



Early diagnosis of liver graft steatosis and fibrosis: are non-invasive tests the answer?

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Background: Graft steatosis and fibrosis detection is a challenge to avoid graft loss. The role of liver biopsy (LB) after liver transplantation (LT) is changing with the emergence of non-invasive tests. Our aim is to evaluate the accuracy of transient elastography (TE) in predicting steatosis and fibrosis post-LT.

Methods: This prospective study was performed on 158 LT patients. Controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) were carried out prior to LB. We built receiver operating characteristic (ROC) curves to evaluate the predictive performance of TE.

Results: Using CAP, the area under the curve (AUC) were 0.872 [95% confidence interval (CI): 0.791–0.953, $P=0.01$] and 0.708 (95% CI: 0.614–0.801, $P<0.001$) for the diagnosis of steatosis $\geq S2$ and $\geq S1$, respectively. Using LSM, the AUC were 0.588 (95% CI: 0.486–0.691, $P=0.10$) and 0.651 (95% CI: 0.480–0.822, $P=0.10$) for the diagnosis of fibrosis $\geq F2$ and $F3-F4$, respectively. Cut-offs for CAP were 246.5 dB/m for $S1$ and 275.5 dB/m for $S2$. Cut-offs for LSM were 7.65 kPa for $\geq F2$ and 9.25 kPa for $\geq F3$.

Conclusions: TE may be useful for screening advanced fibrosis and, interestingly, steatosis after LT. TE might gain relevance to track graft metabolic dysfunction and to propose lifestyle interventions.

Keywords: Liver transplantation (LT); transient elastography (TE); graft fibrosis; graft steatosis

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Introduction

Survival rates of liver transplant recipients have risen over the last decades (1). New concerns in terms of managing post-transplant and immunosuppressive complications and avoiding graft loss are challenging the transplantation teams

worldwide.

With increasing prevalence of obesity and metabolic syndrome worldwide and specifically post liver transplantation (LT), detection and quantification of new-onset graft steatosis is becoming a new subject of investigation. Evolution

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and outcome of this condition seem to be different from recurrent metabolic dysfunction-associated steatotic liver disease (MASLD), with reduced cardiovascular and metabolic complications and mortality (2). Several risk factors influence the occurrence of MASLD after LT, including donor genetic (3-5), environmental (rapid weight gain after LT, alcohol abuse) (6,7), and immunosuppressive therapy, including steroids and calcineurin inhibitors (8).

Fibrosis is one of the main forms of graft injury and emerges much earlier than altered liver function tests. A correlation between chronic inflammation and fibrosis development has been established (9). Multiple triggers have been identified including recurrence of the primary disease, viral diseases, idiopathic post-transplantation hepatitis (10,11), immunomodulatory mechanisms, biliary and vascular post-surgical complications, graft particularities (age of donor, partial graft transplantation) (12), and even dysbiosis (13). Timely detection and good assessment of fibrosis are essential steps forward to a better management of graft and recipient.

Liver biopsy (LB) is the gold standard for histological evaluation after LT (14). The histological analysis of LB offers a comprehensive picture, largely exceeding the sole quantitation of fibrosis and steatosis, which includes assessment of inflammation, rejection, biliary changes, and vascular alterations. However, LB is fraught with procedural risks that include serious post-puncture complications, important discomfort for the patient, hospitalisation costs.

Additionally, the quality of reports depends on specimen size and inter-observer variations (14). The role of LB in the follow-up of the graft is evolving with the emergence of non-invasive tests, including patented and non-patented fibrosis scores, transient elastography (TE) and other imaging techniques (acoustic radiation force impulse, shear wave elastography and magnetic resonance elastography). However, no recommendation of non-invasive methods is currently available concerning the usefulness of these assessment methods after LT (15). Only a few studies about the role of TE in the screening and the diagnosis of fibrosis recurrence after LT have been published, the majority being retrospective. Evidence is even weaker for the non-invasive evaluation of steatosis after LT.

The aims of our study are to ascertain factors associated with graft fibrosis and graft steatosis, to determine if TE is correlated to histological scores and to evaluate the accuracy of TE in predicting steatosis and fibrosis grade in the post-LT period. We present this article in accordance with the STARD reporting checklist (available at <https://tgh.amegroups.com/article/view/10.21037/tgh-24-131/rc>).

Methods

Study design

This prospective analysis was performed on a monocentric cohort including all consecutive liver transplant recipients admitted in the Department of Abdominal Surgery and Transplantation of the University Hospital Saint-Luc, Brussels, Belgium, from February 2021 to September 2022, who underwent per-protocol LB. Patients with history of heart failure (16) and unresolved biliary complications were excluded. In our institution, per-protocol LBs are scheduled 6 months, 1 year, and every 5 years after LT. This study was performed according to the Good Clinical Practice recommendations and the Helsinki Declaration and its subsequent amendments, and was approved by the Ethical Committee of the University Hospital Saint-Luc (EC No. 2021/07JUI/265). All patients signed an informed consent form prior to enrollment into the study.

We recorded all demographic and transplant-related data, including etiology of the native liver disease, its severity according to the Child-Pugh Classification and Model for End-Stage Liver Disease (MELD) (17) at the time of LT, recipient and donor characteristics [ABO blood group, gender, age and body mass index (BMI)], graft and surgical features, post-LT complications, and relevant comorbidities (cardiovascular diseases, renal impairment, concomitant

Highlight box

Key findings

- Transient elastography is an interesting non-invasive tool in screening liver transplant recipients for metabolic dysfunction-associated steatotic liver disease (MASLD) in their follow-up.

What is known and what is new?

- Liver biopsy is considered the gold standard to follow liver grafts. Metabolic syndrome is an important issue post-liver transplantation (LT), occurring in up to 60% of the patients and leads to cardiovascular and metabolic related mortality. MASLD is associated with the development of this metabolic syndrome.
- Liver stiffness measurement should be interpreted with caution in liver transplant recipients. Controlled attenuation parameter (CAP) measurement is a promising tool with good correlation with liver biopsy in screening developing MASLD after LT.

What is the implication, and what should change now?

- Non-invasive tests could find a place in the screening and the follow-up of post-transplant metabolic dysfunction.

kidney transplantation, active alcohol use and neoplasia). A panel of laboratory parameters were also documented.

Liver biopsy

Liver sampling was obtained either by percutaneous or transvenous biopsy depending on the risks of bleeding. LB specimens with a minimum of five complete portal tracts were considered suitable for inclusion. Each histological sample was evaluated by one of our two trained liver-expert pathologists, who were blinded to TE results.

Our pathologists classified histological fibrosis according to both the Metavir score (18) and the liver allograft fibrosis semiquantitative scoring system (LAFSc or Venturi score) (19). Steatosis was classified in three categories (S0 <3%, S1 =3–33%, S2 >33%). Other histological modifications including acute and chronic T-cell-mediated rejection portal tract inflammatory cells infiltration, sinusoidal dilatation, *de novo* autoimmune hepatitis and steatohepatitis were reported.

TE

Controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) were assessed in each patients using Fibroscan® (Echosens®, Paris, France), with an adult M probe or an XL probe (following device recommendation). One out of three independent investigators performed the said measurements a few hours before LB during all the inclusion time. The probe was placed in the standard position (patient laid on the back, positioned in a right intercostal space on the anterior axillary line). An ultrasound probe was used in case of unusual anatomy of the graft. Ten validated measurements were performed in each patient. The mean CAP was indicated in decibel per meter (dB/m). The median LSM was indicated in kilopascal (kPa). Interquartile range (IQR) had to be <30% to validate the measurements (20).

Statistical analysis

Data were processed using the software packages SPSS version 29 (SPSS Inc., Chicago, IL, USA). Numerical variables were expressed as mean $\pm$ standard deviation (SD). We tested for differences in categorical variables using Fisher's exact test or χ^2 test, and in continuous variables using the Mann-Whitney *U* test, Kruskal-Wallis test, or logistic regression were used. Spearman's rank correlation

coefficient was used to assess the relationship between quantitated histological scores and non-invasive methods. To assess the performance of the TE measurement in identifying significant fibrosis and steatosis, the receiver operating characteristic (ROC) curves were conducted and the area under the curve (AUC) along with 95% confidence intervals (CIs) were calculated. The null hypothesis of AUC =0.5 was tested. The cut-off value was set to obtain a minimal specificity of 0.80. The probability level of $P < 0.05$ was considered for statistical significance.

Results

Out of the 173 patients hospitalized for per-protocol LB during the inclusion period, 15 patients were excluded: 10 due to lack of LT history data, 1 due to heart failure, and 4 due to unreliable LSM (<10 valid results or IQR/median >30%). Finally, 158 patients were considered for subsequent analysis. No side effect of TE was noticed. One patient suffered from liver hematoma after LB and needed a hospital readmission a few days after discharge. One hundred and thirty-two liver biopsies with adequate number of portal tracts were included in the uni- and multivariate analyses, correlation, and accuracy analysis of non-invasive methods. Thus, 26 liver biopsies were ultimately excluded because the specimens contained fewer than 5 portal tracts.

Population characteristics

The overall population comprised 95 men (60.1%). The mean age at the time of protocolar LB was 59 years (SD, ± 15 years). The first two major indications for LT were alcohol-related liver disease (19.6%) and liver congenital disorders (19.6%), including congenital biliary atresia, Alagille syndrome, familial intrahepatic cholestasis and metabolic disorders. The third most frequent indication was hepatitis C (7.6%). Hepatocellular carcinoma was detected in 38.0% of surgical specimens, including known and incidental cases. The mean time since transplantation was 5 years (SD, ± 8.1 years). The mean current BMI was 24.68 kg/m² (SD, ± 4.2 kg/m²). Diabetes mellitus (DM) was present in 29.7% of patients and arterial hypertension in 41.8%. Tacrolimus was used as a single immunosuppressive agent in 67.7% of cases. Specific data regarding donors and grafts were only recorded for the 140 patients who underwent LT as adults (over 16 years). The most frequent recipient blood group was A (49.3%)

Table 1 Recipient characteristics

Variables	Value
Recipient sex (male)	95 (60.1)
Recipient age (years)	59 (± 15)
Recipient ABO group	
A	80 (50.6)
B	14 (8.9)
AB	5 (3.2)
O	59 (37.3)
Primary liver disease	
Alcohol-related liver disease	31 (19.6)
Metabolic dysfunction	11 (7.0)
Hepatitis B	12 (7.6)
Hepatitis C	12 (7.6)
APKD	12 (7.6)
Toxic hepatitis	7 (4.4)
Autoimmune diseases	11 (7.0)
Complications of previous transplantation	13 (8.2)
Liver congenital disorders	31 (19.6)
Neoplasia (excluding hepatocarcinoma)	7 (4.4)
Cirrhosis of unknown origin	4 (2.5)
Hepatocarcinoma	60 (38.0)
Number of liver transplantations	
One transplantation	140 (88.6)
Two or more transplantations	18 (11.4)
Follow-up time since last transplantation (years)	5 (± 8.1)
Complications after transplantation	
Vascular complications	24 (15.2)
Biliary complications	57 (36.1)
Infectious complications	72 (45.6)
Acute rejection	54 (34.2)
Chronic rejection	3 (1.9)
De novo autoimmune hepatitis	12 (7.6)
Current recipient BMI (kg/m^2)	24.68 (± 4.2)

Table 1 (*continued*)**Table 1** (*continued*)

Variables	Value
Comorbidities	
Diabetes mellitus	47 (29.7)
Arterial hypertension	66 (41.8)
Chronic kidney failure	48 (30.4)
Neoplasia	8 (5.1)
Dyslipidemia	51 (32.3)
Kidney transplantation	7 (4.4)
Maintenance immunosuppression	
Tacrolimus monotherapy	105 (67.7)
Lipid-lowering medications	36 (22.8)

Data are presented as mean ($\pm$ SD) or n (%). APKD, autosomal dominant polycystic kidney disease; BMI, body mass index; SD, standard deviation.

Table 2 Graft characteristics

Variables	Value
Male donor	80 (57.1)
Mismatch sex	60 (42.9)
Donor age (years)	51 (± 16.8)
Delta donor & recipient age (years)	16 (± 13.4)
Donor CMV IgG positive and recipient CMV IgG negative	33 (23.6)
Total ischemia time (minutes)	459 (± 191)
Cold ischemia time (minutes)	412 (± 189)
Warm ischemia time (minutes)	42 (± 15)
Graft type	
DBD	91 (65.0)
DCD	31 (22.1)
Split or living donor transplant	18 (12.9)
Donor BMI (kg/m^2)	24.69 (± 7.3)
Delta donor & recipient BMI (kg/m^2)	3.27 (± 2.91)

Data are presented as mean ($\pm$ SD) or n (%). BMI, body mass index; CMV, cytomegalovirus; DBD, donation after brainstem death; DCD, donation after circulatory death; IgG, immunoglobulin G.

Table 3 Factors associated with graft steatosis

Characteristics	Histological steatosis					
	Univariate analysis			Multivariate analysis (logistic reg.)		
	Odds ratio	95% CI	P	Odds ratio	95% CI	P
Recipient age (years)	1.073	1.036–1.112	<0.001	1.068	0.996–1.081	0.08
Male gender	1.936	0.929–4.037	0.10			
Primary liver disease						
Alcohol-related liver disease	4.765	1.885–12.044	<0.001	2.993	0.918–9.758	0.07
MASLD	14.364	0.907–36.858	0.03	11.951	1.251–114.609	0.03
Hepatitis B or C	0.536	0.206–1.391	0.26			
Autoimmune diseases	0.577	0.496–0.671	<0.001			
Follow-up time since last transplantation (years)	1.003	0.960–1.049	0.89			
Current recipient BMI	1.189	1.077–1.312	<0.001	1.121	1.101–1.257	0.04
Diabetes mellitus	1.848	0.861–3.969	0.12			
Arterial hypertension	3.520	1.693–7.318	<0.001	1.026	0.370–2.842	0.96
Multiple immunosuppressive medications	0.692	0.316–1.515	0.44			
LDL-cholesterol level	1.016	1.005–1.028	0.04	1.016	1.002–1.030	0.03
Triglycerid level	1.010	1.003–1.017	0.01	1.003	0.994–1.012	0.55

BMI, body mass index; CI, confidence interval; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease; reg., regression.

and we did not observe any ABO mismatch. Gender mismatch between recipient and donor was present in 42.9% of LT. The mean donor BMI was 24.69 kg/m² (SD, ± 7.3 kg/m²). Grafts came from donors after brain death in 65.5% of cases. All the population characteristics are presented in *Tables 1,2*.

Factors associated with graft steatosis

Steatosis was present in 39.4% of the LB specimens (90.4% in up to one-third of the hepatocytes and 9.6% in one to two-thirds of the hepatocytes). Histological findings of steatohepatitis were present in 30.7%. Factors independently associated with the presence of histological steatosis in logistic regression were LT for metabolic dysfunction-associated steatohepatitis (MASH) [odds ratio (OR) 11.951, 95% confidence interval (CI): 1.251–114.609, $P=0.03$], current recipient BMI (OR 1.121, 95% CI: 1.101–1.257, $P=0.04$) and low-density lipoproteins-cholesterol (LDL-cholesterol) level (OR 1.016, 95% CI: 1.002–1.030, $P=0.03$). We found no correlation with donor BMI and the presence of DM. Details of the uni- and multivariate

analyses are displayed in *Table 3*. Finally, we compared *de novo* steatosis and recurrence of MASLD. The group with recurrent MASLD included patients who underwent transplantation due to complications of MASLD. All these patients had a confirmed MASLD/MASH before LT, based on histological evidence from LB and/or liver explant. On the contrary, the *de novo* steatosis group consisted of patients who underwent a LT for complications of liver disease not related to MASLD, but subsequently developed steatosis during the post-LT follow-up period. In the 52 patients with significant steatosis, 44 were classified with *de novo* steatosis, and 8 were classified with recurrent MASLD. We did not find any significant difference between the groups regarding BMI, hypertension, age, LDL-cholesterol and triglyceride levels. The only statistically significant difference between the two groups was the higher prevalence of DM in the recurrent MASLD group (31.6% *vs.* 6.1%, $P=0.04$). Rates of significant fibrosis and steatohepatitis were not different in the two groups.

Factors associated with graft fibrosis

Through univariate analysis, the following factors were

found to be associated with significant histological fibrosis (Metavir $\geq$ F2): LT for autoimmune liver disease ($P=0.01$), low serum LDL-cholesterol level ($P=0.03$) and low serum triglyceride level ($P=0.01$). Factors associated with significant histological fibrosis (LAFSc ≥ 4) were LT for auto-immune liver diseases ($P=0.03$), second LT ($P=0.04$), low serum LDL-cholesterol ($P=0.04$) and low serum triglyceride level ($P=0.03$). We also performed subgroup analysis for LAFSc. Concerning the portal tract (P), LT for auto-immunes diseases were associated with fibrosis development (15.7% with P-score =2 or 3 *vs.* 2.8% with P-score =1) ($P=0.01$). No association was demonstrated with sinusoidal tract (S). Fibrosis in the centrilobular tract (C-score) was associated with median time since LT (8 years in C-score =2–3 *vs.* 5 years in C-score =1) ($P=0.02$) and with history of previous LT (31.3% with C-score =2–3 *vs.* 9.2% with C-score =1) ($P=0.02$).

In the multivariate analysis, factors associated with significant fibrosis (Metavir $\geq$ F2) were LT for autoimmune liver diseases (OR 8.618, 95% CI: 1.546–47.939, $P=0.01$) and low serum triglyceride level (OR 0.989, 95% CI: 0.980–0.999, $P=0.03$). We did not identify any factors associated with significant fibrosis, defined as LAFSc ≥ 4 , although autoimmune liver diseases showed a trend toward significance. We found no significant correlation between the presence of significant fibrosis and the subsequent variables assessed: recipient characteristics [age, positive cytomegalovirus polymerase chain reaction (CMV PCR)], graft quality (donor age, donor/recipient weight ratio, donor/recipient age ratio), surgery (graft type, prolonged ischemia time), and complications (biliary, vascular, infectious complications and rejection episodes). Details of the uni- and multivariate analyses are displayed in *Table 4*.

Performance of non-invasive techniques

Correlation between CAP (dB/m) and histologically graded steatosis was tested and we observed a better positive correlation ($\rho=0.37$, 95% CI: 0.21–0.51, $P<0.001$; $r^2=0.13$, $P<0.001$) (*Figure 1*). Regarding the assessment of graft fibrosis, we observed a weak positive correlation between Metavir score and TE (kPa) ($\rho=0.26$, 95% CI: 0.09–0.42, $P=0.01$; $r^2=0.12$, $P<0.001$), and between LAFSc and TE (kPa) ($\rho=0.29$, 95% CI: 0.12–0.45, $P<0.001$; $r^2=0.10$, $P<0.001$). We found no correlation between serum marker models [Fibrosis-4 Index for Liver Fibrosis (FIB-4) and Aspartate Aminotransferase to Platelet Ratio Index (APRI)] and Metavir score or LAFSc. Fibrosis correlation

analyses are presented in *Figure 2*.

The distribution of the CAP and LSM for each steatosis and fibrosis grades are represented in the *Figure 3*. Using CAP, AUC were 0.872 (95% CI: 0.791–0.953, $P=0.01$) and 0.708 (95% CI: 0.614–0.801, $P<0.001$) for the diagnosis of graft steatosis $\geq$ S2 and $\geq$ S1, respectively. Using LSM, AUC were 0.588 (95% CI: 0.486–0.691, $P=0.10$) and 0.651 (95% CI: 0.480–0.822, $P=0.10$) for the diagnosis of graft fibrosis $\geq$ F2 and F3–F4, respectively. ROC curves and the AUC between each steatosis and fibrosis stage are illustrated in *Figure 4*. The optimal cut-off values for CAP were 246.5 dB/m for S1 and 275.5 dB/m for S2. The cut-offs for LSM were 7.65 for $\geq$ F2 and 9.25 for $\geq$ F3. Performance of CAP and LSM for the diagnostic of steatosis and fibrosis are resumed in *Table 5*. The performance of FIB-4 and APRI were poor in the diagnosis of significant fibrosis ($\geq$ F2) with an AUC of 0.5 and 0.60, respectively.

Discussion

Nowadays, LT yields remarkable long-term outcomes. Nevertheless, liver fibrosis persists as a prominent contributor to graft dysfunction (21). The improvement of the knowledge of its physiopathology, detection and assessment is needed.

With 132 graft biopsies, we present here one of the largest prospective cohort of histological post-LT specimens in an adult population. Della-Guardia *et al.* published in 2017 the results from a cohort of 276 post-LT biopsies, in predominantly hepatitis C virus (HCV) patients, with 46% of significant fibrosis ($\geq$ F2) (22). To date, HCV cohorts have mainly been found in the literature (21,23). Our strength is the inclusion of a larger non-HCV population, in line with the current evolution of hepatology. The proportion of significant fibrosis we detected (32.6% of patients $\geq$ F2) was lower compared to other publications, while mean recipient age was comparable (24). Risk factors for fibrosis development after LT are poorly investigated. The pathophysiology of post-LT fibrosis has been studied mostly in pediatric transplant populations (9,25–27). LAFSc was created and validated to better assess graft fibrosis in a pediatric population. This scoring system was conceived to quantify and discriminate fibrosis in the three areas of the hepatic acinus: the portal tract, the sinusoids and the centrilobular veins. Venturi *et al.* (19) showed a better correlation between LAFSc and morphometric analysis of the allograft fibrosis, compared to Metavir or Ishak (28) scoring systems. The use of LAFSc, with its

Table 4 Factors associated with graft fibrosis

Characteristics	Fibrosis (Metavir score $\geq$ F2)						Fibrosis (LAFSc $\geq$ 4)					
	Univariate analysis			Multivariate analysis (logistic reg.)			Univariate analysis			Multivariate analysis (logistic reg.)		
	Odds ratio	95% CI	P	Odds ratio	95% CI	P	Odds ratio	95% CI	P	Odds ratio	95% CI	P
Recipient age (years)	0.983	0.959–1.007	0.17	–	–	–	0.986	0.962–1.011	0.27	–	–	–
Male gender	0.781	0.373–1.634	0.57	–	–	–	0.691	0.327–1.457	0.34	–	–	–
Primary liver disease												
Alcohol-related liver disease	0.716	0.276–1.862	0.64	–	–	–	0.781	0.299–2.033	0.81	–	–	–
MASLD	0.241	0.029–1.992	0.27	–	–	–	0.615	0.122–3.099	0.72	–	–	–
Hepatitis B or C	0.767	0.293–2.005	0.64	–	–	–	0.651	0.238–1.771	0.48	–	–	–
Autoimmune diseases	8.458	1.676–42.688	0.01	8.618	1.546–47.939	0.01	5.029	1.191–21.228	0.03	3.842	0.817–18.179	0.08
Number of liver transplantations												
Two or more transplantations	1.728	0.597–5.005	0.39	–	–	–	3.375	1.160–9.823	0.04	2.080	0.658–6.572	0.21
Follow-up time since last transplantation (years)	1.022	1.976–1.069	0.36	–	–	–	1.022	0.976–1.070	0.36	–	–	–
Complications after transplantation												
Vascular complications	1.548	0.604–3.966	0.46	–	–	–	1.333	0.511–3.481	0.62	–	–	–
Biliary complications	1.056	0.496–2.245	>0.99	–	–	–	1.846	0.867–3.932	0.12	–	–	–
Infectious complications	1.786	0.852–3.743	0.13	–	–	–	1.133	0.535–2.402	0.85	–	–	–
Acute rejection	1.167	0.540–2.522	0.70	–	–	–	1.298	0.597–2.820	0.56	–	–	–
Current recipient BMI	0.959	0.874–1.053	0.38	–	–	–	0.913	0.827–1.008	0.07	–	–	–
Diabetes mellitus	0.548	0.232–1.292	0.22	–	–	–	0.601	0.254–1.442	0.3	–	–	–
Arterial hypertension	0.378	0.173–0.830	0.02	0.593	0.250–1.404	0.24	0.496	0.228–1.077	0.09	–	–	–
Multiple immunosuppressive medications	1.050	0.474–2.326	>0.99	–	–	–	0.691	0.298–1.595	0.41	–	–	–
LDL-cholesterol level	0.987	0.975–0.999	0.03	0.991	0.978–1.005	0.20	0.987	0.975–0.999	0.04	0.991	0.978–1.004	0.20
Triglycerid level	0.988	0.979–0.996	0.01	0.989	0.980–0.999	0.03	0.991	0.983–0.999	0.03	0.993	0.984–1.001	0.10

BMI, body mass index; CI, confidence interval; LAFSc, liver allograft fibrosis semiquantitative scoring system; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease; reg., regression.

finer description of histological fibrosis, offers clinical translational potential and has given us insights into fibrosis risk factors even in our adult population.

Autoimmune liver diseases are correlated with histological graft fibrosis. Profound immunosuppression, including the use of multiple immunosuppressive drugs, is associated with less fibrosis occurrence. Koshiba *et al.* have

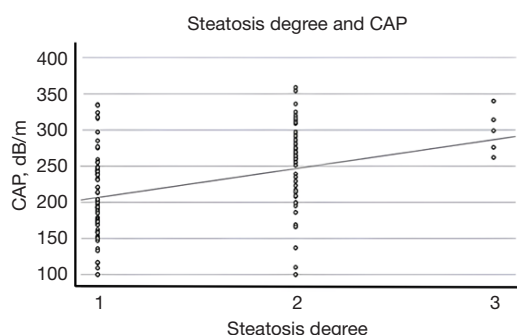


Figure 1 Spearman's correlation between CAP and histological steatosis. CAP, controlled attenuation parameter.

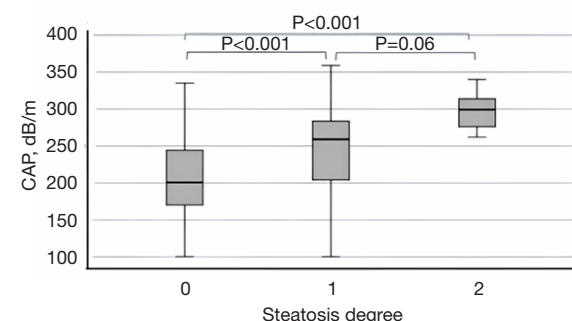
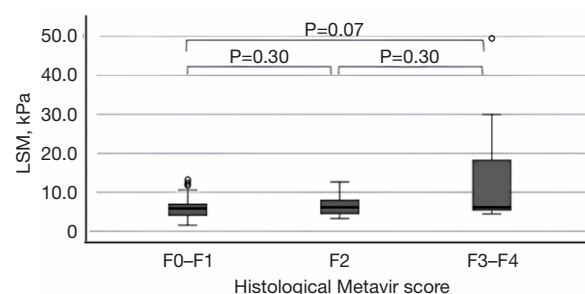


Figure 3 Distribution of LSM and CAP. CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

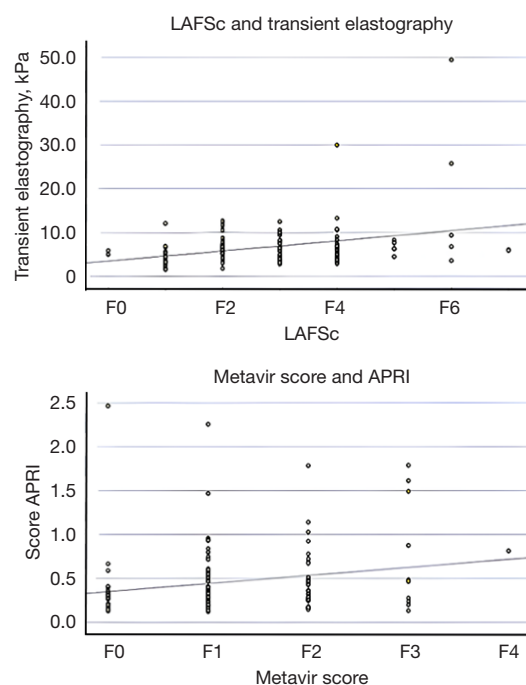
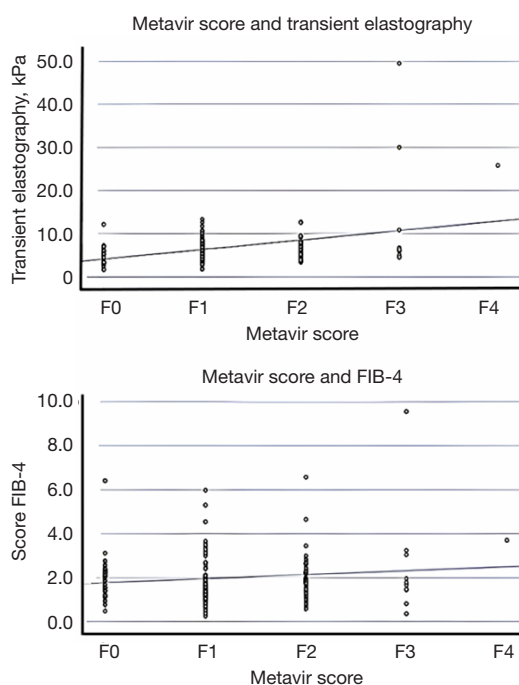


Figure 2 Spearman's correlations of non-invasive assessment techniques of graft fibrosis. APRI, Aspartate Aminotransferase to Platelet Ratio Index; FIB-4, Fibrosis-4 Index for Liver Fibrosis; LAFSc, the liver allograft fibrosis semiquantitative scoring system.

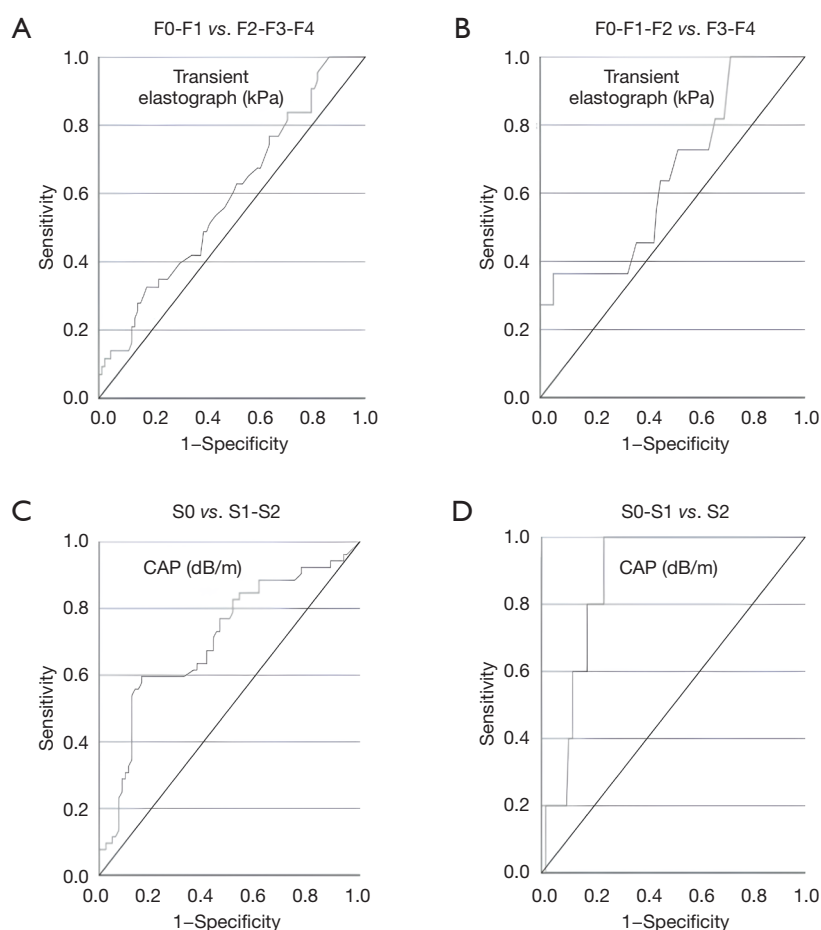


Figure 4 ROC curves and the AUC between each fibrosis and steatosis stage. (A) ROC curves and AUC between fibrosis stages F0-F1 and F2-F3-F4; (B) ROC curves and AUC between fibrosis stages F0-F1-F2 and F3-F4; (C) ROC curves and AUC between steatosis stages S0 and S1-S2; (D) ROC curves and AUC between steatosis stage S0-S1 and S2. AUC, area under the ROC curve; CAP, controlled attenuation parameter; ROC, receiver operating characteristic.

Table 5 Performance of CAP and LSM for the diagnosis of steatosis and fibrosis

Cutt-offs	Sn	Sp	PPV (%)	NPV (%)	AUC (95% CI)	P
S0-S1 vs. $\geq$ S2 (steatosis $>33\%$)						
CAP =275.5 dB/m	0.80	0.83	15.4	99.1	0.872 (0.791–0.953)	0.01
S0 vs. $\geq$ S1-S2 (steatosis $>3\%$)						
CAP =246.5 dB/m	0.60	0.80	66.0	75.3	0.708 (0.614–0.801)	<0.001
F0-F1 vs. F2-F3-F4						
LSM =7.65 kPa	0.33	0.82	45.2	71.3	0.588 (0.486–0.691)	0.10
F0-F1-F2 vs. F3-F4						
LSM =9.25 kPa	0.36	0.88	21.1	93.8	0.651 (0.480–0.822)	0.10

AUC, area under the curve; CAP, controlled attenuation parameter; CI, confidence interval; LSM, liver stiffness measurement; NPV, negative predictive value; PPV, positive predictive value; Sn, sensitivity; Sp, specificity.

already demonstrated that fibrosis develops more extensively in the grafts of immunotolerant patients compared to patients who are still under immunosuppressive therapy (29). This probably suggests the need for a careful follow-up of autoimmune diseases and patients with operational tolerance. Further correlations could be identified by increasing the population size and obtaining a higher rate of significant fibrosis. This also raises the question of the impact of auto- and allo-immune activity on the development of graft fibrosis. Low triglyceride levels have been linked to advanced fibrosis in other studies involving patients with MASH (30). However, it remains unclear whether this is a cause or a consequence of the progression to more advanced liver disease.

The rates of recurrence for both MASLD and MASH, as well as the rates of new-onset MASLD/MASH, vary significantly across different studies (7). The variability can be attributed to several factors, such as the retrospective nature of most of the studies, their single-centre design, and the absence of a universally applied post-LT biopsy regimen, standardized histological criteria, and consistent inclusion/exclusion criteria in these studies. Dumortier *et al.* published a series where the prevalence of histological steatosis was comparable to that of our cohort (31.10%) (6). Definition of histological steatosis, studied population (including age, comorbidities, BMI, immunosuppressive medications), and median delay between LT and LB were also similar to our study. However, the severity of steatosis was milder in our population. Based on our multivariate regression analysis, we found that obesity, LDL-cholesterol level and MASLD cirrhosis, as the primary indication for LT, are risk factors for the occurrence of post-LT steatosis. Vallin *et al.* showed different natural courses for new-onset or recurrent MASLD, with earlier and more rapid onset for recurrent MASLD and more frequent progression to MASH (31). We could not reach a similar conclusion, likely due to a small cohort concerning MASH and significant fibrosis, as well as because of a random inclusion follow-up after LT. The performance of a more extensive study, with long-term follow-up and biopsies at different time points after LT, might as well have added power to find similar evidence.

Because of the potential for severe complications and the high cost of the procedure needing in-hospital surveillance, there is a strong interest in exploring alternatives to LB for detection and follow-up of fibrosis and steatosis. In addition, per-protocol LB cannot be performed too frequently, as opposed to other non-invasive techniques. Studies examining serum markers to detect significant fibrosis after LT have

primarily focused on two scores: APRI (AST/platelet ratio) and FIB-4 (based on age, AST, ALT, and platelet count) (21). We found AUC values similar to a recent meta-analysis, in which AUC ranged from 0.50 to 0.83, for APRI, and from 0.66 to 0.81, for FIB-4 (24). Important limitations hinder the utilisation of serum markers after LT. Evidences and cut-offs are lacking (15). Another significant concern is the impact of other post-LT factors on biological scores. Splenomegaly and thrombocytopenia may persist after LT, even if portal hypertension is resolved, and this can have a negative influence on the biological assessment of liver fibrosis (32). Based on the available literature, it seems that TE outperforms biological markers in the detection of fibrosis and the evaluation of its progression (21,24). Research on the post-LT period is limited. Most studies involve only a small number of patients and intend to evaluate the recurrence of HCV in recipients (33). On this score, we included a large and heterogeneous population of liver transplant recipients. We excluded all patients with biliary complications where effective drainage was not achieved. TE was feasible in 97.5% of our patients. Notably, in our study, TE failed to effectively differentiate between different stages of hepatic fibrosis when compared to the widely accepted gold standard LB. Various confounding factors may play a role in the estimation of fibrosis degree using TE, including diaphragm elevation, mismatch between the graft and the abdominal cavity, hepatic inflammation, and outflow obstruction (34).

To our knowledge, this is the second but largest study that prospectively and systematically assesses the performance of CAP in evaluating graft steatosis after LT in comparison with gold standard LB. The occurrence of steatosis after LT is soaring, and, therefore, non-invasive diagnostic and quantification methods are critically needed (35). Regarding ROC curves, CAP demonstrates an adequate performance in detecting steatosis from 3%. Additionally, the technique well discriminates between two grades of steatosis, suggesting that CAP could be a reliable semi-quantitative method for assessing steatosis. Cut-offs for the detection of S1 and S2, respectively of 246 dB/m and 276 dB/m are distinct. Other non-invasive techniques are employed to evaluate steatosis in the native liver. Abdominal ultrasound offers adequate sensitivity but lacks precision in providing quantitative information (36,37). Magnetic resonance imaging-proton density fat fraction (MRI-PDFF) showed excellent results in detecting steatosis, but is not accessible in daily practice and not yet described in post-LT (38). CAP presents the advantages of repeatability,

cost-effectiveness, and ease of execution. It is essential to bear in mind the strong correlation between MASLD and other complications of the metabolic syndrome, including cardiovascular diseases, DM, and kidney dysfunction. CAP could become a tool to track graft metabolic dysfunction and to propose lifestyle interventions and follow their impact on the liver graft.

Our study suffers from some limitations that could potentially weaken the results. Firstly, we observed a large number of “healthy” grafts, showing either no or very mild fibrosis. Secondly, there was a lack of standardization in the interpretation of LB by different pathologists, particularly in the interpretation of the degree of inflammation and sinusoidal congestion. Thirdly, another weakness of our study is the use of LB at different post-LT times. Additionally, there might have been inter-observer variability in estimating fibrosis on the LB. Lastly, the involvement of multiple investigators in performing TE can have affected the quality of the results despite standardization.

Conclusions

In conclusion, the effective management of post-LT complications, immunosuppressive-related issues, and the prevention of graft loss is still challenging. LB remains unmatched in identifying underlying causes of allograft dysfunction and in grading the severity of pathological pictures. Nonetheless, logistics, costs, and potential complications of LB trigger a need for alternative non-invasive tools for routine graft monitoring. As such, TE might help identify, in routine follow-up, liver transplant recipient who exhibit normal liver tests but are at risk for histological abnormalities. Additionally, while our study emphasizes the importance of careful interpretation of non-invasive tests, particularly for fibrosis detection and grading, CAP could find a place in screening and monitoring post-LT metabolic dysfunction.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed according to the Good Clinical Practice recommendations, the Helsinki Declaration and its subsequent amendments, and was approved by our Ethical committee of University Hospital Saint-Luc (EC No. 2021/07JUI/265). All patients signed an informed consent form prior to enrollment into the study.

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