Transient elastography in the assessment of liver fibrosis in adult thalassemia patients

Mirella Fraquelli,[1] Elena Cassinerio,[2] Alberto Roghi,[3] Cristina Rigamonti,[4] Giovanni Casazza,[5] Massimo Colombo,[4] Sara Massironi,[1] Dario Conte,[1] and Maria Domenica Cappellini[2]

Transient elastography (TE) is a valuable noninvasive technique of measuring liver stiffness and a reliable tool for predicting hepatic fibrosis in patients with chronic liver disease. The role of TE in patients with β-thalassemia has not been extensively investigated. The present study aimed to evaluate the role of TE in the assessment of hepatic fibrosis in 115 adult patients with β-thalassemia major (TM) (#59) or intermedia (TI) (#56). TE was performed according to current practice. Histologic data were obtained in 14 cases. Liver iron concentration was assessed by atomic absorption spectrometry and T2* magnetic resonance. In patients with TM, the proportion of anti-HCV positive viremic patients, median serum ferritin levels, and TE values were significantly higher than in TI. In the group of 14 patients who underwent liver biopsy, a significant positive correlation was observed between liver stiffness and fibrosis stage ($r = 0.73$, $P = 0.003$). Severe fibrosis is diagnosed with a sensitivity of 60% and a specificity of 89%, whereas cirrhosis is detected with a sensitivity of 100% and a specificity of 92%. At multivariate analysis, the variables independently associated with TE were ALT, GGT, and bilirubin levels in both groups and, in patients with TM, HCV RNA positivity. In β-thalassemia patients, TE is a reliable tool for assessing liver fibrosis even if the influence of iron overload has to be clarified. Am. J. Hematol. 85:564–568, 2010. © 2010 Wiley-Liss, Inc.

Introduction

β-Thalassemia syndromes are hereditary anemias caused by the absent or decreased production of β-globin chains, associated with considerable morbidity and mortality [1–6]. Multitransfused adult β-thalassemia patients [thalassemia major (TM)] represent a population with a high prevalence of hepatitis C infection due to transfusions of HCV infected blood units prior to the introduction of anti-HCV screening [6,7]. In these patients in whom the bioptic procedure is associated with a high rate of complications because of many comorbidities, as compared to the other group of patients with chronic HCV infection, the need for noninvasive techniques to stage hepatic fibrosis is particularly pressing. Moreover, in thalassemia patients, frequent blood transfusions and increased iron gastrointestinal absorption can lead to iron overload over time. TM patients undergo regular chelation therapy, whereas there are not yet guidelines for chelation in thalassemia intermedia (TI) patients not regularly transfused, in whom iron overload is mainly due to ineffective erythropoiesis and iron absorption.

Transient elastography (TE) is a recently developed, rapid, noninvasive technique designed to predict liver fibrosis, based on a mechanical wave generated by vibration. The measurement of the speed of propagation of the wave across the hepatic parenchyma provides an estimate of the liver elasticity, which is a surrogate marker of liver fibrosis [8–10]. In studies comparing the results of TE versus liver biopsy as the reference standard, TE showed a satisfactory accuracy in identifying patients with chronic liver disease (CLD) accompanied by significant fibrosis or cirrhosis and it has been shown to have a good interobserver and intraobserver reproducibility [11–23].

The role of TE in evaluating liver fibrosis in patients with β-thalassemia with or without HCV chronic infection has not been extensively investigated. Only recently, two papers reported TE evaluation in 56 homozygous β-thalassemic patients from Southern Italy [24] and in 15 chronically transfused patients from France [25]. Therefore, the present study aimed to assess the role of TE in staging hepatic fibrosis and evaluating factors possibly associated with TE results in a larger cohort of patients with β-TM and TI.

Methods

Cross-sectional assessment of hepatic fibrosis by TE was performed in 115 adult patients with β-thalassemia (TM: 59, median age 33 years, range 21–50 years; TI: 56, median age 40 years, range 17–76 years) and followed up in a single tertiary Thalassemia Care Center in Italy.

Transient elastography. TE (FibroScan®, EchoSens) was performed in all patients. The procedures were performed by two independent investigators (MF and CR) who were blind to clinical, serological, and histological data. The right lobe of the liver was targeted through an intercostal space access while the patient was lying in the dorsal decubitus position with the right arm in maximal abduction. With the assistance of the Fibroscan ultrasound (US), a liver portion of at least 6-cm thickness, free of large vessels, was identified for examination. The rate of successful measurements was calculated as the ratio between the number of those validated and total measurements. The results were expressed as a median value of the total measurements in kPa. Only the examinations with at least 10 validated measurements, a success rate of at least 60%, and an interquartile range (IQR) of the median stiffness value lower than 30% were considered reliable. Patients with ascites were excluded from the study. One operator (MF) performed 66 examinations and the second one (CR) performed the remaining 49 examinations. The intraobserver agreement between the two operators was good, as shown in a previous paper [12]. No liver stiffness measurement failure was observed in the present study. TE

[1]Second Division of Gastroenterology, Foundation IRCCS Cà Granda, Maggiore Policlinico Hospital and University of Milan, Milan, Italy; [2]Hereditary Anemia Center, Department of Internal Medicine, Foundation IRCCS Cà Granda, Maggiore Policlinico Hospital and University of Milan, Italy; [3]Cardiovascular Magnetic Resonance Unit, Department of Cardiology and Cardiac Surgery A. De Gasperis, Azienda Ospedaliera Niguarda Ca'Granda, Milan, Italy; [4]First Division of Gastroenterology, Foundation IRCCS Cà Granda, Maggiore Policlinico Hospital and University of Milan, Milan, Italy; [5]Department of Statistics and Biometry, University of Milan, Milan, Italy

Conflict of interest: Nothing to report.

*Correspondence to: Maria Domenica Cappellini, Hereditary Anemia Center, Pad. Granelli, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Via F. Sforza n.35, 20122 Milano, Italy.
E-mail: maria.cappellini@unimi.it

Received for publication 30 December 2009; Accepted 26 April 2010

Am. J. Hematol. 85:564–568, 2010.

Published online 30 April 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21752

cut-off for diagnosing different stages of hepatic fibrosis was predefined according to the results of a previous study in a cohort of patients with chronic liver disease mainly related to HCV chronic infection [12]. The following TE thresholds were considered: >7.9 kPa for $S \geq 3$; >10.3 kPa for $S \geq 4$; >12.0 kPa for $S \geq 5$.

Histological data. The 14 patients who underwent liver biopsy prior to the biopsy procedure (and in all cases in the previous 2 weeks) also underwent a standard US scan of the abdomen using standard equipment (iU22, Philips, Bothell) with a detailed study of the liver, spleen, and main vessels. Liver biopsy was performed by experienced hepatologists with a 16 G Menghini needle (Biomol, Hospital Service, HS, Rome, Italy) under US guidance. The liver tissue was fixed in formalin and paraffin embedded. Five-μm-thick sections of liver tissue were stained with hematoxylin-eosin and Masson trichrome and read by one expert liver pathologist blinded to results of liver stiffness measurement and clinical data. Only samples with a length of >1.5 cm and including at least twelve complete portal tracts were considered adequate. Necroinflammation and fibrosis were scored by Ishak classification [26]; LIC (mg/g of liver dry weight) was performed on fresh tissue (atomic absorption spectrometry). All patients fulfilling the inclusion criteria were enrolled only after their written informed consent was obtained.

The following variables were collected in all patients: gender, age, body mass index (BMI; kg/m^2), chelation therapy, iron intake [mg/(kg day)], hemoglobin (Hb, normal value 12–16 mg/dL), platelets count (normal value 130–400 $\times$ 10^3/l). Serum alanine aminotransferase (ALT; normal value ≤40 IU/l), aspartate aminostransferase (AST; normal value ≤40 IU/l), gammaglutamiltransferase (GGT; normal value ≤50 IU/l), bilirubin (normal value 0.12–1.1 mg/dl), alkaline phosphatase (ALP; normal value 35–104 UI), anti HCV determination, and serum ferritin (SF; normal value 15–150 ng/ml) were measured with validated methods. Commercially available enzyme immunoassays were used to determine serum hepatitis B surface antigen, antibodies to hepatitis B core antigen and anti-HCV. Serum HCV-RNA was detected in house by nested reverse transcription (RT)-PCR, using primers of the 5′ noncoding region. Using a panel of infected sera calibrated to the WHO International Standard, the minimum detectable level was ~20 IU/ml. The diagnosis of concomitant alcohol abuse was based on the consumption of greater than 30 g ethanol per day in women and 50 g ethanol per day in men.

Cardiological follow up. Each patient underwent echocardiographic examination once yearly. Patients' parameters were evaluated according to recent guidelines published by Derchi et al. in 2008 [27].

T2 Magnetic Resonance Imaging.* Seventy-three (47 TM and 26 TI) patients underwent T2* magnetic resonance imaging (MRI) at the Cardiology and MRI Department "A. De Gasperis" at Niguarda Ca' Granda Hospital in Milan. Cardiac T2*, cardiac function, and liver T2* were assessed using validated techniques [28,29]. Patients were scanned with 1.5-T Magnetom Avanto Siemens; each scan lasted ~20 min and included heart and liver T2* assessment with gradient-echo sequences and biventricular function evaluation with steady-state free procession (true-FISP) sequences. Images were analyzed using post-processing software (CMR Tools, Imperial College, London). In patients who did not undergo liver biopsy, LIC was calculated from liver T2* according the formula [1/(T2*/1000)] $\times$ 0.0254 + 0.202 [30]. Normal LIC was defined for values under 4.2 mg/(g dry weight).

Statistical analysis. The main clinical and demographic characteristics of TM and TI patients were compared with the *t*-test, the Mann-Whitney test, or the χ^2 square test, as appropriate. Univariate and multivariate analysis (linear regression analysis) [31] were performed to investigate the possible relationship between log transformed TE values and demographic, biochemical, and RMN parameters. Univariate analyses considered these independent variables: gender, age, BMI, chelation therapy, Hb, platelets, alkaline phosphatase, anti-HCV, HCV-RNA, ferritin, T2*MRI, ALT, AST, GGT, and bilirubin. The final multivariate models contained only statistically significant independent variables.

The correlation between TE and histological features and TE operative characteristics in predicting hepatic fibrosis stage were also assessed in the subgroup with available liver biopsies. All statistical analyses were performed with statistical software SAS, release 9.1.

The study was conducted according to the guidelines of the Helsinki declaration and approved by the Ethics Committee of our hospital.

TABLE I. Main Clinical and Demographic Characteristics of 59 Patients With Thalassemia Major and 56 With Thalassemia Intermedia

Feature	T. major, $N = 59$	T. intermedia, $N = 56$	P value
Males, No.	20 (34%)	26 (46%)	NS
Age, years[a]	33 (19–50)	40 (14–75)	<0.0001
Anti-HCV positive, No.	55 (93%)	13 (23%)	<0.0001
HCV-RNA positive, No.	29 (49%)	6 (11%)	<0.0001
BMI (kg/m^2)[a]	22.5 (17.6–32.7)	21.6 (17–29.5)	0.04
ALT (IU/L)[a]	33 (7–286)	22.5 (6–122)	NS
AST (IU/L)[a]	31 (15–258)	26 (11–94)	NS
GGT (IU/L)[a]	17 (7–242)	16 (7–134)	NS
Platelet, $n \times 10^3$/mm^{3a}	375 (139–949)	369 (140–1006)	NS
Ferritin (ng/mL)[a]	1032 (410–6092)	583 (27–2600)	<0.0001
TE value (kPa)[a]	6.1 (4.2–49.6)	5.7 (3.3–17.6)	0.01

[a] Median (range).

Results

Clinical data

Fifty-nine patients (20 male, median age 33 years, range 21–50 years) with TM and 56 patients (26 male, median age 40 years, range 17–76 years) with TI were studied. The main clinical and laboratory characteristics of the patients are given in Table I. All patients affected by TM are transfusion-dependent: they transfused packed red cells every 15–21 days [about 160 ml/(kg years)], with pretrasfusional Hb levels from 9 to 10 g/dl. In the TI group, 10 patients were regularly transfused from adulthood and 18 patients had been occasionally transfused. Fifty-five patients with TM (93%) were anti HCV positive and 29 of them (49%) were viremic (HCV RNA positive), whereas 13 patients with TI (23%) were anti HCV positive, of whom six (11%) were HCV RNA positive ($P < 0.001$ for both comparisons).

In the two groups, median BMI were 22.5 (range 17–33 kg/m^2) and 21.6 (range 17–29 kg/m^2), respectively ($P = 0.04$). Median AST, ALT, and GGT levels did not differ significantly between the two groups, whereas ferritin levels were significantly higher in patients with TM as compared to those with TI (1032 ng/ml versus 583 ng/ml, respectively, $P < 0.001$).

None of the patients studied showed decompensated cardiac insufficiency. In detail, only three patients affected by TM presented a reduction in left ventricular ejection fraction (LVEF) (respectively, of 19, 45, and 46%); they had no signs or symptoms of acute heart failure and were clinically stable with therapeutic support.

Transient elastography

Median TE values were significantly higher in patients with TM (6.1 kPa; range 4.2–49.6 kPa) as compared to those with TI (5.7 kPa; range 3.3–17.6 kPa) ($P = 0.01$). Table II details the distribution of TE values in the two groups according to the predefined TE cut-off values to diagnose different stages of liver fibrosis. According to the TE results, a significant fibrosis (TE > 7.9 kPa corresponding to $S \geq 3$) was present in 21 patients (35%) with TM and in 10 patients (18%) with TI, a severe fibrosis (TE > 10.3 kPa corresponding to $S \geq 4$) was detected in 14 patients with TM (24%) and eight patients with TI (15%), and cirrhosis (TE ≥ 12 corresponding to $S \geq 5$) was present in 10 patients with TM (17%) and five with TI (9%). Anti-HCV positive viremic patients showed significantly higher median TE values (median value 7.8 kPa in TM and 8.3 kPa in TI) as compared to non-viremic ones (median value 6.1 kPa in TM and 6.7 kPa in TI) and as compared to anti-HCV nonreactive patients (median value 4.6 kPa in TM and 5.2 kPa in TI) (Table III). TE values were also influenced by the association of high ferritin levels and HCV-RNA positivity

TABLE II. Distribution of TE Values in 59 Patients With Thalassemia Major and 56 With Thalassemia Intermedia

TE cut-off (kPa)	T. major (# 59), *N* (%)	T. intermedia (# 56), *N* (%)
≤7.9 ($S < 3$)	38 (65%)	46 (82%)
>7.9 ($S \geq 3$)	21 (35%)	10 (18%)
>10.3 ($S \geq 4$)	14 (24%)	8 (15%)
>12.0 ($S \geq 5$)	10 (17%)	5 (9%)

TABLE III. Influenced of HCV Positivity and Viremia in TM and TI Patients on TE Values

HCV	TM patients (*n*)	TE (kPa)
AbHCV-/HCV-RNA-	4	4.6
AbHCV+/HCV-RNA-	26	6.1
AbHCV+/HCV-RNA+	29	7.8
HCV	TI patients (*n*)	TE (kPa)
AbHCV-/HCV-RNA-	43	5.2
AbHCV+/HCV-RNA-	7	6.7
AbHCV+/HCV-RNA+	6	8.3

TABLE IV. Iron Overload Parameters, HCV Viremia and TE Values in TM and TI Patients

Iron intake [mg/(kg die)]	TM patients (*n*)	TE (kPa)
<0.3	15	6.85
0.3–0.5	44	5.95
>0.5	0	/

Ferritin (ng/ml)	TM patients (*n*)	TE (kPa)	TE (kPa) HCV-RNA−	TE (kPa) HCV-RNA+
<500	6	6.1	6.1	7.3
500–1000	22	8.2	6.1	6.9
>1000	31	9.9	5.7	10.9

Transfusion regimen	TI patients (*n*)	TE (kPa)
Never	28	5.1
Occasionally	18	5.9
Regular	10	6.6

Ferritin (ng/ml)	TI patients (*n*)	TE (kPa)
<500	25	5.8
500–1000	19	5.3
>1000	12	7.3

(Table IV). In TM patients with higher ferritin levels, TE increased progressively; viremic patients with higher ferritin levels (ferritin above 1000 ng/ml) showed a higher increase of TE as compared with non viremic ones (TE 5.7 kPa vs. 10.9 kPa). In TI patients with higher ferritin levels or on regular transfusion regimen, TE values were increased.

The results of multivariate analysis are summarized in Table V. The variables significantly and independently associated with TE values were ALT ($P = 0.01$), GGT ($P = 0.001$), bilirubin levels ($P = 0.01$), and HCV-RNA positivity ($P = 0.03$) in patients with TM, and ALT ($P = 0.007$), GGT ($P = 0.01$), bilirubin levels ($P = 0.04$), previous cholecystectomy ($P = 0.011$), and splenectomy ($P = 0.018$) in those with TI, respectively.

T2 magnetic resonance imaging (MRI)*

A T2* MRI was performed in 73 patients (47 with TM and 26 with TI) to quantify LIC. Median LIC values were 4.58 mg/(g dw) (range 1.02–19.7) in patients with TM and 5.98 mg/(g dw) (range 1.11–19.02) in those with TI. In both groups (see Fig. 1), no correlation was found between LIC and TE results ($r = -0.14257$ and $r = 0.09$).

TABLE V. Variables Indipendently Associated With log-Transformed TE Values

Variables	β	*P* value
	Thalassemia major[a]	
ALT (IU/l)	0.33	0.01
GGT (IU/l)	0.63	0.001
Bilirubin total (mg/dl)	0.44	0.01
HCV-RNA positivity	0.28	0.03
	Thalassemia intermedia[b]	
ALT (IU/l)	0.22	0.007
GGT (IU/l)	0.23	0.01
Bilirubin total (mg/dL)	0.38	0.04

[a] R^2 for the multivariate model = 0.564; [b] R^2 for the multivariate model = 0.588.

Liver histology

Fourteen out of 115 patients underwent US-guided liver biopsy: 11 patients did it because they had good clinical conditions and they were able to start antiviral therapy. In three patients, liver biopsy was performed during surgical procedures (cholecistectomy or splenectomy). After the procedure, no major complications were observed. The mean length of liver cores was 34 mm (range 15–42). The median LIC value in these patients was 308 ng/mL (range 15–900). As shown in Fig. 2, the histological stage of liver fibrosis was significantly related to TE results ($r = 0.73$, $P = 0.003$), whereas the histological score did not correlate with LIC values. Table VI details the diagnostic performances of TE in diagnosing severe fibrosis and cirrhosis. In particular, a TE cut-off of 10.3 kPa diagnosed severe fibrosis with a sensitivity of 60% (95%CI: 15–95) and a specificity of 89% (95%CI: 52–99) (corresponding LR +5.4 and LR −0.4), whereas a TE cut-off of 12 kPa diagnosed cirrhosis with a sensitivity of 100% (95% CI: 23–100) and a specificity of 92% (95% CI: 62–99), LR +12 and LR −0.1, respectively.

Discussion

The present study indicates that TE is reliable in detecting severe fibrosis and cirrhosis in thalassemia patients in whom TE results are independently associated with biochemical activity and severity of liver disease, related to both chronic HCV infection and iron overload, both consequences of chronic transfusion regimen and increased gastrointestinal absorption [1,2,5,6].

To define the hepatic damage in thalassemia patients, mainly those with active HCV chronic infection, the main steps are the assessment of the degree of hepatic fibrosis (to decide the need of an antiviral regimen) and the evaluation of the amount of iron overload (to tailor iron chelation therapy). Iron overload and HCV infection represent independent risk factors for progression of liver fibrosis in thalassemia patients following bone marrow transplantion [32], and data in transfusion-dependent thalassemia patients have shown a mild liver necroinflammation due to HCV infection but frequent significant fibrosis, the progression of which is largely influenced by iron overload [6].

TE, a recently developed, simple and noninvasive technique to measure hepatic stiffness, has been shown to be a reliable tool to estimate the degree of hepatic fibrosis in patients with chronic liver disease, mainly in those with chronic HCV infection [11–22]. In β-thalassemia patients (especially those HCV infected) at higher risk of liver biopsy related complications as compared to other chronically HCV-infected patients, the availability of a noninvasive method to measure hepatic fibrosis is crucial. Current data in this setting are scattered, even if preliminary studies in 13 patients with β-TM and post-transfusional iron overload

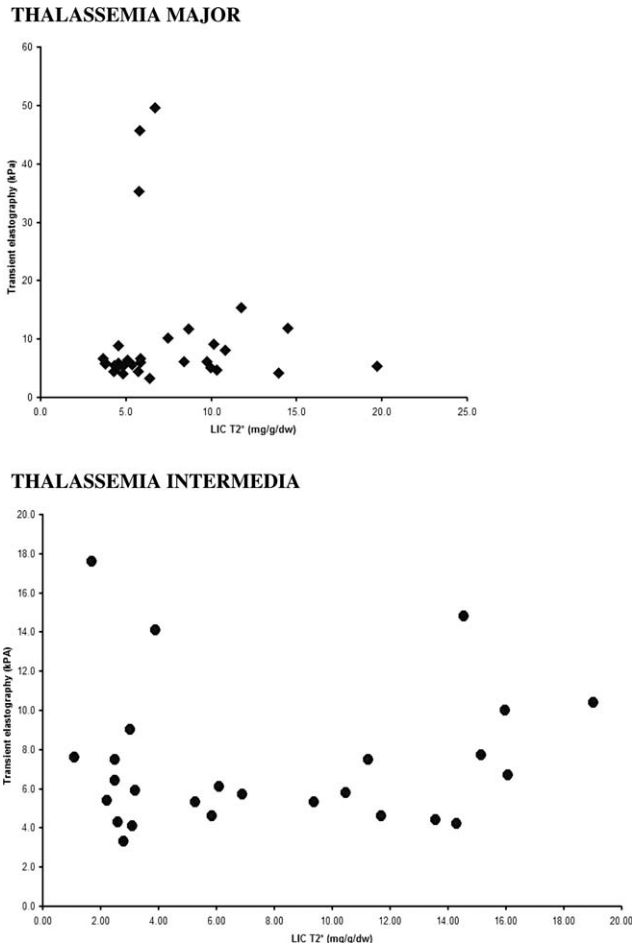

Figure 1. Correlation between TE results and liver T2* (LIC T2*) [mg/(g dw)].

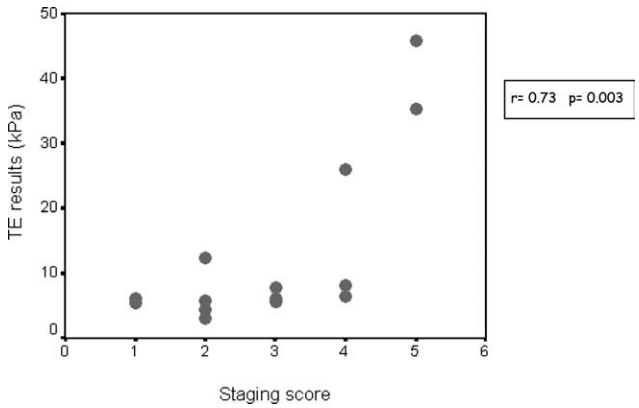

Figure 2. Relationship between TE and fibrosis stage.

TABLE VI. TE Operative Characteristics in Detecting Severe Fibrosis ($S \geq 4$) and Cirrhosis ($S = 5–6$) in 14 Patients With Thalassemia Major Who Concurrently Underwent TE and Liver Biopsy

TE values	$S \geq 4$	
	Yes	No
$>$ 10.3 kPas	3	
$\geq$ 10.3 kPas	2	8
Total (n. 14)	5 (35%)	9 (65%)

Sensitivity: 60% (95%CI: 15–95), −LR: 0.4, NPV: 80%.
Specificity: 89% (95%CI: 52–99), +LR:5.4, PPV: 75%.
95% confidence intervals (95%CI) are given in parenthesis.

[25] and in 56 consecutive patients with homozygous β-thalassemia [24] reported that TE can reliably diagnose advanced liver fibrosis.

In contrast to previous series, which reported a rate of failure ranging from 2 to 8% [33], we did not experience any TE measurement failure in our group of patients. The possible reason for this success rate is represented by the low median BMI in patients with β-TM (22.5 kg/m^2) and TI (21.6 kg/m^2), with only five cases with a BMI higher than 28 kg/m^2. This is in line with recent data [33] indicating a high BMI ($>$28 kg/m^2) as the main factor negatively interfering with TE measurement.

A further possible confounding factor that has recently been demonstrated to increase TE values is increased venous pressure, as in the presence of cardiac insufficiency [34]. In our series, none of the patients showed clinical signs of severe cardiac insufficiency.

Interestingly, as reported in chronic liver disease of different etiologies, liver fibrosis is the most relevant factor influencing liver stiffness [11–22], although other variables were found to independently and significantly affect TE results [12–14,20,21,23]. In the present study, focused on hepatic necroinflamation in TM and TI patients, the severity of the underlying liver disease and, in only the subset with TM, the activity of concomitant HCV chronic infection, played a pivotal role. A relationship between TE results and necroinflammatory hepatic activity has already been reported in acute viral hepatitis [12,14,20,35] and in patients with chronic hepatitis B during a hepatitis flare up [13].

Interestingly, in thalassemic patients, ALT levels were also related to liver stiffness value, whereas it is less clear from the literature if a correlation exists between serum ALT levels, a sensitive marker of necroinflammation of the liver, and liver stiffness in patients with chronic hepatitis C. Also, gammaglutamyltranspeptidase (GGT) levels have been reported to affect TE values, as observed in graft recipients with recurrent HCV infection [20]. A relationship between GGT levels and TE levels could further support the high predictive value of GGT, a marker used in different prognostic models for staging hepatic fibrosis [36,37].

Bilirubin levels were also found to be significantly associated with TE values in β-thalassemics, possibly reflecting the rate of ineffective erythropoiesis.

An additional relevant finding of our study was the lack of correlation between liver iron concentration and the other iron-related parameters and TE values. This is of particular interest in view of the realistic employment of TE in thalassemic patients. Usually, a correlation is observed between the degree of hepatic fibrosis and iron overload: Jensen et al. in 2003 showed a critical LIC range above which hepatocellular injury development may exist [38]. Usually, iron overload may be concomitantly present in advanced fibrosis stages; on the other hand, iron overload could act as a confounding factor in the measurement of liver stiffness, thus rendering the actual TE prediction of hepatic fibrosis less reliable. The finding that LIC unaffected liver stiffness turns into a more consistent use of TE in thalassemic patients, as in patients with primary or secondary iron overload of different etiology and it is in accord with previous findings in homozygous β-thalassemia patients [24]. As seen in the present study, increased values of TE were observed in TM patients with higher ferritin levels and in transfused TI patients. Moreover, splitting TM patients on the basis of both ferritin values and HCV viremia showed that the highest TE values were observed in patients with serum ferritin $>$1000 ng/ml and HCV-RNA positivity, thus

suggesting a possible association between iron overload and ongoing HCV.

A possible limitation of this paper is related to the lack of liver biopsy in most patients. According to current clinical practice, however, this procedure is limited to only HCV viremic patients mainly in order to define the degree of both necroinflammation and fibrosis, as the recent introduction of valid non invasive technique (such as T2* MRI) allows us to precisely define the amount of iron overload [28–30].

In conclusion, TE represents a reliable tool for assessing liver fibrosis in patients with β-thalassemia in whom TE values have been shown to be independently associated with the biochemical activity and the severity of liver disease. Iron overload, in association with HCV infection, may lead to an increased rate of fibrosis detected at TE, but further investigations are needed in a larger cohort of patients before this can be determined.

References

1. Olivieri NF. The β-thalassemias. N Engl J Med 1999;341:99–109.
2. Rund D, Rachmilewitz E. β-Thalassemia. N Engl J Med 2005;353:1135–1146.
3. Higgs DR, Thein SL, Wood WG.The pathophysiology of the thalassaemias. In:Weatherall DJ,Clegg B, editors. The Thalassaemia Syndromes,4th ed. Oxford, England: Blackwell Science; 2001. pp 192–236.
4. Cohen AR, Galanello R, Pennell DJ, et al. Thalassemia. Hematology (Am Soc Hematol Educ Program) 2004;1:14–34.
5. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89:1187–1193.
6. Prati D, Maggioni M, Milani S, et al. Clinical and histological characterization of liver disease in patients with transfusion-dependent β- thalassemia. A muticenter study of 117 cases. Haematologica 2004;89:1179–1186.
7. Cunningham MJ, Macklin EA, Neufeld EJ, et al. Complications of β-thalassemia major in North America. Blood 2004;104:34–39.
8. Sanada M, Ebara M, Fukuda H, et al. Clinical evaluation of sonoelasticity measurement in liver using ultrasonic imaging of internal forced low-frequency vibration. Ultrasound Med Biol 2000;26:1455–1460.
9. Sandrin L, Tanter M, Gennisson JL, et al. Shear elasticity probe for soft tissues with 1-D transient elastography. IEEE Trans Ultrason Ferroelectr Freq Control 2002;49:436–446.
10. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: A new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705–1713.
11. Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology 2005;41:48–54.
12. Fraquelli M, Rigamonti C, Casazza G, et al. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. Gut 2007;56:968–973.
13. Coco B, Oliveri F, Maina AM, Ciccorossi P, et al. Transient elastography: A new surrogate marker of liver fibrosis influenced by major changes of transaminases. J Viral Hepat 2007;14:360–369.
14. Arena U, Vizzutti F, Corti G, et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. Hepatology 2008;47:380–384.
15. Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343–350.
16. Foucher J, Chanteloup E, Vergniol J, et al. Diagnosis of cirrhosis by transient elastography Fibroscan(R): A prospective study. Gut 2006;55:403–408.
17. Colletta C, Smirne C, Fabris C, et al. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. Hepatology 2005;42:838–845.
18. Saito H, Tada S, Nakamoto N, et al. Efficacy of non-invasive elastometry on staging of hepatic fibrosis. Hepat Res 2004;29:97–103.
19. Ganne-Carrié N, Ziol M, de Ledinghen V, et al. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. Hepatology 2006;44:1511–1517.
20. Rigamonti C, Donato MF, Fraquelli M, et al. Transient elastography predicts fibrosis progression in patients with recurrent hepatitis C after liver transplantation. Gut 2008;57:821–827.
21. Talwalkar JA, Kurtz DM, Schoenleber SJ, et al. Ultrasound-based transient elastography for the detection of hepatic fibrosis: Systematic review and meta-analysis. Clin Gastroenterol Hepatol 2007;5:1214–1220.
22. Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient elastography for the staging of liver fibrosis: A meta-analysis. Gastroenterology 2008;134:960–974.
23. Millonig G, Reimann FM, Friedrich S, et al. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. Hepatology 2008;48:1718–1723.
24. Di Marco V, Bronte F, Cabibi D, et al. A noninvasive assessment of liver fibrosis in thalassaemia major patients by transient elastography (TE) - lack of interference by iron deposition. Br J Haematol 2010;148:476–479.
25. Mirault T, Lucidarme D, Turlin B, et al. Non-invasive assessment of liver fibrosis by transient elastography in post transfusional iron overload. Eur J Haematol 2008;80:337–340.
26. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–699.
27. Cogliandro T, Derchi G, Mancuso L, et al. Guidelines recommendations for heart complications in thalassemia major. J Cardiovasc Med 2008;9:515–525.
28. Mavrogeni SI, Gotsis ED, Markussis V, et al. T2 relaxation time study of iron overload in β-thalassemia. MAGMA 1998;6:7–12.
29. Anderson LJ, Holden S, Davies B, et al. Cardiovascular T2* (T2 star) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001;22:2171–2179.
30. Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle-cells disease patients. Blood 2005;106:1460–1465.
31. Fleiss JL. The Design and Analysis of Clinical Experiments. New York: Wiley; 1986. p 7.
32. Angelucci E, Muretto P, Nicolucci A, et al. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100:17–21.
33. Castera L, Foucher J, Bernard PH, et al. Pitfalls of liver stiffness measurement: A 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828–835.
34. Millonig G, Friedrich S, Adolf S, et al. Liver stiffness is directly influenced by central venous pressure. J Hepatol 2010;52:206–210.
35. Sagir A, Erhardt A, Schmitt M, et al. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatology 2008;47:592–595.
36. Adams LA, Bulsara M, Rossi E, et al. Hepascore: An accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem 2005;51:1867–1873.
37. Imbert-Bismut F, Messous D, Thibault V, et al. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and references ranges in healthy blood donors. Clin Chem Lab Med 2004;42:323–333.
38. Jensen PD, Jensen FT, Christensen T, et al. Relationship between hepatocellular injury and transfusional iron overload prior to and during iron chelation with desferrioxamine: A study in adult patients with acquired anemias. Blood 2003;101:91–96.