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Highlight	◇Fontan 術後には従来の検査手法（超音波エラストグラフィや血液検査）では正確な評価が難しい特異な肝線維化の進展が高い比率でみられる。 ◇Fontan 術後の患者には、少なくとも術後 10 年以降は肝臓の合併症の有無について専門的な医療機関で定期的な診療を受けていただくことが望ましい。
Description	【概要】 研究グループは、Fontan 術後の長期経過における合併症として、従来の検査手法（超音波エラストグラフィや血液検査）では的確な評価が難しい特異な肝線維化の進展が認められることを明らかにしました。 先天性の複雑心疾患に対する Fontan 手術は、国内で年間 400 件近く行われる頻度の高い手術です。22 名の術後患者（術後中央値 14.7 年）の方々から肝組織を採取し、従来の検査手法（超音波エラストグラフィや血液検査）が組織学的な線維化の程度を反映するのか検討しました。 その結果、中程度の線維化が半数以上に、高度な線維化が約 3 割の方々に認められ、それらの線維化は一般的な肝硬変とは異なり類洞域を中心とした特異なパターンを示すことがわかりました。さらに、肝硬度は術後経時的に上昇するにもかかわらず、門脈圧の影響を受け組織学的な線維化を的確に反映せず、また、線維化マーカーとして用いられる 4 型コラーゲンや FIB-4 index などの血液マーカーも組織学的線維化を反映しないことがわかりました。 ‘従来の検査手法では評価が困難　Fontan 術後に特異な肝線維化が生じることを明らかに’. 大阪市立大学. https://www.osaka-cu.ac.jp/ja/news/2020/210307. (参照 2021/03/07)

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ORIGINAL ARTICLE

Hepatology Research

Assessing liver stiffness with conventional cut-off values overestimates liver fibrosis staging in patients who received the Fontan procedure

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Abstract

Aim: Patients who undergo the Fontan procedure for complex congenital heart disease are prone to liver cirrhosis. Liver stiffness (LS) reflects liver fibrosis stage in patients with chronic viral hepatitis; however, its accuracy in predicting liver fibrosis stage in Fontan patients is controversial. We aimed to clarify the correlation between LS and liver fibrosis stage in Fontan patients.

Methods: Fifty-eight Fontan patients were prospectively measured for LS with transient elastography. We undertook liver biopsy, cardiac catheterization, and

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; CHFS, congestive hepatic fibrosis score; CVP, central venous pressure; FIB-4, Fibrosis-4; GGT, γ-glutamyl transpeptidase; HVPG, hepatic venous pressure gradient; LS, liver stiffness; M2BPGi, Mac-2 binding protein glycan isomer.

laboratory tests in 22 of these patients (median age, 14.7 years; range, 9.9–32.1 years) with LS > 11.0 kPa (median, 19.2 kPa; range, 12.2–39.8 kPa); these elevated LS values suggest liver cirrhosis.

Results: Histologically, all patients showed mild-to-severe portal and sinusoidal fibrosis but no cirrhosis. Statistically, LS did not predict histological liver fibrosis scores ($p = 0.175$). Liver stiffness was not correlated with central venous pressure ($p = 0.456$) or with the hepatic venous pressure gradient (HVPG; $p = 0.062$), although the p value for HVPG was only slightly above the threshold for significance.

Conclusions: Fontan patients are prone to developing both portal and sinusoidal fibrosis. Liver stiffness could be influenced by HVPG, and using the conventional cut-off values for LS overestimates and overtreats liver fibrosis in these patients.

KEYWORDS
Fontan procedure, hepatic venous pressure gradient, liver fibrosis, liver stiffness, transient elastography

INTRODUCTION

The Fontan procedure directly connects the inferior vena cava to the pulmonary artery (Figure 1) and is one of the most frequent surgical repairs for children with a functional or anatomic single ventricle.[1–3]

This surgical management increases CVP, leading to liver congestion.[4,5] Thus, as a long-term consequence of the procedure, Fontan patients are prone to developing liver fibrosis that is histologically characterized by portal and sinusoidal fibrosis associated with sinusoidal dilatation (Figure 1).[6,7] This histology differs from that observed with conventional fibrosis caused by chronic viral hepatitis.[8,9] Despite the importance of carefully monitoring the development of liver fibrosis in Fontan patients, currently there is no established method for doing so non-invasively.

Liver stiffness measured with transient elastography[10,11] is an established parameter for predicting liver fibrosis stages in patients with chronic viral hepatitis,[12,13] and cut-off values ranging from 11.0 to 13.6 kPa have been proposed for predicting liver cirrhosis in those patients.[14] In Fontan patients, LS values are mostly elevated,[15–17] but the ability of LS to predict histological liver fibrosis stage is controversial.[16,18] Liver stiffness has also been found to correlate with the HVPG in chronic liver diseases and is therefore able to detect the presence of significant portal hypertension.[19–21] Liver stiffness was also found to increase with elevated CVP in an animal model,[22] but there is no knowledge of whether LS is influenced by HVPG and CVP in Fontan patients. The aim of the present study was to clarify the ability of LS to predict the liver fibrosis stage and to assess the influence of CVP and HVPG on the LS measurement in patients after the Fontan procedure.

METHODS

Patients

Patients who had undergone the Fontan procedure were examined for LS at intervals of 6–12 months between 1 March 2015 and 30 September 2019. Those Fontan patients whose LS values were >11.0 kPa, suggesting liver cirrhosis based on the conventional cut-off values,[14] were recruited for liver biopsy and cardiac catheterization. All experimental protocols were approved by the Osaka City University's and Osaka City General Hospital's Ethics Committee (approval number: 2610) and the study was designed and carried out in accordance with the tenets of the Declaration of Helsinki.

Written informed consent was obtained from each adult patient and each pediatric patient's parent or legal guardian, and written assent was obtained from pediatric patients who were at least 6 years old.

Evaluation of LS

Liver stiffness values were obtained by using FibroScan (EchoSens, Paris, France). Each examination was undertaken by using the 3.5-MHz standard M probe (diameter, 7 mm) after the patient had fasted for at least 3 h, as previously described.[10,11] Liver stiffness measurements were carried out with the patient lying flat. In most cases, the M probe was placed in a right intercostal space under the guide of abdominal ultrasound. In patients with heterotaxy or abdominal situs inversus, the M probe was placed in a left intercostal space if readings could not be detected in the usual location. We

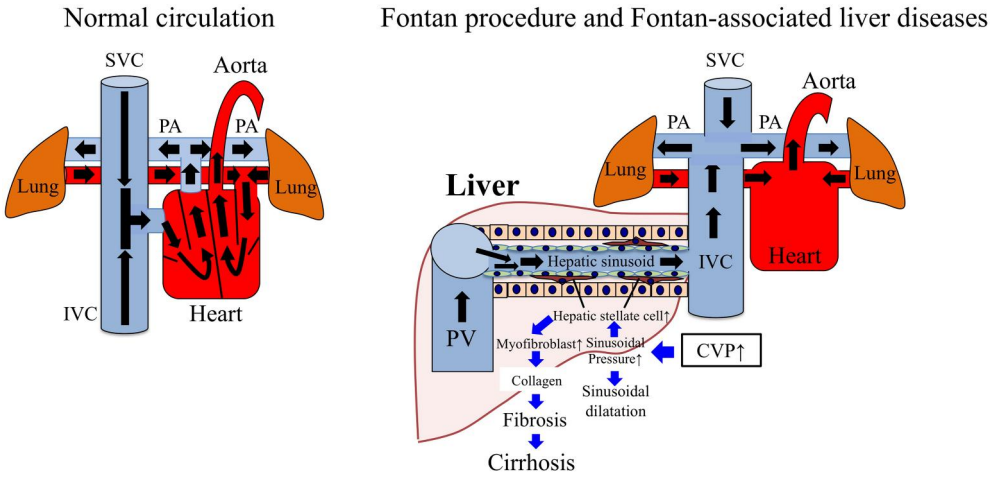

FIGURE 1 Fontan procedure and Fontan-associated liver diseases. CVP, central venous pressure; IVC, inferior vena cava; PA, pulmonary artery; PV, portal vein; SVC, superior vena cava

excluded from the study patients having ascites, a known cause of LS measurement failure. The median value of 10 individual valid measurements was taken as the final LS value and expressed in kilopascals (kPa). For each patient, the success rate was calculated as the ratio of the number of successful measurements to the total number attempted (expressed as a percentage). An examination was considered successful when 10 valid measurements with a success rate of at least 60% were taken and the interquartile range was ≤30% of the median LS value.

Biochemical parameters

The following parameters were assessed at the time of the LS measurement: AST, ALT, GGT, total bilirubin, platelet count, albumin, hyaluronic acid, type IV collagen 7S, M2BPGi, α2-macroglobulin, apolipoprotein-A1, and haptoglobin.[23–28] The APRI, FIB-4 index (based on age, AST, ALT, and platelet count), and FibroTest score (based on age, gender, bilirubin, GGT, α2-macroglobulin, apolipoprotein-A1, and haptoglobin) were calculated as previously described.

Cardiac catheterization and liver biopsy

Cardiac catheterization and liver biopsy were carried out within 3 months of the LS assessment. With the patient under local anesthesia, a venous introducer was placed in the right internal jugular vein by using the Seldinger technique. In cases where accessing the internal jugular vein proved difficult, the femoral vein was selected for cardiac catheterization. During cardiac catheterization, CVP was measured at the inferior vena cava. A balloon-tipped catheter was guided into the right hepatic vein to allow measurement of the wedged and free hepatic venous pressures. Adequacy of occlusion was checked by injecting a small amount of radiological contrast medium. The portal pressure gradient was measured as the HVPG (i.e., the difference between the wedged and free hepatic venous pressures). A transjugular or transfemoral liver biopsy was then carried out with the Liver Access and Biopsy Set and Quick Core Biopsy needle (18G) (both from Cook Japan, Tokyo, Japan) by conventional means. In patients who lacked the necessary venous access for the transjugular or transfemoral liver biopsy, percutaneous needle liver biopsy was carried out by using a 16G or 18G Tru-Cut needle (Merit Medical) through the ultrasound-guided subcostal route. As a result, among 22 patients who received liver biopsy, transjugular biopsy, percutaneous biopsy, and transfemoral biopsy were undertaken in 19, two, and one case, respectively.

Histological evaluation

Biopsy specimens were processed routinely and stained using hematoxylin–eosin and Azan stain. Histological analysis was independently carried out by two pathologists with special training in gastrointestinal and liver pathology; these pathologists were blinded to the LS and clinical results. In cases of discrepancies, liver biopsy specimens were reanalyzed by a third experienced pathologist to unify the histological scores. Liver fibrosis in Fontan patients differs from that caused by chronic viral hepatitis and is histologically characterized by dilated and congested sinusoids with formation of broad, fibrous septa that bridge central veins.[6,7] We therefore used the CHFS score to assess the presence and severity of portal and sinusoidal liver fibrosis as well as sinusoidal dilatation as described previously.[29] Briefly, CHFS was scored: 0, no fibrosis; 1, central zone fibrosis; 2A, central zone and mild portal fibrosis, with accentuation at central zone; 2B, at least moderate portal fibrosis and central zone fibrosis, with accentuation at portal zone; 3, bridging fibrosis; and 4, cirrhosis.

Statistical analyses

All statistical analyses were undertaken by a professional statistician. To assess changes in LS over time, we carried out a non-linear regression analysis with a Huber–White robust sandwich estimator. This model included the value of LS evaluated at each time point as a function of the elapsed time from the Fontan surgery with adjustment for the baseline values of LS, age, and gender. The robustness estimator accounts for dependence in repeated measures within a single patient. This model contained non-linear restricted cubic splines to assess the non-linear association between a value of LS and elapsed time. We assessed the relationship between congestive hepatic fibrosis score and LS, indirect biochemical markers (FibroTest score, FIB-4 index, APRI, type IV collagen 7s, M2BPGi, and hyaluronic acid) or time since surgery for using the C-index estimated by a binary logistic regression model. The model included the expression of fibrosis as a dependent variable and LS, indirect biochemical markers, or time since surgery as independent variables with non-linearity among the biopsy patients. To examine the associations between LS and CVP/or HVPG, linear regression analyses were carried out with CVP or HVPG as functions of LS. All statistical analyses were undertaken with two-sided tests at the 5% significance level using the "rms" package and R software version 3.5.0 (https://www.r-project.org/foundation/).

RESULTS

Liver stiffness increases with time since Fontan procedure

A total of 168 LS examinations were carried in 58 Fontan patients (median age, 11.1 years; range, 4.2–32.0 years) with a median time since surgery of 9.4 years (range, 1.3–18.7 years). Liver stiffness showed a significant non-linear association with the time since surgery ($p = 0.007$, p for non-linearity = 0.037) (Figure 2).

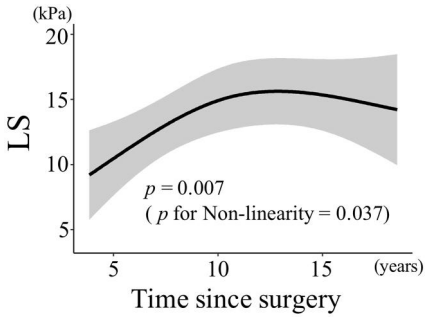

FIGURE 2 Relationship between liver stiffness (LS) and time since surgery in Fontant patients. Relationship between LS and the time since surgery was analyzed by using a robust estimation that included two baseline covariates (sex and age at the time of the Fontan procedure). A total of 168 valid LS examinations were carried out in 58 patients. Data from all examinations are included in this analysis

Liver stiffness does not accurately predict liver fibrosis staging in Fontan patients

Among the 58 Fontan patients, 22 patients (median age, 14.7 years; range, 9.9–32.1 years) whose LS values were more than 11.0 kPa (median, 19.2 kPa; range, 12.2–39.8) underwent liver biopsy and measurement of CVP and HVPG. All 22 patients showed both portal and sinusoidal fibrosis to some extent (Figure 3). Predominant sinusoidal dilatation was observed, and portal inflammation was absent or mild. Twenty-one of the 22 (95.5%) patients had CHFS (≥2A), 12 (54.5%) had CHFS (≥2B), and six (27.3%) had CHFS (≥3) (Table 1). None of the patients had cirrhosis (Table 1).

In terms of the predictive impact of the log odds of LS in CHFS, LS did not show significant associations with CHFS ($p = 0.496$, p for non-linearity = 0.750) (Figure 4a). When LS was analyzed for the relationship with moderate-to-severe liver fibrosis (CHFS ≥ 2B) and severe liver fibrosis (CHFS ≥ 3), LS did not show significant associations with CHFS (≥2B) ($p = 0.428$, p for non-linearity = 0.808), and CHFS (≥3) ($p = 0.614$, p for non-linearity = 0.383) (Figure 4b,c).

Relationships between conventional biochemical markers and liver fibrosis staging in Fontan patients

We also examined whether conventional biochemical markers (FibroTest score, FIB-4 index, APRI, M2BPGi, hyaluronic acid, and type IV collagen 7s) predict histological liver fibrosis stages in Fontan patients. As shown in Figure 5, hyaluronic acid, type IV collagen 7s, FibroTest, FIB-4, and APRI did not show significant associations with CHFS in the 22 patients who had a liver biopsy. However, we found a significant but inverse U-shaped association between CHFS and M2BPGi ($p = 0.011$, p for non-linearity = 0.005).

Time since surgery does not have a significant association with liver fibrosis staging in Fontan patients

We examined whether time since surgery has a significant relationship with congestive hepatic fibrosis score. As shown in Figure 6, time since surgery did not show a significant association with congestive hepatic fibrosis scores in the 22 patients who had a liver biopsy with a median of 12.8 years (range, 7.5–19.4 years) since the Fontan procedure.

Relationship between LS and CVP or HVPG in Fontan patients

In the 22 patients who had both LS examination and cardiac catheterization, the median CVP was 10.5 mmHg (range, 5–17 mmHg;

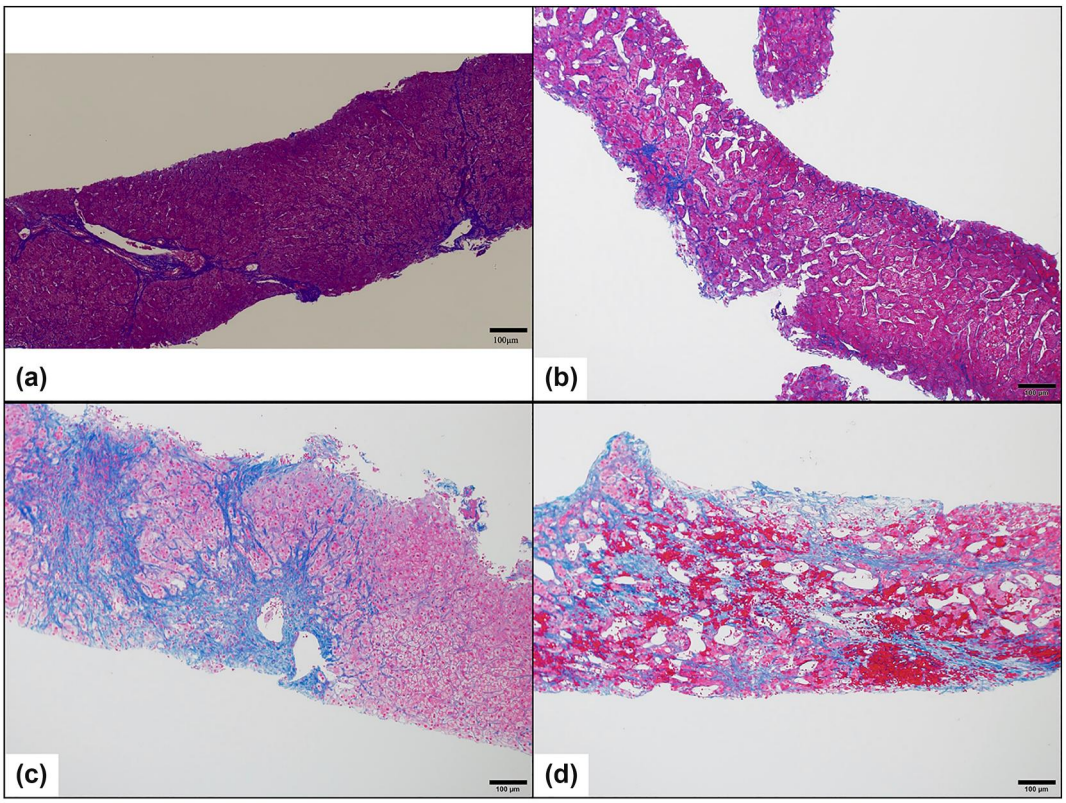

FIGURE 3 Representative microscopic images of Azan staining of liver biopsy specimens. (a) A patient who had the Fontan procedure 12.2 years earlier. Congestive hepatic fibrosis score (CHFS) was 2A. (b) A patient who had the Fontan procedure 11.7 years earlier. CHFS was 1. (c) A patient who had the Fontan procedure 12.3 years earlier. CHFS was 2B. (d) A patient who had the Fontan procedure 16.8 years earlier. CHFS was 3. Bar, 100 μm

reference value, <10 mmHg), and 13 (59.1%) of the 22 patients showed CVP ≥ 10 mmHg (Table 1). The median HVPG was 2 mmHg (range, 1–17 mmHg; reference value, <5 mmHg), and two (9.1%) of the 22 had portal hypertension (Table 1).

Liver stiffness did not show a significant association with CVP (p = 0.456, p for non-linearity = 0.642) (Figure 7a) or with HVPG, although the p value for HVPG was not far above the threshold for significance (p = 0.062, p for non-linearity = 0.352) (Figure 7b).

DISCUSSION

The usefulness of measuring LS has been established in chronic liver diseases (e.g., chronic hepatitis C);[12,13] however, its reliability for assessing liver fibrosis in Fontan patients has been uncertain. Our data clarified that LS does not accurately indicate histological liver fibrosis stages in Fontan patients if the conventional cut-off values designed for chronic viral hepatitis are used. None of our patients who underwent liver biopsy showed evidence of liver cirrhosis (CHFS 4) even if they had LS values suggestive of liver cirrhosis (>11.0 kPa). In addition, logistic regression showed no statistical significance between LS elevation and the development of moderate or severe fibrosis (CHFS ≥ 2B), although we observed severe and moderate-to-severe liver fibrosis in 27.3% and 54.5% of Fontan patients, respectively. These results are alarming in that liver fibrosis stage in Fontan patients could be overestimated or overdiagnosed with liver cirrhosis in spite of actually having a milder stage of liver fibrosis if these patients are assessed based on their LS value alone using the conventional cut-off established in chronic viral hepatitis. Overestimation of liver fibrosis will cause overtreatment associated with unnecessary medical care. In addition, our results showed that conventional indirect biochemical markers (e.g., hyaluronic acid and type IV collagen 7s) do not accurately predict histological liver fibrosis stages in Fontan patients. We consider that the possible explanation for the poor relationship between those conventional indirect biochemical markers (e.g., hyaluronic acid and type IV collagen 7s) and liver fibrosis in Fontan patients is the influence of myocardial fibrosis and hemodynamics. In terms of type IV collagen 7s, it is not a tissue-specific marker and previous studies suggest the relationship between type IV collagen 7s and cardiac diseases.[30,31] A previous study found elevated type IV collagen 7s levels in patients with idiopathic dilated cardiomyopathy and secondary myopathy without liver diseases, thus suggested the involvement of type IV collagen 7s in the myocardial tissue damage.[30] In spite of the fact that the degree of liver congestion at an early stage after Fontan

TABLE 1 Clinical, histological, and hemodynamic data of 22 Fontan patients who underwent liver biopsy and cardiac catheterization

No.	Sex	Age[a] (years)	Time since surgery (years)	LS (kPa)	Congestive hepatic fibrosis score	Hemodynamic data	
						CVP (mmHg)	HVPG (mmHg)
1	F	19.4	16.8	20.5	3	10	2
2	M	18.4	14.1	24.5	2B	8	5
3	F	13.6	11.9	27	2A	13	1
4	M	14.4	12.8	26.4	2B	5	2
5	F	32.1	18.8	21.1	3	13	2
6	M	19.5	17.7	35.3	3	14	17
7	F	20.3	17.2	20.4	2A	13	2
8	M	13.9	12.3	39.8	2B	12	2
9	M	17.7	15.3	17.2	3	8	2
10	F	11.5	10.3	15.5	2B	9	4
11	F	14.9	12.3	13.1	2B	7	2
12	F	22.4	12.2	15.1	2A	6	1
13	M	23.5	19.4	12.2	2A	11	1
14	M	12.9	11.7	17.6	1	12	1
15	F	14.2	12.9	20.9	3	13	2
16	F	30.0	19.3	12.4	2A	12	4
17	M	12.1	10.0	17	2B	8	1
18	F	13.3	11.3	14.1	3	9	1
19	M	14.3	12.1	12.6	2A	10	1
20	M	9.9	7.5	19.8	2A	8	3
21	M	15.7	13.3	18.6	2A	17	4
22	M	14	12.8	21.5	2A	13	1

Abbreviations: CVP, central venous pressure; HVPG, hepatic venous pressure gradient; F, female; LS, liver stiffness value; M, male.

[a]Age at liver biopsy and cardiac catheterization.

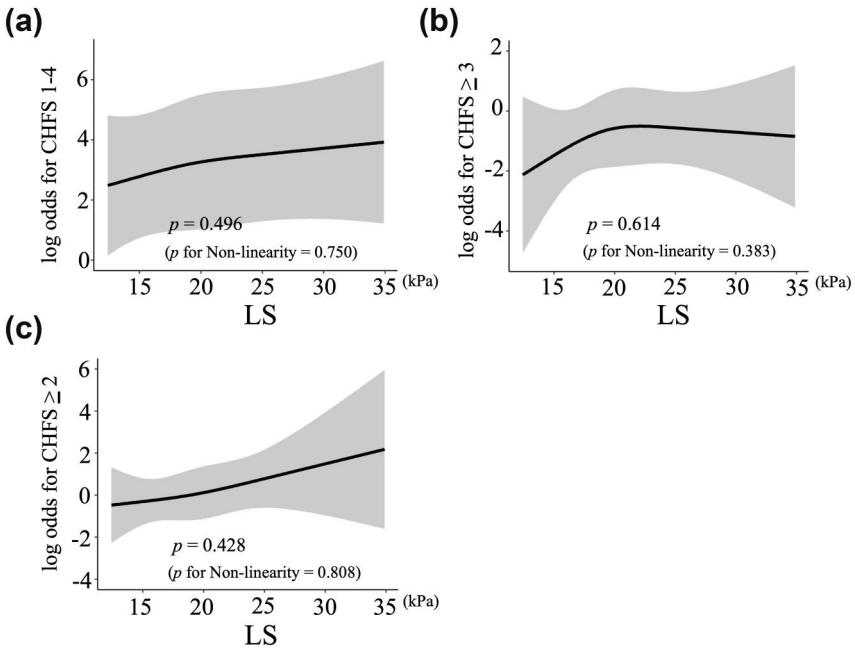

FIGURE 4 Relationship between liver stiffness (LS) and liver fibrosis scores in Fontan patients. The ability of LS to predict: (a) congestive hepatic fibrosis score (CHFS) 1–4, (b) CHFS ≥ 3, or (c) CHFS ≥ 2B was analyzed by using the C-index estimated by a binary logistic regression model

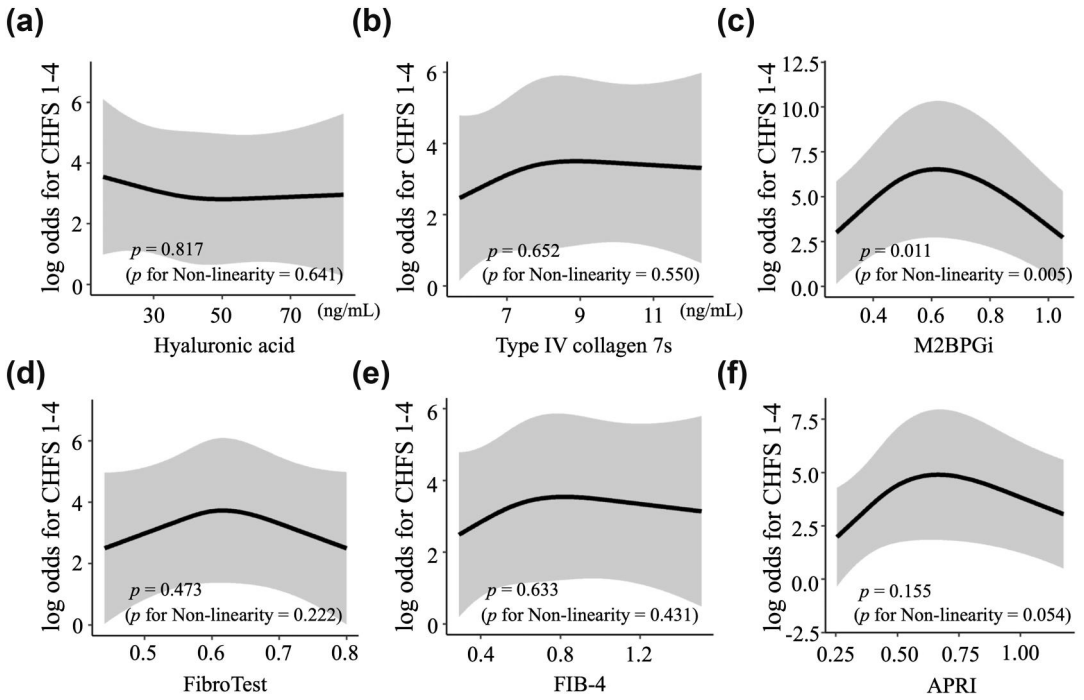

FIGURE 5 Relationship between indirect biochemical markers and liver fibrosis scores in Fontan patients. Ability of indirect biochemical markers to predict congestive hepatic fibrosis score (CHFS) was analyzed by using the C-index estimated by a binary logistic regression model. APRI, aspartate aminotransferase to platelet ratio index; FIB-4, Fibrosis-4; M2BPGi, Mac-2 binding protein glycan isomer

procedure was not sufficiently severe to cause liver fibrosis, type IV collagen 7s was elevated in patients after Fontan procedure and correlated with central venous pressure.[31] Hyaluronic acid has also been reported to be included in cardiac diseases.[32–34] A previous study showed the elevation of hyaluronic acid with myocardial fibrosis in patients after Fontan procedure.[32] Another study reported increased serum concentrations of hyaluronic acid in patients with congestive heart failure.[33,34] Therefore, overlap from concurrent myocardial fibrosis and the worsening hemodynamics could limit the diagnostic value of biomarkers in screening specifically for liver fibrosis.

Among conventional indirect biochemical markers, we found an inverse U-shaped association between CFHS and M2BPGi in patients after Fontan procedure. A previous study has reported that histological liver fibrosis stage increased according to the elevation of M2BPGi in patients with hepatitis C virus infection, hepatitis B virus infection, non-alcoholic fatty liver disease, autoimmune hepatitis, and primary biliary cholangitis.[35] In the current study, either higher or lower values of M2BPGi was associated with reduction of liver fibrosis score, therefore suggesting the inappropriateness of M2BPGi as a fibrotic marker of patients after Fontan procedure. It is necessary to further elucidate the potential influences of other factors on the relationship between higher value of M2BPGi and the reduced fibrosis score in the future large cohort study. In addition, there is a great need to develop novel biomarkers or other methods to specifically predict liver fibrosis in Fontan patients.

LS increased in patients with time after the Fontan procedure. Why does LS increase but not accurately predict liver fibrosis stage

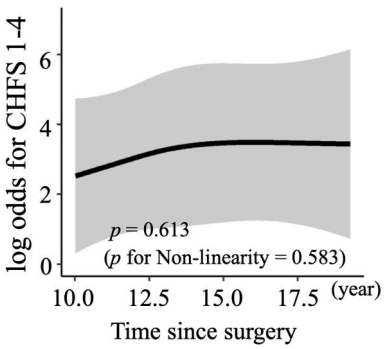

FIGURE 6 Relationship between time since surgery and liver fibrosis scores in Fontan patients. Relationship between time since surgery and congestive hepatic fibrosis score was analyzed using the C-index estimated by a binary logistic regression model

in Fontan patients? Previous studies have found that LS can be increased by factors such as dietary intake, acute liver inflammation (e.g. acute hepatitis), increased CVP, congestive heart failure, and portal hypertension.[19,20,22,36,37] In the present study, LS was measured after the patients fasted for at least 3 h, thus minimizing the influence of diet. The histological finding that portal inflammation was absent in most of the biopsies suggests that the increase in LS cannot be attributed to liver inflammation in our patients. However, CVP was elevated in half of our cases. In addition, predominant sinusoidal dilatation suggesting elevated CVP was found in 13.6% of the patients. These data suggest that increased CVP might increase LS. However, there was no

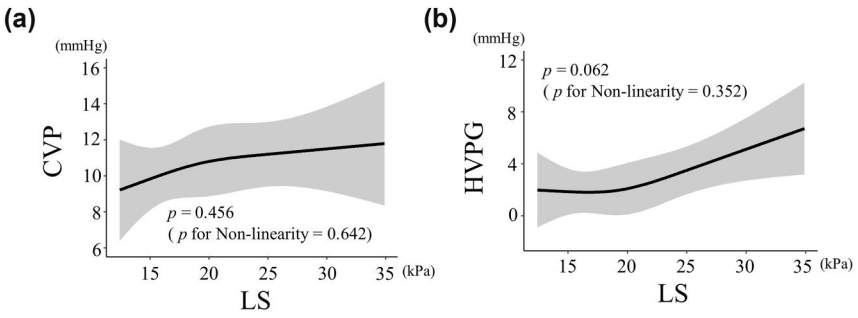

FIGURE 7 Relationship between liver stiffness (LS) and central venous pressure (CVP) or hepatic venous pressure gradient (HVPG) in Fontan patients. Correlations between LS and CVP or HVPG were analyzed by linear regression analyses. (a) Association between LS and CVP ($p = 0.456$). (b) Association between LS and HVPG ($p = 0.062$)

statistically significant association between LS and CVP, and we therefore cannot determine the exact relationship between LS and CVP from the present study. Hepatic venous pressure gradient, an accurate indicator of portal hypertension, showed an association with LS just beyond the threshold for significance; therefore, HVPG might be considered as a potential contributing factor to the increased LS in our patients. Several studies in chronic liver diseases (e.g. chronic viral hepatitis C) have reported a positive association between LS and HVPG with a proposed LS cut-off value of 13.6–21 kPa for predicting clinically significant portal hypertension (HVPG $\geq$ 12 mmHg),[19,20] which is higher than the cut-off value of LS for predicting liver cirrhosis (11.0–13.6 kPa).[14] A future large cohort study could determine whether LS has a significant role in predicting portal hypertension in Fontan patients and reveal the appropriate cut-off values.

Our study has several limitations: it included a relatively small number of subjects with liver biopsy and cardiac catheterization, limiting its statistical power. Nonetheless, our 22 liver biopsies represent the largest cohort in a prospective study of the use of transient elastography for Fontan patients. Another limitation is the difficulty of interpreting the relationship between LS and liver fibrosis that includes both the portal and sinusoidal regions. In chronic hepatitis C, LS is used to predict the portal fibrosis stage, whereas in Fontan patients, LS needs to reflect both the portal and sinusoidal fibrosis stage. There is no consensus on evaluating histological grading in Fontan patients; this study will contribute to develop the histological scoring system in Fontan patients. Other limitation is the interpretation of the relationship between the time intervals since Fontan surgery and the development liver fibrosis. Accumulated study have suggested the development of liver fibrosis according to the time since surgery.[38] A previous study has histologically examined 13 patients and disclosed all of cases had at least some degree of sinusoidal liver fibrosis at mean time of 16.9 years (range 6.9–25) after Fontan procedure.[6] Another histological study found that the time since Fontan procedure >18 years was associated with a higher degree of liver fibrosis as compared with ≤18 years.[39] A large retrospective study with liver function and imaging data reported that 40/195 patients (20.5%) had liver cirrhosis at a mean time of 23.4 years since Fontan procedure.[40] Although our study did not find a significant relationship between liver fibrosis severity and the time since surgery, it did not indicate that the time since surgery has no relationship with the development of liver fibrosis. On the contrary, our study showed that mild-to-severe liver fibrosis will be found in 95.5% of Fontan patients when we focused on those patients whose time interval since surgery was approximately 10 years. We analyzed the relationship between time since surgery and the development of liver fibrosis in a relatively small number of subjects with a range of 12.2–38.9 years since surgery; therefore, if we include patients with earlier or longer time since surgery, the relationship between time since surgery and the development of liver fibrosis could become clearer. In this regard, it is necessary to undertake a large cohort study.

In conclusion, LS measurement overestimates and overtreats liver fibrosis in Fontan patients if conventional cut-off values established for chronic viral hepatitis are used.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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