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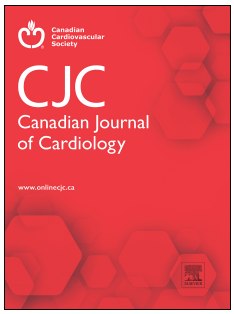

Determinants of advanced liver fibrosis in adult patients after Fontan-palliation: Usefulness of ultrasound transient elastography

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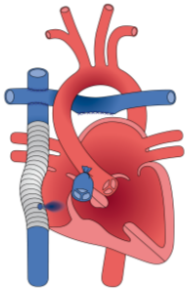
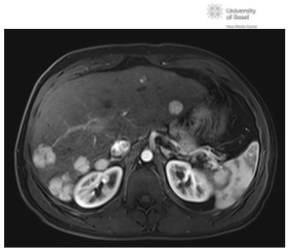
97 Fontan patients

- 94 with liver ultrasound
- 50 transjugular liver biopsy

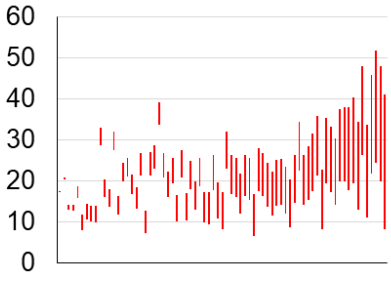




Fontan associated liver disease (FALD)

- Liver stiffness: 20.9 kPa, range: 6.6-75.0 kPa
- 70% advanced fibrosis (CHFS 3/4)

High variability of liver stiffness individual pts



Range of liver stiffness (kPa)
in 73 individual patients with serial measurements of liver stiffness

Predictors of advanced liver fibrosis

- ☑ MELD-XI-Score ≥11
- ☑ Splenomegaly
- ☑ Younger age at Fontan-OP

- ☒ Age, time since Fontan-OP
- ☒ Liver stiffness

Determinants of advanced liver fibrosis in adult patients after Fontan-palliation: Usefulness of ultrasound transient elastography

Short title: Utility of liver stiffness for assessment of FALD

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Word count: 5178

Abstract

Background: Fontan-associated liver disease is an increasing concern. Our aim was to assess prevalence and predictors of advanced liver fibrosis with a specific focus on utility of liver stiffness measurement by ultrasound transient elastography.

Methods: 97 adult Fontan-patients (55% males, median age: 23.1 years, IQR: 18.7-30.6), 92 (95%) were evaluated with transient elastography and 50 (52%) underwent transjugular liver biopsy. Advanced liver fibrosis was defined as congestive hepatic fibrosis score 3 or 4.

Results: Only four patients (4%) had liver stiffness values <10kPa. Liver stiffness measurements correlated weakly with peak oxygen uptake on exercise testing and Fontan-pressure but not with Model for End-stage Liver Disease excluding INR (MELD-XI)-score or spleen size. Serial follow-up liver stiffness measurements in 73 clinically stable patients showed large variability among individual patients. Advanced liver fibrosis was present in 35/50 (70%) patients on liver biopsy and was associated to MELD-XI-Score ≥11 and splenomegaly but not to liver stiffness measurements. Advanced liver fibrosis was not associated with patient age or time since Fontan-operation but with younger age at Fontan-completion (3.7 years, IQR: 2.3-6.3 versus 6.8 years, IQR: 3.5-12.1, p = 0.037).

Conclusions: In our cohort, advanced liver fibrosis was present in the majority of adult Fontan-patients. Liver stiffness as measured by transient elastography was not associated with the degree of liver fibrosis. Due to its high variability on serial measurements it seems not to be useful for clinical decision-making. The unexpected finding that younger age at Fontan-completion was associated with advanced liver fibrosis merits further evaluation.

Key words: Fontan operation, Fontan associated liver disease (FALD), transjugular liver biopsy, transient elastography, liver stiffness

Introduction

The Fontan palliation has emerged as the standard surgical procedure for patients with univentricular physiology and has considerably improved their survival. [1] Thus, the number of adult patients with univentricular physiology after Fontan palliation is continuously growing. [2] Fontan-associated liver disease (FALD) has emerged as a major contributor to long-term morbidity and mortality in these cohorts. [3-6] Advanced liver fibrosis is associated with adverse outcomes and has an important impact on clinical decision-making. [7-11] The evaluation of liver stiffness by transient elastography is well established for non-invasive staging of liver fibrosis in various chronic liver diseases such as chronic viral hepatitis and metabolic liver disease. [12] However, liver stiffness is not only increased by hepatic fibrosis but also due to hepatic congestion, which is a feature of FALD. Yet, non-invasive determinants and predictors of advanced fibrosis in Fontan patients are not well defined and conflicting data exist regarding the utility of measurements of liver stiffness in this setting. [7, 13, 14]

The aims of our study were therefore to assess the prevalence and predictors of advanced liver fibrosis in a well-defined cohort of adult Fontan patients with a specific focus on the utility of non-invasive measurements of liver stiffness as determined by transient elastography.

Material and methods

In the absence of a generally accepted surveillance strategy for FALD, a structured FALD surveillance protocol was implemented at the University Hospital Zurich by a multi-disciplinary team of specialized cardiologists, hepatologists and radiologists in 2013. This surveillance protocol was also adopted by the program at the University Hospital of Basel.

Standard follow-up procedures according to the FALD surveillance protocol include: Liver / abdominal ultrasound, liver stiffness measurement by transient elastography, liver function tests and serologic screening for viral hepatitis at first visit. In patients with abnormal findings on ultrasound or transient elastography, transjugular liver biopsy with measurement of Fontan-pressure and hepatic venous pressure gradient (HVPG) was recommended. In case of elevated Fontan-pressures (≥ 15mmHg) a full invasive work-up with left- and right heart catheterization is recommended. Since 2019 routine liver magnetic resonance imaging or multi-phase contrast computed tomography (in case of contraindications for magnetic resonance imaging) has been recommended for all patients at least once for assessment and screening of liver nodules. Follow-up intervals, follow-up (imaging) modalities and further testing (e.g. endoscopic screening for esophageal varices) were regularly discussed and determined at our institution's multi-disciplinary Fontan-Liver board.

Patient cohort

All Fontan-patients followed at the University Hospitals of Basel and Zurich that previously had been enrolled into the Swiss SACHER-registry by the end of 2022 were identified (ClinicalTrials.gov Identifier NCT 2258724). For the purpose of this analysis, we included all patients that had undergone liver ultrasound and transient elastography and/or a transjugular liver biopsy. All patients had given written informed consent for analysis of clinical data at the time of enrolment into the registry. [15] The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the responsible ethics committee (KEK-ZH 2020-01042).

Aims

The study aims were: 1.) to assess the association between liver stiffness as measured by transient elastography with clinical parameters and liver fibrosis in the subset of patients with transjugular liver biopsies; 2.) to assess the value of serial measurements of liver stiffness in clinically stable patients and 3.) to assess determinants of advanced liver-fibrosis / cirrhosis in the subset of patients with transjugular liver biopsy.

Study procedures

Liver ultrasound and transient elastography

Abdominal ultrasound examination was performed by experienced hepatologists after the patients were fasting for at least six hours. Examination focused on liver parenchyma and surface, focal liver lesions, as well as liver vessels and signs of portal hypertension (spleen size, flow portal vein, diameter of portal vein and ascites).

Liver stiffness (in kPa) was measured by transient elastography (FibroScan®, Echosens, Paris, France) after fasting of at least 6 hours according to current guideline recommendations. [16] In patients with a BMI <30 kg/m^2 the standard M probe (3.5 MHz) was used and the XL probe (2.5 MHz) for BMI ≥ 30 kg/m^2. [17] Liver stiffness measurement results were defined as reliable when ≥10 valid measurements with a success rate ≥ 60% and an interquartile/median ratio (IQR/Med) ≤ 30% were achieved as recommended. [16] The median value of liver stiffness measurements of each individual patient and the IQR/Med were used for further analysis.

Transjugular liver biopsy with hepatic venous pressure gradient measurement

The intervention was performed in an outpatient setting without discontinuation of oral anticoagulation. After local anesthesia a 6-French sheath was introduced in the internal jugular vein under ultrasonographic guidance. Pressure measurements were performed with a dedicated balloon catheter. Thereafter the transvenous needle-biopsy was performed through the same vascular access. Liver tissue was stained with hematoxylin and eosin (H&E), Sirius red, Elastin van Gieson and/or Masson's trichrome. Severity of tissue fibrosis was graded according to the congestive hepatic fibrosis score (CHFS: 0-4). [18] In challenging cases glutamine synthetase (GS; mouse monoclonal antibody, clone 6/Glutamine Synthetase; dilution 1:1000) immunohistochemistry was applied, as it is regarded useful to clarify the architecture in small or fragmented specimens and to evaluate the loss of metabolic zonation for a more reliable scoring of congestive hepatic fibrosis. [19] All samples were evaluated by two experienced hepatic pathologists (EMM or DL). Fibrosis scores of 3 or 4 were considered advanced fibrosis.

Routine cardiology follow-up and baseline characteristics

All patients are under regular follow-up by dedicated teams of specialists for adult congenital heart disease. Routine echocardiography, clinical assessment and cardio-pulmonary exercise-testing follow standardized institutional protocols, based on recommendations of national and international guidelines. [20, 21] For the purpose of this analysis, clinical data, cardiac imaging data and exercise data were extracted from the electronic patient record, using data from within three months before or after hepatic ultrasound and / or transjugular liver biopsy.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Categorical variables are reported as frequencies and percentages and continuous variables as mean and standard deviation or median and range or interquartile range (IQR) as appropriate. For comparisons between groups, we used Chi-square or Fisher's exact tests and for continuous variables Student's t-test (independent-samples or paired-samples as appropriate) or Mann-Whitney tests as appropriate. For the analysis of correlation between liver stiffness, demographic and clinical variables, Pearson-correlation and linear or logistic regression were used. A two-sided p-value of <0.05 was considered statistically significant.

Results

Patient cohort

A total of 97 adult Fontan patients were included, 50 (52%) of which had undergone transjugular liver biopsy. Data from liver ultrasound and transthoracic echocardiography were available in 94 patients (97%) and data from cardio-pulmonary exercise testing in 88 patients (91%). Baseline-characteristics are displayed in ***table 1***, stratified for patients with and without transjugular liver biopsy. Within the cohort, 27 patients (28%) had single ventricle physiology of right ventricular type, including 12 patients (12% of the entire cohort) with Norwood palliation for hypoplastic left heart syndrome. Patients without liver biopsy were younger, more commonly underwent a staged Fontan-operation, were less likely to be on oral anticoagulation and had lower NT-proBNP-levels. Serology for hepatitis A, B and C was negative in all patients. The MELD-XI-Score (Model for End-stage Liver

Disease excluding INR Score) was calculated in 96/97 patients (99%). Of these, 30 patients (31%) had a MELD-XI-score of ≥ 11, previously found to be associated with adverse outcomes. [22]

Liver ultrasound and transient elastography

Data from liver ultrasound was available in 94 patients (97%) and measurement of liver stiffness by means of transient elastography (FibroScan®) was technically feasible in 92 of these patients (98%). Main findings are presented in ***table 1***. Median liver stiffness measurement values were not significantly different between patients with mild fibrosis (CHFS 0-2) and advanced fibrosis (CHFS 3-4) (***Table 4, Figure 2***). Only four patients (4%) had liver stiffness measurement values of < 10 kPa.

Assessment of correlations and associations between liver stiffness, demographic and clinical variables are depicted in ***table 2*** and ***table 3***. Table 3 shows a bivariate analysis of liver stiffness for several demographic or hepatologic characteristics (e.g. male versus female patients, patients with single ventricles of right ventricular type versus single ventricle of left ventricular type, etc.). Weak, albeit statistically significant correlations were found for conduit size in patients with extracardiac total cavo-pulmonary connection, platelet count, peak oxygen uptake on cardio-pulmonary exercise testing and Fontan-pressures in patients with transjugular liver biopsy. No association was found for other non-invasive and sonographic characteristics of liver disease (irregular liver surface, spleen size and MELD-XI-score).

Serial measurements of liver stiffness

In 73 patients (78%) serial transient elastography measurements were available with a median of 4 (range: 2 – 11) measurements per patient and a median follow-up duration of 3.3 years (IQR: 2.0-4.7 years). On average, there was no change from first to last

measurement of liver stiffness (20.9 ± 8.2 kPa versus 20.8 ± 8.3 kPa, p = 0.963) with relatively large variability in individual patients (***figure 1, Panel A***). ***Figure 1, Panel B*** depicts maximal range of measurements of liver stiffness in all 73 individual patients in whom serial data were available.

Liver biopsy

In 7/50 patients (14%) liver biopsy was performed for a specific clinical indication (in 4 patients as part of transplant assessment, 2 patients prior to major cardiac re-operation and in 1 patient in preparation before embarking on pregnancy). In all other patients, liver biopsy was recommended as part of routine hepatologic surveillance. The majority of transjugular liver biopsies (90%) were performed in an outpatient setting without discontinuation of anticoagulation and none of the patients experienced procedure-related complications. The majority of patients (35/50, 70%) had advanced fibrosis according to the congestive hepatic fibrosis score, including six patients (12%) with findings consistent with liver cirrhosis (CHFS 4). Of patients without advanced fibrosis, only one patient had findings consistent with CHFS 1, nine (18%) were in CHFS 2A and five patients (10%) in CHFS 2B.

A comparison of patients with and without advanced fibrosis is displayed in ***table 4***. Neither type of Fontan-procedure, type of single ventricle, ventricular function, exercise capacity, patient age nor time since Fontan-operation were associated with advanced liver fibrosis. Patients with advanced fibrosis had their Fontan-completion at significantly younger age compared to those without advanced fibrosis (3.7 years, IQR: 2.3-6.3 versus 6.8 years, IQR: 3.5-12.1, p = 0.037). Hepatologic features associated with advanced fibrosis were a MELD-XI-score of ≥11 and a larger spleen size. Noteworthy, no differences were found in liver stiffness measurements (***see figure 2***) and other sonographic features

of liver disease in patients with and without advanced fibrosis. Patients with advanced fibrosis tended to have higher Fontan-pressures (14mmHg, IQR: 10-16 versus 11mmHg, IQR: 9-13, p = 0.055) but this did not reach statistical significance.

Discussion

In our cohort of adult Fontan patients from two tertiary care centers, a substantial proportion (70% of those who underwent biopsy) were found to have advanced liver fibrosis, including six patients (12%) classified as cirrhosis. Liver stiffness, as measured by ultrasound transient elastography, was not associated with the degree of liver fibrosis on liver biopsy. Due to its high intra-patient variability on serial follow-up measurements in a clinically stable patient population, serial transient elastography measurements do not seem to be clinically useful for the detection or follow-up of FALD.

Utility of transient elastography

Over the last decade, there has been an increasing interest in FALD and numerous studies have been published on the topic. Reported patient cohorts and methodology of these studies were very heterogeneous, which hampers interpretation and generalizability of some of the results. For example, reported cohorts often included pediatric and adult populations and in many studies definitions of advanced liver fibrosis were not determined by histologic analysis of liver biopsies but rather by non-invasive findings, such as findings on hepatic ultrasound.

Previous reports on the utility of liver stiffness measurements by means of transient elastography were ambiguous. While some reports found an association between liver stiffness and advanced liver fibrosis, other studies did not find such an association. [7, 13]

Most series included pediatric and adult patients. In our cohort of adult Fontan patients, markedly elevated liver stiffness was present in almost all patients. We found a weak correlation of liver stiffness with exercise capacity and elevated Fontan-pressures in the subgroup of patients that underwent liver biopsy but liver stiffness was not associated with liver fibrosis or other ultrasound characteristics of liver disease. In a substantial proportion of clinically stable patients within our cohort, serial measurements of liver stiffness were available. While there was no change in average liver-stiffness over a follow-up duration of more than three years in the entire cohort, large changes of liver stiffness in individual patients were observed. We believe that our findings challenge the utility of liver stiffness measurements by means of transient elastography in clinical practice for identification of adult Fontan patients at risk for advanced liver fibrosis. Whether other measures of liver stiffness, such as shear-wave measurements or determinants of liver stiffness determined by magnetic resonance imaging provide better prediction remains to be elucidated.

Advanced liver fibrosis and its predictors

Among the herein reported cohort of adult Fontan patients, about half underwent transjugular liver biopsy. Most liver biopsies were performed as part of routine follow-up, due to abnormal findings on liver ultrasound. Patients that had not (yet) undergone liver biopsy were younger and thus more commonly had total cavo-pulmonary connection. Otherwise, there were no major differences among baseline characteristics and importantly no differences among non-invasive determinants of advanced Fontan-associated liver disease, such as enlarged spleen size, MELD-XI-score, liver stiffness or abnormal liver appearance on ultrasound. Noteworthy, apart from spleen size, none of the other ultrasound characteristics of liver disease were associated with advanced fibrosis. As outlined above, particularly liver stiffness measured with transient elastography showed

no association to the degree of liver fibrosis. This contradicts early reports in small patient cohorts but is in agreement with the most comprehensive series, published by Munsterman and colleagues, which did also not find an association of liver stiffness and advanced fibrosis. [7] Baseline-characteristics of the cohort reported by Munsterman were comparable to the cohort reported in this study. Advanced fibrosis was found in roughly two thirds of all patients within the two cohorts. In contrast to the study by Munsterman, we found an association of advanced fibrosis with spleen size and MELD-XI-Score of ≥11. While some studies suggested a correlation of advanced liver fibrosis with age or time since Fontan operation, surprisingly, in our cohort this association was not present. As outlined above, heterogeneity among studies, particularly in terms of definition of advanced Fontan associated liver disease hamper comparison of reported findings. Thus, all efforts for unifying surveillance protocols for FALD must be applauded. [3]

Age at Fontan operation predicted advanced fibrosis

Interestingly, while we did not observe an association between age or time since the Fontan operation and advanced fibrosis, patients with advanced liver fibrosis were significantly younger at the time of their Fontan-completion compared to those without advanced fibrosis. We believe that this observation merits further exploration in larger cohorts. Contemporarily the vast majority of children with univentricular hearts will undergo total cavo-pulmonary connections with use of extra-cardiac prosthetic conduits. One may speculate that earlier Fontan-completion limits conduit size and thus with somatic (out-) growing of small conduits, patients with earlier completion may have less favorable long-term hemodynamics with higher Fontan-pressures, promoting accelerated hepatic fibrosis. While it did not reach statistical significance, there was a trend towards higher Fontan-pressures at the time of biopsy in patients with advanced liver fibrosis in our cohort.

Transjugular versus percutaneous liver biopsy

While percutaneous liver biopsies may provide slightly better tissue samples compared to transjugular biopsies, the latter technique offers several potential advantages. First, the risk for complications, particularly the risk of bleeding, is likely lower in transjugular biopsies. [23] Second, transjugular biopsy allows assessment of Fontan-pressures and may thus be a valuable screening tool for identification of patients with unfavorable Fontan hemodynamics that may be amenable to further testing and treatment. In our clinical practice, we offer full hemodynamic assessment by means of left and right heart catheterization with or without vasoreactivity testing to all patients with Fontan-pressures ≥15mmHg at time of transjugular liver biopsy.

Our data do not allow generalizable recommendations for hepatic follow-up in Fontan-patients. However, we believe that establishing an institutional surveillance protocol with close inter-disciplinary collaboration among adult congenital heart disease specialists, hepatologists, radiologists and pathologists is important. It may help identifying patients at risk for hepatic complications and may help with early detection of hepatic complications once they occur.

Limitations

Although well defined, the herein reported cohort of adult Fontan patients is relatively small and ideally, findings have to be confirmed in larger cohorts. Although baseline characteristics were comparable in patients with and without liver biopsy, only about half of all patients underwent biopsy. While findings of liver stiffness were not associated with advanced liver fibrosis, we cannot exclude a prognostic value, as it may to some extent

reflect Fontan-hemodynamics. Given the very high variability of liver stiffness measurements among individual patients, however, limits its use for serial follow-up assessment. Transjugular liver biopsies may be hampered by sampling errors and thus a comprehensive surveillance strategy needs to include imaging modalities. The yield of additional imaging modalities, such as liver magnetic resonance imaging and computed tomography requires careful analysis in the future, in order to develop optimal and comprehensive liver surveillance protocols in Fontan patients.

Conclusions

In our cohort, the majority of adult Fontan patients were found to have advanced liver fibrosis. Liver stiffness as measured by ultrasound transient elastography was not associated with the degree of liver fibrosis and due to its high variability on serial measurements seems not to be useful for clinical decision-making. The surprising finding that younger age at Fontan completion was associated with advanced liver fibrosis merits further evaluation.

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Tables

Table 1: Baseline characteristics, stratified for patients with and without liver biopsy

	All patients (n = 97)	**Biopsy** (n = 50)	**No biopsy** (n = 47)	*p value*
Male gender (%)	53 (55)	25 (50)	28 (60)	0.416
Anatomy				
Single right ventricle (%)	27 (28)	10 (36)	17 (20)	0.112
HLHS (%)	12 (12)	6 (12)	6 (13)	0.396
Age at Fontan completion (years)	4.1 (2.6-6.4)	4.2 (2.4-7.0)	3.8 (2.7-5.3)	0.462
Age at first liver ultrasound (years)	23.1 (18.7-30.6)	25.9 (19.5-34.0)	20.3 (18.2-26.5)	**0.002**
Time since Fontan operation (years)	18.3 (15.3-25.8)	21.8 (16.7-27.8)	16.5 (15.0-22.0)	**0.003**
Type of Fontan operation				
Staged Fontan operation (%)	55 (57)	21 (42)	34 (72)	**0.004**
Atrio-pulmonary Fontan (%)	28 (29)	19 (38)	9 (19)	0.117
TCPC-Fontan (%)	69 (71)	31 (62)	38 (81)	**0.047**
Previous IART (%)	38 (40)	25 (50)	13 (28)	0.060
Oral anticoagulation (%)	39 (40)	28 (57)	11 (23)	**0.001**
Body mass index (kg/m^2)	23.0 (20.8-25.0)	23.1 (21.0-25.8)	22.8 (20.7-24.6)	0.352
Oxygen saturation (%)	94 (91-96)	93 (91-95)	94 (91-96)	0.264
Impaired ejection fractions (%)	32 (34)	20 (40)	12 (27)	0.197
>Mild AV-valve regurgitation (%)	19 (20)	10 (20)	9 (20)	1.00
Diameter of inferior vena cava (mm)	20 (18-22)	20 (18-22)	20 (18-21)	0.329
Peak % predicted oxygen uptake (%)	64 (54-73)	61 (51-72)	67 (55-74)	0.249
NT-proBNP (ng/l)	169 (75-330)	287 (117-490)	120 (49-206)	**<0.0001**
Platelet count (G/l)	171 (140-227)	167 (135-228)	180 (145-226)	0.286
MELD-XI score ≥ 11 (%)	30 (31)	18 (37)	12 (26)	0.275
Liver stiffness (kPa)	20.9 (14.3-26.2)	21.2 (14.4-26.0)	18.7 (14.1-26.2)	0.317
Spleen size (cm)	12.5 +/- 2.0	12.6 +/- 2.3	12.3 +/- 1.5	0.609
Spleen size > 13.0cm	28 (32)	19 (40)	9 (23)	0.110
Irregular liver surface (%)	46 (49)	24 (50)	22 (48)	0.840
Blunt liver edge (%)	69 (73)	34 (71)	35 (76)	0.644

Continuous variables are expressed as mean with Standard deviation or median and interquartile range (IQR) as appropriate. Categorical variables are expressed as n (%). Abbreviations: HLHS: Hypoplastic left heart syndrome; TCPC-Fontan: Total cavo-pulmonary connection (lateral tunnel or extra-cardiac Fontan); IART: Intra-atrial reentrant tachycardia; AV-valve: Atrio-ventricular valve; NT-proBNP: N-terminal pro natriuretic peptide; MELD-XI-score: Model for End-stage Liver Disease excluding INR (MELD-XI)

Table 2: Correlation of liver stiffness with demographic and clinical variables

Variable	R-value	p-value
Age at Fontan completion	0.097	0.357
Years since Fontan completion	-0.030	0.774
Conduit size in patients with TCPC-Fontan	0.332	**0.020**
Body mass index	0.104	0.325
Oxygen saturation	-0.135	0.200
Spleen size	0.124	0.258
MELD-XI-Score	0.127	0.228
NT-proBNP value	0.006	0.955
Platelet count	-0.278	**0.008**
Diameter of inferior caval vein	0.212	0.056
Ejection fraction of single ventricle	0.092	0.478
Peak oxygen uptake on exercise testing	-0.315	**0.004**
Fontan-pressure at time of transjugular biopsy	0.360	**0.012**
Hepatic venous pressure gradient	-0.009	0.954

Abbreviations: TCPC: Total cavo-pulmonary connection, NT-proBNP: N-terminal pro natriuretic peptide; MELD-XI-score: Model for End-stage Liver Disease excluding INR (MELD-XI)

Table 3: Bivariate comparison of liver stiffness with clinical variables

Variable / characteristics	Comparison of liver stiffness (kPa) depending on presence of absence of demographic or hepatologic characteristics		p-value
	Variable present	**Variable absent**	
Male gender	22.7 ± 11.6	20.3 ± 8.0	0.254
Single ventricle of right ventricular type	21.0 ± 7.6	21.8 ± 11.0	0.725
Total cavo-pulmonary connection	21.0 ± 9.8	23.1 ± 11.1	0.356
History of intra-atrial re-entrant tachycardia	23.1 ± 13.6	20.7 ±- 7.1	0.266
Abnormal single ventricle systolic function	19.8 ±- 7.7	22.5 ± 11.3	0.239
> mild atrio-ventricular valve regurgitation	23.6 ± 15.0	21.1 ± 8.7	0.345
MELD-XI score <11	21.4 ± 8.2	22.1 ± 14.0	0.768
Irregular liver surface	23.6 ± 11.9	19.9 ± 8.0	0.086
Blunt liver edge	22.2 ± 11.0	20.2 ± 7.0	0.433

Abbreviations: MELD-XI: Model for End-stage Liver Disease excluding INR Score

Table 4: Comparison of patients with and without advanced liver fibrosis (Congestive Hepatic Fibrosis Score 1/2 versus 3/4)

	CHFS 1 / 2 n = 15 (30%)	**CHFS 3 / 4** n = 35 (70%)	p-value
Single ventricle of right ventricular type, n = 10 (%)	4 (40)	6 (60)	0.462
Single ventricle of left ventricular type, n = 40 (%)	11 (28)	29 (72)	
Atrio-pulmonary Fontan, n = 19 (%)	5 (26)	14 (74)	0.757
Total cavo-pulmonary connection, n = 31 (%)	10 (32)	21 (68)	
Conduit size (mm), n = 21	19 (15-21)	17 (16-20)	0.905
Staged Fontan, n = 21 (%)	5 (24)	16 (76)	0.537
Single Stage Fontan, n = 29 (%)	10 (35)	19 (65)	
Male gender, n = 25 (%)	4 (16)	21 (84)	0.062
Female gender, n = 25 (%)	11 (44)	14 (56)	
Body mass index (kg/m^2)	23.1 (20.3-26.1)	23.2 (21.0-25.8)	0.828
History of intra-atrial reentrant tachycardia, n = 25 (%)	8 (32)	17 (68)	1.0
No previous arrhythmia, n = 25 (%)	7 (28)	18 (72)	
Age at Fontan completion (years)	6.8 (3.5-12.1)	3.7 (2.3-6.3)	**0.037**
Age at liver biopsy (years)	30.2 (22.4-38.7)	26.3 (21.8-31.0)	0.193
Time since Fontan completion (years)	23.5 (17.7-27.3)	24.3 (17.8-27.8)	0.649
Normal single ventricle function, n = 30 (%)	11 (37)	19 (63)	0.345
Abnormal single ventricle function, n = 20 (%)	4 (20)	16 (80)	
Normal AV-valve function, n = 40 (%)	11 (28)	29 (72)	0.462
> Mild AV-valve regurgitation, n = 10 (%)	4 (40)	4 (60)	
Diameter of inferior vena cava, n = 44 (mm)	20 (18-22)	20 (18-22)	0.806
NT-proBNP-value, n = 49 (ng/l)	308 (163-501)	232 (108-496)	0.696
Oxygen saturation at rest, n = 48 (%)	95 (92-98)	93 (91-94)	0.171
Peak oxygen uptake, n = 48 (% predicted)	62 +/-11	60 +/-16	0.747
MELD-XI score <11, n = 31 (%)	13/31 (42)	18/31 (58)	**0.029**
MELD-XI score ≥11, n = 18 (%)	2/18 (11)	16/18 (89)	
Platelet count, n = 47 (G/l)	171 (112-229)	167 (135-226)	0.675
Liver stiffness, n = 46 (kPa)	23.4 (13.0-33.6)	21.3 (17.3-26.0)	0.911
Spleen size, n = 48 (cm)	11.3 +/- 1.9	13.2 +/- 2.3	**0.009**
Spleen size > 13.0cm, n = 19 (%)	2/19 (11)	17/19 (90)	**0.026**
Spleen size ≤ 13.0cm, n = 29 (%)	12/29 (41)	17/29 (59)	
Irregular liver surface, n = 24 (%)	7/24 (29)	17/24 (71)	1.0
No irregular liver surface, n = 24 (%)	7/24 (29)	7/2414 (71)	
Blunt liver edge, n = 34 (%)	8/34 (24)	26/34 (74)	0.124
No blunt liver edge, n = 14 (%)	6/14 (43)	8/14 (57)	
Fontan-pressure (mmHg)	11 (9-13)	14 (10-16)	0.055
Hepatic venous pressure gradient (mmHg)	2.0 (1.0-2.0)	2.0 (2.0-3.0)	0.186

Abbreviations: CHFS: Congestive Hepatic Fibrosis Score; AV-valve: Atrio-ventricular valve; NT-proBNP: N-terminal pro natriuretic peptide; MELD-XI-score: Model for End-stage Liver Disease excluding INR (MELD-XI)

Figure legends

Figure 1: Liver stiffness in patients with serial follow-up data

Panel A: Average difference between first and last measurement of liver stiffness by means of transient elastography
Panel B: Maximum range of lowest and highest measurements of liver stiffness among all 73 individual patients with serial follow-up data. Each bar represents the range of minimal and maximal liver stiffness measurement for each of the 73 individual patients

Figure 2: Comparison of liver stiffness in patients with and without advanced liver fibrosis

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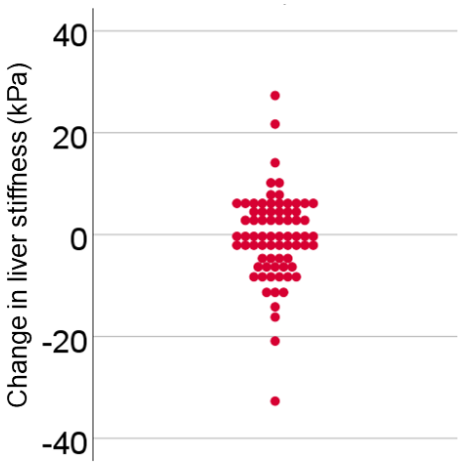

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