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Hepatic and renal end-organ damage in the Fontan circulation: A report from the Australian and New Zealand Fontan Registry

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ABSTRACT

Background: Hepatic and renal dysfunction have been observed in survivors of the Fontan procedure, however their incidence and associated factors remain poorly defined.
Methods: A total of 152 participants from a Registry of 1528 patients underwent abdominal ultrasound, transient elastography (FibroScan), serum fibrosis score (FibroTest), in vivo Tc-99m DTPA measurement of glomerular filtration rate (mGFR), and urine albumin-creatinine ratio (ACR).
Results: Mean age and time since Fontan were 19.8 ± 9.3 and 14.1 ± 7.6 years, respectively. Features suggestive of hepatic fibrosis were observed on ultrasound in 87/143 (61%) and no patient was diagnosed with hepatocellular carcinoma. FibroScan median kPa was ≥10 in 117/133 (88%), ≥15 in 75/133 (56%), and ≥20 in 41/133 (31%). Fifty-four patients (54/118, 46%) had a FibroTest score ≥0.49 (equivalent to ≥F2 fibrosis). FibroTest score correlated with FibroScan value ($r = 0.24$, $p = 0.015$) and ACR ($r = 0.29$, $p = 0.002$), and patients with ultrasound features of hepatic fibrosis had a higher FibroScan median kPa (19.5 vs 15.4, $p = 0.002$). Renal impairment was mild (mGFR 60–89 ml/min/1.73 m^2) in 46/131 (35%) and moderate (mGFR 30–59 ml/min/1.73 m^2) in 3/131 (2%). Microalbuminuria was detected in 52/139 participants (37%). By multivariable analysis, time since Fontan was associated with increased FibroScan median kPa ($\beta = 0.89$, 95% CI 0.54–1.25, $p = 0.002$) and decreased mGFR ($\beta = -0.77$, 95% CI −1.29–0.24, $p = 0.005$).
Conclusions: In the second decade after Fontan hepatic and renal structure and function are abnormal in a significant number of patients: close to 60% have ultrasonographic evidence of structural hepatic abnormalities, 46% have elevated serum hepatic fibrosis scores, and 57% have either reduced glomerular filtration rate or microalbuminuria. Hepatic and renal function should be monitored for potential impacts on outcomes after Fontan completion.

Abbreviations: DTPA, diethylene-triamine-pentaacetate; NYHA, New York Heart Association; PVP, peripheral venous pressure; BSA, body surface area; mGFR, measured glomerular filtration rate; eGFR, estimated glomerular filtration rate; ACR, albumin-creatinine ratio; ACE, angiotensin-converting enzyme; IVC, inferior vena cava.

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1. Introduction

Survival and quality of life after the Fontan procedure are exceeding previous expectations [1]. Successive surgical modifications have improved the efficiency of the Fontan circulation while reducing the burden of arrhythmias [2]. The greatest unknown moving forward is how the Fontan physiology will affect end-organ function in the 3rd and 4th decades. The characteristic findings of systemic venous hypertension and decreased cardiac output have been linked to hepatic congestion, hepatic fibrosis, and hepatocellular carcinoma [3–6]. Although very little has been published about the incidence of renal disease among the Fontan population, increased levels of microalbuminuria and a reduction in glomerular filtration rate (GFR) have been described [7, 8]. The incidence of hepatic and renal dysfunction, the best methods of screening and the precise determinants of these changes after Fontan, however, remain largely unknown.

2. Methods

2.1. Patients

Patients were recruited for this cross-sectional study from the Australian and New Zealand Fontan Registry, the full design and administration of which has been described previously [1]. Patients were eligible for recruitment if they were a minimum of 8 years of age, a minimum of 5 years post Fontan, had consented to participate in Registry substudies and could attend one of three study centres. The full design of the present study was approved by the national and local hospital ethics committees of each centre. Baseline characteristics and follow-up data were obtained from the Registry database [1]. History of arrhythmia was defined as either tachyarrhythmia (supraventricular tachycardia, atrial flutter, atrial fibrillation, or intra-atrial re-entry tachycardia) or bradyarrhythmia (complete heart block, sinus node dysfunction, or sinus bradycardia [resting HR < 50 bpm]). The presence of arrhythmia was determined using cardiologist clinical review documentation and hospital medical documentation. The time point at which an arrhythmia was first recognized was recorded as the time of onset.

2.2. Clinical assessment

All participants underwent an initial clinical assessment including a targeted history and physical examination. Height, weight, heart rate, blood pressure, oxygen saturation, and the presence of jaundice, hepatomegaly, ascites or other manifestations of chronic liver disease and/or portal hypertension were recorded. Palpable splenomegaly was subjectively determined by the examining clinician on abdominal palpation. Body surface area (BSA) was calculated using Haycock's formula, and functional capacity was categorized by New York Heart Association (NYHA) Class I–IV. The order of testing was as follows: (1) blood sampling, (2) peripheral venous pressure, (3) Tc-99m DTPA clearance, (4) FibroScan, (5) abdominal ultrasound, (5) echocardiography.

2.3. Measurement of peripheral venous pressures

Peripheral venous pressure (PVP) was measured at the time of cannulation for Tc-99m DTPA clearance using a portable pressure monitor with a standard pressure transduction setup. The cannula was inserted in the cubital fossa or cephalic vein, and measurements were taken with participants lying supine. The pressure transduction circuit was connected using three 3-way taps with a transducer between the second and third taps from the patient. Zero pressure was levelled with the transducer at the level of the mid-axillary line. End-expiratory, end-inspiratory and pressures at rest were recorded and used to calculate a mean PVP, which was used as a surrogate marker of central venous pressure [9].

2.4. Assessment of the hepatic structure and function

Patients underwent transabdominal ultrasonography with hepatic and portal venous Doppler using a Siemens or Acuson S2000 ultrasound machine. Assessment of liver surface, echotexture, echogenicity, and sonographic features of portal hypertension were all included in the imaging protocol. Patients were fasted for at least 8 h prior to imaging. Spleen length was converted to a percentage of the upper limit of normal based on age and gender.

A FibroScan® (Echosens, France) was performed at the time of the clinical assessment using age-appropriate transducers. The FibroScan utilizes transient elastography to measure liver stiffness, and has been validated as a non-invasive marker of liver fibrosis [10]. A series of ten measurements were used to calculate a median kPa value, and these were stratified according to the METAVIR scoring system for stages of estimated fibrosis [11]. Cut-off values ranging from 7.1 to 8.8 kPa have been used to diagnose ≥F2 fibrosis in patients with other etiologies of chronic liver disease [12].

Laboratory measurements were conducted on fasting venous blood samples (minimum 8 h fasting) immediately prior to measurement of Tc-99m DTPA clearance. A total of 6 haemolysed samples were excluded from the study.

A panel of six biochemical markers (alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, γ-GT, total bilirubin and ALT) were used to calculate a FibroTest® score (BioPredictive, Paris), a validated serum measurement of liver fibrosis [13]. Bilirubin and creatinine were used to calculate a MELD-XI score using the formula MELD-XI = 11.76 ($\log_e$ creatinine) + 5.112 ($\log_e$ total bilirubin) + 9.44 [14]. Creatinine and total bilirubin values <1 mg/dl were set to 1 mg/dl for the purpose of calculating the score. Creatinine was set at 4 mg/dl for patients with measured creatinine values >4 mg/dl.

2.5. Assessment of renal function

Measurement of GFR (mGFR) was performed using vivo Tc-99m DTPA clearance using multiple time point blood sampling and a single reference standard. All patients were pre-hydrated with 20 ml/kg of oral water which was consumed in the 1 h preceding the study. Serum creatinine was measured using an enzymatic assay at two of the three centres, and by the Jaffe method at the other centre, and values were converted from μmol/l to mg/dl for the purpose of eGFR calculation. An eGFR was calculated from serum creatinine using the Schwartz equation (0.413 × (height [Ht] / serum creatinine [Scr]), with Ht in centimetres, and Scr in mg/dl) in children (<18 years) and the MDRD equation ($175 \times S_{Cr}^{-1.154} \times age^{-0.203}$) × ($0.742^{female}$) × ($1.212^{Black}$) in adults (≥18 years) [15]. An early morning spot urine sample was collected to calculate an albumin-creatinine ratio (ACR), and microalbuminuria was defined as an ACR ≥ 2.5 mg/mmol in males and ≥3.5 mg/mmol in females.

2.6. Assessment of cardiac function

Standard transthoracic echocardiography was undertaken using a GE Vivid 7 ultrasound machine with age-appropriate transducers. Chest leads were placed during the study to correlate events with electrocardiogram (ECG) and respiratory tracings. Participants were classified as having moderate/severe valve regurgitation if RV, LV, or common AV valve regurgitation was moderate or severe; both right and left AV valve regurgitation grades were mild; native aortic valve or native pulmonary valve regurgitation was moderate or severe; or both native aortic valve and native pulmonary valve regurgitation grades were mild. The images were acquired and interpreted at each of the three centres using a standard protocol. Qualitative assessment of cardiac function was obtained by focused 2D and colour Doppler imaging.

2.7. Statistical analysis

Variables are presented as count (percentage), mean (standard deviation (SD)) or median (interquartile range (IQR)). Laboratory results for which different references ranges apply for different subgroups are reported as categorical variables (lower/within/higher than reference range). Laboratory measurements were included in the analysis as both absolute values and/or as percentages of an upper limit of normal. Correlations between normally distributed variables were calculated using Pearson's correlation (r). *t*-Tests for 2 groups or ANOVA were used to compare means for normally distributed variables and for non-normally distributed variables the Wilcoxon rank sum test for 2 groups or the Kruskal-Wallis test were used to compare medians. Multiple linear regression analyses were performed to identify independent variables associated with mGFR, FibroScan (median kPa), logarithmic transformed urine ACR and FibroTest score. Logistic regression analysis was performed to identify factors affecting the presence of hepatic fibrosis on ultrasound. Patient characteristics, including baseline clinical and surgical data collected in the Registry were utilized within the analyses, along with data collected as part of the study assessment. Variables found to be associated with the outcome in the univariable setting (using a threshold of $p < 0.05$) were included in what we called a "full model". We allowed up to 16 variables to be included in the "full model" and then using backward elimination (with a threshold of $p < 0.05$) and taking into account collinearity among variables, reduced the model such that all included variables were independently associated with the outcome variable using a significance level of 5%. Variables considered to be possible confounders (i.e. time since Fontan) were included in the final multivariable model regardless of their association with the outcome. Warfarin was excluded from the final multivariable model for Tc-99m DTPA mGFR based on clinical significance. All statistical analyses were performed in R (Version 3.3.2, http://www.r-project.org/).

3. Results

A total of 152 patients were recruited for participation across three study centres from a total of 1129 patients that were assessed for eligibility (Appendix G). Patient characteristics compared to the total Registry population are displayed in Table 1.

3.1. Clinical assessment

Clinical features suggestive of portal hypertension and/or chronic liver disease were observed in 35 patients (23%) at the time of the initial assessment, including hepatomegaly in 18 (12%), palpable splenomegaly in 11 (7%), spider naevi in 14 (9%), jaundice in 4 (3%) and ascites in 4 (3%). Eighteen patients (12%) had clinically evident

Table 1
Patient characteristics.

Characteristics	Participants (n = 152)	Total (n = 1129)[a]	p-Value
Male, n (%)	80 (53%)	655 (58%)	0.4
Age in years, mean (SD)	19.8 (9.3)	19.5 (10.5)	0.7
Years post-Fontan, mean (SD)	14.1 (7.6)	13.3 (9.4)	0.2
Anatomical comorbidities, n (%)			
Dextrocardia/mesocardia	16 (11%)	102 (9%)	0.4
Isomerism/heterotaxy	13 (9%)	78 (7%)	0.4
Ventricular morphology, n (%)			
Left	103 (68%)	677 (60%)	0.1
Right	38 (25%)	361 (32%)	
Biventricular/Indeterminate	11 (7%)	91 (8%)	
Primary morphological diagnosis, n (%)			
TA	39 (26%)	247 (22%)	0.1
DILV	32 (21%)	194 (17%)	
DORV	16 (11%)	147 (13%)	
CAVC	16 (11%)	97 (9%)	
HLHS	15 (10%)	134 (12%)	
ccTGA	13 (9%)	78 (7%)	
PA-IVS	9 (6%)	89 (8%)	
Other	12 (8%)	143 (13%)	
Prior BCPS, n (%)	111 (73%)	734 (65%)	0.05
Age at Fontan, mean (SD)	5.7 (4.3)	5.8 (4.0)	0.99
Fontan type, n (%)			
AP	20 (13%)	125 (11%)	0.7
LT	31 (20%)	226 (20%)	
ECC	101 (67%)	778 (69%)	
Fenestrated[b], n (%)	52 (34%)	406 (36%)	0.5
Ventricular systolic dysfunction ≥ moderate after Fontan, n (%)	14 (9%)	105 (9%)	–
Current medications, n (%)			
ACE inhibitor	60 (39%)	406 (36%)	0.2
Aspirin	61 (40%)	431 (38%)	0.6
Warfarin	85 (56%)	599 (53%)	0.4
NYHA classification, n (%)			
I	104 (68%)	791 (70%)	0.06
II	40 (26%)	309 (27%)	
III	8 (5%)	29 (3%)	
Pacemaker in situ, n (%)	16 (11%)	68 (6%)	–

[a] Includes all patients assessed for study eligibility. SD: standard deviation; TA: tricuspid atresia; DILV: double-inlet left ventricle; DORV: double-outlet right ventricle; CAVC: common atrioventricular canal; HLHS: hypoplastic left heart syndrome; ccTGA: congenitally-corrected transposition of the great arteries; PA-IVS: pulmonary atresia with intact ventricular septum; BCPS: bidirectional cavopulmonary shunt; AP: atriopulmonary; LT: lateral tunnel; ECC: extracardiac conduit; ACE: angiotensin-converting enzyme; NYHA: New York Heart Association.
[b] Fenestrated at time of Fontan operation.

peripheral edema. Seven patients (5%) reported a history of hematemesis. Mean resting oxygen saturations were 94 ± 4.3%. The NYHA status and proportion of patients taking aspirin, warfarin and angiotensin-converting enzyme (ACE) inhibitors are displayed in Table 1. Additional medications at the time of enrolment included beta blockers (23,15%), diuretics (16,11%), and digoxin (5,3%).

A total of 129 patients (85%) underwent measurement of PVP at the time of the clinical assessment. Mean PVP was 14.3 ± 4.6 mm Hg and did not differ significantly between Fontan types (ECC 14.2 ± 3.9 mm Hg, LT 13.0 ± 4.5 mm Hg, and AP 14.1 ± 4.9 mm Hg, p = 0.3), or between fenestrated and non-fenestrated patients (14.4 ± 4.7 vs 13.7 ± 3.9, p = 0.4). PVP was not associated with increased time after Fontan (r = 0.01, p = 0.8).

3.2. Liver assessment

3.2.1. Abdominal ultrasound

A total of 143 patients underwent an abdominal ultrasound. Features suggestive of hepatic fibrosis (parenchymal changes, heterogeneous echotexture, surface nodularity or hyperechoic lesions) were observed in 87 patients (61%). Liver surface nodularity was identified in 19 patients (13%). Features suggestive of portal hypertension were observed in 41 patients (29%) (splenomegaly in 35, ascites in 4, and portal vein flow reversal in 3). No patient had varices identified on ultrasound. No discrete liver lesions suspicious of hepatocellular carcinoma (HCC) were demonstrated. Nine of the 11 patients (82%) identified as having palpable splenomegaly on clinical examination had splenomegaly on abdominal ultrasound, with the remaining two patients having a spleen length just below the upper limit of normal for age. After adjustment for time since Fontan, history of arrhythmia was associated with features of hepatic fibrosis on ultrasound (odds ratio (OR) = 3.79, 95% CI 1.38–12.4, p = 0.02). Resting oxygen saturations did not differ between those with or without evidence of hepatic fibrosis on ultrasound (93.8 ± 5.0 vs 94.5 ± 3.4, p = 0.37). Patients with ultrasonographic evidence of portal hypertension did not have significantly increased mean PVP measurements compared to those without (14.2 ± 5.1 vs 13.8 ± 3.8, p = 0.6).

3.2.2. FibroScan

A FibroScan was undertaken in 133 patients (88%), with a median kPa of 16.5 (11.7–21.3). Median kPa based on Fontan type was 18.4 (11.3–22.9), 17.0 (12.3–21.4) and 16.0 (11.5–21.1) for AP, LT and ECC, respectively (p = 0.7). Median kPa was ≥10 in 117 patients (88%), ≥15 in 75 (56%), and ≥20 in 41 (31%). Patients with clinical features suggestive of chronic liver disease (hepatomegaly, palpable splenomegaly, jaundice, ascites or spider naevi) had a higher median kPa compared to those without (19.0 (15.1–23.3) vs 15.1 (11.2–20.9), p = 0.02). Higher median kPa was associated with a higher mean PVP (r = 0.23, p = 0.01).

By multivariable analysis, increased time since Fontan was associated with a higher median kPa (increase of 0.84 kPa per year increase, 95% CI 0.44–1.24, p < 0.001). Median kPa versus time since Fontan is displayed in Fig. 1. After adjustment for time since Fontan, other factors associated with a higher median kPa were ECC Fontan type (16.4 kPa higher than AP Fontan type, 95% CI 8.1–24.26, p < 0.001), presence of fenestration (β = 4.3, 95% CI 0.7–7.9, p = 0.02) and history of arrhythmia (β = 5.3, 95% CI 1.3–9.3, p = 0.01).

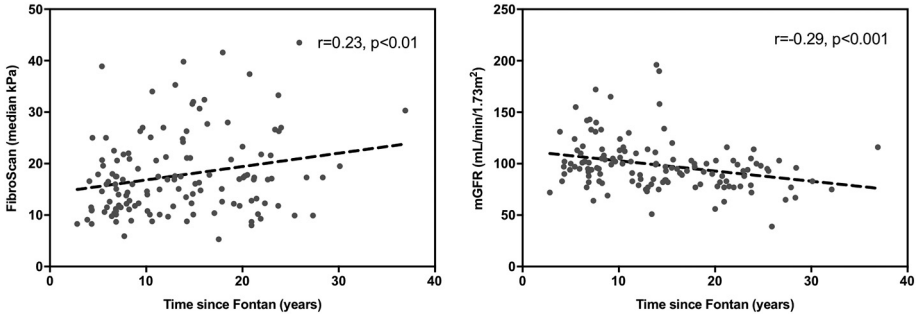

Fig. 1. FibroScan (median kPa) & glomerular filtration rate as measured by Tc-99m DTPA clearance (mGFR) vs time since Fontan (n = 133, n = 131).

3.2.3. Laboratory measurements of hepatic function (Table 2)

A FibroTest score was generated for a total of 118 patients. Nine patients had a haptoglobin value below assay, and a FibroTest score was not calculated for these patients. Median FibroTest score was 0.46 (0.34–0.64) and 54 patients (46%) had a FibroTest score equivalent to ≥F2 fibrosis based on the METAVIR staging system [11]. By multivariable analysis, factors associated with a higher FibroTest score were male gender ($\beta = 0.13$, 95% CI 0.06–0.19, $p < 0.001$), palpable splenomegaly ($\beta = 0.16$, 95% CI 0.05–0.27, $p < 0.01$), increased serum BNP (analysed as log (BNP); $\beta = 0.113$ per 100 unit increase, 95% CI 0.034–0.192, $p < 0.01$), and decreased platelet count ($\beta = -0.067$ per 100 unit increase, 95% CI −0.118–0.017, $p = 0.01$).

A MELD-XI score was calculated for 123 patients. Patients with a MELD-XI score >12 had a higher median kPa than those with a score <10 (24 ± 10.7 vs 16.7 ± 7.5, $p = 0.02$). MELD-XI score was higher in those with ultrasonographic features of hepatic fibrosis (10.4 ± 1.8 vs 9.8 ± 1.1, $p = 0.04$).

3.3. Kidney assessment

3.3.1. Renal ultrasound

Findings of increased parenchymal echogenicity were seen in 8 patients (6%), cortical thinning in 4 (3%), and discrete scarring in 6 (4%). Pelvicalyceal system dilatation was observed in 2 patients (1%). One patient had a solitary kidney. Five patients (3%) had enlarged kidneys measured as >95th centile in length.

3.3.2. Measurement of GFR

A total of 131 patients underwent measurement of glomerular filtration rate (mGFR) by method of Tc-99m DTPA clearance. Three patients (2%) had an mGFR <60 ml/min/1.73 m^2, 46 (35%) had an mGFR of 60–90 ml/min/1.73 m^2, and 82 (63%) had an mGFR of >90 ml/min/1.73 m^2. The median age of those with an mGFR <90 was 17.9 (12.3–24.7) years. Rate of decline in mGFR was 9.92 ml/min/1.73 m^2 per decade following Fontan (Fig. 1). By multivariable analysis, factors associated with a lower mGFR were increasing time since Fontan ($\beta = -0.77$, 95% CI −1.29–0.24, $p = 0.005$), and NYHA Class III relative to NYHA Class I ($\beta = -22.1$, 95% CI −38.0–6.2, $p < 0.01$). HLHS was associated with a higher mGFR, even after adjustment for age ($\beta = 25.6$, 95% CI 12.7–38.6, $p < 0.001$) and time since Fontan ($\beta = 26.4$, 95% CI 13.0–39.8, $p < 0.001$).

Table 2
Laboratory results.

Parameters	Median (IQR)	Normal values[a]	Number abnormal (%)
Platelets (×109/l)	192 (155–245)	150–600	26 (20%)
Liver enzymes			
ALT (U/l)	33 (25–39)	10–40	32 (24%)
GGT (U/l)	73 (52–112)	8–78	58 (44%)
AST (U/l)	30 (25–37)	0–40	23 (19%)
ALP (U/l)	64 (38–82)	30–120	8 (6%)
AFP (ng/ml)	1.7 (1.0–2.0)	0–10	0 (0%)
Albumin (g/l)	43 (41–46)	35–50	5 (4%)
Total bilirubin (μmol/l)	13 (10–18)	0–15	42 (32%)
LDH (U/l)	511 (420–603)	313–618	29 (24%)
Creatinine (μmol/l)	60 (50–71)	50–110	2 (2%)
Urea (mmol/l)	4.9 (4.1–5.8)	2.1–6.5	9 (7%)

ALT: alanine transaminase; GGT: γ-glutamyl-transpeptidase; AST: aspartate transaminase; ALP: alkaline phosphatase; AFP: alpha-fetoprotein; LDH: lactate dehydrogenase; eGFR: estimated glomerular filtration rate.

[a] Values with differing age-dependent ranges were adjusted to those presented above based on the percentage of the upper limit of normal.

The mGFR of patients who were currently taking an ACE inhibitor did not significantly differ from to those who were not (101.9 ± 29.2 vs 96.8 ± 21.5, $p = 0.26$).

3.3.3. Laboratory measurements of renal function (Table 2)

Mean serum creatinine was 60 ± 17 μmol/l using the enzymatic method and 64 ± 14 μmol/l using the Jaffe method ($p = 0.2$). Mean eGFR (Schwartz) in children was 158 ± 31 ml/min/1.73 m^2, and mean eGFR (MDRD) in adults was 108 ± 22 ml/min/1.73 m^2. None of the children had an eGFR (Schwartz) <90 ml/min/1.73 m^2. Fifteen of the 66 adult patients (23%) had an eGFR (MDRD) <90 ml/min/1.73 m^2 and one adult patient (2%) had an eGFR (MDRD) <60 ml/min/1.73m^2. There was a positive correlation between mGFR and eGFR (MDRD) ($r = 0.67$, $p < 0.0001$) in adults, however eGFR (Schwartz) did not correlate significantly with mGFR in the children ($r = 0.22$, $p = 0.1$). Bland-Altman plots comparing Tc-99 m DTPA GFR (mGFR) with eGFR are shown in Appendix F. Both equations overestimated mGFR (eGFR (Schwartz): bias 50.8, 95% CI 41.1–60.5 ml/min/1.73 m^2, $p < 0.0001$; eGFR (MDRD): bias 16.1, 95% CI 11.8–20.4 ml/min/1.73 m^2, $p < 0.0001$).

A total of 52 of 139 patients (37%) had an increased ACR. Median ACR was 2.2 (1.1–3.9) and 2.3 (1.4–4.1) for male and female patients, respectively. Higher mean PVP was associated with an increased ACR ($r = 0.18$, $p = 0.05$), however there was no significant correlation between PVP values and GFR (measured via Tc-99m DTPA clearance or estimated from serum creatinine). By multivariable analysis, factors associated with a higher log ACR were increased total serum bilirubin ($\beta = 1.73$ per 100-unit increase, 95% CI 0.20–3.26, $p = 0.03$) and decreased serum haptoglobin ($\beta = -0.62$, 95% CI −1.04–0.20, $p < 0.01$). ECC Fontan type was associated with a lower ACR relative to AP Fontan type ($\beta = -1.16$, 95% CI −2.17–0.14, $p = 0.03$). There was no significant difference in ACR between patients taking and not taking an ACE inhibitor at the time of the study (ACR elevated in 38.1% vs 37.9%, $p = 0.95$).

Of the 124 patients who underwent measurement of both Tc-99m DTPA clearance and urine ACR, 18 (15%) had both an mGFR <90 and an elevated ACR, and 71 (57%) had either an mGFR <90 or an elevated ACR.

3.4. Echocardiographic assessment of cardiac function

Echocardiography was performed in 142 participants (93%). Ventricular systolic function was mildly impaired in 19 patients (13%) and moderately impaired in 5 (4%). Atrioventricular valve (AVV) regurgitation was mild in 52 patients (37%) and moderate in 15 (11%). There were no significant associations between degree of ventricular systolic dysfunction or atrioventricular valve regurgitation and measurements of liver fibrosis. An increased E-A ratio was associated with increased median kPa ($r = 0.32$, $p < 0.001$) and patients with an E-A ratio >2 had a higher median kPa compared to those with an E-A ratio <1 (22.4 ± 10.5 vs 15.1 ± 5.0, $p = 0.02$).

Both decreased deceleration time (DT) and decreased percentage change in IVC diameter from inspiration to expiration were associated with an increased mean PVP ($r = -0.26$, $p = 0.006$, and $r = -0.21$, $p = 0.02$, respectively).

3.5. Correlations between major endpoints

Correlations between major outcome variables are displayed in Table 3.

4. Discussion

This cross-sectional study provides a detailed analysis of the determinants of hepatic and renal function in a representative sample of the Australian and New Zealand Fontan population. It confirms the

Table 3
Pearson correlations between major outcome variables.

	mGFR (Tc-99m DTPA clearance)	FibroScan (median kPa)	Urine ACR[a]
FibroScan (median kPa)	0.00 (1)		
Urine ACR[a]	−0.15 (0.1)	0.14 (0.1)	
FibroTest score	−0.03 (0.8)	0.24 (0.015)	0.29 (0.002)

Values expressed as r (p-value). mGFR: measured glomerular filtration rate; Tc-99m: Technetium-99m; DTPA: diethylene-triamine-pentaacetate; ACR: albumin-creatinine ratio.
[a] Log.

high prevalence of end-organ damage associated with the Fontan circulation [8, 16, 17]. After a median of 14 years, close to two-thirds of patients had sonographic evidence of structural hepatic damage, and a significant proportion had elevated elastography measurements and/or serum fibrosis scores. Our data, demonstrating changes in a number of different non-invasive markers of liver and renal damage, serves as a basis for future longitudinal studies defining the best predictors of Fontan failure and screening methods for end-organ damage. Our findings may also facilitate targeting of early interventions to prevent deterioration of the Fontan circulation.

The utility of FibroScan in Fontan associated liver disease remains controversial. We demonstrated an association between FibroScan values and both ultrasonographic features and serum-based measures of hepatic fibrosis, and therefore suspect that it may be of some use for estimating the degree of hepatic fibrosis in Fontan patients. The potential influence of raised venous pressures must be taken into account when interpreting results, however we are not currently in a position to define an exact method of correction based on venous pressures. Serological markers appear to be often only mildly deranged and may underestimate the magnitude of end-organ damage in some cases, however trends over time may be useful. The association between elevated transient elastography values and the ECC Fontan is difficult to understand. It could be explained by an undetected selection bias towards the more severe end of the single ventricle spectrum in more recent years, or alternatively, differences in stiffness and flow impedance in the Fontan circuitry between the ECC and earlier modifications.

Our study is one of the first to highlight the high prevalence of renal dysfunction in this population, with more than half of the patients having a reduced glomerular filtration rate or microalbuminuria. This is consistent with the recent findings of Lee et al., which demonstrated evidence of CKD in close to half of an adult-aged Fontan cohort using combined criteria based on mGFR and urine ACR [18]. The precise mechanisms leading to renal impairment in Fontan patients remain unclear, but it is possible that these changes are progressive and related to the physiological stress imposed by the Fontan state. Although no conclusive link has been established between creatinine-based eGFR and late adverse clinical events, cystatin C-based eGFR has been linked to adverse outcomes after Fontan [19]. This suggests that creatinine-based equations may inaccurately estimate true GFR in Fontan patients, which in turn may explain the lack of correlation between mGFR and creatinine-based eGFR in children. Creatinine-based GFR estimation proved more accurate in the adult patients despite a tendency to overestimate mGFR, although this was much less marked than seen in the children. The finding of an increased mGFR in HLHS patients even after adjustment for age and time since Fontan is surprising, however a convincing explanation for this remains elusive at this time.

There is currently no available medical therapy to prevent end-organ damage in the Fontan circulation, which may lead one to question the rationale for monitoring these changes. We would argue that the potential for malignant transformation of hepatic lesions, as well as the possible impacts on eligibility for cardiac transplantation both validate the monitoring of end-organ consequences. Based on this, and to further build on our understanding of the aetiology and temporality of these changes, hepatic and renal function should be followed serially in all patients after Fontan completion. The finding of abnormal hepatic and renal function should also, in some cases, prompt investigation to exclude reversible impediment to the passive pulmonary blood flow in the Fontan circuit in the form of thrombi, stenosis and valve regurgitation. Despite the fact that none of the patients in the current study had malignant hepatic lesions identified on imaging, we believe that the established risk of hepatocellular carcinoma warrants some form of mandatory surveillance imaging of the liver. The choice of modality is likely to be influenced by further studies comparing a variety of non-invasive techniques with histological findings. Further insights into the nature and mechanisms of renal impairment in the Fontan population will aid the ongoing management of these patients.

5. Limitations

This study provides a cross-sectional measurement of the impact of the Fontan circulation on hepatic and renal function at a single time point; to more precisely evaluate the incidence of these issues over time, prospective repeat measurements are necessary. The lack of histological findings is also a limitation of this study.

6. Conclusions

In the second decade after Fontan, hepatic and renal structure and function are abnormal in a significant number of patients: close to two-thirds have ultrasonographic evidence of structural hepatic abnormalities, half have elevated serum hepatic fibrosis scores, and half have renal dysfunction with either reduced glomerular filtration rate or microalbuminuria. The hepatic and renal function of patients with a Fontan circulation should be monitored for potential impacts on outcomes after Fontan completion. Further studies will focus on defining the ideal methods of surveillance and identification of potential treatment options or strategies to alleviate end-organ dysfunction after the Fontan procedure.

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Disclosures

All authors have approved the final version of this manuscript.

Conflicts of interest

Yves d'Udekem is consultant for MSD and Actelion. Robert Weintraub serves on an advisory board for Actelion. Andrew Bullock reports consulting fees from Actelion. The remaining authors have nothing to disclose.

Appendix A. Pearson correlations between FibroScan median kPa (n = 133) and clinical, biochemical, echocardiographic and ultrasonographic features

	n	r	p value
Platelet count	117	−0.33	<0.001
Neutrophil count	117	−0.31	<0.001
Systemic AVV EA ratio[a]	116	0.26	0.004
MELD-XI score	110	0.25	0.007
Time since Fontan (years)	133	0.21	0.01
Age (years)[a]	133	0.24	0.006
Body surface area	133	0.24	0.006
Spleen length[b]	125	0.3	0.008
HR	133	−0.24	0.01
Total bilirubin[a]	117	0.23	0.01
GGT[a]	117	0.22	0.01
Peripheral venous pressure (mean)[a]	115	0.22	0.02
Conduit VTI (mean)[a]	116	0.2	0.04
Total protein	112	0.19	0.04

AVV: atrioventricular valve; HR: heart rate; IVC: inferior vena cava; GGT: γ-glutamyl transpeptidase; VTI: velocity time integral.
[a] Log.
[b] Spleen length as measured on abdominal ultrasonography and expressed as a percentage of the upper limit of normal based on age ranges.

Appendix B. Pearson correlations between FibroTest score (n = 118) and clinical, biochemical, echocardiographic and ultrasonographic features

	n	r	p value
Haemoglobin	113	0.33	<0.001
Haematocrit	113	0.33	<0.001
Platelet count	114	−0.3	0.001
Albumin	112	0.3	0.001
White cell count	114	−0.29	0.002
Serum creatinine	110	0.28	0.003
Total protein	112	0.27	0.003
Time since Fontan (years)	118	0.23	0.01
Age (years)	118	0.20	0.03
Alpha-fetoprotein[a]	118	0.22	0.02
LDH	104	0.23	0.02
Peripheral venous pressure (mean)	108	0.22	0.02
Neutrophil count	114	−0.22	0.02
Serum urea	110	0.22	0.02
Hepatic vein VTI (inspiration)[a]	96	−0.22	0.02
AST[a]	116	0.21	0.03
Arrhythmia, time since onset	31	0.46	0.03

AVV: atrioventricular valve; LDH: lactate dehydrogenase; VTI: velocity time integral; AST: aspartate transaminase; IVC: inferior vena cava.
[a] Log.

Appendix C. Pearson correlations between Tc-99m DTPA mGFR (n = 131) and clinical, biochemical, echocardiographic and ultrasonographic features

	n	r	p value
Serum creatinine	119	−0.40	<0.001
Age (years)	131	−0.37	<0.001
Conduit VTI (expiration)[a]	96	0.39	<0.001
eGFR[b]	119	0.35	<0.0001
Descending aorta mean gradient (mm Hg)[a]	115	0.35	0.001
Age at BCPS (months)[a]	95	−0.31	0.002
Time since Fontan (years)	131	−0.29	<0.001
Duration of shunt or band (years)[a]	88	−0.32	0.003
Conduit VTI (mean)[a]	96	0.29	0.005
Serum ure[a]	119	−0.25	0.007
Age at Fontan (years)	131	−0.26	0.003
Descending aorta Vmax	115	0.21	0.02
Number of pre-Fontan procedures	131	0.19	0.03

VTI: velocity time integral; eGFR: estimated glomerular filtration rate; BCPS: bidirectional cavopulmonary shunt; AVV: atrioventricular valve; VTI: velocity time integral.
[a] Log.
[b] eGFR calculated using the Schwartz formula in children and the MDRD formula in adults.

Appendix D. Pearson correlations between log urine ACR (n = 139) and clinical, biochemical, echocardiographic and ultrasonographic features

	n	r	p value
LDH[a]	113	0.32	<0.001
Haptoglobin[a]	129	−0.29	0.001
Total bilirubin[a]	133	0.27	0.002
Peripheral venous pressure (inspiration)	121	0.24	0.01
AST[a]	121	0.21	0.03
Haematocrit	133	0.18	0.04

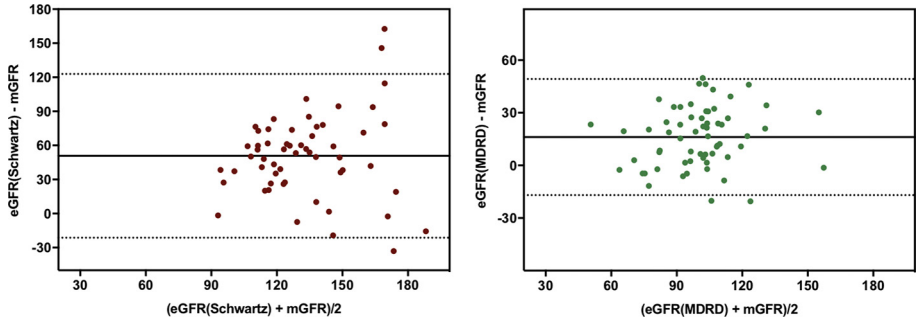
LDH: lactate dehydrogenase; AST: aspartate transaminase.
[a] Log.

Appendix E. Reduced multivariable models for major endpoints

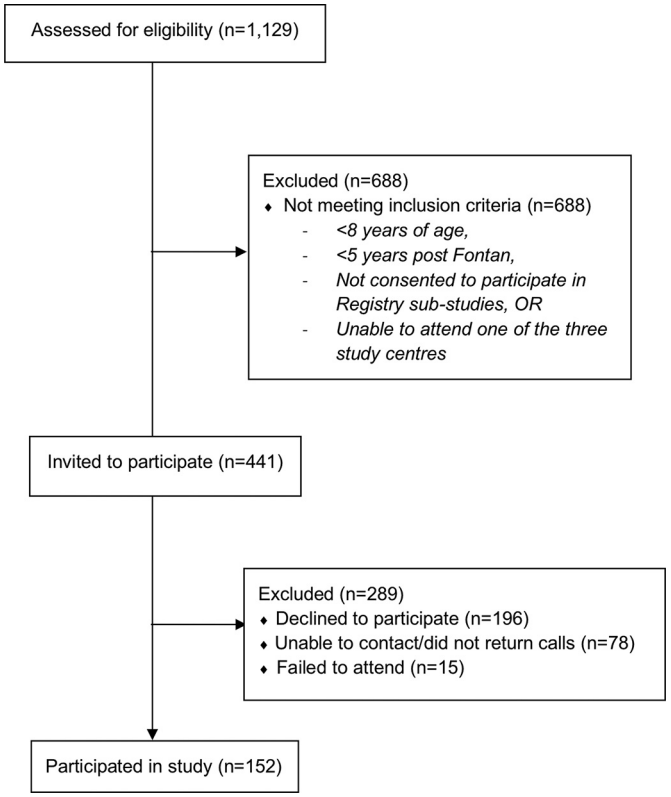
	Regression coefficient, β (standard error)	95% CI for β	p value (β = 0)
Tc-99m DTPA clearance (mGFR) (ml/min/1.73 m²), n = 131			
Intercept	110.1 (4.5)	(101.3, 118.9)	<0.001
HLHS	26.4 (6.8)	(13, 39.8)	<0.001
Time since Fontan (years)	−0.77 (0.27)	(−1.29, −0.24)	0.005
Mild limitation	−6.8 (4.6)	(−15.7, 2.2)	0.14
Moderate limitation	−22.1 (8.1)	(−38, −6.2)	0.007
FibroScan (median kPa), n = 120			
Intercept	−12.7 (7.8)	(−28, 2.7)	0.1
Time since Fontan (years)	0.84 (0.21)	(0.44, 1.24)	<0.001
ECC Fontan type	16.4 (4.2)	(8.1, 24.6)	<0.001
LT Fontan type	7.8 (3.0)	(1.9, 13.8)	0.011
History of arrhythmia	5.3 (2.0)	(1.3, 9.3)	0.01
Fenestration	4.3 (1.8)	(0.7, 7.9)	0.02
Mean IVC diameter[a] (per unit increase)	0.4 (0.3)	(−0.3, 1.1)	0.2
Urine ACR (mg/mmol)[b], n = 122			
Intercept	2.5 (0.7)	(1.0, 3.9)	0.001
Time since Fontan (per 10 years)	−0.42 (0.25)	(−0.92, 0.08)	0.1
ECC Fontan type	−1.16 (0.52)	(−2.17, −0.14)	0.03
LT Fontan type	−0.54 (0.36)	(−1.26, 0.17)	0.1
Total bilirubin (per 100 units)	1.73 (0.78)	(0.20, 3.26)	0.03
Haptoglobin	−0.62 (0.21)	(−1.04, −0.20)	0.005
FibroTest score, n = 112			
Intercept	0.475 (0.077)	(0.323, 0.627)	<0.001
Time since Fontan (per 10 years)	0.032 (0.025)	(−0.018, 0.082)	0.2
Platelet count (per 100 units)	−0.067 (0.026)	(−0.119, −0.016)	0.01
Log (BNP) (per log unit increase)	0.035 (0.018)	(0.000, 0.071)	0.05
Male gender	0.53 (0.08)	(0.37, 0.69)	<0.001
Palpable splenomegaly	0.16 (0.06)	(0.05, 0.28)	0.005

DTPA = diethylene-triamine-pentaacetate; mGFR = measured glomerular filtration rate; HLHS = hypoplastic left heart syndrome; NYHA = New York Heart Association; AP = atriopulmonary; ECC = extracardiac conduit; LT = lateral tunnel; ACR = albumin-creatinine ratio; BNP = brain natriuretic peptide.
[a] Adjusted for body surface area (millimetres per m²).
[b] Log.

Appendix F. Bland-Altman plots comparing Tc-99m DTPA GFR (mGFR) with eGFR (Schwartz) in children, and eGFR (MDRD) in adults, using bias plots showing mean bias and upper & lower limits of agreement



Appendix G. CONSORT diagram detailing the recruitment of study participants



Appendix H. Pathology techniques for major study parameters

Parameter	Sample	Processing/storage	Assay
AFP	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Chemiluminescent immunoassay
Haptoglobin	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Immunoturbidimetric or rate nephelometry
Apolipoprotein A1	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Immunoturbidimetric or rate nephelometry
LDH	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Pyruvate to lactate multiple point rate reaction (dry slide)
Urea	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Colorimetric – urease/ NH4 substrates
Creatinine	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Enzymatic two-point rate reaction (amidohylase/ oxidase) or Jaffe method[a]
Bilirubin	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Colorimetric (end point) caffeine sodium benzoate
ALT	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Multiple point rate reaction with pyridoxal-5-phosphate cofactor
ALP	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Multiple point rate (*p*-nitrophenol substrate)
AST	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Multiple point rate reaction with pyridoxal-5-phosphate cofactor
GGT	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Multiple point rate reaction (glutamyl-nitroanilide glycylglycine substrate)
Total protein	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Colorimetric (biuret)
Albumin	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Colorimetric (bromocresol green)
BNP	Serum	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Chemiluminescent microparticle immunoassay
Urine albumin	Urine	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Immunoturbimetric
Urine creatinine	Urine	Centrifuged at 5300 rpm at 4 °C for 5 min. Assayed on arrival – no storage	Enzymatic – two point rate reaction (amidohylase/oxidase)

AFP: alpha-fetoprotein; LDH: lactate dehydrogenase; ALT: alanine transaminase; ALP: alkaline phosphatase; AST: aspartate transaminase; GGT: γ-glutamyl-transpeptidase; BNP: brain natriuretic peptide.

[a] The Jaffe method was used at one of the three study centres.

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