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Prevalence and predictors of liver fibrosis evaluated by vibration controlled transient elastography in type 1 Gaucher disease

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Abstract

Background & aims

Long-term liver-related complications of Gaucher disease (GD) include cirrhosis, portal hypertension and hepatocellular carcinoma. Although liver fibrosis is the main determinant of adverse liver-related clinical outcomes, it has rarely been evaluated in previously published cohorts of GD patients. We aimed at: assessing the prevalence of significant liver fibrosis in a cohort of patients with type 1 GD; identifying its predictors among GD-related variables, enzyme replacement therapy (ERT) and metabolic features.

Methods

37 adult type 1 GD patients from two Italian academic referral centers were prospectively submitted to vibration controlled transient elastography (Fibroscan®); significant fibrosis was defined as liver stiffness ≥7 kPa.

Results

Median liver stiffness was 4.6 [3–15.1] kPa and 7 patients (19%) had significant fibrosis. Significant fibrosis was associated with splenectomy ( $p=0.046$ ) and with scores (DS3:  $p=0.002$ ; SSI:  $p=0.026$ ) and biomarkers (ACE:  $p=0.016$ ; HDL cholesterol:  $p=0.004$ ) of GD severity. Length of ERT was significantly lower in GD patients with significant fibrosis. In the subgroup of 29 patients who were on stable ERT for at least 24 months, further to splenectomy, GD severity and non-N370S GBA1 genotypes, also diastolic blood pressure, BMI and the number of metabolic syndrome (MetS) components emerged as factors significantly associated with significant fibrosis.

Conclusions

Significant fibrosis is present in a remarkable proportion of adult type 1 GD patients. Splenectomy, GD severity and GBA1 genotypes are major GD-related predictors of liver fibrosis. Length of ERT is inversely correlated with liver disease in GD patients, suggesting a beneficial effect of ERT on liver fibrosis. However, GD patients on stable ERT should be monitored for metabolic complications, since MetS features may enhance liver

disease progression despite optimal GD control.

**Keywords**: Enzyme replacement therapy; Glucocerebrosidase deficiency; Liver stiffness; Metabolic syndrome; Vibration controlled transient elastography

**Abbreviations**: GD, Gaucher Disease; GBA, glucocerebrosidase; HCC, hepatocellular carcinoma; VCTE, vibration controlled transient elastography; ERT, enzyme replacement therapy; GD1 DS3, Disease Severity scoring system for type 1 Gaucher disease; SSI, Severity Scoring Index; MRI, magnetic resonance imaging; ACE, angiotensin converting enzyme; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotrasferase; GGT, gamma-glutamyltranspeptidase; HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high-density lipoprotein; MetS, metabolic syndrome; ATPIII, Adult Treatment Panel III; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease

## 1.1 Introduction

Gaucher disease (GD) is a rare lysosomal storage disorder caused by deficiency of the enzyme glucocerebrosidase (GBA), which leads to glycosphingolipids accumulation in macrophages (Gaucher cells) of several parenchymal tissues, predominantly the bone marrow, spleen and liver. The most prevalent clinical form, type 1 or non-neuronopathic GD, is mainly characterized by bone marrow infiltration with skeletal disease and visceral involvement with marked splenomegaly [1, 2]. Several patterns of liver involvement have been reported in type 1 GD [3]. Hepatomegaly, due to the hepatic infiltration by Gaucher cells, with or without liver enzymes abnormalities, is found in the majority of GD patients and frequently has no significant clinical consequences [4–6]. However, long-term liver-related complications, including cirrhosis, portal hypertension and hepatocellular carcinoma (HCC), may occur in some individuals and are associated with a worse prognosis [4, 6, 7]. Since it has been shown that the risk for liver-related complications in GD patients is mainly driven by the presence of liver fibrosis [4, 6, 7], the assessment of fibrosis represents an important step in identifying those patients at increased risk for developing clinical sequelae of advanced liver disease.



Liver biopsy represents the gold standard for diagnosing and staging liver fibrosis. However, the systematic evaluation of liver fibrosis by liver biopsy in GD patients, who have an increased bleeding tendency, is unsuitable since this procedure is limited by a non-negligible rate of complications. Several imaging methods, which allow a non-invasive and accurate estimation of liver fibrosis, are now available [8]. Vibration controlled transient elastography (VCTE) is the prototypic and most popular example of ultrasound-based elastographic methods. It relies on the estimation of the velocity of shear waves propagating through the liver to assess liver stiffness, which provides an indication on fibrosis [9]. Several studies including patients with chronic liver disease of different etiology consistently reported that VCTE has a good diagnostic performance in the identification of clinically relevant liver fibrosis [10], and may reliably predict the presence and the development of liver-related complications [11]. Current guidelines recommend the use of ultrasound-based elastography techniques, including VCTE, for assessing disease severity in chronic liver disease [8]. As such, VCTE could be a useful tool for screening GD patients for the presence of liver fibrosis.


To date it is unknown which GD patients are at higher risk for developing liver fibrosis and consequentially are more likely to progress to long-term liver-related complications. GD patients with more severe and longstanding disease and multi-organ manifestations have been reported to have a higher degree of liver involvement [4, 6]. Splenectomy, a procedure frequently performed in the past to alleviate symptoms of severe splenomegaly, has been suggested as a potential risk factor for advanced liver involvement [4, 6, 7]. Several treatment strategies, enzyme replacement therapy (ERT) and substrate reduction therapy, are currently available and have been proven to be effective in reversing clinical signs and symptoms of GD [12]. However, the impact of treatment on liver fibrosis is unknown. Moreover, the presence of concurrent liver diseases, such as chronic viral hepatitis or vascular liver diseases, frequently observed in GD patients who underwent blood transfusions and surgical procedures, and metabolic abnormalities, such as insulin resistance or increased adiposity, associated with GD itself, treatment or lifestyle habits, may contribute to the development and progression of liver fibrosis [13].

In this prospective study, we aim to assess the prevalence and the predictors of significant liver fibrosis evaluated by VCTE (Fibroscan®) in adult type 1 GD patients from two Italian GD referral centers.

## 2.2 Patients and methods

### 2.1.2.1 Patients

This study enrolled consecutive adult patients with a diagnosis of type 1 GD, confirmed on the basis of deficient GBA activity in leukocytes and defined mutations in the human GBA1 gene, monitored at two Italian academic referral centers (Emilia-Romagna Regional Referral Centre for Lysosomal Storage Diseases, Division of Internal Medicine and Metabolism, Civil Hospital, AOU Modena; Centre of Rare Diseases, Division of Internal Medicine, Foundation IRCCS  Ca' Granda  Ospedale Maggiore Policlinico, Milan) between 2015 and 2017.

A complete medical history was retrieved.  All patients underwent careful assessment of GD severity and evaluation of anthropometric and metabolic parameters. Moreover, VCTE with Fibroscan® for evaluation of liver stiffness was performed. Concurrent causes of liver disease were checked through medical history, imaging and biochemical examinations. Dietary and lifestyle habits, including the amount of alcohol consumption, were also

registered.

The study was approved by the local ethics committees, and all participants gave written informed consent according to the Helsinki Declaration.

## 2.2.2.2 Gaucher disease severity evaluation

Various GD parameters, including age at diagnosis, GBA1 genotype, history of splenectomy, dose and duration of ERT, and GD severity, were assessed. GBA1 genotypes were divided in three groups according to the number of N370S mutant variants (homozygous N370S/N370S, compound heterozygous N370S/other mutant variant, two non-N370S mutant variants). In GD patients receiving ERT the cumulative dose of ERT (U/kg) was calculated by multiplying the total months of ERT by the mean dose of ERT (U/kg/months) since the start of treatment. GD severity at the time of evaluation was assessed using two validated scores: the Disease Severity scoring system for type 1 GD (GD1 DS3) [14] and the Zimran’s Severity Scoring Index (SSI) [5]. Moderate-marked GD was defined as DS3 ≥ 3 or SSI > 10, respectively. Liver and spleen volume were measured by magnetic resonance imaging (MRI). Serum angiotensin converting enzyme (ACE) and chitotriosidase activity were also determined.

## 2.3.2.3 Anthropometric and metabolic parameters

Weight, height and waist circumference were measured in all GD patients at the time of evaluation. The body weight and height were obtained without shoes and clothes. Waist circumference was measured midway between the lowest rib margin and the iliac crest. Body Mass Index (BMI) was calculated according to the formula: weight (kg) divided by height squared (m<sup>2</sup>). Central obesity was defined as waist circumference > 102 cm in men and ≥ 88 cm in women. Blood pressure was measured in the sitting position after 10 minutes of rest. High blood pressure was defined as diastolic blood pressure ≥ 130 mm Hg and/or systolic blood pressure ≥ 85 mm Hg and/or use of anti-hypertensive drugs. On the same day of the study visit a fasting blood sample was obtained from each patient for measurements of glucose, insulin, glycated hemoglobin (HbA1c), lipid profile, alanine and aspartate aminotransferases (ALT and AST), gamma-glutamyltranspeptidase (GGT), ferritin, ACE, chitotriosidase, hemoglobin, platelets count and albumin. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: [fasting insulin (mIU/L) × fasting glucose (mg/dl)] / 405. Altered glucose homeostasis was defined as a fasting glucose ≥ 110 mg/dl, hypertriglyceridemia as fasting triglycerides levels ≥ 150 mg/dl and low high-density lipoprotein (HDL) cholesterol as HDL cholesterol levels < 40 mg/dl in men and < 50 mg/dl in women. Metabolic syndrome (MetS) was diagnosed according to Adult Treatment Panel III (ATPIII) criteria by the concomitant presence of at least 3 conditions among central obesity, high blood pressure, altered glucose homeostasis, hypertriglyceridemia and low HDL cholesterol [15].

## 2.4.2.4 Vibration controlled transient elastography

VCTE (Fibroscan®) combines a vibrator and an ultrasound transducer probe mounted on its axis. The vibrating device generates an elastic shear wave that propagates through the liver parenchyma; the pulse-echo ultrasound acquisition follows the propagation of the shear wave and measures its velocity, which directly correlates with liver stiffness: the stiffer the liver, the faster the shear wave propagates. VCTE measures liver stiffness in a cylinder 1 cm wide and 4 cm long, a volume that is at least 100 times bigger than a biopsy sample. Two Fibroscan® probes are available: the M probe and the XL probe; the latter has been designed specifically for use in obese patients. Results, which are automatically generated by the software, are expressed in kiloPascals (kPa) and correspond to the median of 10 valid measurements. Liver stiffness values range from 2.5 to 75 kPa [9]. VCTE has an excellent inter- and intra-operator reproducibility, and variability has been shown to further improve when the experience of the operator is higher than 100 exams [16].

All patients were submitted to VCTE for evaluation of liver stiffness on the same day as the study visit. Liver stiffness measurements were performed by using the M probe, or the XL probe when indicated, after at least 3 hours of fasting, by two highly experienced operators (>500 exams). Measurements were conducted on the right lobe of the liver in an area without focal lesions, obvious scarring or large vessels, through intercostal spaces with the patient lying in dorsal decubitus with the right arm in maximal abduction. In all patients we were able to obtain successful measurements defined by the median of at least 10 valid shots with a success rate of at least 60% and an interquartile range lower than 30% of the median of measurements, as suggested by the manufacturing company. None of the patients presented with aminotransferases flares, cholestasis, congestive heart failure or other medical conditions known to affect the interpretation of VCTE measurements.

Studies reported that normal liver stiffness values range from 3 to 6 kPa in healthy populations [9, 17]. Since a liver stiffness cut-off of 7 kPa has been considered consistent with significant fibrosis in several studies enrolling patients with different etiologies of liver disease, including a previous study among patients with GD [18], we also defined significant fibrosis (stage F2 or higher) as liver stiffness values ≥ 7 kPa.

## 2.5.2.5 Statistical analysis

Results were expressed as median [range] for numerical variables and frequencies (percentages) for categorical variables. Medians were compared by the Mann-Whitney *U* test and nominal variables by the Pearson’s Chi-square test. Spearman’s rho was calculated for correlations between liver stiffness values and other parameters. Because treatment with ERT may significantly affect GD progression over time, sensitivity analyses according to the length of ERT were conducted. A two-sided *p* value < 0.05 was considered significant. Statistical analyses were performed using the statistical software package SPSS, version 17.0 for Windows (SPSS Inc., Illinois, USA).

### 3.3 Results

#### 3.1.3.1 General features of the overall study population

Thirty-seven adult patients with type 1 GD were recruited for this study: 20 GD patients from Modena and 17 from Milan. The main clinical features of the overall study population are shown in [Tables 1 and 2](#). Twenty-one patients were males; median age was 46 [18–78] years. The majority of patients (89%) were carriers of at least one N370S mutant variant of GBA1 and had mainly mild GD severity according to DS3 (1.1 [0–6.7]) and SSI (5 [1–16]) scores. Ten patients had undergone a splenectomy in the past. All but 2 patients were currently on ERT; the median ERT duration was 98 [0–294] months and 29 patients were treated for at least 24 months.

**Table 1.** General characteristics of 37 adult patients with Gaucher Disease.

alt-text: Table 1	
Study cohort	
Modena	20 (54)
Milan	17 (46)
Demographic data	
Age (years)	46 [18–78]
Male sex	21 (57)
Gaucher disease-related data	
Age at diagnosis (years)	28 [2–62]
Genotype	
N370S/N370S	10 (27)
N370S/other	23 (62)
Other/other	4 (11)
Gaucher disease severity	
DS3	1.1 [0–6.7]
Moderate-marked GD (DS3)	11 (30)
SSI	5 [1–16]
Moderate-marked GD (SSI)	6 (16)
Splenectomy	10 (27)
Liver volume (cc) <sup>a</sup>	1450 [1106–2826]
Spleen volume (cc) <sup>a</sup>	426 [203–975]
On ERT	35 (95)
Age at start of ERT (years)	38 [4–76]
Months of ERT	98 [0–294]
Dose of ERT (U/kg/month)	60 [0–120]
Cumulative dose of ERT (U/kg)	5250 [0–16,200]

Anthropometric data		
Weight (kg)		69 [46-104]
BMI (kg/m²)		24 [18-31]
BMI ≥ 25 kg/m²		15 (41)
Waist circumference (cm) <sup>a</sup>		91 [69-120]
Metabolic features		
Central obesity		6 (16)
Altered glucose homeostasis		4 (11)
High blood pressure		14 (38)
Diastolic blood pressure (mm Hg)		75 [60-90]
Systolic blood pressure (mm Hg)		120 [100-150]
Hypertriglyceridemia		5 (14)
Low HDL cholesterol		16 (43)
No of factors of metabolic syndrome		1 [0-4]
Metabolic syndrome (ATPIII)		6 (16)
Biochemical data		
ACE (IU/L) <sup>a</sup>		31 [5-191]
Chitotriosidase (nmol/ml/h) <sup>a</sup>		1333 [10-59,312]
Ferritin (ng/ml)		144 [4-1013]
Platelets (1000/mm <sup>3</sup> )		185 [88-453]
AST (IU/L)		21 [11-36]
ALT (IU/L)		20 [8-58]
GGT (IU/L)		25 [5-143]
Albumin (g/dl)		4.4 [3.5-5]
Glucose (mg/dl)		89 [72-375]
Insulin (mIU/L)		6.7 [2.1-43]
HOMA-IR (%)		1.5 [0.5-33]
HbA1c (%) <sup>a</sup>		5.2 [3.8-8.7]
Triglycerides (mg/dl)		115 [25-275]
Total cholesterol (mg/dl)		178 [91-282]
HDL cholesterol (mg/dl)		44 [30-125]
LDL cholesterol (mg/dl)		111 [39-208]

Transient elastography data	
Liver stiffness (kPa)	4.6 [3–15.1]
Significant fibrosis	7 (19)
Other data	
Alcohol consumption (g/day)	0 [0–50]
Known concurrent liver disease	4 (11) <sup>b</sup>

<sup>a</sup> Data available for n° patients: liver volume 20 patients; spleen volume 13 patients; waist circumference 35 patients; ACE 20 patients; chitotriosidase 20 patients; HbA1c 33 patients.

<sup>b</sup> 2 patients with cleared HCV infection and 2 patients with post-splenectomy portal vein thrombosis.

**Table 2.** Main characteristics of 37 adult patients with Gaucher disease.

alt-text: Table 2

Patient Number	Age (yrs); Sex (M/F)	Genotype	GD Severity (DS3; SSI)	Sx (Y/N)	On ERT (Y,N; type; months; mean dose (U/kg/month))	Liver stiffness (kPa)	Known concurrent liver disease	ALT (U/L); AST (U/L); GGT (U/L)	Ferritin (ng/ml)	Alcohol consumption (g/day)	BMI (Kg/m²)	Metabolic syndrome (Y/N)
MO1	42; M	N370S/N370S	DS3: 0.4 SSI: 6	N	Y; Imiglucerase; 236; 35	6.8	N	24; 17; 37	70	50	31.4	Y
MO2	61; M	N370S/N370S	DS3: 3.3 SSI: 8	Y	Y; Imiglucerase; 191; 55	4.3	N	17; 18; 65	146	20	24.1	N
MO3	55; F	N370S/RecNcil	DS3: 0.4 SSI: 3	N	Y; Imiglucerase; 244; 50	3.8	N	12; 16; 13	37	0	24.1	N
MO4	37; M	N370S/RecNcil	DS3: 4.4 SSI: 12	N	Y; Imiglucerase; 9; 120	4.9	N	58; 36; 24	518	20	25.0	N
MO5	56; F	N370S/N370S	DS3: 0.4 SSI: 2	N	Y; Imiglucerase; 216; 45	3.8	N	22; 21; 21	38	0	19.1	N
MO6	54; F	R353G/R353G	DS3: 4.1 SSI: 14	Y	Y; Imiglucerase; 150; 35	11.3	N	19; 26; 54	35	0	28.7	N
MO7	49; M	L444P/RecNcil	DS3: 3.3 SSI: 9	Y	Y; Imiglucerase; 275; 45	6.8	Post-splenectomy portal thrombosis	22; 22; 88	203	30	29.0	Y
MO8	74; M	N370S/L444P	DS3: 0 SSI: 4	N	Y; Imiglucerase; 83; 45	3.0	N	21; 17; 33	67	0	24.7	N
MO9	64; F	N370S/N370S	DS3: 0 SSI: 4	N	Y; Imiglucerase; 110; 50	4.5	N	20; 22; 34	318	0	26.0	Y
MO10	78; M	N370S/L385P	DS3: 3.6 SSI: 11	N	Y; Imiglucerase; 24; 60	3.6	N	10; 26; 27	342	25	26.0	N
MO11	76; F	N370S/L444P	DS3: 1.7 SSI: 5	Y	N (previously treated with Imiglucerase); 108; 30	6.6	N	21; 19; 13	29	0	21.1	N
MO12	38; M	N370S/H255Q+L409H	DS3: 1.1 SSI: 6	N	Y; Imiglucerase; 98; 50	4.6	N	25; 21; 46	117	0	25.0	N
MO13	42; M	N370S/R170P	DS3: 0.4 SSI: 2	N	Y; Imiglucerase; 148; 65	4.4	N	19; 25; 34	69	5	21.6	N
MO14	27; F	N370S/N370S	DS3: 3.4	N	Y; Imiglucerase; 15; 120	7.8	N	8; 15; 5	4	0	20.5	N





MI16	57; F	N370S/L444P	DS3: 3.6 SSI: 7	N	Y; Imiglucerase; 138; 60	3.4	N	14; 20; 18	34	12	20.0	N
MI17	33; M	N370S/RecNcil	DS3: 0.6 SSI: 4	N	Y; Imiglucerase; 18; 30	7.2	N	43; 29; 30	526	0	26.5	N

Sx~~+~~: Splenectomy; MO~~+~~: Modena; MI~~+~~: Milan; M~~+~~: male; F~~+~~: female; Y~~+~~: yes; N~~+~~: No; GD~~+~~: Gaucher Disease; ERT~~+~~: enzyme replacement therapy; BMI~~+~~: body mass index; LTR~~+~~: long-term responder; IFN~~+~~: interferon; HCV~~+~~: hepatitis C virus

The median liver stiffness value was 4.6 [3~~-~~15.1] kPa; 7 patients (19%) showed a liver stiffness  $\geq$ 7 kPa consistent with the presence of significant fibrosis. Other etiologies of liver disease, including HCV and HBV infections, autoimmune liver diseases, vascular liver diseases and other inherited liver diseases were evaluated in all the patients. Four patients had potentially concurrent liver diseases: 2 patients developed post-splenectomy portal vein thrombosis and were on anticoagulant medications; 1 patient had spontaneously cleared HCV infection and 1 patient was a long-term responder to interferon-based therapy for post-transfusion chronic HCV infection. Liver enzymes were generally in the normal range or only mildly elevated. Ferritin levels exceeded 300 ng/ml in 11 patients. A significant proportion of patients (41%) were overweight or obese with a BMI  $\geq$  25 kg/m<sup>2</sup>, had high blood pressure (38%) and low HDL cholesterol (43%), but the full-blown MetS was present only in 6 patients. Alcohol consumption was null or mild in the majority of patients; 6 patients consumed  $\geq$ 20 g/day of alcohol.

### 3.2.3.2 Factors associated with liver stiffness

Liver stiffness values were not associated with the study cohort ( $p_i$ =~~0~~.478) and demographic factors, such as male gender ( $p_i$ =~~0~~.457) and age.

Several GD-related variables were significantly associated with liver stiffness (Table 3). In particular, there was a positive and significant correlation between liver stiffness and GD severity as assessed by both DS3 (rho<sub>i</sub>=0.499,  $p_i$ =~~0~~.002) and SSI (rho<sub>i</sub>=0.385,  $p_i$ =~~0~~.019) scores (Figure 1, panels A and B). Consistently, patients with moderate-marked GD had significantly higher liver stiffness values than patients with mild disease according to DS3 (6.8 [3.4~~-~~11.3] vs. 4.4 [3~~-~~15.1] kPa,  $p_i$ =~~0~~.031). A similar although not statistically significant trend was also observed for patients with moderate-marked disease according to SSI (6.3 [3.6~~-~~11.3] vs. 4.4 [3~~-~~15.1] kPa,  $p_i$ =~~0~~.125). Liver stiffness was also significantly correlated with some, but not all, biomarkers of GD activity. Indeed, liver stiffness resulted positively correlated with ACE (rho<sub>i</sub>=0.516,  $p_i$ =~~0~~.020) and negatively correlated with HDL cholesterol (rho<sub>i</sub>=~~-~~0.423,  $p_i$ =~~0~~.009) (Figure 1, panels C and D); however, we were not able to demonstrate a significant correlation between liver stiffness and chitotriosidase activity ( $p_i$ =~~0~~.798), probably due to the small sample size and missing values. Similarly, ferritin ( $p_i$ =~~0~~.836) and platelets ( $p_i$ =~~0~~.649) were not associated with liver stiffness. With regards to GBA1 genotype, patients carrying two non-N370S mutant variants showed a not statistically significant higher liver stiffness than patients carrying at least one N370S mutant variant (7.3 [3.1~~-~~11.3] vs. 4.5 [3~~-~~15.1] kPa,  $p_i$ =~~0~~.262). Patients with a past history of splenectomy presented significantly higher liver stiffness than patients without (6.7 [3.3~~-~~11.3] vs. 4.4 [3~~-~~15.1] kPa,  $p_i$ =~~0~~.021). Age at start, dose and length of ERT were not correlated with liver stiffness. Patients receiving a mean dose  $\geq$ 60 U/kg/month of ERT had liver stiffness values similar to those receiving <60 U/kg/month of ERT ( $p_i$ =~~0~~.845). Of note, the 8 patients who had received ERT for ~~less than~~  $\leq$ 24 months had significantly higher values of liver stiffness than the 29 patients who were on stable ERT for at least 24 months (7.5 [4.4~~-~~15.1] vs. 4.3 [3.0~~-~~11.3] kPa,  $p_i$ =~~0~~.002).

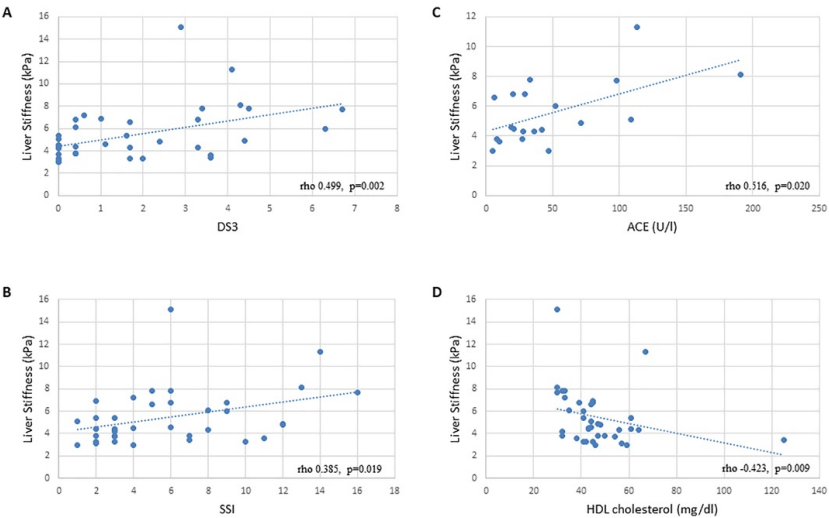
**Table 3.****Table 3** Correlation of Liver Stiffness with other relevant variables.

alt-text: Table 3

	Spearman's rho	p
Demographic data		
Age (years)	<del>-</del> 0.025	0.884
Gaucher Disease-related data		
Age at diagnosis (years)	<del>-</del> 0.083	0.626
DS3	0.499	<b>0.002</b>
SSI	0.385	<b>0.019</b>
Liver volume (cc) <sup>a</sup>	0.066	0.782
Spleen volume (cc) <sup>a</sup>	0.322	0.283

Age at start of ERT (years)	0.079	0.644
Months of ERT	<div><div></div><div></div><div></div></div> -0.156	0.356
Dose of ERT (U/kg/month)	<div><div></div><div></div><div></div></div> -0.059	0.728
Cumulative dose of ERT (U/kg)	<div><div></div><div></div><div></div></div> -0.221	0.190
Anthropometric data		
Weight (kg)	0.045	0.792
BMI (kg/m²)	0.154	0.364
Waist Circumference (cm) <sup>a</sup>	0.060	0.732
Metabolic features		
Diastolic blood pressure (mm Hg)	0.264	0.115
Systolic blood pressure (mm Hg)	0.037	0.829
N° of factors of metabolic syndrome	0.367	<b>0.026</b>
Biochemical data		
ACE (IU/L) <sup>a</sup>	0.516	<b>0.020</b>
Chitotriosidase (nmol/ml/h) <sup>a</sup>	0.061	0.798
Ferritin (ng/ml)	<div><div></div><div></div><div></div></div> -0.035	0.836
Platelets (1000/mm <sup>3</sup> )	0.077	0.649
AST (IU/L)	0.229	0.173
ALT (IU/L)	0.221	0.188
GGT (IU/L)	0.289	0.082
Albumin (g/dl)	<div><div></div><div></div><div></div></div> -0.174	0.302
Glucose (mg/dl)	0.211	0.210
Insulin (mIU/L)	<div><div></div><div></div><div></div></div> -0.045	0.790
HOMA-IR (%)	<div><div></div><div></div><div></div></div> -0.005	0.977
HbA1c (%) <sup>a</sup>	0.032	0.859
Triglycerides (mg/dl)	<div><div></div><div></div><div></div></div> -0.133	0.431
Total cholesterol (mg/dl)	<div><div></div><div></div><div></div></div> -0.504	<b>0.001</b>
HDL cholesterol (mg/dl)	<div><div></div><div></div><div></div></div> -0.423	<b>0.009</b>
LDL cholesterol (mg/dl)	<div><div></div><div></div><div></div></div> -0.353	<b>0.032</b>
Other data		
Alcohol consumption (g/day)	<div><div></div><div></div><div></div></div> -0.048	0.777

<sup>a</sup> Data available for n° patients: liver volume 20 patients; spleen volume 13 patients; waist circumference 35 patients; ACE 20 patients; chitotriosidase 20 patients; HbA1c 33 patients.



**Figure 1.** Fig. 1 Correlations between liver stiffness and scores and biomarkers of Gaucher disease severity in the overall cohort.

Panel A. Correlation between liver stiffness and DS3 score; Panel B. Correlation between liver stiffness and SSI score; Panel C. Correlation between liver stiffness and ACE; Panel D. Correlation between liver stiffness and HDL cholesterol.

alt-text: Fig. 1

Liver and spleen volumes and liver enzymes were not significantly correlated with liver stiffness. Among anthropometric and metabolic variables, only the number of factors of MetS was associated with liver stiffness ( $\rho=0.367$ ,  $p=0.026$ ); this association was largely conveyed by low HDL cholesterol, probably as a biomarker of GD activity [19]. Indeed, the parameters of adiposity, blood pressure, glucose profile and the presence of full-blown MetS ( $p=0.506$ ) were not associated with liver stiffness in the overall study population.

Liver stiffness was not correlated with the amount of alcohol consumption, whereas the 4 patients with potentially concurrent liver diseases had higher, although not statistically significant, liver stiffness values than patients without (7.3 [4.8–8.1] vs. 4.4 [3–15.1] kPa,  $p=0.051$ ).

### 3.3.3.3 Factors associated with significant fibrosis

A comparison of the main features of GD patients with and without significant fibrosis as evaluated by VCTE is shown in Table 4. The factors associated with the presence of significant fibrosis were consistent with the findings obtained for liver stiffness. In particular, patients with significant fibrosis presented statistically significant higher values of DS3 and SSI scores and more frequently had moderate-marked GD as defined by both scores than patients without significant fibrosis. Patients with significant fibrosis also showed higher levels of ACE and lower levels of HDL cholesterol, and were more often splenectomized than those without significant fibrosis. Moreover, there was a non-statistically significant overrepresentation of carriers of two non-N370S mutant variants among patients with significant fibrosis (29% vs. 7%,  $p=0.093$ ). Notably, while age at start and monthly dose of ERT were not associated with significant fibrosis, length and cumulative dose over time of ERT were significantly lower in GD patients with significant fibrosis than in those without. Five out of 7 patients with significant fibrosis (71%) and 3 out of 30 patients (10%) without significant fibrosis had received ERT for less than ≤24 months ( $p<0.001$ ). Among metabolic variables only low HDL cholesterol was significantly associated with significant fibrosis. GGT was the only liver enzyme significantly higher in patients with significant fibrosis.

**Table 4.** Table 4 Comparison between Gaucher Disease patients with and without significant fibrosis.

alt-text: Table 4

	Patients without significant fibrosis (n=30)	Patients with significant fibrosis (n=7)	p§
Study cohort			
Modena	16 (53)	4 (57)	0.855

Milan	14 (47)	3 (43)	
Demographic data			
Age (years)	48 [18-78]	44 [27-54]	0.312
Male Sex	18 (60)	3 (43)	0.410
Gaucher Disease-related data			
Age at diagnosis (years)	28 [2-62]	25 [4-42]	0.608
Genotype			0.218
N370S/N370S	8 (27)	2 (29)	
N370S/Other	20 (67)	3 (43)	
Other/Other	2 (7)	2 (29)	
Gaucher Disease severity			
DS3	0.4 [0-6.3]	4.1 [0.6-6.7]	<b>0.002</b>
Moderate-marked GD (DS3)	6 (20)	5 (71)	<b>0.007</b>
SSI	3.5 [1-12]	6 [4-16]	<b>0.026</b>
Moderate-marked GD (SSI)	3 (10)	3 (43)	<b>0.034</b>
Splenectomy	6 (20)	4 (57)	<b>0.046</b>
Liver volume (cc) <sup>a</sup>	1494 [1108-2567]	1289 [1106-2826]	0.494
Spleen volume (cc) <sup>a</sup>	403 [203-862]	975 [975-975]	0.154
On ERT	29 (97)	6 (86)	0.249
Age at start of ERT (years)	38 [4-76]	42 [25-52]	0.747
Months of ERT	109 [6-294]	17 [0-244]	<b>0.044</b>
Dose of ERT (U/kg/month)	60 [30-120]	60 [0-120]	0.985
Cumulative dose of ERT (U/kg)	5450 [360-16200]	1020 [0-15860]	<b>0.029</b>
Anthropometric data			
Weight (kg)	70 [46-104]	65 [47-86]	0.482
BMI (kg/m²)	24.1 [18.1-31.4]	23.3 [17.7-28.7]	0.894
BMI ≥ 25 kg/m²	12 (40)	3 (43)	0.890
Waist Circumference (cm) <sup>a</sup>	91.5 [69-120]	88 [75-104]	0.702
Metabolic features			
Central obesity	4 (13)	2 (29)	0.325
Altered glucose homeostasis	4 (13)	0 (0)	0.306
High blood pressure	11 (37)	3 (43)	0.761

Diastolic blood pressure (mmHg)	75 [60-90]	80 [70-90]	0.690
Systolic blood pressure (mmHg)	123 [100-150]	120 [100-140]	0.413
Hypertriglyceridemia	5 (100)	0 (0)	0.245
Low HDL cholesterol	10 (33)	6 (86)	<b>0.012</b>
N° of factors of metabolic syndrome	1 [0-4]	1 [1-3]	0.199
Metabolic syndrome (ATPIII)	5 (17)	1 (14)	0.878
Biochemical data			
ACE (IU/L) <sup>a</sup>	28 [5-109]	106 [33-191]	<b>0.016</b>
Chitotriosidase (nmol/ml/h) <sup>a</sup>	900 [96-6920]	12450 [10-59312]	0.290
Ferritin (ng/ml)	140 [29-1013]	144 [4-782]	0.985
Platelets (1000/mm <sup>3</sup> )	180 [88-387]	200 [96-453]	0.805
AST (IU/L)	20 [11-36]	26 [15-31]	0.077
ALT (IU/L)	20 [9-58]	27 [8-46]	0.128
GGT (IU/L)	22 [12-88]	52 [5-143]	<b>0.029</b>
Albumin (g/dl)	4.4 [3.8-5]	4 [3.5-4.9]	0.458
Glucose (mg/dl)	88 [73-375]	91 [72-99]	0.719
Insulin (mIU/L)	6.8 [2.1-43]	6.7 [3.2-15.3]	0.582
HOMA-IR (%)	1.5 [0.5-32.7]	1.4 [0.6-3.6]	0.556
HbA1c (%) <sup>a</sup>	5.2 [4.2-8.7]	5.3 [3.8-5.9]	0.914
Triglycerides (mg/dl)	116 [25-275]	115 [57-128]	0.690
Total cholesterol (mg/dl)	183 [95-282]	119 [91-193]	<b>0.021</b>
HDL cholesterol (mg/dl)	45 [32-125]	32 [30-67]	<b>0.004</b>
LDL cholesterol (mg/dl)	113 [51-208]	86 [39-138]	0.199
Transient elastography data			
Liver stiffness (kPa)	4.4 [3-6.9]	7.8 [7.2-15.1]	<b>&lt;0.001</b>
Other data			
Alcohol consumption (g/day)	0 [0-50]	0 [0-0]	0.227
Known concurrent liver disease	2 (7)	2 (29)	0.093

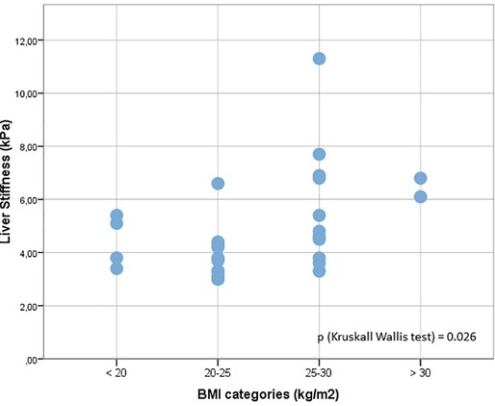
<sup>a</sup> Data available for no patients: liver volume 20 patients; spleen volume 13 patients; waist circumference 35 patients; ACE 20 patients; chitotriosidase 20 patients; HbA1c 33 patients.

<sup>§</sup> Pearson's Chi-square test or Mann-Whitney *U* test, where appropriate.

### 3.4.3.4 Sensitivity analyses according to the length of ERT

When considering only the 8 patients who had received ERT for **less than**  $\leq 24$  months, HDL cholesterol remained the single factor strongly and negatively associated with liver stiffness ( $\rho = -0.976$ ;  $p < 0.001$ ).

Analyses were also repeated among the 29 patients who were on stable ERT for at least 24 months. A past history of splenectomy ( $p = 0.009$ ), having moderate-marked GD according to DS3 ( $p = 0.004$ ) and SSI ( $p < 0.001$ ), carrying two non-N370S mutant variants ( $p < 0.001$ ) were all confirmed as factors significantly and positively associated with significant fibrosis in this subgroup of patients. Moreover, liver stiffness was significantly associated also with diastolic blood pressure ( $\rho = 0.424$ ,  $p = 0.022$ ), BMI ( $\rho = 0.419$ ,  $p = 0.024$ ) and the number of factors of MetS ( $\rho = 0.417$ ,  $p = 0.025$ ). Notably, liver stiffness increased across BMI categories ( $p = 0.026$ ) (Figure 2).



**Figure 2.** Fig. 2 Liver stiffness values across body mass index categories in the subgroup of patients on stable enzyme replacement therapy for at least 24 months

alt-text: Fig. 2

## 4.4 Discussion

In this study, we assessed the prevalence of significant liver fibrosis by measuring liver stiffness by VCTE (Fibroscan®) in a cohort of Italian adult patients with type 1 GD. Moreover, we tried to identify which factors, among GD-related variables, ERT treatment and metabolic features, were associated with liver stiffness and significant liver fibrosis. We showed that significant liver fibrosis was present in a remarkable proportion of adult GD patients, nearly 1 in 5 individuals. Splenectomy status, GD severity as assessed either by scores or biomarkers, and non-N370S GBA1 genotypes were found to be major GD-related predictors of liver stiffness and significant liver fibrosis. We also demonstrated that length of ERT treatment was inversely correlated with liver disease in patients with GD. Another interesting finding was that metabolic features, including blood pressure, BMI and the number of MetS components, were directly associated with liver stiffness in the subgroup of GD patients on stable ERT for at least 2 years.

Despite the clinical relevance of liver disease in GD, liver involvement and particularly liver fibrosis have rarely been evaluated in previously published cohorts of GD patients. In the pre-ERT era, James et al. conducted a comprehensive examination of liver abnormalities in 25 patients with GD, 22 of whom with available liver biopsy [4]. At least pericellular fibrosis was present in the vast majority of patients (19 out of 22), 6 of which presented fibrous septa and 3 liver cirrhosis; of note, fibrosis was associated with the accumulation of storage cells in the liver [4]. More recently, two separate groups from the Netherlands and Israel have assessed liver fibrosis by VCTE in GD patients [18, 20]. In the first study, Bohte et al. evaluated 14 type 1 GD patients and found a median liver stiffness of 6.9 kPa (range 3.4–16 kPa); the Authors were able to identify 7 patients, 50% of the entire cohort, with significant fibrosis by using our same cut-off of 7.0 kPa [18]. Similarly, in the second cohort comprising 42 type 1 GD patients, the median liver stiffness was as high as 7.1 kPa [20]. These figures are significantly higher than those reported in our cohort, where the median liver stiffness was 4.6 kPa and significant fibrosis was detected in 19% of patients. Substantial differences in the prevalence of splenectomy and genotypes, GD severity and treatment strategies between our cohort and the abovementioned studies may easily account for this seemingly discrepancy. Indeed, in the Dutch cohort 50% of GD patients were splenectomized (vs. 27% in our cohort) and there was a very high prevalence of non-N370S GBA1 genotypes [18]; whereas, in the cohort described by Webb et al. only 75% of patients were on ERT (vs. 95% in our cohort) [20]. Taken together with these studies, our findings confirm that VCTE is a feasible screening tool and allows the identification of clinically relevant liver fibrosis in a significant proportion of GD patients, net of the differences between GD cohorts.

For what concerns the predictors of liver fibrosis in GD, James et al., in their seminal study, were the first to report that the degree of liver involvement correlated with the severity of extrahepatic manifestations of GD [4]. A subsequent case series of 4 patients with GD and severe liver involvement confirmed that liver disease was strictly associated with severe GD with multi-organ involvement. In particular, all had undergone splenectomy and had high values of SSI score, and the 3 who had their GBA1 genotypes determined had non-N370S mutations [6]. Similar associations were found in the few studies evaluating liver stiffness, obtained either from VCTE or magnetic resonance elastography (MRE), in GD patients. Bohte et al. showed that liver stiffness values measured by both VCTE and MRE were significantly higher in patients with a past history of splenectomy than in those without; moreover, VCTE-

measured liver stiffness correlated with biomarkers of GD severity (chitotriosidase and ACE) [18]. Consistently, a very recent study also reported that MRE-measured liver stiffness was significantly higher in patients with splenectomy than in those without and positively correlated with DS3 score [21]. Finally, Webb et al. found a greater liver stiffness measured by VCTE in GD patients being compound heterozygous, than in those being homozygous for the N370S mutation [20]. Our findings further substantiate the role of GD severity, splenectomy and GBA1 genotypes in GD-related liver fibrosis. Several hypotheses might explain the association between these GD-related variables and liver involvement in GD. Liver fibrosis may be due to the accumulation of Gaucher cells in the liver, which is more marked in severe GD and may act through the local release of cytotoxic, proinflammatory and fibrogenic factors in the early steps of fibrosis development and through the induction of ischemia and infarction once dense fibrous bands have developed [4, 6]. GD patients with non-N370S GBA1 genotypes usually show a more severe phenotype than patients homozygous for the N370S mutation [5]. Similarly, the indication for therapeutic splenectomy usually reflects disease severity. Moreover, it has also been hypothesized that splenectomy may foster more severe disease in other organ compartments; the spleen has been proposed as the preferred storage organ in GD, and once removed, it may enhance liver injury by increasing hepatic infiltration [3]. As such, patients with one or more of these GD-related risk factors, further to GD patients with concurrent chronic liver diseases, are those at the highest risk of liver fibrosis and deserve careful evaluation of their liver involvement and close follow-up for the progression of liver fibrosis and the development of liver-related complications. Unfortunately, there are currently no guidelines for the assessment of liver involvement in GD. Of note, a very recent international case series describing 16 type 1 GD patients who developed HCC suggested to investigate all GD patients for the presence of liver fibrosis by VCTE, since fibrosis emerged as the most prominent risk factor for HCC development [7].

Our study also revealed that the length of ERT treatment was inversely correlated with liver involvement, thus suggesting a beneficial effect of ERT on liver fibrosis progression. ERT is highly effective in reducing liver volume and it has been suggested that it may delay disease progression even when liver fibrosis is advanced [6]. Here we have to acknowledge that liver stiffness is not only sensitive to fibrosis, but it is also affected by other factors including inflammation. Due to lack of liver biopsy and longitudinal data, we are unable to demonstrate that the lower liver stiffness values seen in GD patients who have received ERT for a longer time are the reflection of reduced liver fibrosis rather than infiltration of storage cells. Nevertheless, this finding supports a positive effect of ERT on GD-related liver disease, if it holds true that fibrosis parallels the accumulation of storage cells in the liver. Several ERT products are commercially available. Currently, the different ERTs have not shown evidence-based differences in their efficacy on liver volume [22], and the effect of ERT on the risk of long-term liver-related complications, including HCC, is not clear [23]. Our study was not designed to assess if the different ERTs differently affect liver stiffness.

Several studies suggested that long-term ERT treatment is associated with weight gain, increase in BMI and in fat mass and high prevalence of diabetes and MetS [13, 24]. Whether these metabolic disturbances, caused either by ERT itself or by unhealthy lifestyle, contribute to liver disease development and progression in treated GD patients is unknown so far. An important finding of the current study is that several components of the MetS, notably including BMI, were directly associated with liver stiffness in the subgroup of GD patients on long-term ERT. This finding may suggest that the metabolic abnormalities described in treated GD patients do affect the severity of liver fibrosis. Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disease worldwide, is closely related to obesity, diabetes and MetS, and may lead to fibrosis progression and end-stage liver disease, including HCC [25, 26]. Disappointingly, the prevalence of NAFLD in treated GD patients has never been evaluated to our knowledge. Future studies are needed to investigate if NAFLD is the missing piece explaining why liver disease may progress and liver complications may develop also in stable GD patients.

There are some limitations of this study that should be acknowledged. First, data were not complete for some investigated variables. This may partly account for the fact that we were not able to show any correlation between liver stiffness or significant fibrosis and chitotriosidase activity. However, it should be also emphasized that chitotriosidase is better used for monitoring over time than for one-time assessment of GD severity, since up to 30–40% of individuals are carriers of a duplication in chitotriosidase gene that results in a reduced activity [27, 28]. Second, liver biopsy, which is the gold standard for liver fibrosis assessment, was not performed, hence it was not possible to demonstrate that liver stiffness actually represented fibrotic changes in GD patients. However, in one case (patient MO6) surgical liver biopsy performed during cholecystectomy for symptomatic cholelithiasis showed the presence of bridging fibrosis, a finding fully consistent with her liver stiffness value (11.3 kPa) measured by VCTE. Moreover, all VCTE exams were performed by experienced operators and none of the patients had factors known to affect the interpretation of VCTE measurements.

**In conclusion**, significant liver fibrosis as evaluated by VCTE is present in a remarkable proportion of adult type 1 GD patients. Since liver fibrosis is the main predictor of the risk of liver-related complications and HCC, all GD patients should be investigated for the presence of liver fibrosis by VCTE. The identification of major GD-related predictors of liver fibrosis (splenectomy status, GD severity and non-N370S GBA1 genotypes) may contribute to select GD patients at increased risk of complications who deserve careful monitoring and close follow-up. Length of ERT is inversely correlated with liver disease in GD patients, suggesting a beneficial effect of ERT on liver fibrosis. However, GD patients on stable ERT should be monitored for metabolic complications, since features of the MetS, in particular increased adiposity, may enhance liver disease progression despite optimal GD control.

## Conflict of interest:

We disclose that some of the authors have been consultants or have received grants or honoraria from Sanofi-Genzyme and Shire.

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## Authors' contributions:

FN: study concept and design; acquisition, analysis and interpretation of data; statistical analysis; drafting of the manuscript; EC, ADS, IM, SB, SD: acquisition and interpretation of data; critical revision of the manuscript for important intellectual content; VS: critical revision of the manuscript for important intellectual content;; MDC: study concept and design; critical revision of the manuscript for important intellectual content; FC: study concept and design; critical revision of the manuscript for important intellectual content; study supervision. All Authors read and approved the final manuscript.

## REFERENCES:References

- [1] [PKPK](#) Mistry, [MDMD](#) Cappellini, [EE](#) Lukina, [HH](#) Ozsan, [SS](#) Mach Pascual, [HH](#) Rosenbaum, [MM](#) Helena Solano, et al., A reappraisal of Gaucher disease-diagnosis and disease management algorithms, [Am J Hematol](#) [Am. J. Hematol](#) **86**, 2011, 110-115.
- [2] [IL](#) Motta, [MM](#) Filocamo, [EE](#) Poggiali, [MM](#) Stroppiano, [AA](#) Dragani, [DD](#) Consonni, [WW](#) Barcellini, et al., A multicentre observational study for early diagnosis of Gaucher disease in patients with Splenomegaly and/or Thrombocytopenia, [Eur J Haematol](#), [Eur. J. Haematol](#) **96**, 2016, 352-359.
- [3] [FT](#) Adar, [YY](#) Ilan, [DD](#) Elstein and [AA](#) Zimran, Liver involvement in Gaucher disease - review and clinical approach, [Blood Cells Mol Dis](#) [Blood Cells Mol. Dis](#) **68**, 2018, 66-73.
- [4] [SPSP](#) James, [FWFW](#) Stromeyer, [CC](#) Chang and [JAIA](#) Barranger, Liver abnormalities in patients with Gaucher's disease, *Gastroenterology* **80**, 1981, 126-133.
- [5] [AA](#) Zimran, [AA](#) Kay, [FT](#) Gelbart, [PP](#) Garver, [DD](#) Thurston, [AA](#) Saven and [EE](#) Beutler, Gaucher disease. Clinical, laboratory, radiologic, and genetic features of 53 patients, *Medicine (Baltimore)* **71**, 1992, 337-353.
- [6] [RRRH](#) Lachmann, [DGDG](#) Wight, [DDDJ](#) Lomas, [NEN.C](#) Fisher, [JPI.P](#) Schofield, [EE](#) Elias and [FMTM](#) Cox, Massive hepatic fibrosis in Gaucher's disease: clinico-pathological and radiological features, *QJM* **93**, 2000, 237-244
- [7] [MM](#) Regenboog, [EL](#) van Dussen, [JJ](#) Verheij, [NN.NI](#) Weinreb, [DD](#) Santosa, [SS](#) Vom Dahl, [DD](#) Haussinger, et al., Hepatocellular carcinoma in Gaucher disease: an international case series, [J Inherit Metab Dis](#) [J. Inherit. Metab. Dis](#) 2018.
- [8] European Association for Study of L, Asociacion Latinoamericana para el Estudio del H, EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis, [J Hepatol](#) [J. Hepatol](#) **63**, 2015, 237-264.
- [9] [EL](#) Castera, [XX](#) Forns and [AA](#) Alberti, Non-invasive evaluation of liver fibrosis using transient elastography, [J Hepatol](#) [J. Hepatol](#) **48**, 2008, 835-847.
- [10] [MM](#) Friedrich-Rust, [MFM.F](#) Ong, [SS](#) Martens, [CC](#) Sarrazin, [JJ](#) Bojunga, [SS](#) Zeuzem and [EE](#) Herrmann, Performance of transient elastography for the staging of liver fibrosis: a meta-analysis, *Gastroenterology* **134**, 2008, 960-974.
- [11] [SS](#) Singh, [LLLL](#) Fujii, [MHM.H](#) Murad, [ZZ](#) Wang, [SKSK](#) Asrani, [RRLI](#) Ehman, [PSPS](#) Kamath, et al., Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis, *Clin. Gastroenterol. Hepatol.* **11**, 2013, 1573-1584, (e1571-1572; quiz e1588-1579).
- [12] [FMTM](#) Cox, [GG](#) Drelichman, [RR](#) Cravo, [MM](#) Balwani, [TATA](#) Burrow, [AMAM](#) Martins, [EE](#) Lukina, et al., Eliglustat compared with imiglucerase in patients with Gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial, *Lancet* **385**, 2015, 2355-2362.
- [13] [FF](#) Nascimbeni, [AA](#) Dalla Salda and [FF](#) Carubbi, Energy balance, glucose and lipid metabolism, cardiovascular risk and liver disease burden in adult patients with type 1 Gaucher disease, [Blood Cells Mol Dis](#) [Blood Cells Mol. Dis](#) **68**, 2018, 74-80.
- [14] [NN.NI](#) Weinreb, [MDMD](#) Cappellini, [FMTM](#) Cox, [EHE.H](#) Giannini, [GAGA](#) Grabowski, [WLWL](#) Hwu, [HH](#) Mankin, et al., A validated disease severity scoring system for adults with type 1 Gaucher disease, *Genet. Med.* **12**, 2010, 44-51.
- [15] National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report, *Circulation* **106**, 2002, 3143-3421.



- [16] [HH](#) Perazzo, [FFEF](#) Fernandes, [JGIC](#) Soares, [JI](#) Fittipaldi, [SWSW](#) Cardoso, [BB](#) Grinsztejn and [VGVG](#) Veloso, Learning curve and intra/interobserver agreement of transient elastography in chronic hepatitis C patients with or without HIV co-infection, *Clin. Res. Hepatol. Gastroenterol.* **40**, 2016, 73-82.
- [17] [FF](#) Conti, [RR](#) Vukotic, [FEGG](#) Foschi, [MM](#) Domenicali, [PP](#) Giacomoni, [SS](#) Savini, [AA](#) Lanzi, et al., Transient elastography in healthy subjects and factors influencing liver stiffness in non-alcoholic fatty liver disease: an Italian community-based population study, *Dig Liver Dis Dig. Liver Dis.* **48**, 2016, 1357-1363.
- [18] [AEA.E](#) Bohte, [EL](#) van Dussen, [EMEM](#) Akkerman, [AJA.I](#) Nederveen, [RR](#) Sinkus, [PLPL](#) Jansen, [JI](#) Stoker, et al., Liver fibrosis in type I Gaucher disease: magnetic resonance imaging, transient elastography and parameters of iron storage, *PLoS One* **8**, 2013, e57507.
- [19] [PP](#) Stein, [RR](#) Yang, [JI](#) Liu, [GMGM](#) Pastores and [PKPK](#) Mistry, Evaluation of high density lipoprotein as a circulating biomarker of Gaucher disease activity, *J Inherit Metab Dis J. Inherit. Metab. Dis.* **34**, 2011, 429-437.
- [20] [MM](#) Webb, [AA](#) Zimran, [FT](#) Dinur, [OO](#) Shibolet, [SS](#) Levit, [DMD.M](#) Steinberg and [OO](#) Salomon, Are transient and shear wave elastography useful tools in Gaucher disease?, *Blood Cells Mol Dis Blood Cells Mol. Dis.* **68**, 2018, 143-147.
- [21] [SDS.D](#) Serai, [APAP](#) Naidu, [TT](#) Andrew Burrow, [CECE](#) Prada, [SS](#) Xanthakos and [AJA.I](#) Towbin, Correlating liver stiffness with disease severity scoring system (DS3) values in Gaucher disease type 1 (GD1) patients, *Mol Genet Metab Mol. Genet. Metab.* **123**, 2018, 357-363.
- [22] [EE](#) Shemesh, [EL](#) Deroma, [BB](#) Bembi, [PP](#) Deegan, [GC](#) Hollak, [NHN.I](#) Weinreb and [FMT.M](#) Cox, Enzyme replacement and substrate reduction therapy for Gaucher disease, *Cochrane Database Syst Rev Cochrane Database Syst. Re* 2015, CD010324.
- [23] [BEB.E](#) Rosenbloom and [NHN.I](#) Weinreb, Gaucher disease: a comprehensive review, *Crit Rev Oncog Crit. Rev. Oncog.* **18**, 2013, 163-175.
- [24] [MM](#) Langeveld, [MM](#) de Fost, [JMJ.M](#) Aerts, [HPH.P](#) Sauerwein and [CECE](#) Hollak, Overweight, insulin resistance and type II diabetes in type I Gaucher disease patients in relation to enzyme replacement therapy, *Blood Cells Mol Dis Blood Cells Mol. Dis.* **40**, 2008, 428-432.
- [25] [ZMZ.M](#) Younossi, [ABAB](#) Koenig, [DD](#) Abdelatif, [YY](#) Fazel, [EL](#) Henry and [MM](#) Wymer, Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology* **64**, 2016, 73-84.
- [26] Italian Association for the Study of the L, AISF position paper on nonalcoholic fatty liver disease (NAFLD): updates and future directions, *Dig Liver Dis Dig. Liver Dis.* **49**, 2017, 471-483.
- [27] [PP](#) Giraldo, [AA](#) Cenarro, [PP](#) Alfonso, [JLI](#) Perez-Calvo, [DD](#) Rubio-Felix, [MM](#) Giralt and [MM](#) Pocovi, Chitotriosidase genotype and plasma activity in patients type 1 Gaucher's disease and their relatives (carriers and non carriers), *Haematologica* **86**, 2001, 977-984.
- [28] [PP](#) Irun, [PP](#) Alfonso, [SS](#) Aznarez, [PP](#) Giraldo and [MM](#) Pocovi, Chitotriosidase variants in patients with Gaucher disease. Implications for diagnosis and therapeutic monitoring, *Clin Biochem Clin. Biochem.* **46**, 2013, 1804-1807.

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## HIGHLIGHTS

- Significant liver fibrosis is prevalent in patients with type 1 Gaucher disease.
  - Splenectomy and severity of Gaucher disease are strong predictors of liver fibrosis.
  - Length of enzyme replacement therapy is inversely correlated with liver fibrosis.
  - Features of metabolic syndrome may enhance liver fibrosis in stable Gaucher disease.
-

## Queries and Answers

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**Answer:** p-values in bold denote statistical significance

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### Query:

Please provide the volume number and page range for the bibliography in Ref. [7].

**Answer:** Regenboog M, van Dussen L, Verheij J, Weinreb NJ, Santosa D, Vom Dahl S, Häussinger D, Müller MN, Canbay A, Rigoldi M, Piperno A, Dinur T, Zimran A, Mistry PK, Salah KY, Belmatoug N, Kuter DJ, Hollak CEM. Hepatocellular carcinoma in Gaucher disease: an international case series. J Inherit Metab Dis. 2018 Feb 8. doi: 10.1007/s10545-018-0142-y. [Epub ahead of print]

### Query:

Please provide the volume number and page range for the bibliography in Ref. [22].

**Answer:** Shemesh E, Deroma L, Bembi B, Deegan P, Hollak C, Weinreb NJ, Cox TM. Enzyme replacement and substrate reduction therapy for Gaucher disease. Cochrane Database Syst Rev. 2015 Mar 27;(3):CD010324. doi: 10.1002/14651858.CD010324.pub2.