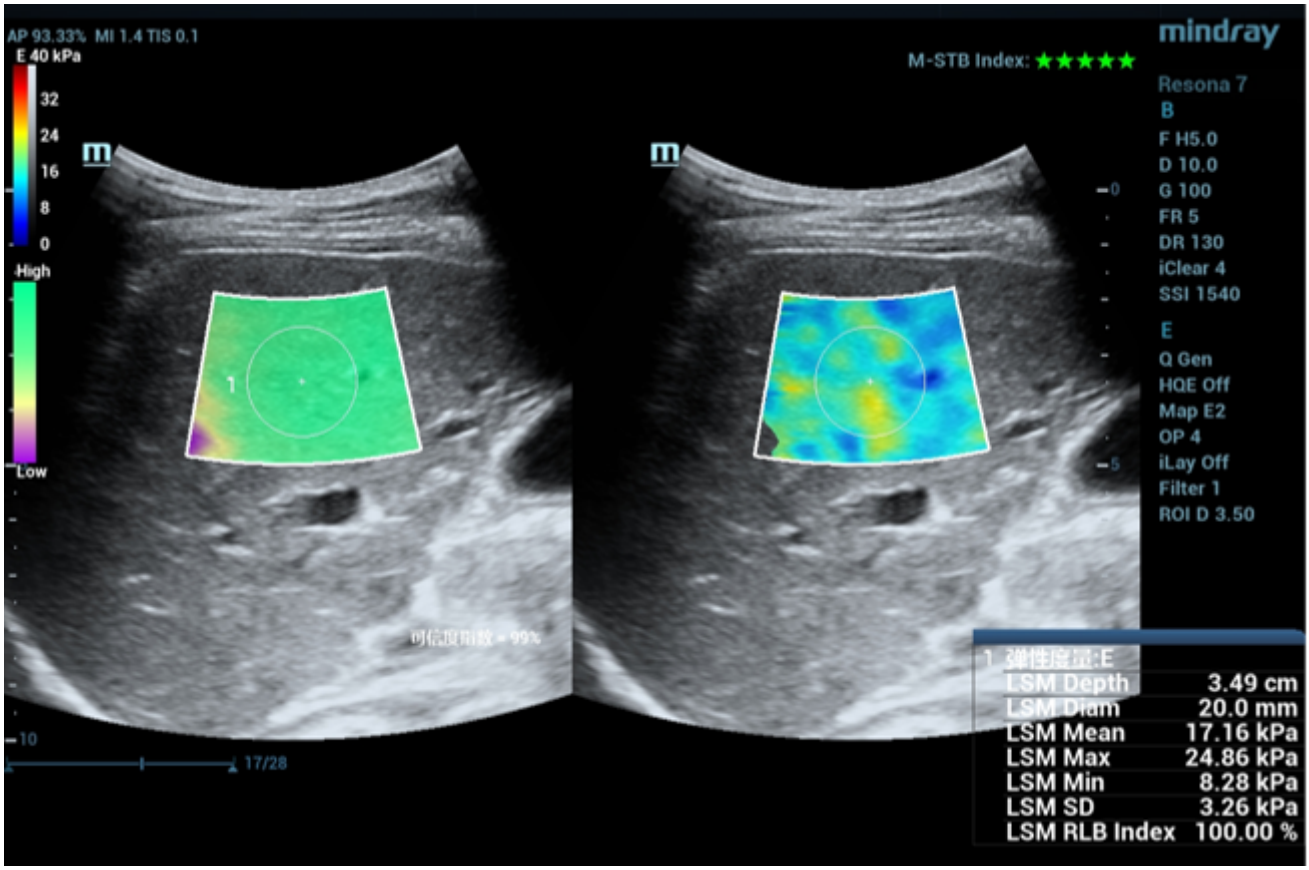

Figure 1

A 26-year-old male WD patient, with an average elasticity of 17.16 kPa in the right liver lobe

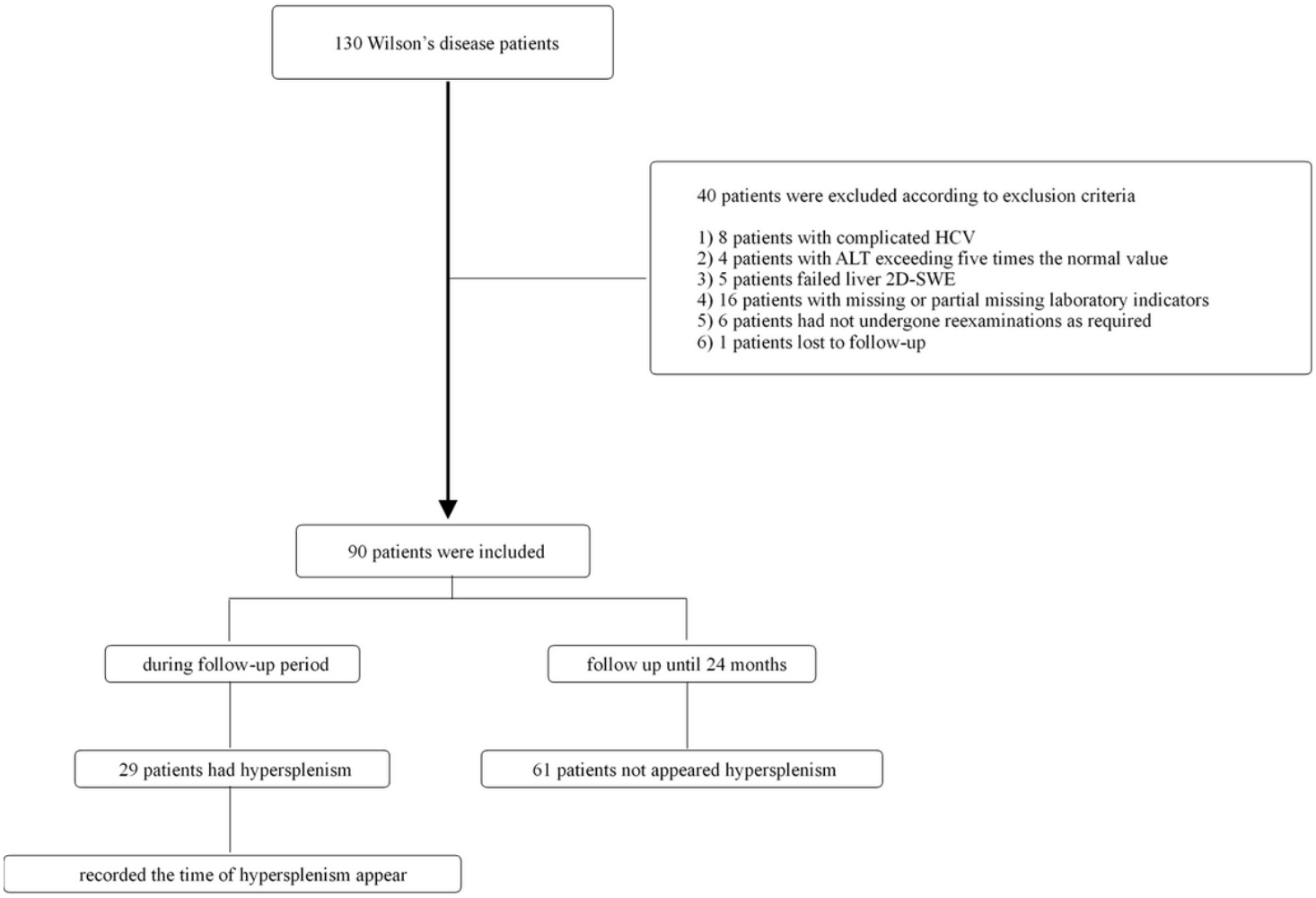

Figure 2

Flowchart of study participants

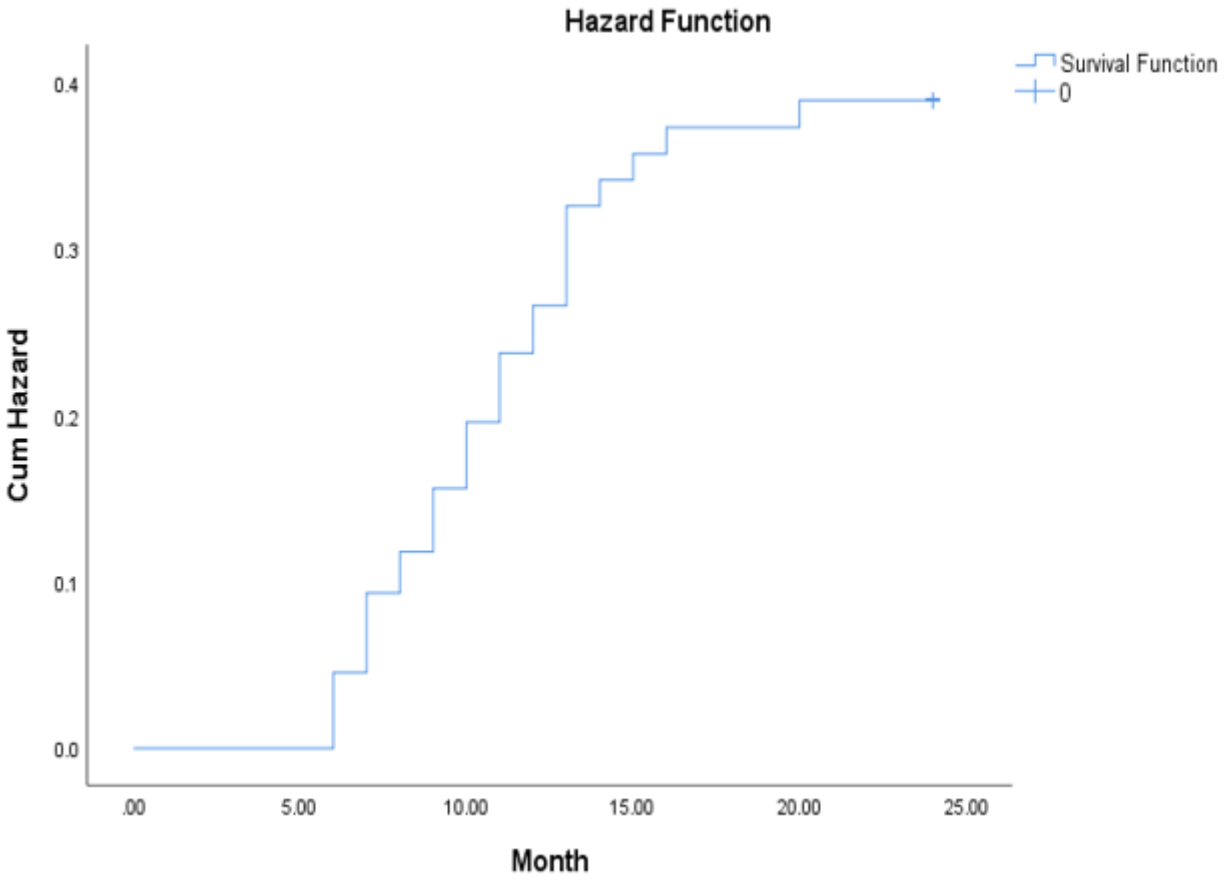

Figure 3

Cumulative incidence of hypersplenism in WD patients

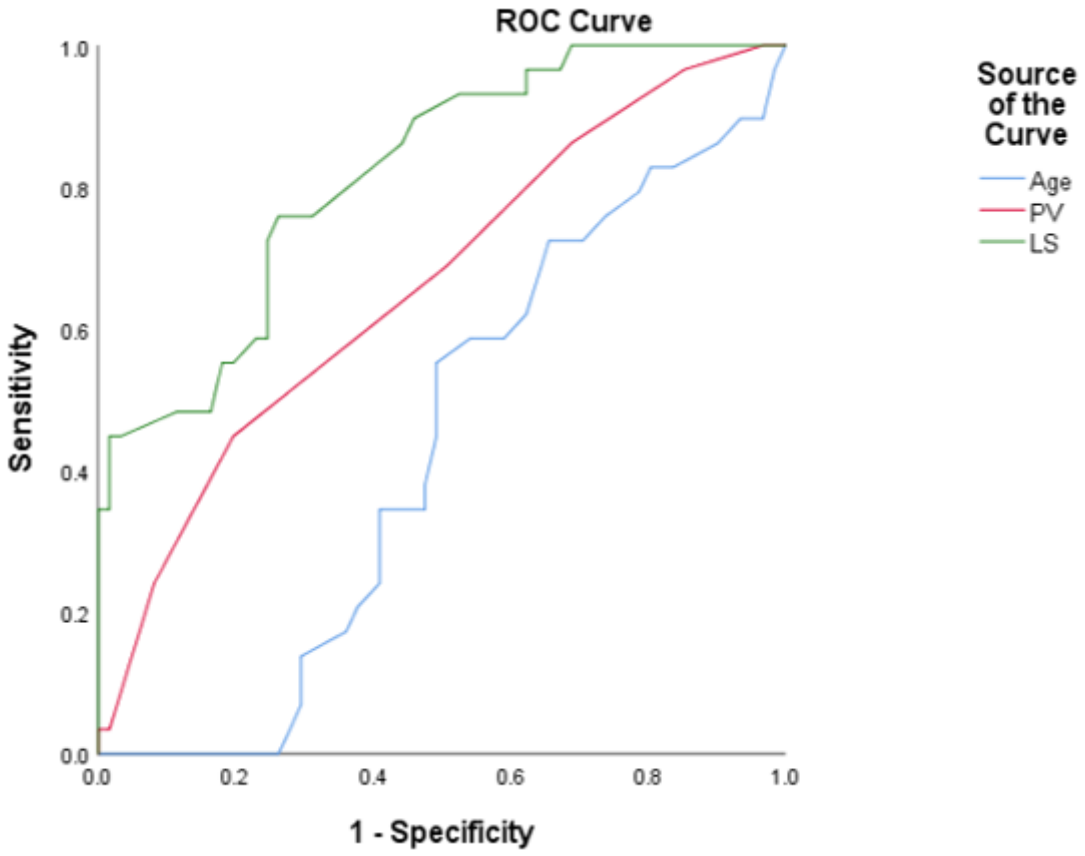

Figure 4

Value of age, diameter of the portal vein and liver stiffness measured using 2D-SWE in predicting hypersplenism in WD patients

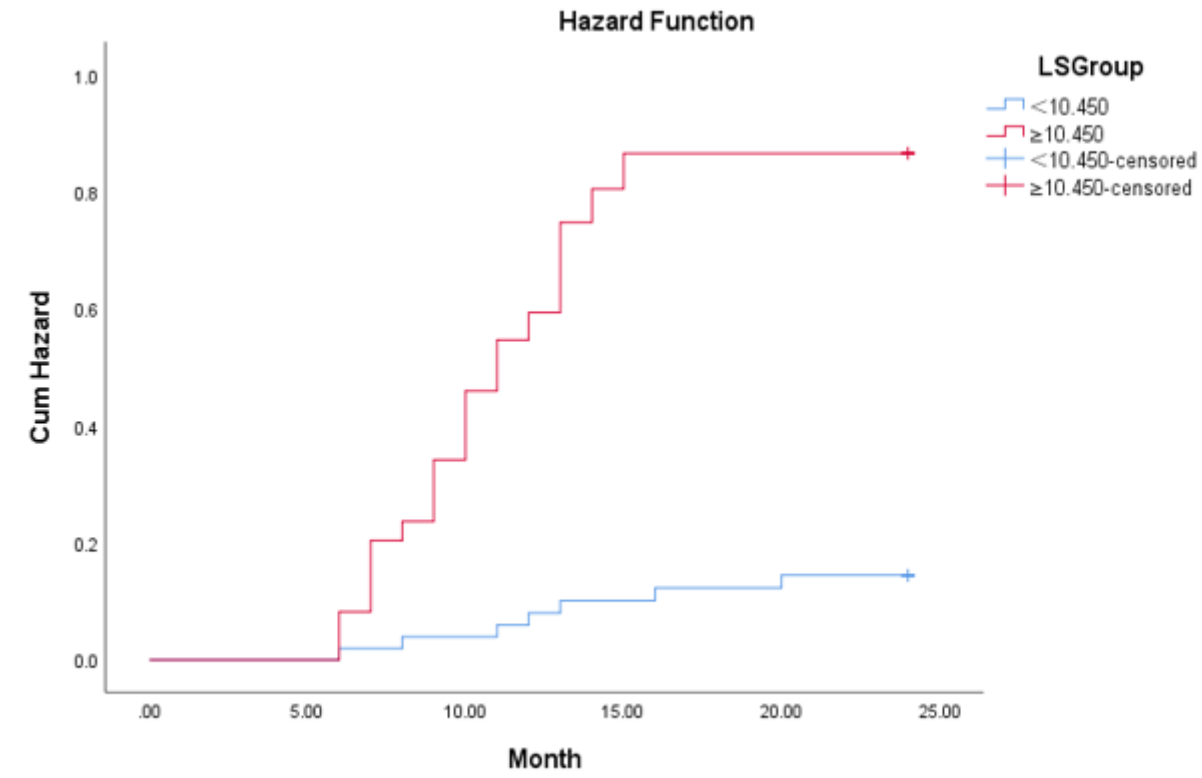

Figure 5

Risk of hypersplenism in WD patients in both high-risk (≥10.45 kPa) and low-risk(<10.45kPa) groups

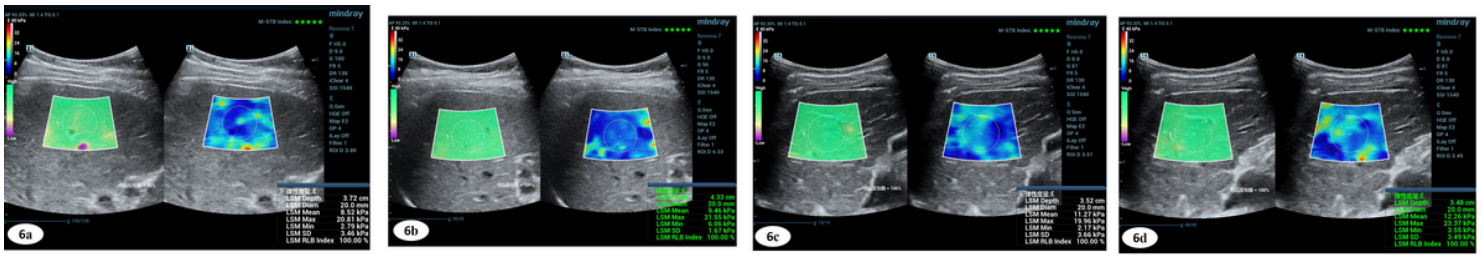

Figure 6

Baseline 2D-SWE measurements of the liver in WD patients a, The WD patient elasticity of right liver lobe was 8.52 kPa, which was followed up for 24 months, and no hypersplenism occurred b, The WD patient elasticity of right liver lobe was 9.46 kPa, which was followed up for 24 months, and no hypersplenism occurred c, The WD patient elasticity of right liver lobe was 11.27 kPa, which was occurred hypersplenism at the 13th month of follow-up d, The WD patient elasticity of right liver lobe was 12.26 kPa, which was occurred hypersplenism at the 13th month of follow-up