Original Article

Accuracy of non-invasive liver stiffness measurement and steatosis quantification in patients with severe and morbid obesity

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Background: Vibration controlled transient elastography (VCTE) and controlled attenuation parameter (CAP™) have shown reliable performance predicting fibrosis and steatosis in normal- to overweight patients but have not been validated in severe to morbid obesity. This study aimed at determining the accuracy of VCTE, CAP™ and the composite score FibroScan-AST (FAST) in patients with a body mass index (BMI) of ≥35 kg/m².

Methods: Patients scheduled for bariatric-metabolic surgery underwent preoperative VCTE/CAP™ measurement, and intraoperative liver biopsy. The feasibility and accuracy of VCTE, CAP™ and the composite score FAST were retrospectively analysed to evaluate fibrosis, steatosis and active fibrotic non-alcoholic steatohepatitis [NASH + non-alcoholic fatty liver disease (NAFLD) activity score ≥4 + fibrosis grade ≥2] using per protocol (PP) and intent to diagnose (ITD) calculation.

Results: In total, 170 patients (median BMI 44.4 kg/m²) were included in the study. Liver biopsy showed NASH, simple steatosis, and normal livers in 60.6% (n=103), 28.8% (n=49), and 10.6% (n=18), respectively. VCTE and CAP™ delivered reliable results in 90.6% (n=154/170) and 90.5% (n=134/148). The AUC (PP) of VCTE, CAP™, and FAST were 0.687 (≥F2), 0.786 (≥F3), 0.703 (≥S2), 0.738 (S3), and 0.780 (active fibrotic NASH). The AUC increased to 0.742 (≥F2), 0.842 (≥F3), 0.712 (≥S2), 0.780 (S3), and 0.836 (active fibrotic NASH) in patients below the median BMI of 44.4 kg/m².

Conclusions: VCTE, CAP™ and FAST show acceptable accuracy for the detection of fibrosis, steatosis and NASH in a real-life cohort of patients with obesity. Accuracy improves in patients with a BMI <44.4 kg/m².

Keywords: Transient elastography; controlled attenuation parameter (CAP™); FibroScan-AST score (FAST score); obesity; non-alcoholic fatty liver disease (NAFLD)

Submitted Nov 04, 2020. Accepted for publication Dec 24, 2020.
doi: 10.21037/hbsn-20-787
View this article at: https://dx.doi.org/10.21037/hbsn-20-787

Introduction

Obesity and the concomitant metabolic syndrome are associated with a high risk of non-alcoholic fatty liver disease (NAFLD). Up to 90% of patients with obesity are affected by NAFLD (1). In about half of the patients with NAFLD and obesity with a body mass index (BMI) ≥35 kg/m^2 non-alcoholic steatohepatitis (NASH) is present (2), and up to 5% of patients who undergo bariatric-metabolic surgery suffer from undiagnosed cirrhosis at the time of surgery (3). As obesity becomes an increasing burden, NAFLD has become the most common chronic liver disease worldwide, and is predicted to become the most frequent cause for liver transplantation in the Western World (4).

Bariatric-metabolic surgery has been shown to reduce obesity-related comorbidities in patients with severe (BMI ≥35–39.9 kg/m^2) and morbid obesity (BMI ≥40 kg/m^2) including liver steatosis and fibrosis in the majority of patients (5). Thus, NAFLD/NASH itself is increasingly discussed as an explicit indication for bariatric-metabolic surgery (6), and was shown to be cost-effective for patients with obesity and NASH regardless of fibrosis stage, as well as in patients with overweight and advanced fibrosis (≥F3) (7). Bariatric-metabolic surgery has also been performed in compensated and decompensated cirrhosis. However, in these patient cohorts, the perioperative mortality was increased to 0.9%, and 16.3%, respectively, in comparison to patients without cirrhosis (0.3%) (8). Data is still inconclusive upon the choice of the optimal bariatric-metabolic surgical technique in patients with advanced fibrosis or cirrhosis, and preoperative assessment of liver alterations is essential to avoid unnecessary risks for the patient.

In order to optimally select candidates for bariatric-metabolic surgery non-invasive tools are required, which also allow to assess currently applied endpoints in clinical trials for the treatment of NAFLD/NASH, i.e., resolution of NASH without worsening of fibrosis, or reduction of fibrosis without worsening of NASH.

Vibration controlled transient elastography (VCTE; FibroScan® Echosens, Paris, France) has shown promising results as a non-invasive screening tool for significant and advanced liver fibrosis by liver stiffness measurement (LSM; kPa) in normal to overweight patients with various liver diseases (9,10). Furthermore, controlled attenuation parameter (CAP™), which was developed to quantify ultrasound attenuation during LSM by VCTE (FibroScan® CAP™) has been used to non-invasively quantify liver steatosis in lean patients. A recent individual patient data based meta-analysis on CAP™ in 2,735 patients with mainly viral hepatitis and NAFLD/NASH and a mean BMI of 25.0 kg/m^2 reported good accuracy for the detection of significant (≥S2, AUC 0.865) and severe (S3, AUC 0.882) steatosis (11).

With regard to non-invasive diagnosis of NASH by LSM/CAP™, a recent prospective UK multicentre study reported only poor to fair accuracy (LSM: AUC 0.68, CAP™: AUC 0.71) in a patient cohort with a median BMI of 33.8 kg/m^2 (12). Of note, LSM by FibroScan® and CAP™ have considerable limitations in patients with a BMI ≥30 kg/m^2 (9,13,14). An increased subcutaneous fat proportion or waist circumference were identified as independent predictors of measurement failure using the M-probe (15,16), which lead to the development of the XL probe to improve reliability of LSM in patients with overweight and obesity (17,18). Recently, a new composite score for NAFLD consisting of LSM by VCTE, CAP™ and aspartate aminotransferase (AST), the so-called FAST score, was evaluated and tested on the outcome parameter of active fibrotic NASH (NASH + NAS ≥4 + F ≥2). In the study by Newsome *et al.* FAST identified patients with an AUC of 0.80 (median BMI 34.2 kg/m^2), who were at the highest risk of disease progression and had an excellent AUC of 0.95 for active fibrotic NASH in patients with severe and morbid obesity (median BMI 43.0 kg/m^2) (19).

Therefore, the aim of this study was to evaluate the accuracy of LSM, CAP™ and FAST score for non-invasive detection of significant and advanced liver fibrosis, significant and severe steatosis, and diagnosis of NASH in a patient cohort with severe and morbid obesity undergoing bariatric-metabolic surgery, in a real life setting.

Methods

The study was performed in consecutive patients with severe and morbid obesity scheduled for bariatric-metabolic surgery from September 2014 through November 2018. Surgery was performed in the presence of BMI ≥35 kg/m^2 in combination with one or more obesity-related comorbidities (i.e., metabolic disorders, cardiorespiratory disease, severe joint disease), or in the presence of a BMI ≥40 kg/m^2 according to the European Guidelines on Metabolic and Bariatric Surgery (20). Roux-en-Y gastric bypass, one-anastomosis gastric bypass, sleeve gastrectomy and other bariatric-metabolic procedures were performed in 55 (32.4%), 94 (55.3%), 15 (8.8%) and 6 (3.5%) patients.

Feasibility and accuracy of LSM, steatosis quantification and detection of NASH was assessed by VCTE, CAP™, and FAST score prior to surgery using intraoperative liver biopsy as a reference standard.

Laboratory parameters reflecting relevant components of the metabolic syndrome [i.e., total cholesterol, triglycerides, high-density lipoprotein (HDL); low-density lipoprotein (LDL), glycosylated haemoglobin A1 (HbA1c), homeostatic model assessment for insulin resistance (HOMA-IR)] and liver alteration [AST, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), platelets, ferritin, albumin] were assessed within 14 days prior to surgery and three and 12 months after surgery.

Comorbidities as signs of the metabolic syndrome were documented according to the International Diabetes Federation consensus on the metabolic syndrome (21), including HOMA-IR as a measure of impaired glucose tolerance (22).

Patients with liver disease other than NAFLD were excluded by assessing daily ethanol consumption (<20 g ethanol in females, <30 g ethanol in males) and standard laboratory testing.

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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of the Medical University of Vienna (vote number 2297/2017) and individual consent for this retrospective analysis was waived. We present the following article in accordance with the Liver-FibroSTARD reporting checklist for data (23) and completed the STARD reporting checklist as required (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-20-787/rc).

VCTE

VCTE was performed according to manufacturer's recommendations (FibroScan®, Echosens, Paris, France) by independent, experienced examiners in fasted patients using the XL probe prior to surgery at the out-patient service of a tertiary university medical centre. Patients with VCTE examinations performed >6 months prior to surgery were excluded from analysis. At least 10 valid measurements were performed. LSM values (kPa) were considered unreliable if IQR/median was >30% for values above 7.0 kPa (23). CAP™ (dB/m), calculated simultaneously with LSM measurements, was used as a quantitative indicator of hepatic steatosis using the median of 10 valid measurements.

FAST score

In line with the publication on FibroScan-AST (FAST) score for NASH detection by Newsome *et al.*, FAST was calculated using the equation below and tested on the outcome parameters significant fibrosis (≥F2), significant steatosis (≥S2), NASH according to SAF and active fibrotic NASH (19).

$$FAST = \frac{e\left(-1.65+1.07\,x\,\ln\left(LSM\right)+2.66*10^{-8}\,x\,CAP^{3}-63.3\,x\,AST^{-1}\right)}{1+e\left(-1.65+1.07\,x\,\ln\left(LSM\right)+2.66*10^{-8}\,x\,CAP^{3}-63.3\,x\,AST^{-1}\right)} \quad [1]$$

Liver biopsy

Liver biopsy was routinely performed intraoperatively during bariatric-metabolic surgery on the left liver lobe under direct visualization using a 14-gauge 17-cm Tru-cut™ needle (ACHIEVE programmable automatic biopsy system CareFusion Waukegan, IL, USA). Biopsy specimens were at least 1.5 cm long and displayed ≥6 portal fields. Histological specimens were analysed and graded with regard to steatosis, hepatocyte ballooning, lobular inflammation and fibrosis by two independent experienced pathologists according to NAFLD Activity Score (NAS) and Steatosis, Activity and Fibrosis (SAF) Score (24,25). Briefly, steatosis grades were divided by percentage of macrovesicular steatosis in S1 (5–33% of hepatocytes), S2 (34–66% of hepatocytes) and S3 (>66% of hepatocytes). Fibrosis was grouped accordingly in F0–F1 (no fibrosis or portal fibrosis without septa), F2 (portal fibrosis with few septa), F3 (numerous septa without cirrhosis) and F4 (cirrhosis).

Statistics

Patient characteristics and clinical data were descriptively reported as medians [quartile 1 (Q1); quartile 3 (Q3)] and frequencies (%). Group comparisons were performed by Chi-Square or Fisher's exact tests for categorical variables as appropriate, and ANOVA and student's *t*-test (after checking for normal distribution by Levene test) or Mann-Whitney U-test (for non-normal distributed variables) for parametric and non-parametric continuous variables. The diagnostic accuracy of VCTE and CAP™ as compared to liver histology was determined using areas under the receiver operating characteristics (AUC) at a confidence

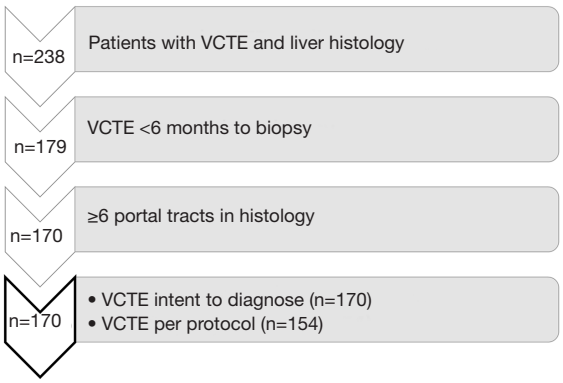

Figure 1 Flow chart of study population. Patients with VCTE examinations >6 months prior to surgery, as well as <6 portal tracts in histology were excluded from analysis. VCTE, vibration controlled transient elastography.

interval of 95% (95% CI). We performed a per protocol (PP) analysis excluding unreliable results as well as an intent-to-diagnose (ITD) analysis based on all VCTE, CAP™ and FAST examinations including failed measurements. The diagnostic accuracy for significant fibrosis (≥F2) and advanced fibrosis (≥F3), significant steatosis (≥S2) and advanced steatosis (S3) and NASH according to SAF were examined, and sensitivity and specificity were calculated. The optimal cut offs were analysed according to Youden Index (26). LSM/CAP™ results (PP and ITD) were further stratified according to BMI. In a subgroup analysis we aimed to evaluate the influencing factors of steatosis and BMI on VCTE by assessing its accuracy in patients with steatosis <S3 and/or < median BMI of the study cohort.

Factors influencing LSM and CAP values were identified using uni- and multivariate linear regression analysis. Available laboratory parameters, patients' age and BMI were tested by univariate linear regression. Values with a significance level <0.02 were further analysed by multivariate linear regression using the enter method.

All statistical analyses were performed using SPSS Version 23 (SPSS Inc., Chicago, IL, USA). The significance level was set at P<0.05.

Results

Study population

In total, in 238 patients VCTE was performed prior to bariatric-metabolic surgery. Study patients were excluded either if LSM was performed >6 months prior to surgery (n=59), or evaluability of liver biopsy was limited (<6 portal fields present, n=9) (*Figure 1*). Consequently, 170 patients (64.7% female, median age 42.0 years) were included in the final analysis. Patients with missing data were excluded from analysis and the number of patients is stated accordingly. The median BMI at the time of VCTE was 44.4 kg/m^2 (Q1; Q3: 41.3; 48.8). Although patients were asked to lose 5 to 10 kg prior to surgery, the majority did not succeed, so that the median BMI at surgery was similar to that at the time of VCTE (44.2 kg/m^2, Q1; Q3: 41.0; 48.4, P=0.911). Patients with a BMI ≥ or < the median BMI of 44.4 kg/m^2 at VCTE did not differ in terms of comorbidities (T2DM P=0.449, aHTN P=0.376, hypertriglyceridemia P=0.387, hypercholesterolaemia P=0.277, hyperuricaemia P=0.165). Patients' characteristics are demonstrated in *Table 1*.

Liver histology

In total, 152 (89.4%) patients had features of NAFLD, 49 (28.8%) patients were classified as NAFL, 103 (60.6%) patients were classified as NASH. Significant to advanced fibrosis (≥F2) was present in 37 (21.8%) patients, advanced fibrosis (≥F3) was present in 14 (8.2%) patients, significant to severe steatosis was present in 72 (42.4%) patients, severe steatosis was present in 39 (22.9%) patients. The results of liver biopsy are presented in *Table 2*.

Feasibility and accuracy of LSM and CAP™

Data on diagnostic accuracy for LSM by VCTE (ITD and PP) as well as diagnostic accuracy of quantitative steatosis measurement by CAP™ (ITD and PP) are presented in *Figure 2*. The results of LSM and CAP™ measurement stratified according to liver histology are displayed in *Figure 3* and Table S1.

VCTE/CAP™ results were stratified according to the median BMI of 44.4 kg/m^2. The median time interval between VCTE/CAP™ and liver biopsy was 2.9 months (Q1; Q3: 1.6; 4.6).

ITD analysis, taking all LSM measurements into account (n=170), showed an AUC for ≥F2 and ≥F3 of 0.670, and 0.758. Stratified according to BMI, in patients with a BMI <44.4 kg/m^2 (n=84) an AUC for ≥F2 and ≥F3 of 0.727, and 0.831 was reached, respectively. In patients with a BMI ≥44.4 kg/m^2 (n=85) the AUC for ≥F2 and ≥F3 was 0.614, and 0.677.

PP analysis, including only valid VCTE measurements (n=154), showed an AUC for ≥F2 and ≥F3 of 0.687 and

Table 1 Patient characteristics at the time of surgery stratified according to the presence of significant fibrosis (≥F2), significant steatosis (≥S2) and NASH based on histologic assessment of intraoperative liver biopsy

Study population	Number (n=170)	Significant fibrosis			Significant steatosis			NASH		
		<F2 (n=133)	≥F2 (n=37)	P value	<S2 (n=98)	≥S2 (n=72)	P value	No NASH (n=67)	NASH (n=103)	P value
Sex, n (%)				0.714			0.244			0.029
Male	60 (35.3)	46 (76.7)	14 (23.3)		31 (51.6)	29 (48.3)		17 (28.3)	43 (71.7)	
Female	110 (64.7)	87 (79.1)	23 (20.9)		67 (60.9)	43 (39.0)		50 (45.5)	60 (54.5)	
Age (y), median (Q1; Q3)	42.0 (33.5; 50.7)	40.6 (32.4; 50.1)	46.0 (37.2; 53.2)	0.112	41.1 (31.8; 50.6)	43.8 (35.2; 50.8)	0.329	37.4 (30.9; 49.6	45.0 (35.1;52.2)	0.052
BMI at VCTE (kg/m²), median (Q1; Q3)	44.4 (41.3; 48.8)	44.4 (41.3; 49.2)	45.0 (41.4; 48.7)	0.762	44.3 (41.3; 47.8)	45.1 (41.6; 49.7)	0.398	43.9 (40.3; 45.7)	45.2 (41.7; 50.1)	0.01
BMI at operation (kg/m²), median (Q1; Q3)	44.2 (41.0; 48.4)	44.2 (41.0; 49.2)	43.1 (40.9; 48.3)	0.926	41.0 (43.6; 47.0)	45.1 (41.5; 49.7)	0.305	43.3 (40.6; 46.4)	44.8 (41.3; 50.4)	0.008
Severe obesity (BMI ≥35–39.9 kg/m²), n (%)	23 (13.5)	18 (13.5)	5 (13.5)	0.997	12 (12.3)	11 (15.3)	0.568	12 (17.9)	11 (10.7)	0.178
Morbid obesity (BMI ≥40–49.9 kg/m²), n (%)	115 (67.6)	89 (66.9)	26 (70.3)	0.700	70 (71.4)	45 (62.5)	0.219	49 (73.1)	66 (64.1)	0.260
Super obesity (BMI ≥50 kg/m²), n (%)	32 (18.8)	26 (17.3)	6 (16.2)	0.646	16 (16.3)	16 (22.2)	0.331	6 (9.0)	26 (25.2)	0.008
T2DM, n (%)	48 (28.2)	26 (17.3)	22 (59.5)	<0.001	22 (22.4)	26 (36.6)	0.051	9 (13.4)	39 (37.9)	0.001
aHTN, n (%)	73/164 (44.5)	51/129 (39.5)	22/35 (62.9)	0.014	38/96 (39.5)	36/69 (52.2)	0.092	21/63 (33.3)	52/101 (51.5)	0.023
Hypercholesterolemia, n (%)	40/162 (24.7)	32/128 (0.25)	8/34 (23.5)	0.860	22/95 (23.2)	18/68 (26.5)	0.655	16/65 (26.6)	24/97 (24.7)	0.985
Hypertriglyceridemia, n (%)	61/163 (37.4)	49/129 (38.0)	12/34 (35.3)	0.773	27/95 (28.4)	35/69 (50.7)	0.003	20/65 (30.8)	41/98 (41.8)	0.153
Hyperuricemia, n (%)	47/119 (39.5)	36/95 (37.9)	11/24 (45.8)	0.477	19/66 (28.8)	28/54 (51.8)	0.012	12/43 (27.9)	35/76 (46.1)	0.052

BMI, body mass index; VCTE, vibration controlled transient elastography; T2DM, type 2 diabetes mellitus; aHTN, arterial hypertension.

Table 2 Liver histology of intraoperative liver biopsy

Histologic findings	Number (n=170)
Fibrosis stage, n (%)	
F0–1	133 (78.2)
F2	23 (13.5)
F3	12 (7.1)
F4	2 (1.2)
Steatosis grade, n (%)	
S0	18 (10.6)
S1	80 (47.1)
S2	33 (19.4)
S3	39 (22.9)
NAS, n (%)	
NAS 0–2	56 (32.9)
NAS 3–4	67 (39.4)
NAS ≥5	47 (27.7)
NAS, median (Q1; Q3)	3 (2; 5)
SAF, n (%)	
No steatosis	18 (10.6)
NAFL	49 (28.8)
NASH	103 (60.6)

NAFLD was classified according to NAS and SAF. In liver biopsies, a median of 12 (Q1; Q3: 8; 15.8) portal tracts were present. NAS, NAFLD Activity Score; SAF, Steatosis Activity and Fibrosis score; NAFLD; non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; F, fibrosis grade; S, steatosis grade.

0.786, respectively. Stratified according to BMI, in patients with a BMI <44.4 kg/m^2 (n=82) an AUC for ≥F2 and ≥F3 of 0.742, and 0.842 was achieved. In patients with a BMI ≥44.4 kg/m^2 (n=72) the AUC for ≥F2 and ≥F3 was 0.616, and 0.702, respectively. The cut off for ≥F2 was 6.3 kPa with a sensitivity of 78.8% and specificity of 53.7%. The cut off for ≥F3 was 12.6 kPa with a sensitivity and specificity of 53.8% and 91.5%, respectively.

CAP™ showed an AUC (ITD/PP) for ≥S2 and S3 of 0.690/0.703, and 0.725/0.738, respectively. Stratifying according to the median BMI of <44.4 kg/m^2, CAP™ achieved an AUC (ITD/PP) for ≥S2 and S3 of 0.706/0.712, and 0.783/0.780. The cut off (PP) for ≥S2 was 350.0 dB/m and for S3 it was 353.5 dB/m, respectively.

As LSM values were significantly higher in advanced steatosis and NASH (NAS and SAF, see Table S1) we aimed to assess the influence of S3 on the accuracy of VCTE. Therefore, we performed a subgroup analysis of patients with <S3. In patients with <S3 the AUC (ITD/PP) for ≥F2 and ≥F3 was 0.673/0.699, and 0.747/0.801. In a further subgroup analysis, patients with <S3 and a BMI of <44.4 kg/m^2 had an improved AUC for ≥F2 (0.702/0.721) and ≥F3 (0.842/0.856) compared to patients with a BMI ≥44.4 kg/m² (≥F2: 0.647/0.668, ≥F3 0.621/0.706).

In total, 16 (9.4%) VCTE/CAP™ measurements delivered unreliable results. The patients concerned had a significantly higher median BMI [50.1 kg/m² (Q1; Q3: 45.3; 56.4) *vs.* 44.2 kg/m² (Q1; Q3: 41.2;47.8), P<0.001]. In addition, the rate of unreliable results was higher in patients with a BMI of ≥44.4 kg/m² (16.3% *vs.* 2.4%, P=0.002). However, severity of steatosis (P=0.654) and fibrosis (P=0.932) according to histology did not differ between patients with valid and invalid measurements. Median LSM and CAP™ values of invalid measurements were significantly higher [LSM 19.4 kPa (Q1; Q3: 12.2; 26.4) *vs.* 6.5 kPa (Q1; Q3: 5.0; 9.43), P<0.0001, CAP™ 385 dB/m (Q1; Q3: 325.5; 400) *vs.* 336 dB/m (Q1; Q3: 305.8; 375), P=0.036].

Non-invasive diagnosis of NASH

The diagnostic accuracy of VCTE for the detection of NASH according to SAF was assessed. VCTE detected NASH with an AUC (ITD/PP) of 0.628/0.640. In patients with a BMI <44.4 kg/m² AUC (ITD/PP) was 0.540/0.547 and was increased in patients with ≥44.4 kg/m² (AUC 0.696/0.745).

CAP™ predicted NASH with an AUC (ITD/PP) of 0.758/0.758. In patients with a BMI <44.4 kg/m² AUC (ITD/PP) improved (0.786/0.793).

FAST score was available in 147 (ITD; n=133 PP) patients. In total, 23 patients (17.3%) were found at risk of progressive NASH (active fibrotic NASH). The AUC (ITD/PP) for ≥F2 and ≥F3 was 0.668/0.666 and 0.628/0.729, respectively. For the detection of ≥S2 and S3 the AUC was 0.817/0.838 and 0.786/0.812, respectively. For NASH according to SAF and active fibrotic NASH the AUC was 0.784/0.781, and 0.764/0.780. Stratification for BMI <44.4 kg/m² improved the AUC (ITD/PP) for the diagnosis of active fibrotic NASH (0.828/0.836) but had no effect on NASH according to SAF for PP analysis (AUC 0.788/0.787).

VCTE (PP) (Fibrosis)	AUC ≥F2	Cut off (kPa)	Youden Index	Sens.	Spec.	AUC ≥F3	Cut off (kPA)	Youden Index	Sens.	Spec.
All patients (n=154)	0.687	6.3	0.325	78.8	53.7	0.786	12.6	0.453	53.8	91.5
BMI <44.4 kg/m² (n=82)	0.742	8.3	0.424	61.1	81.3	0.842	8.4	0.630	85.7	77.3
BMI ≥44.4 kg/m² (n=72)	0.616	6.3	0.288	86.7	42.1	0.702	14.1	0.394	50.0	89.4
<S3 (n=117)	0.699	6.3	0.345	76.2	58.3	0.801	8.4	0.513	70.0	81.3
<S3, BMI <44.4 kg/m² (n=65)	0.721	9.7	0.360	41.7	94.3	0.856	8.4	0.681	83.3	84.7
CAP™ (PP) (Steatosis)	AUC ≥S2	Cut off (dB/m)	Youden Index	Sens.	Spec.	AUC S3	Cut off (dB/m)	Youden Index	Sens.	Spec.
All patients (n=134)	0.703	350.0	0.367	63.0	73.8	0.738	353.5	0.466	75.0	71.6
BMI <44.4 kg/m² (n=68)	0.712	311.5	0.398	84.0	55.8	0.780	325.5	0.574	100.0	57.4
BMI ≥44.4 kg/m² (n=68)	0.687	333.5	0.407	75.9	64.9	0.701	369.5	0.472	72.2	75.0
FAST (PP) (Fibrosis)	AUC ≥F2	Cut off (kPa)	Youden Index	Sens.	Spec.	AUC ≥F3	Cut off (kPA)	Youden Index	Sens.	Spec.
All patients (n=133)	0.666	0.301	0.453	73.1	57.0	0.729	0.714	0.380	44.4	93.5
FAST (PP) (Steatosis)	AUC ≥S2	Cut off (dB/m)	Youden Index	Sens.	Spec.	AUC S3	Cut off (dB/m)	Youden Index	Sens.	Spec.
All patients (n=133)	0.838	0.261	0.611	85.5	75.6	0.812	0.303	0.568	87.5	69.3
VCTE (PP) (NASH)	AUC	Cut off (kPa)	Youden Index	Sens.	Spec.					
NASH acc. SAF (n=154)	0.640	6.0	0.270	53.6	46.4					
NASH, BMI <44.4 kg/m² (n=82)	0.547	6.0	0.270	80.6	46.4					
NASH, BMI ≥44.4 kg/m² (n=72)	0.745	8.0	0.479	52.1	95.8					
CAP™ (PP) (NASH)	AUC	Cut off (dB/m)	Youden Index	Sens.	Spec.					
NASH acc. SAF (n=134)	0.758	323.5	0.423	80.3	62.1					
NASH, BMI <44.4 kg/m² (n=68)	0.793	311.5	0.506	84.8	65.7					
NASH, BMI ≥44.4 kg/m² (n=68)	0.719	369.5	0.381	51.2	87.0					
FAST (PP) (NASH)	AUC	Cut off	Youden Index	Sens.	Spec.					
NASH acc. SAF (n=133)	0.781	0.366	0.483	58.7	89.7					
NASH, BMI <44.4 kg/m² (n=68)	0.787	0.128	0.509	90.9	60.0					
NASH, BMI ≥44.4 kg/m² (n=65)	0.772	0.377	0.604	69.0	91.3					
FAST (PP) (active fibrotic NASH)	AUC	Cut off	Youden Index	Sens.	Spec.					
Active fibrotic NASH (n=133)	0.780	0.206	0.453	100.0	45.3					
NASH, BMI <44.4 kg/m² (n=68)	0.836	0.261	0.661	100.0	66.1					
NASH, BMI ≥44.4 kg/m² (n=65)	0.754	0.562	0.490	71.4	77.6					

VCTE (ITD) (Fibrosis)	AUC ≥F2	Cut off (kPA)	Youden Index	Sens.	Spec.	AUC ≥F3	Cut off (kPA)	Youden Index	Sens.	Spec.
All patients (n=170)	0.670	6.3	0.294	80.6	48.9	0.758	12.6	0.436	57.1	86.5
BMI <44.4 kg/m² (n=84)	0.727	8.4	0.399	61.1	78.8	0.831	8.4	0.610	85.7	75.3
BMI ≥44.4 kg/m² (n=85)	0.614	6.3	0.247	88.9	358.0	0.677	14.1	0.392	57.1	82.1
<S3 (n=131)	0.673	6.3	0.306	78.3	52.3	0.747	8.4	0.433	70.0	73.3
<S3, BMI <44.4 kg/m² (n=67)	0.702	9.7	0.326	41.7	90.9	0.842	8.4	0.653	83.3	82.0
CAP™ (ITD) (Steatosis)	AUC ≥S2	Cut off (dB/m)	Youden Index	Sens.	Spec.	AUC S3	Cut off (dB/m)	Youden Index	Sens.	Spec.
All patients (n=148)	0.690	350	0.338	63.3	70.5	0.725	353.5	0.449	76.5	68.4
BMI <44.4 kg/m² (n=70)	0.706	311.5	0.392	84.6	54.5	0.783	325.5	0.554	100.0	55.4
BMI ≥44.4 kg/m² (n=78)	0.666	371.5	0.368	61.8	75.0	0.680	371.5	0.457	75.0	70.7
FAST (ITD) (Fibrosis)	AUC ≥F2	Cut off (kPA)	Youden Index	Sens.	Spec.	AUC ≥F3	Cut off (kPA)	Youden Index	Sens.	Spec.
All patients (n=147)	0.668	0.642	0.295	41.4	88.1	0.728	0.714	0.405	50.0	90.5
FAST (ITD) (Steatosis)	AUC ≥S2	Cut off (dB/m)	Youden Index	Sens.	Spec.	AUC S3	Cut off (dB/m)	Youden Index	Sens.	Spec.
All patients (n=147)	0.817	0.261	0.578	86.9	57.8	0.786	0.303	0.538	88.6	65.2
VCTE (ITD) (NASH)	AUC	Cut off (kPa)	Youden Index	Sens.	Spec.					
NASH acc. SAF n=170	0.628	6.0	0.270	53.6	46.4					
BMI <44.4 kg/m² (n=84)	0.540	6.0	0.270	80.6	46.4					
BMI ≥44.4 kg/m² (n=85)	0.696	8.0	0.417	58.9	82.8					
CAP™ (ITD) (NASH)	AUC	Cut off (dB/m)	Youden Index	Sens.	Spec.					
NASH acc. SAF (n=148)	0.758	323.5	0.415	81.2	60.3					
BMI <44.4 kg/m² (n=70)	0.786	311.5	0.492	85.3	63.9					
BMI ≥44.4 kg/m² (n=78)	0.727	369.0	0.420	56.9	85.2					
FAST (ITD) (NASH)	AUC	Cut off	Youden Index	Sens.	Spec.					
NASH acc. SAF (n=147)	0.784	0.366	0.492	61.9	87.3					
BMI <44.4 kg/m² (n=70)	0.788	0.128	0.498	91.4	58.3					
BMI ≥44.4 kg/m² (n=77)	0.776	0.377	0.587	73.5	85.2					
FAST (ITD) (active fibrotic NASH)	AUC	Cut off	Youden Index	Sens.	Spec.					
active fibrotic NASH (n=147)	0.764	0.206	0.415	100.0	41.5					
BMI <44.4 kg/m² (n=70)	0.828	0.261	0.629	100.0	62.9					
BMI ≥44.4 kg/m² (n=77)	0.744	0.366	0.492	61.9	87.3					

Figure 2 Diagnostic accuracy of VCTE, CAP™ and FAST according to per protocol and intent to diagnose-analysis. Diagnostic accuracy was calculated of VCTE for ≥F2, ≥F3 and NASH according to SAF, was calculated of CAP™ for ≥S2, S3 and NASH according to SAF and further calculated of FAST for ≥F2, ≥F3, ≥S2, S3, NASH according to SAF and active fibrotic NASH according to per protocol and intent to diagnose – analysis. Patients were stratified according to ≥/< median BMI of 44.4 kg/m² (VCTE, CAP™ and FAST) and steatosis <S3 (VCTE). In patients with <S3 and below the median BMI of 44.4 kg/m² the accuracy of VCTE improved. VCTE, vibration controlled transient elastography; CAP™, Controlled Attenuation Parameter; AUC, area under the receiver operating characteristic, Sens, sensitivity, Spec, specificity; ≥F2, significant fibrosis; ≥F3, advanced fibrosis; ≥S2, significant steatosis; S3, severe steatosis; kPA, kilopascal; PP, per protocol; ITD, intent-to-diagnose; BMI, body mass index; NASH, non-alcoholic steatohepatitis; active fibrotic NASH, definition according to Newsome *et al.* (NASH + NAS ≥4 + F ≥2).

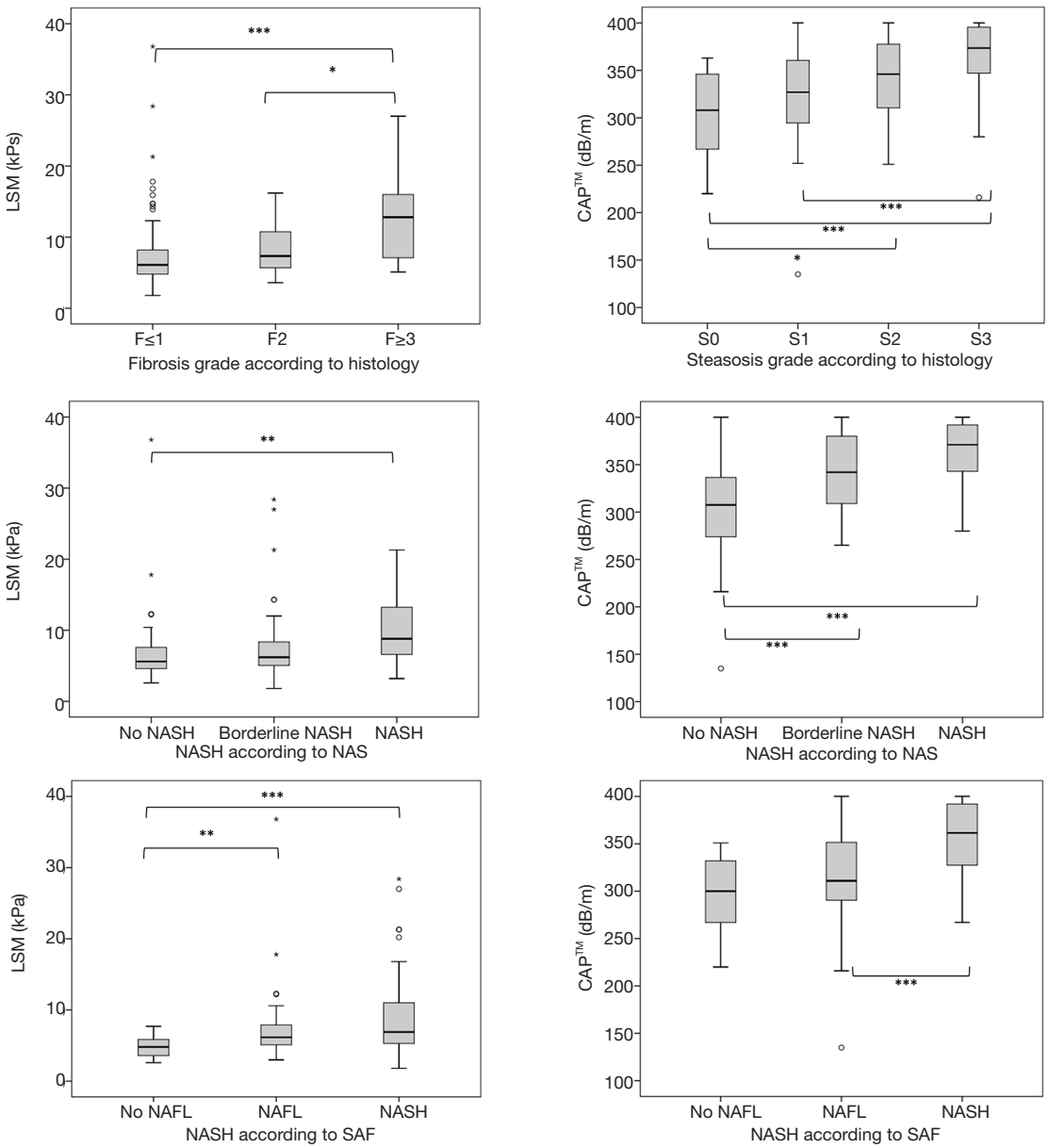

Figure 3 Per protocol liver stiffness measurement (LSM) and CAP™ distribution stratified for fibrosis, steatosis, and NASH according to histology. All groups were statistically compared, and significant differences marked by asterisks *, P<0.05; **, P<0.01; ***, P<0.001. F, fibrosis grade; S, steatosis grade; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; SAF, Steatosis, Activity and Fibrosis score; NAS, NAFLD Activity Score; kPA, kilopascal; dB/m, decibel per meter.

The influence of comorbidities on the diagnostic potential of VCTE and CAP™

To determine factors influencing VCTE and CAP™ univariate and multivariate linear regressions were calculated (Table S2). In the univariate analysis, BMI (P=0.008), macrovesicular (P=0.001) and total steatosis (P=0.008), HbA1c (P=0.042), GGT (P=0.036), HDL (P=0.026), and LDL (P=0.039) were independent confounders of LSM. BMI (P=0.004), fibrosis grade (P=0.023), HbA1c (P=0.011), HOMA-IR (P=0.026), GGT (P=0.035), HDL (P=0.008), and triglycerides (P=0.003) were influencing factors for CAP™. In multivariate linear regression there was a trend

for BMI (P= 0.061) influencing LSM values. Concerning CAP™, BMI (P=0.005) and HbA1c (P=0.018) remained significant in the adjusted regression.

Comparing patients above and below the median BMI of 44.4 kg/m^2 (50.6% *vs.* 49.4%), there was no significant difference in ≥F2 (22.1% *vs.* 21.4%, P=0.916), ≥S2 (44.2% *vs.* 40.5%, P=0.625), NASH according to SAF (66.3% *vs.* 54.8%, P=0.124) or active fibrotic NASH according to FAST score (14.0% *vs.* 13.1%, P=0.870). In contrast, patients with and without diabetes showed differences in significant fibrosis (≥F2 45.8% *vs.* 12.3%, P<0.001), significant steatosis (54.2% *vs.* 37.7%, P=0.051), NASH according to SAF (81.3% *vs.* 52.5%, P=0.001) and active fibrotic NASH (31.3% *vs.* 6.6%, P<0.001).

Laboratory parameters

Laboratory values assessed prior to the operation and three and 12 months thereafter are reported in Table S3 and Figure S1. Blood lipids (total cholesterol, triglycerides, LDL), as well as HbA1c (P<0.001) and HOMA-IR (P<0.001) significantly decreased 3 and 12 months after surgery, while HDL significantly increased 12 months after surgery (P<0.001). Median values of transaminases decreased after surgery, but this was only significant in GGT (P<0.001 after 3 and 12 months). There was no significant difference in transaminases between patients with or without fibrosis as according to liver histology or LSM/CAP™ (≥F2 *vs.* <F2 or ≥F3 *vs.* <F3, data not shown) at the time of surgery. In patients with ≥F2, GGT significantly decreased over time. In contrast, in hepatic steatosis (≥S2 *vs.* <S2) significant differences in GGT were already present at the time of operation. However, independently of steatosis grade, GGT significantly decreased after surgery over time.

Discussion

We analysed the accuracy of VCTE, CAP™ and FAST score in a real life setting for their utility to non-invasively assess liver fibrosis, steatosis and NASH in patients with severe and morbid obesity undergoing bariatric-metabolic surgery. LSM by VCTE, CAP™, and FAST achieved an acceptable accuracy (PP). However, in patients with a BMI <44.4 kg/m^2 accuracy was improved.

The importance of non-invasive liver fibrosis detection and surveillance has continuously increased especially as obesity and concomitant NAFLD/NASH have reached considerate significance as a widespread disease. In our patient cohort the prevalence of biopsy proven NASH was 60.6%, which is in line with previously published data on the prevalence of NASH in patients with obesity (27). However, to date, intraoperative liver biopsy has not been widely applied as a standard procedure during bariatric - metabolic surgery, and non-invasive screening tools have not been independently validated for patients with severe to morbid obesity. Therefore, NASH and advanced fibrosis is at risk to be overlooked in bariatric-metabolic patients, but may lead to an increased intra- and perioperative risk (28).

Previously, increased failure rates of VCTE were reported to be associated with higher BMI (29). In a study with obese patients with a median BMI of 30 kg/m², the XL-probe for LSM was superior compared to the M-probe, but reliable measurements were less likely in patients with a BMI ≥40 kg/m^2 (16). Furthermore, studies that investigate exclusively patients with severe or morbid obesity (BMI ≥35.0 kg/m^2) are scarce, have high heterogeneity, small sample size and consider only few numbers of patients with significant to advanced fibrosis. In our study, the feasibility of LSM was 90.6% comparable to previously reported data (88.5–91.3%) (30-32). Patients with unreliable VCTE results had a significantly higher BMI, and thus in the group above the median BMI of 44.4 kg/m^2 a higher percentage of unreliable results was found. In a study including 87 patients with a mean BMI of 51.6 kg/m² the feasibility of LSM was even only two thirds, and an association between increased subcutaneous fat and LSM failure was reported (33). However, in our study not only feasibility was significantly altered but also the diagnostic accuracy of VCTE was strongly affected especially in patients above the median BMI. In contrast, in patients with a BMI <44.4 kg/m^2 we achieved an AUC of 0.842 for the diagnosis of advanced fibrosis, comparable to previously published results (29,30).

When the XL-probe was first introduced, thresholds for fibrosis grading had to be modified as LSM by the XL-probe led to lower values, thereby underscoring fibrosis (17). Up to now, only a small amount of studies analysed patients with obesity and NAFLD, and report a wide cut off range between 7.6–12.5 kPa for the detection of advanced fibrosis (29,30,34). Our findings are in line with previously published data (cut off for ≥F3: 12.6 kPa). On the contrary, in patients with a BMI ≥44.4 kg/m² we found a considerably higher cut off of 14.1 kPa. Thus, for patients with severe to morbid obesity and NAFLD further studies are required to better define LSM cut off values or cut off ranges, respectively, for significant to advanced fibrosis (35-37). Too low cut off values in patients with obesity

may further contribute to underestimation of liver fibrosis and low diagnostic accuracy of LSM (37). In our patients with morbid obesity, LSM levels increased significantly with progressive steatosis and NASH, and the predictive potential of VCTE was improved when patients with advanced steatosis were excluded. As previously reported, fatty liver itself may cause scattering artefacts and therefore lead to a reduced accuracy of LSM (15). This may be one of the causes for reduced diagnostic accuracy in patients with obesity as they have a higher degree of steatosis (38). For the detection of significant and severe steatosis CAP™ was a mediocre parameter achieving an AUC of 0.703 and 0.738, respectively. Stratification for BMI moderately improved accuracy (AUC 0.712 and 0.780, respectively). In a study by Ooi *et al.* evaluating 82 patients prior to bariatric-metabolic surgery comparable diagnostic accuracy for significant steatosis (AUC 0.688), but inferior accuracy for severe steatosis (AUC 0.540) was reported (39).

After bariatric-metabolic surgery, steatosis and NASH resolve in the majority of cases (40). Nevertheless, accurate non-invasive NASH diagnosis has gained importance as NASH *per se* may become an indication for bariatric-metabolic surgery in the near future. Currently, a randomized controlled trial investigates the treatment of severe NASH with advanced liver fibrosis by bariatric-metabolic surgery in patients with mild obesity (41). In our study, we analysed the diagnostic accuracy of VCTE, CAP™ and FAST for the non-invasive detection of NASH. Whereas VCTE was not a useful parameter, CAP™ achieved an acceptable accuracy (AUC 0.758) similar to findings reported by Eddowes *et al.*, investigating a patient cohort with a median BMI of 33.8 kg/m^2 (AUC of 0.710) (12). Notably, accuracy for the detection of steatosis (≥S2 0.838, S3 0.812) and NASH (0.781) was improved when the composite score FAST comprising LSM, CAP™ and AST was used (19). FAST had a similar diagnostic performance for the outcome parameter of active fibrotic NASH as in the original publication's derivation cohort [0.780 (PP) *vs.* 0.800; median BMI 34.2 kg/m^2]. The AUC for FAST substantially improved in patients with a BMI <44.4 kg/m^2 (AUC 0.836). In contrast, in the French bariatric surgery cohort (n=110) of the above-mentioned study (median BMI 43.0 kg/m^2) FAST achieved an AUC of even 0.95 for active fibrotic NASH, which could not be reproduced in our cohort of 133 patients (PP analysis). As an explanation, this discrepancy could have occurred due to spectrum bias based on a lower prevalence of significant to advanced fibrosis, significant to severe steatosis, as well as comorbidities in the French cohort.

The laboratory parameters, AST and ALT were not useful to detect patients with significant to advanced fibrosis prior to surgery, but GGT was significantly higher in patient with significant steatosis. GGT significantly decreased after surgery. This may allude to the improvement of hepatic inflammation, fibrosis, and NASH as reported in a study by Dixon *et al.* that identified GGT as a predictor of histologic improvement as proven by follow up biopsies performed 29.5±10 months after surgery (42). Unfortunately, a verification of this finding was not possible in our cohort, as consecutive follow up biopsies were not available.

A further limitation of our study was that the sample size was not sufficient to allow further stratifications for T2DM and the BMI classes "severe obesity", "morbid obesity" and "super obesity". Furthermore, an independent validation cohort would be required to confirm our results.

In conclusion, VCTE and CAP™ show acceptable accuracy for the detection of advanced fibrosis and significant to severe steatosis in patients with severe obesity. FAST was a good predictor of hepatic steatosis and active fibrotic NASH. Accuracy of VCTE, CAP™ and FAST improved in the cohort of patients below the median BMI of 44.4 kg/m^2. VCTE may be used as an additional tool for identifying patients at risk for advanced fibrosis, significant and severe steatosis and NASH, particularly when applied as the composite score of FAST.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-20-787/rc

Data Sharing Statement: Available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-20-787/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-20-787/coif). Dr. MT reports personal fees from BMS, grants and personal fees from Falk Foundation, grants, personal fees and other from Gilead, grants, personal fees and other from Intercept,

grants and personal fees from MSD, personal fees from Albireo, personal fees from Boehringer lngelheim, personal fees from BiomX, personal fees from Genfit, personal fees from Novartis, personal fees from Phenex, personal fees from Regulus, other from Abbvie, grants from Albireo, grants from Cymabay, grants from Takeda, outside the submitted work. In addition, Dr. MT has a patent Medical use of nor-UDCA (W02006119803 and W020099013334) licensed to Medical University of Graz. Dr. KS serves as an unpaid editorial board member of *Hepatobiliary Surgery and Nutrition*. The other authors have no conflicts of interest to declare.

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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of the Medical University of Vienna (vote number 2297/2017) and individual consent for this retrospective analysis was waived.

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Cite this article as: Eilenberg M, Munda P, Stift J, Langer FB, Prager G, Trauner M, Staufer K. Accuracy of non-invasive liver stiffness measurement and steatosis quantification in patients with severe and morbid obesity. HepatoBiliary Surg Nutr 2021;10(5):610-622. doi: 10.21037/hbsn-20-787

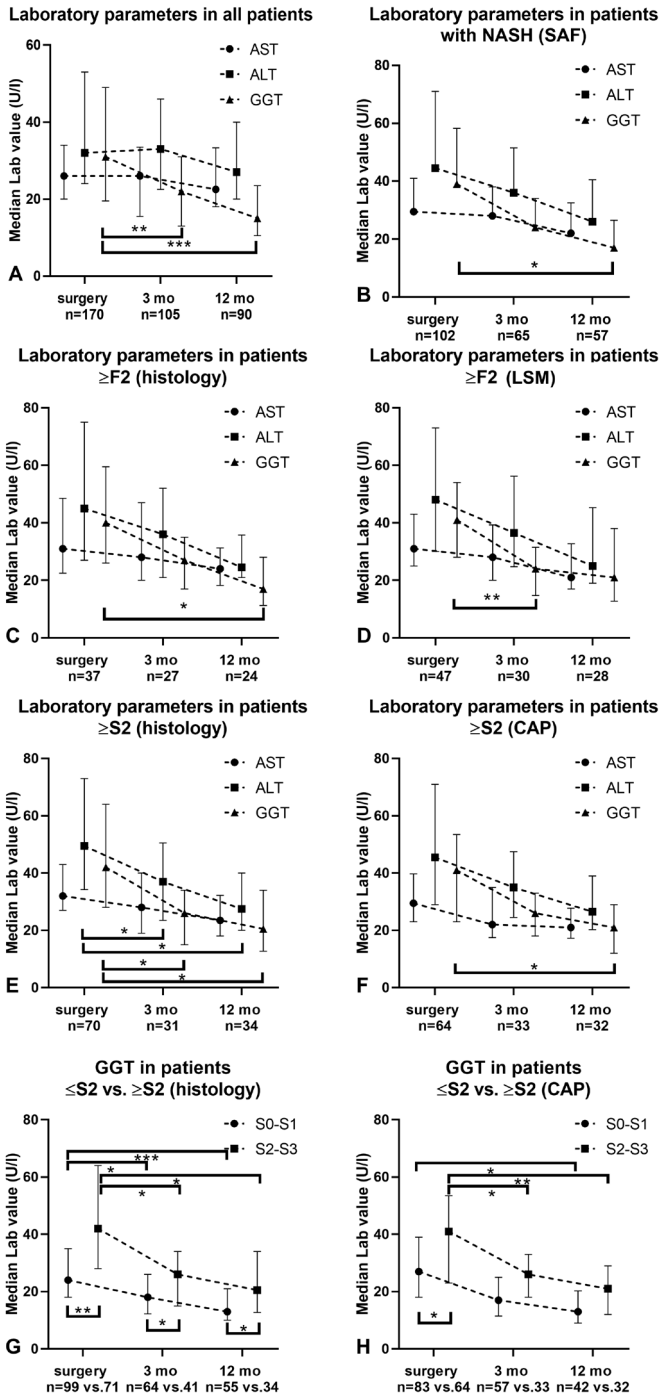

Figure S1 Evolution of laboratory values in NASH, significant fibrosis ≥F2 and steatosis ≥S2 according to histology, LSM and CAP™. All groups were statistically compared, and significant differences marked by asterisks *, P<0.05; **, P<0.01; ***, P<0.001. GGT was significantly decreased over time, in NASH patients, significant fibrosis and steatosis. Laboratory courses were similar in stratifications according to histology versus LSM or CAP™. CAP™ for steatosis grades ≥S2 was calculated by determination of a cutoff (350.0 d/m) by Youden Index. The cutoff (8.3 kPa) for ≥F2 was calculated by Youden Index as for patients with a BMI <44.4 kg/m² due the higher sensitivity and specificity. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; S, steatosis grade; NASH, Non-alcoholic steatohepatitis, SAF, Steatosis, Activity and Fibrosis score.

http://dx.doi.org/10.21037/hbsn-20-787

Table S1 LSM and CAP™ results stratified according to histopathology

	LSM (kPA, PP), N=154		CAP™ (dB/m, PP), N=134
Liver histology (CRN)		Liver histology (CRN)	
Fibrosis		Fibrosis	
F0-1; median (Q1; Q3)	6.1 (4.8; 8.3)	F0-1; median (Q1; Q3)	332.0 (300.0;375.0)
F2; median (Q1; Q3)	7.4 (5.5; 10.9)*	F2; median (Q1; Q3)	345.0 (326.3;379.3)
≥F3; median (Q1; Q3)	12.8 (7.0; 18.1)*	≥F3; median (Q1; Q3)	333.0 (301.5;360.5)
P value (ANOVA) Post hoc analysis	P=0.001 F1/3 P=0.001; F2/3 P=0.029	P value (ANOVA)	P=0.478
≥F2; median (IQR)	8.5 (6.5; 13.3)	≥F2; median (IQR)	342.0 (318.0;375.0)
≥F3; median (IQR)	12.8 (7.0; 18.1)	≥F3; median (IQR)	333.0 (301.5;360.5)
P value (t-test)	≥F2 P=0.004 ≥F3 P=0.011	P value (t-test)	≥F2 P=0.362 ≥F3 P=0.920
Steatosis		Steatosis	
S0; median (Q1; Q3)	5.0 (3.6;6.5)	S0; median (Q1; Q3)	308.0 (267.0;347.5)
S1; median (Q1; Q3)	6.1 (4.8;7.9)	S1; median (Q1; Q3)	327.0 (294.0;362.0)
S2; median (Q1; Q3)	7.6 (6.1;11.1)*	S2; median (Q1; Q3)	346.0 (309.0;380.0)*
S3; median (Q1; Q3)	8.7 (5.8;13.0)*	S3; median (Q1; Q3)	373.5 (342.5; 395.8)*
P value(ANOVA) Post hoc t-test	P=0.013 S0/2: P=0.042, S0/3: P=0.02	P value (ANOVA) Post hoc t-test	<0.001 S0/2: P=0.034, S0/3: P<0.001, S1/3:P=0.002
NAFLD according to NAS		NAFLD according to NAS	
0-2 (no NASH)	5.6 (4.5;7.6)	0-2 (no NASH)	307.5 (270.5; 338.8)
3-4 (Borderline)	6.2 (5.0;8.4)	3-4 (Borderline)	342.0 (308.5; 384.0)*
5-8 (NASH)	8.8 (6.6;13.5)*	5-8 (NASH)	371.0 (340.5; 393.5)*
P value (ANOVA) Post hoc analysis	P=0.009 No NASH *vs.* NASH: P=0.009	P- value (ANOVA) Post hoc analysis	<0.001 No NASH *vs.* Borderline: P<0.001 No NASH *vs.* NASH: P<0.001
NAFLD according to SAF (FLIP Algorithm)		NAFLD according to SAF (FLIP Algorithm)	
No steatosis/NAFL	4.8 (3.6; 6.1)	no Steatosis/NAFL	300.0 (261.8; 334.8)
NAFL	6.2 (5.1;8.1)*	NAFL	311.0 (290.3; 354.3)
NASH	6.9 (5.3;11.1)*	NASH	361.5 (327.3; 392.0)
P value (ANOVA) Post hoc analysis	P=0.013 No NAFL *vs.* NAFL: P=0.007 No NAFL *vs.* NASH <0.001	P value (ANOVA) Post hoc analysis	<0.001 NAFL *vs.* NASH: P<0.001

VCTE values increased with higher fibrosis, but also with higher steatosis grades according to CRN. LSM was increased with higher NAS (NASH) and SAF (NAFLD, NASH) (per protocol analysis). CAP™ values increased with higher steatosis grades according to CRN, with NAS (NASH and borderline NASH) and SAF (NASH) (per protocol analysis. * indicates significant differences in relation to baseline. SAF, Steatosis, Activity and Fibrosis score; NAS, NAFLD Activity Score; NAFL, Non-alcoholic fatty liver; NASH, Non-alcoholic steatohepatitis; CAP™, Controlled Attenuation Parameter; kPA, kilopascal.

http://dx.doi.org/10.21037/hbsn-20-787

Table S2 Factors influencing liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) assessed by multivariate linear regression analysis

	LSM					CAP			
	Unadjusted regression coefficient (95% CI)	P value	Adjusted regression coefficient (95% CI)	P value		Unadjusted regression coefficient (95% CI)	P value	Adjusted regression coefficient (95% CI)	P value
BMI	0.212 (0.046; 0.307)	0.008	0.115 (-0.005; 0.236)	0.061	BMI	0.250 (0.676; 3.368	0.004	1.749 (0.542; 2.956)	0.005
Steatosis (total)	0.214 (0.009; 0.059)	0.008	0.023 (-0.027; 0.073)	0.112	Fibrosis	0.196 (1.504; 20.379)	0.023		
Macrovesicular steatosis	0.292 (0.02; 0.73)	0.001	0.02 (-0.035; 0.075)	0.750	HbA1c	0.236 (1.92; 14.615)	0.011	8.128 (1.442; 14.814)	0.018
HbA1c	0.175 (0.027; 1.480)	0.042			HOMA-IR	0.210 (0.116; 1.758)	0.026		
GGT	0.170 (0.001; 0.037)	0.036			GGT	0.183 (0.014; 370)	0.035		
HDL	0.190 (-128; -0.008)	0.026			HDL	0.243 (-1.342; -0.207)	0.008	-0.528 (-1.138; 0.082)	0.089
LDL	0.180 (-0.055; -0.001)	0.039			TG	0.262 (0.052; 0.241)	0.003	0.025 (-0.071; 0.12)	0.609

Following parameters were analyzed by the univariate linear regression analysis: platelets, aspartate aminotransferase; alanine aminotransferase; gamma-glutamyl transferase (GGT); High-density lipoprotein (HDL); Low-density lipoprotein (LDL); glycosylated hemoglobin (HbA1c); Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), ferritin, albumin, total cholesterol, triglycerides (TG), fibrosis grade (for CAP™); total, microvesicular and macrovesicular steatosis (for LSM), patients' age and body mass index (BMI). Only significant values from the regression are listed. Adjusted regression coefficient was then assessed for all parameters with a significance level of P<0.02. All P values are given for parameters that were included in the multivariate linear regression. Statistical significance was reached at P≤0.05. CI, confidence interval.

Table S3 Changes of laboratory parameters over time

Laboratory parameters	Prior to surgery (N=170)	3 months post-surgery (N=106)	P value baseline *vs.* 3 months	12 months post-surgery N=91	P value baseline *vs.* 12 months
Platelets (G/L), median (Q1; Q3)	269.5 (225.8; 319.0)	250.5 (217.5; 293.0)	0.017	252.0 (212.0; 282.0)	0.003
AST (U/L), median (Q1; Q3)	26.0 (20.0; 34.0)	26.0 (18.5; 33.5)	0.541	22.5 (18.0; 33.3)	0.631
ALT (U/L), median (Q1; Q3)	32.0 (24.0; 53.0)	33.0 (22.5; 46.0)	0.508	27.0 (20.0; 40.0)	0.211
GGT (U/L), median (Q1; Q3)	31 (19.5; 49.0)	22.0 (13.0; 31.0)	<0.001	15.0 (10.5; 23.5)	<0.001
Ferritin (ng/mL), median (Q1; Q3)	86.4 (48.0; 167.0)	110.6 (53.9; 147.0)	0.742	77.1 (29.1; 126.0)	0.055
Albumin (g/L), median (Q1; Q3)	42.4 (40.4; 44.5)	42.4 (40.5; 44.8)	0.344	42.3 (39.6; 44.1)	0.061
Total cholesterol (mg/dl), median (Q1; Q3)	177.5 (150.8; 200.5)	156.5 (137.0; 178.0)	<0.001	154.0 (139.3; 171.0)	<0.001
HDL (mg/dL), median (Q1; Q3)	42.0 (35.0; 50.0)	40.0 (34.0; 48.0)	0.045	52.5 (45.0; 61.5)	<0.001
LDL (mg/dL), median (Q1; Q3)	111.8 (87.7; 129.8)	89.6 (74.6; 106.8)	<0.001	81.9 (69.4; 102.6)	<0.001
Triglycerides (mg/dL), median (Q1; Q3)	132 (100.5; 195.0)	110.0 (86.0; 143.5.0)	<0.001	85.0 (67.0; 111.5)	<0.001
HbA1c (%), median (Q1; Q3)	5.6 (5.4; 6.4)	5.2 (4.9; 5.5)	<0.001	5.0 (4.8; 5.3)	<0.001
HbA1c >6.5%, % (n)	20.9 (31/148)	2.0 (2/100)	<0.001	1.23 (1/81)	<0.001
HOMA-IR, median (Q1; Q3)	6.0 (3.7; 12.1)	2.3 (1.4; 3.6)	<0.001	1.5 (1.0; 2.3)	<0.001
HOMA-IR >2.5, % (n)	87.6 (129/146)	47.9 (45/94)	<0.001	21.1 (16/76)	<0.001

Statistical differences between preoperatively at the day of surgery were compared to 3 and 12 months postoperatively, respectively (T- Test for parametric, Mann-Whitney U – Test for non-parametric data). In our study we were able to reproduce previously reported postoperative improvement of blood lipids, glucose homeostasis and GGT as typical for patients after bariatric-metabolic surgery (43-45). The transaminases AST and ALT did not significantly decrease three and 12 months after bariatric surgery in contrast to the Swedish Obese Subjects (SOS) Study (45). GGT was significantly reduced over time as in line with the study by Dixon et al that identified GGT as a predictor of histologic improvement (42). VCTE, Vibration controlled transient elastography; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; HbA1c, glycosylated hemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

http://dx.doi.org/10.21037/hbsn-20-787

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http://dx.doi.org/10.21037/hbsn-20-787