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Non-invasive fibrosis tools lack clinical utility for identifying advanced fibrosis in Fontan-associated liver disease: a retrospective cohort study

Paul Armstrong ,[1,2] Aoife Moriarty,[1,2] Robert Hughes,[1] Niamh Mehigan,[1] Rhona Savage,[3] Kevin Walsh,[2,3] Jennifer Russell,[1] Stephen Stewart[1,2]

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[1]The Liver Centre, Mater Misericordiae University Hospital, Dublin, Ireland
[2]School of Medicine, University College Dublin, Dublin, Ireland
[3]Department of Cardiology, Mater Misericordiae University Hospital, Dublin, Ireland

Correspondence to
Dr Paul Armstrong;
paul.armstrong@ucdconnect.ie

ABSTRACT

Objective Fontan-associated liver disease (FALD) results from haemodynamic changes following the Fontan procedure for congenital heart disease and is associated with poorer outcomes. The prevalence of Fontan is rising due to improved survival; however, little is known about predictors of advanced liver fibrosis in adult patients. This study aimed to determine the accuracy of non-invasive fibrosis assessment tools (NIT) in predicting histologically confirmed advanced liver fibrosis in an adult Fontan cohort attending Mater Misericordiae University Hospital.

Methods Patient demographics, congenital cardiac variables and fibrosis biomarkers were recorded including liver stiffness measurement (LSM) via transient elastography, Fibrosis-4 (FIB-4) and Aspartate aminotransferase-to-Platelet Ratio Index (APRI) scores. Biopsies, taken between 2017 and 2024, were staged using the congestive hepatic fibrosis score. Analysis was performed using SPSS.

Results 71 patients (58% male) were included. The median age was 25 years. 62% had histological advanced fibrosis. There were no significant bleeding events post biopsy. Overall, advanced fibrosis was associated with a closed Fontan fenestration (p=0.022) and higher LSM, although with a weak correlation (p=0.04, r=0.25, area under the curve (AUC) 0.65), but not with APRI or FIB-4. There was no difference in rates of advanced fibrosis between sex (p=0.84). In females, higher APRI was associated with advanced fibrosis (p=0.045, r=0.41, AUC 0.73).

Conclusions The majority of Fontan patients have advanced liver fibrosis in their third decade. A patent Fontan fenestration appears to reduce the risk of advanced fibrosis. Despite an association with higher LSM, there was no cut-off which could negate the need for biopsy in a significant population. Our data suggest that the discriminatory ability of NIT may vary according to sex. Liver biopsy is safe and remains the only method of reliably diagnosing advanced fibrosis in FALD.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The development of Fontan-associated liver disease (FALD) is associated with poorer outcomes in Fontan patients; however, little is known about prevalence and predictors of advanced liver fibrosis in adult Fontan patients.

WHAT THIS STUDY ADDS

⇒ In a relatively large adult cohort, we demonstrate that 62% of Fontan patients have advanced liver fibrosis at a median age of 25 years. We find that non-invasive fibrosis tools are largely unreliable for diagnosis; however, importantly, the appropriate use of liver biopsy is safe in Fontan patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We show that liver biopsy is safe, effective and remains the only method of reliably diagnosing a condition with rising prevalence worldwide. As patients with Fontan live longer, effective diagnosis of advanced fibrosis in FALD is essential to reduce the complications of undiagnosed liver disease and aid decision-making in organ transplant.

INTRODUCTION

The Fontan procedure has led to improved long-term survival in children with functionally univentricular congenital heart disease, and results in a surgical atriopulmonary or cavopulmonary connection. Systemic venous blood, therefore, bypasses the heart and goes directly to the lungs, leading to significant haemodynamic changes including elevated central venous pressure and diminished cardiac output.[1] The prevalence of Fontan is estimated to be around 7 per 100 000 and is projected to increase into the future as survival improves.[2] Fontan-associated liver disease (FALD) represents a broad spectrum of liver dysfunction ranging from mild fibrosis to established cirrhosis and has been identified in up to 80% of patients post Fontan.[3] This represents an area of growing concern in hepatology.

The extent of the relationship between Fontan circulation haemodynamics and risks of FALD is unclear;[4] however, hepatic

ischaemia originates due to compression from elevated central venous pressure and tissue hypoxia from low cardiac output, with subsequent release of profibrotic factors. This results in fibrosis and cirrhosis in both a centrilobular and portal venous pattern, with potential formation of regenerative nodules which could predispose to hepatocellular carcinoma (HCC).[5] The presence of FALD is associated with increased mortality[6] and the incidence of HCC has been found to be equivalent to other aetiologies of chronic liver disease with a particular impact on years of life lost.[7] Simply enrolling every Fontan patient in 6 monthly HCC surveillance will represent a significant resource burden and likely contribute to unnecessary patient anxiety[8] as well as economic loss due to time off school, study and work. Similarly, an HCC surveillance programme at reduced frequencies of annually or every 2 years could lead to late diagnoses due to known tumour doubling time.[9] Although at slightly lower rates than seen in other aetiologies, cirrhosis is present in the majority of cases of HCC in FALD.[10 11] Identification of cirrhosis in FALD has, therefore, been suggested as a method of instituting effective HCC surveillance.[12] The authors propose that safe and effective methods of staging fibrosis in FALD could be used to identify patients at higher risk of liver-related events, HCC and all-cause mortality post Fontan.

Liver biopsy is the gold standard when it comes to quantification of liver disease in FALD.[13] Liver biopsy does, however, carry potential morbidity and risks relating to time off anticoagulation, sedation and general anaesthesia. There are also issues with sampling error and interobserver variability. A recent meta-analysis has identified some association between Aspartate aminotransferase to Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) and advanced fibrosis in FALD; however, without establishing a clinical utility, and with the exception of FIB-4, data were pooled including children and adults.[14] Currently, data are lacking regarding effective use of non-invasive fibrosis assessment tools (NIT) to diagnose or exclude advanced fibrosis in this patient cohort.[13 15 16] Furthermore, studies have demonstrated that radiological findings which carry high sensitivity and specificity for cirrhosis and portal hypertension in other forms of liver disease are unreliable for diagnosing cirrhosis in FALD.[16] The aim of this single-centre retrospective cohort study was to determine if patient demographics, congenital cardiac factors or non-invasive liver biomarkers were associated with the presence of histologically confirmed advanced liver fibrosis in an adult Fontan population.

METHODS

Using hospital electronic records, we undertook a pseudonymised retrospective review of all patients with Fontan aged ≥18 years attending Mater Misericordiae University Hospital (MMUH) who had undergone a liver biopsy for assessment of FALD between 2017 and 2024. Data relevant to the patient's cardiac history, including most recent cardiac magnetic resonance (CMR) and transthoracic echocardiography (TTE), and the presence of patent Fontan fenestration at time of liver biopsy, were collected. Ejection fraction (EF) on CMR was formally reported as a percentage. TTE was performed by one of two experienced congenital cardiologists and due to the complex circulation was reported as a binary outcome of either preserved or reduced EF. Laboratory testing included liver enzymes alanine aminotransferase and aspartate aminotransferase (AST) which along with platelet count were used to calculate APRI and FIB-4. For APRI calculation, the upper limit of normal for AST was 40 iu/L. Viral and autoimmune liver serological panels and alcohol histories were carried out on all patients as routine to exclude cofactors for liver disease.

Adequate biopsy samples were defined as the presence of ≥11 portal tracts. Liver histological data were obtained from the pathology reports following review by two experienced histopathologists. Fibrosis was staged according to the Congestive Hepatic Fibrosis Score (CHFS) staging system. The validity of each transient elastography (TE) assessment was ensured by performance being limited to experienced operators enforcing acceptable criteria for reliability; at least 10 valid measurements, an IQR/median liver stiffness measurement (LSM) ≤30% and a success rate ≥60%. Body mass index (BMI) was recorded on the day of TE. Data were analysed using SPSS V.29. Descriptive statistics were used to assess differences in characteristics of the study population. Mann-Whitney U test was used to assess strength of relationships between non-normally distributed values. Independent samples median test was used to assess whether two separate groups differed in central tendency. Independent samples t-test was used to compare normally distributed continuous variables. Strengthening the Reporting of Observational Studies in Epidemiology cohort study reporting guidelines were adhered to and a checklist is available as a online supplemental file 1.

RESULTS

Patient demographics and clinical characteristics

71 Fontan patients' cases were reviewed. 41 patients (58%) were male. The median age at Fontan was 6 years (4–12). The median age at biopsy was 25 years (21–31). The median duration of Fontan circulation at the time of biopsy was 17 years (15–22). Median LSM was 21.1 kPa (16.6–25.8). 67 patients (94%) were Caucasian. The liver biopsy procedure was well tolerated. No patient developed any significant postprocedural complications such as bleeding requiring further scanning or intervention. 5 (7%) of patients underwent transjugular liver biopsy, with all others via ultrasound (US) guided percutaneous liver biopsy. As AST is not part of the routine liver panel, only 65 patients had AST performed and were eligible for FIB-4 and APRI measurements. The median FIB-4 and APRI scores were 0.86 (0.62–1.1) and 0.5 (0.3–0.6), respectively. The median controlled attenuated

parameter score was 213 (198–251). The median BMI was 22.9 kg/m^2 (20.6–25.3). Five (7%) of patients met the criteria for obesity (BMI >30 kg/m^2). Three patients (4%) had evidence of steatosis on biopsy. Three patients (4%) included in the review subsequently died from complications of congenital cardiac disease, all of whom had at least advanced fibrosis on biopsy. One patient in the cohort developed HCC, having had advanced fibrosis on biopsy identified previously.

Laboratory values and fibrosis markers by severity of histological fibrosis

Three patients (4%) had no evidence of fibrosis on biopsy. 12 (17%) had F1 fibrosis and F2 fibrosis, respectively. 25 (35%) had F3 fibrosis and 19 (27%) had F4 (cirrhosis) on biopsy. The median CHFS was F3 (online supplemental table 1). Neither APRI, FIB-4 nor LSM associated with CHFS stage (online supplemental figure 1).

Evaluation of patient demographics, laboratory values and fibrosis markers by the presence of biopsy-proven advanced fibrosis

62% of patients had evidence of at least advanced fibrosis. The authors found that higher LSM had a weak association with advanced fibrosis (p=0.04, r=0.25) (table 1). The median LSM of patients with advanced fibrosis was 23.1 (17–29.7) vs 18.3 (15.4–24) in those without. Neither APRI nor FIB-4 readings were associated with greater likelihood of advanced fibrosis. There was no association between age at Fontan (p=0.37), age at biopsy (p=0.64) or duration of Fontan circulation (p=0.87) and presence of advanced fibrosis. There was no association between serum bilirubin (p=0.53), albumin (p=0.91) or platelet count (p=0.74) and the presence of advanced fibrosis. Albumin-bilirubin index (ALBI) grade could not differentiate between presence or absence of advanced fibrosis (p=0.76) or cirrhosis (p=0.55).

Association of congenital cardiac factors with severity of fibrosis

42 patients (59%) had had a TTE within 12 months of liver biopsy. 30 (42%) underwent a formal CMR. Only nine patients underwent both CMR and TTE; however, in these patients, reduced EF as reported on TTE correlated well with lower percentage of EF as formally quantified on CMR (p=0.027, r=0.73). There were associations identified between the presence of at least significant fibrosis and both lower EF percentage quantified on CMR (p=0.006) and reduced EF on TTE (p=0.001). Median EF (%) measured on CMR was 61 (53–64.5) in the group without significant fibrosis compared with 45 (39-54) in the group with at least significant fibrosis. There was no association between EF measured on either TTE (p=0.32) or CMR (p=0.33) and the presence of advanced fibrosis (table 2). Median EF (%) measured on CMR was 53.5 (42.8–63.3) in the group without advanced fibrosis compared with 47.5 (40.5–57) in the group with at least advanced fibrosis. There was no association identified between higher LSM and lower quantified EF on CMR (p=0.69) or reduced EF on TTE (p=0.66).

The presence of a patent Fontan fenestration at time of biopsy had a negative association with the presence of advanced fibrosis (p=0.022). Neither the ventricular morphology (p=0.97) nor the type of Fontan performed (p=0.28) was associated with advanced fibrosis. The long-term use of amiodarone in patients, likely reflecting a more complex Fontan circulation, exhibited a tendency towards associating with increased likelihood of advanced fibrosis, although it did not achieve statistical significance (p=0.09) (table 2).

Sex differences in variables

61% of males and 63% of females had advanced fibrosis on biopsy (p=0.84). Females were more likely to be older at Fontan (p=0.014) and older at biopsy (p=0.04). They tended to have higher BMI measured at time of TE (p=0.017). There was no difference in LSM between males and females. Females had significantly higher platelet counts (p=0.039), but lower APRI scores (p=0.011) and bilirubin (p=0.001) (table 3).

Assessing diagnostic accuracy of non-invasive markers for advanced fibrosis in Fontan patients

In the male subgroup, there was no NIT which could reliably identify advanced fibrosis. In females, APRI had a predictive ability to identify advanced fibrosis (p=0.045, Spearman rr=0.41) (table 1).

The respective diagnostic performances of LSM, APRI and FIB-4 for detecting the presence of advanced fibrosis were assessed using receiver operating characteristic curves (ROC). For LSM, the area under the ROC (AUROC) was 0.65 (95% CI 0.51 to 0.78) (p=0.048). For APRI, the AUROC was 0.64 (95% CI 0.5 to 0.78) (p=0.06). For FIB-4, the AUROC was 0.56 (95% CI 0.42 to 0.7) (p=0.4) (figure 1a).

Using the coordinates along the AUROC, an LSM of ≥26 kPa optimally predicted advanced fibrosis in the overall population, with specificity of 93%, sensitivity of 33%, positive predictive value (PPV) of 88% and negative predictive value (NPV) of 46%. From the perspective of excluding advanced fibrosis non-invasively, an LSM of ≥16 kPa carried an NPV of 57% (table 4).

The diagnostic performance of APRI for predicting advanced fibrosis in females was also assessed using AUROC (figure 1b). For females, APRI had an AUROC 0.73 (95% CI 0.54 to 0.92) p=0.044), LSM AUROC 0.62 (95% CI 0.4 to 0.83) (p=0.3) and FIB-4 had an AUROC of 0.56 (95% CI 0.34 to 0.78) (p=0.62).

DISCUSSION

In this relatively large study of non-invasive markers of liver fibrosis against histology in a solely adult Fontan population, we have found that the presence of fibrosis is almost universal at a median age of 25, with 96% having at least F1 fibrosis. More concerningly, 79% have at least significant fibrosis and 62% advanced fibrosis, a

Table 1 Relationship between variables and presence of biopsy-proven advanced liver fibrosis in a Fontan cohort

Variables	Overall cohort (median) (IQR)	Advanced fibrosis (median) (IQR)	No advanced fibrosis (median) (IQR)	P value (R)
n (overall)	71	44	27	
n (male)	41	25	16	
n (female)	30	19	11	
Age at Fontan (years)	6 (4–12)	6 (4–11)	7 (5–12)	0.37
Male	6 (4–8)	5 (4–7)	6 (4–8)	0.45
Female	9 (5–12)	9 (5–12)	7 (5–15)	0.53
Age at biopsy (years)	25 (21–32)	25 (20–31)	25 (22–33)	0.64
Male	22 (20–31)	22 (20–31)	23 (20–29)	0.95
Female	26 (22–33)	25 (22–30)	31 (22–34)	0.53
Fontan duration (years)	17 (15–22)	17 (15–24)	17 (15–19)	0.87
Male	17 (15–22)	17 (15–24)	17 (15–19)	0.53
Female	17 (14–23)	17 (13–23)	18 (15–27)	0.77
BMI (kg/m^2)	22.9 (20.6–25.3)	23 (20.8–26.3)	21.9 (20–24.7)	0.17
Male	22.1 (19.8–23.5)	22.6 (19.4–23.4)	21.7 (19.9–24.4)	0.81
Female	24.3 (21.7–27)	25.3 (22–29.2)	22.1 (20.1–25)	0.068
LSM (kPa)	21.1 (16.6–25.8)	23.1 (17–29.7)	18.3 (15.4–24)	0.04 (0.25)
Male	22.1 (17–26.8)	23.2 (18.2–32.5)	18.6 (15.9–23.8)	0.051
Female	19.2 (15.6–24.8)	23.1 (16.4–25.9)	18.3 (14.9–24.1)	0.445
APRI	0.5 (0.3–0.6)	0.5 (0.3–0.7)	0.4 (0.3–0.55)	0.075
Male	0.5 (0.4–0.65)	0.6 (0.4–0.73)	0.5 (0.4–0.6)	0.32
Female	0.3 (0.3–0.58)	0.45 (0.3–0.7)	0.3 (0.28–0.35)	0.045 (0.41)
FIB-4	0.86 (0.62–1.1)	0.91 (0.6–1.3)	0.84 (0.63–0.99)	0.51
Male	0.87 (0.71–1.1)	0.91 (0.62–1.35)	0.86 (0.77–0.95)	0.66
Female	0.82 (0.55–1.1)	0.89 (0.52–1.19)	0.7 (0.57–1.17)	0.62
Platelets (per 10^{9}L)	179 (146–218)	179 (143–221)	177 (150–212)	0.74
Male	174 (134–205)	179 (134–218)	164 (130–192)	0.32
Female	193 (168–251)	179 (145–253)	201 (175–232)	0.64
Bilirubin (µmol/L)	19 (12–31)	19 (11–31)	19 (13–33)	0.53
Male	27 (17–37)	21 (10–39)	28 (20–36)	0.33
Female	15 (11–21)	17 (11–23)	13 (10–19)	0.29
Albumin (g/L)	46 (45–49)	47 (45–49)	46 (45–49)	0.91
Male	48 (45–49)	48 (44–49)	48 (45–49)	0.83
Female	45 (45–48)	46 (45–48)	45 (44–48)	0.64

LSM was the only variable to associate with the presence of advanced fibrosis among the entire cohort. In females, the APRI score was associated with the presence of advanced fibrosis.
APRI, AST-to-Platelet Ratio Index; BMI, body mass index; FIB-4, Fibrosis-4; LSM, liver stiffness measurement.

statistic which is in keeping with previous publications.[5] Unlike previously published literature focused on paediatric populations, we saw no association between age at Fontan and risk of advanced fibrosis in this adult population.[17] We saw no difference in advanced fibrosis according to duration of Fontan, a finding corroborated by subgroup analysis of adults in a recent meta-analysis.[14] Healthcare providers have traditionally been reluctant to electively arrange liver biopsies in suspected FALD.[18] Our data suggest that US-guided percutaneous liver biopsy in Fontan can be safe and effective, as from 71 biopsies performed in our cohort there were no significant bleeding events recorded.

The purpose of Fontan fenestration in Fontan is to allow for shunting of deoxygenated blood from the Fontan conduit into the systemic circulation via the right atrium. This increases cardiac output by increasing the preload, which reduces short-term morbidity.[19] A

Table 2 Congenital cardiac factors and their association with biopsy-proven advanced fibrosis in a Fontan cohort

		n	Advanced fibrosis	No advanced fibrosis	P value
Entire cohort		71	44	27	
Ventricular morphology					
	LV	53	34	19	0.97
	RV	16	10	6	
	Unknown	2	0	2	
Type of Fontan					
	AP	11	8	3	0.28
	Extracardiac TCPC	53	33	20	
	Lateral tunnel	3	1	2	
	AP converted to extracardiac TCPC	4	2	2	
Anticoagulation					
	Aspirin	32	19	13	0.59
	Warfarin	26	15	11	
	NOAC	12	9	3	
	Nil	0	1	0	
Long term amiodarone					
	Exposed	12	10	2	0.09
	Unexposed	59	34	25	
Fontan Fenestration at time of biopsy					
	Patent	8	2	6	0.022 (r=−0.27)
	Closed	63	42	21	
EF (TTE)					
	Preserved	32	19	13	0.32
	Reduced	10	6	4	

This table illustrates the relationship between congenital cardiac variables and the presence of biopsy-proven advanced fibrosis. Two patients had unclear ventricular morphology. One patient was not on any antiplatelet or anticoagulant.
AP, atriopulmonary; EF, ejection fraction; LV, left ventricle; NOAC, novel oral anticoagulant; RV, right ventricle; TCPC, total cavopulmonary connection; TTE, transthoracic echocardiography.

considerable proportion of Fontan fenestrations will close naturally, with some requiring mechanical closure due to systemic desaturation and exertional dyspnoea secondary to persistent right to left shunting.[20] Oka *et al* had previously suggested that a patent Fontan fenestration was protective against FALD; however, they defined the presence of FALD using laboratory biochemistry.[21] In our cohort, the presence of a patent Fontan fenestration at the time of liver biopsy appears protective against biopsy-proven advanced fibrosis. The reduced rates of advanced fibrosis observed in patients with a patent Fontan fenestration are likely due to the reduction of pressure within the Fontan circulation that arises with a patent Fontan fenestration. It should also be noted that the apparent trend towards amiodarone use associating with presence of advanced fibrosis is likely confounded by the use of amiodarone in more complex and severe cases of congenital cardiac disease.

CMR is the gold standard for assessing ventricular function in Fontan patients[22]; however, its use can be limited in some patients due to incompatible intracardiac devices. All TTEs were performed by experienced congenital cardiologists; however, due to the complex circulation, these were simply reported as preserved versus reduced EF. Regardless, although with small numbers, we saw excellent correlation between reduced EF on TTE and lower quantitatively expressed EF% on CMR in those who had both tests, supporting the validity of EF measured via TTE in this cohort. Previous research supports this finding.[23] Lower EF in Fontan physiology associates with higher pressure circulation and hence a poorer performing Fontan.[24] In this study, we saw that a reduced EF on CMR or TTE was associated with greater risk of at least significant liver fibrosis, further linking reduced cardiac output and increased systemic venous pressure to the pathophysiology of FALD.[25] Previous

Table 3 Comparison of distribution of variables according to sex in a Fontan cohort

Variable	Sex	Median (IQR)	P value (R)
Age at Fontan (years)	Male	6 (4–8)	0.014 (–0.29)
	Female	9 (5–12)	
Age at biopsy (years)	Male	22 (20-31)	0.04 (–0.25)
	Female	26 (22–33)	
Fontan duration (years)	Male	17 (15–22)	0.59
	Female	17 (14–23)	
BMI (kg/m^2)	Male	22.1 (19.8–23.5)	0.017 (–0.3)
	Female	24.3 (21.7–27)	
LSM (kPa)	Male	22.1 (17–26.8)	0.31
	Female	19.2 (15.6–24.8)	
CAP	Male	211 (191–249)	0.45
	Female	216 (201–254)	
APRI	Male	0.5 (0.4–0.65)	0.011 (0.32)
	Female	0.3 (0.3–0.58)	
FIB-4	Male	0.87 (0.71–1.1)	0.39
	Female	0.82 (0.55–1.1)	
Platelets (per 10^9 L)	Male	174 (134–205)	0.039 (–0.25)
	Female	193 (168–251)	
Bilirubin (µmol/L)	Male	27 (17–37)	0.001 (0.39)
	Female	15 (11–21)	
Albumin (g/L)	Male	48 (45–49)	0.12
	Female	45 (45–48)	

This table illustrates the differences in distribution of variables between males and females. Variables are presented as median (IQR).
APRI, AST-to-Platelet Ratio Index; BMI, body mass index; CAP, controlled attenuated parameter; FIB-4, Fibrosis-4; LSM, liver stiffness measurement.

studies suggested a link between higher LSM and higher circulatory pressures in Fontan[26]; however, in this study, we did not see any association between higher LSM and reduced EF.

We did not identify any differences in laboratory values, serum bilirubin, albumin or platelet count in patients with and without advanced fibrosis. Nor did we see a role for ALBI Grade in identifying patients with advanced fibrosis or cirrhosis. Thrombocytopaenia is often seen in Fontan patients as a consequence of the right to left cardiac shunting creating abnormalities in blood flow, endothelial function and platelet maturation.[27] Mild thrombocytopaenia was frequently identified in our cohort with a median platelet count of 179. However, unlike Emamaullee *et al*[15] who studied an adolescent population, we found no difference in platelet counts of those with and without advanced fibrosis. Perhaps this lack of difference is due to cardiac causes being the primary mechanism of thrombocytopaenia in our cohort, rather than due to portal hypertension. It has been hypothesised that cardiac cirrhosis results in less frequent portal hypertension than traditional cirrhosis.[5]

Analysis of sex differences

58% of patients included in this review were male, which is broadly in keeping with other data.[3 15] We found that females tended to be older at both Fontan and liver biopsy. These findings are similar to a recent study which identified that females underwent Fontan-type surgery at a higher median age than males,[28] which also found that females had lower weight-for-length scores than males, which may explain the delay in completing surgery. Females were also more likely to have a higher BMI than males, the inverse of which is often the case in healthy populations.[29] Females tended to have higher platelet counts than males, which has been described in the general literature;[30] however, not previous Fontan studies.[31] Males had significantly higher serum bilirubin levels. Mild hyperbilirubinaemia is often seen in congestive hepatopathies and can be multifactorial. To date, we have not identified any literature which describes a sex difference in bilirubin levels in FALD.[31] We did not see any significant difference in rates of advanced fibrosis between sexes, with 61% of males and 63% of females being affected. One previous study has found that in a cohort where age at Fontan and biopsy were equal, females had higher fibrosis scores,[31] whereas in our cohort females were older at both.

Non-invasive predictors of fibrosis in FALD

Our review of 71 liver biopsies in adult Fontan patients finds a weak positive association between LSM and presence of advanced fibrosis. However, following the review of the AUROC curve, we do not identify a cut-off which could exclude or confirm the presence of advanced fibrosis reliably, thus negating the need for biopsy in a meaningful population. The presence of increased systemic venous pressures in Fontan can lead to hepatic congestion and with it falsely elevated LSM.[32] With a median LSM of 21.1 kPa, clearly the majority of patients in our cohort had TE readings that would strongly suggest the presence of advanced fibrosis in patients with alternative aetiologies of liver disease. Two small studies with only ten patients each had suggested an association between higher elastography readings and presence of fibrosis.[26 33] Other studies have found no association between LSM via TE and degree of histological fibrosis, such as Shin *et al* who looked at 45 patients who had biopsy plus elastography,[34] or Munsterman *et al* who had a cohort of 36 patients.[16] The variability in results described by different cohort studies looking at

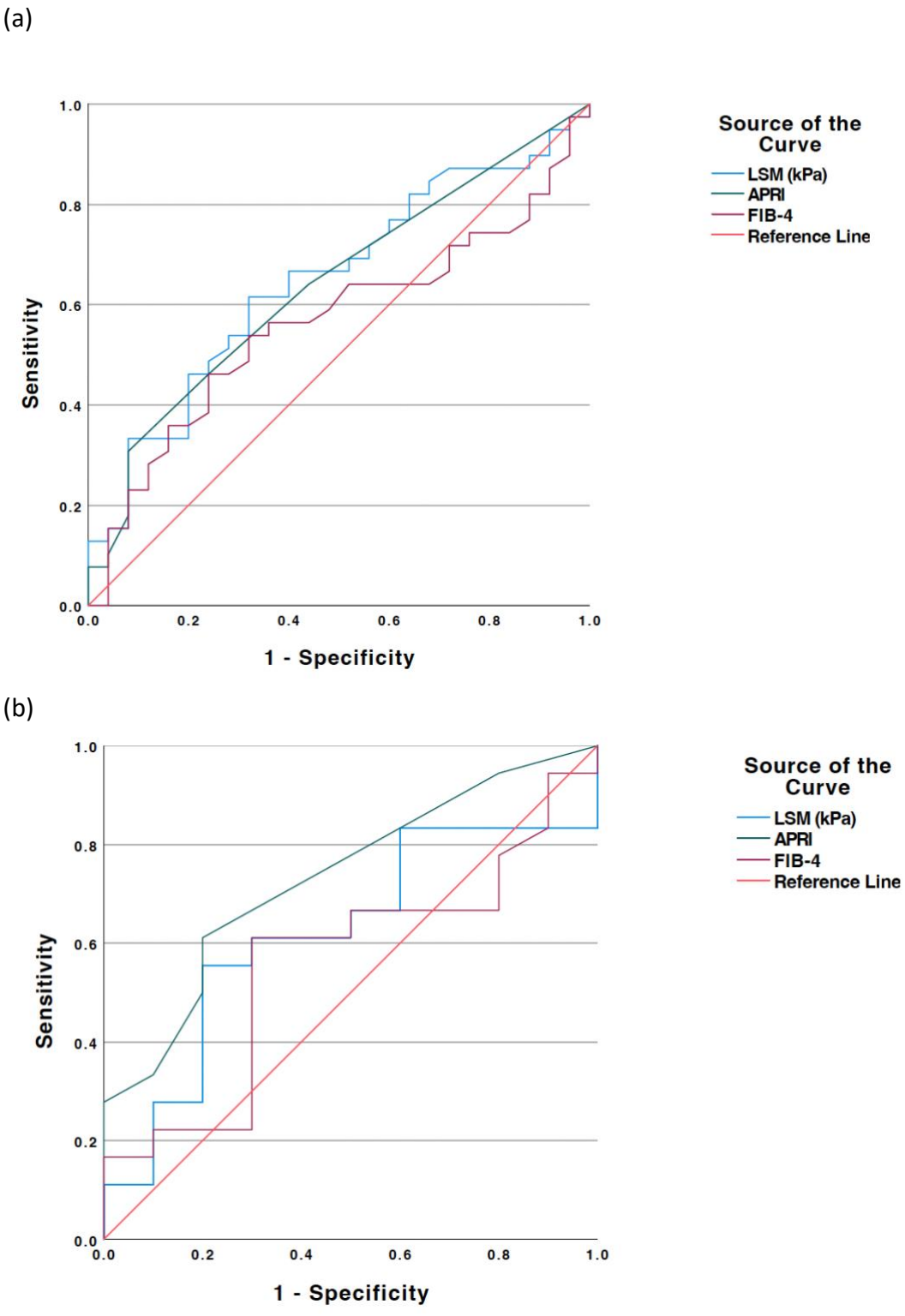

Figure 1 (a) AUROC curve illustrating diagnostic performance of NIT for biopsy-proven advanced fibrosis in Fontan patients and (b) AUROC curves illustrating diagnostic performance of NIT for biopsy-proven advanced fibrosis in female Fontan patients. LSM had the best predictive capacity for the presence of advanced fibrosis in Fontan patients AUROC 0.65 (95% CI 0.51 to 0.78) (p=0.048). For APRI, the AUROC was 0.64 (95% CI 0.5 to 0.78) (p=0.06). For FIB-4, the AUROC was 0.56 (95% CI 0.42 to 0.7) (p=0.4). For females, APRI has a good discriminative ability for predicting advanced fibrosis (AUROC 0.73 (95% CI 0.54 to 0.92) p=0.044). APRI, AST-to-Platelet Ratio Index; AUROC, area under the receiver operating characteristic curve; FIB-4, Fibrosis-4; LSM, liver stiffness measurement; NIT, non-invasive fibrosis assessment tool.

Table 4 AUROC for the diagnostic performance of LSM and APRI in specific Fontan cohorts

Tool	Cohort	AUROC (95% CI) (p value)	Cut-off	Sens.	Spec.	PPV	NPV
LSM	Overall	0.65 (0.51 to 0.78) (0.048)	≥26 kPa	33%	93%	88%	46%
LSM	Overall	0.65 (0.51 to 0.78) (0.048)	≥16 kPa	86%	30%	66%	57%
APRI	Female only	0.73 (0.54 to 0.92) (0.044)	≥0.7	28%	100%	100%	43%
APRI	Female only	0.73 (0.54 to 0.92) (0.044)	≥0.4	58%	78%	85%	47%

This table outlines the sensitivity, specificity, PPV and NPV of LSM in predicting the presence of biopsy-proven advanced fibrosis in the overall cohort, and of APRI for predicting the presence of advanced fibrosis in females only. Proposed cut-offs are only discussed for tests which demonstrated an association with outcome in the specified cohort ($p<0.05$). Higher cut-offs indicate the test's value as a 'rule in' method of predicting those likely to have advanced fibrosis; lower cut-offs indicate the test's value as a 'rule out' method of excluding those unlikely to have advanced fibrosis.
APRI, AST-to-Platelet Ratio Index; AUROC, area under the receiver operating characteristic curve; LSM, liver stiffness measurement; NPV, negative predictive value; PPV, positive predictive value.

the relationship between elastography and fibrosis stage in FALD is a manifestation of the complex effect of the Fontan circulatory pathway on the HVPG, which has been shown to have no predictive value for severity of liver disease in FALD.[35]

The use of APRI and FIB-4 as predictive tools in FALD has been studied with varying results. In our overall cohort analysis, although there is perhaps a trend towards differences, we do not identify any statistically significant association between APRI scores and advanced fibrosis. Similarly, we did not see any association between FIB-4 and advanced fibrosis. One possible explanation for this is the use of age as a variable in the FIB-4 calculator, and the majority of our patients were under 35 years old at the time of biopsy. We hypothesise that APRI's more simple emphasis on AST and platelet count may be more likely to associate with advanced fibrosis than FIB-4. Emamaullee *et al* identified a modest discriminatory role for both APRI and FIB-4 in a relatively large cohort of 106 adolescent patients.[15] However, it should be noted that over 30% of that cohort fit the criteria for obesity, with 10% of biopsies showing evidence of steatosis, suggesting the possibility of MASLD as a confounder. In our study, only 7% were obese and 4% had steatosis on biopsy. Munsterman *et al* in a study of 38 adult patients with a similar median BMI to this review found no differences in APRI or FIB-4 between those with and without advanced fibrosis.[16] Interestingly, despite finding that females had lower APRI scores than males, we identified a moderate correlation between APRI scores and the presence of advanced fibrosis in females only, but not males. We also identified that LSM was less useful in females. Conversely, on analysis of the male cohort only, none of the NITs correlated with the presence of advanced fibrosis.

This study is subject to limitations, primarily that our data were obtained retrospectively from a single centre with some minor variations in the timing and availability of clinical data. The biopsies, however, were performed over a relatively short time period from 2017 to 2024, and as such, biopsy technique and pathological interpretation was similar throughout. Not every patient had either a TTE or CMR, and these were not uniformly performed within the same timeframe in relation to liver biopsy. The sample size, although larger than most published studies on FALD, is relatively small, and as such, larger prospective studies are required in order to ensure that a true effect is not being missed. Also, a liver biopsy, although the gold standard, may not always accurately represent the severity of FALD due to sampling variation.

CONCLUSIONS

In a disease of increasing relevance among adults, we identify a significant FALD morbidity burden. 62% of Fontan patients have histologically confirmed advanced fibrosis at a median age of 25. A patent Fontan fenestration appears protective against advanced fibrosis, and reduced EF associates with presence of at least significant fibrosis. We demonstrate that although there is an association between LSM and presence of advanced fibrosis, no significant clinical utility has been identified to reduce the need for histological assessment. In females only, APRI had a moderate association with advanced fibrosis and higher APRI scores may have utility in 'ruling in' advanced fibrosis in this group. Due to smaller numbers, a validation cohort is required to further investigate this. Liver biopsy is safe and remains the only reliable method of diagnosing or excluding advanced fibrosis in FALD.

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Ethics approval This review was approved by the Mater Misericordiae University Hospital (MMUH) ethics committee (Institutional Review Board Ref: 1/378/2432). Informed consent from individual participants was not required due to the

retrospective nature of the study. All research was conducted in accordance with the Declaration of Helsinki and Istanbul.

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ORCID iD

Paul Armstrong http://orcid.org/0000-0001-7520-9050

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