Hepatic changes in the Fontan circulation: identification of liver dysfunction and an attempt to streamline follow-up screening.

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Promotor: Prof. dr. K. François

Dissertation presented in the 2nd Master year in the programme of
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List of abbreviations

αFP   alfa-fetoprotein
ALT   alanine transaminase
AP    alkaline phosphatase
APC   atrioventricular connection
APRI  aspartate transaminase-to-platelet ratio index
ARFI  acoustic radiation force impulse
AST   aspartate transaminase
AVVI  atrioventricular valve insufficiency
AUROC area under the receiver operating characteristic
BCPA  bidirectional cavopulmonary anastomosis
CHF   chronic heart failure
CI    confidence interval
CO    cardiac output
CVP   central venous pressure
FALD  Fontan associated liver disease
FIB-4 fibrosis-4
FNH   focal nodular hyperplasia
FU    follow-up
γGT   gamma-glutamyl transferase
HA    hepatic artery
HABR  hepatic artery buffer response
HCC   hepatocellular carcinoma
HCT   hematocrit
HV    hepatic veins
HVPG  hepatic venous pressure gradient
INR   international normalised ratio
IVC   inferior vena cava
LA    left atrium
LB    liver biopsy
LC    liver cirrhosis
LS    liver stiffness
LV    left ventricle
NYHA  New York heart association
NPV   negative predictive value
PA    pulmonary artery
PE    pleural effusions
PI    pulsatility index
PLE   protein losing enteropathy
PPV   positive predictive value
PR    pulsatility ratio
Pro-BNP pro-brain natriuretic peptide
PV    portal vein
PVR   pulmonary vascular resistance
RA    right atrium
RI    resistance index
ROI   region of interest
RV    right ventricle
SMA   superior mesenteric artery
SVC   superior vena cava
SWE   shear wave elastography
TCPC  total cavopulmonary connection
TE    transient elastography
US    ultrasound
UVH   univentricular heart disease
WHVP  wedge hepatic venous pressure
# Abstract

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Abstract

Background: Since the introduction of Fontan palliation for univentricular heart (UVH) disease patients are surviving into adulthood. As a consequence end-organ dysfunction might occur, influencing morbidity and mortality. The liver is most vulnerable for developing late complications after Fontan repair due to physiological changes inherent to the construction of a Fontan circulation.

Objective: Since structural and functional liver abnormalities, including hepatic congestion, fibrosis, cirrhosis and eventually hepatocellular carcinoma, are more and more recognised, we tried to identify liver aberrances in adolescent and adult Fontan patients. Subsequently we sought to determine potential useful serum markers and liver imaging modalities for follow-up screening to detect patients at risk.

Methods: 29 patients, median age 23.7 years (interquartile range: 20.5 – 27.2), were prospectively studied with echocardiography, blood analysis and liver imaging. Mean Fontan interval was 18.26 ± 4.98 years. Blood analysis included platelet count, liver enzymes and α-fetoprotein. Fibrosis scoring systems APRI, FIB-4 and Forns index were calculated. Liver morphology using ultrasound (US) and Doppler of hepatic vein (HV), portal vein (PV), hepatic artery (HA) and superior mesenteric artery (SMA) were examined, and resistance (RI) and pulsatility indices (PI) calculated. With shear wave elastography we determined liver stiffness (LS). Correlations between time (age at operation, age at follow-up and Fontan interval), serum markers and imaging results were searched for.

Results: Laboratory tests showed abnormal values for γGT, total bilirubin, direct bilirubin, ALT, platelet count and pro-BNP in 74%, 31%, 50%, 36%, 29% and 39% of the patients, respectively. APRI and FIB-4 were unindicative for liver fibrosis. Forns index indicated moderate fibrosis in 29% of patients and correlated with Fontan interval (p=0.034). US liver morphology was deviant in 46% of patients. Surface nodularity was found in 21% and nodular hyperplasia in 29%. Doppler assessment of flow velocities and resistance/pulsatility indices for most patients were within normal ranges. A trend towards lower SMA RI was seen with increasing Fontan interval (p=0.08). LS (mean 10.4 ± 3.7 kPa) was elevated in 96% of our population. Higher LS values were significantly related to longer Fontan interval (p=0.018), lower SMA RI (p=0.008), larger spleen (p=0.025), lower albumin (p=0.015), lower total protein (p=0.002) and higher AST (p=0.031).
Abstract

**Conclusion:** Adolescent an adult Fontan patients show moderate signs of liver dysfunction. Usefulness of serum parameters and serum fibrosis scores in post-Fontan screening remains ambiguous in the absence of significant changes in liver enzymes and in the absence of correlations with morphologic alterations. The high percentage of morphologic liver changes in palliated patients with UVH disease support the use of US in periodic follow-up. This is in contrast to Doppler indices. Only for SMA RI there are arguments to include this parameter in FU screening. LS is elevated in almost every Fontan patient and increasing values with time and lower SMA resistance were reported. However, LS values in this population are likely an overestimation due to liver congestion, arguing for the need of validation through sequential measurements. We suggest a follow-up strategy where liver screening in palliated UVH disease starts 10 years after Fontan completion. Screening minimally encompasses the following tests: measurement of serum parameters (thrombocyte count, γGT, total and direct bilirubin, serum transaminases) and Forns score and assessment of liver and spleen morphology with US in combination with LS measurement.

**Samenvatting**

**Achtergrond:** Sinds kinderen met een aangeboren univentriculair hartdefect adequaat behandeld kunnen worden door middel van de Fontan palliatie is de overleving sterk verbeterd. Als gevolg daarvan kan eindorgaan dysfunctie optreden, met een belangrijke impact op morbiditeit en mortaliteit. De lever lijkt het meest gevoelig om na Fontan herstel laattijdige complicaties te ontwikkelen als gevolg van de fysiologische veranderingen die optreden na de aanleg van een Fontan circulatie.

**Doelstelling:** Omdat structurele en functionele leverafwijkingen, inclusief levercongestie, fibrose, cirrose en uiteindelijk zelfs hepatocellulair carcinoma meer en meer (h)erkend worden, werd getracht om deze lever abnormaliteiten te identificeren in adolescente en volwassen Fontan patiënten. Vervolgens werd gezocht naar potentiële serum merkers en beeldvormingsmodaliteiten om patiënten te screenen in follow-up op deze levercomplicaties.

**Methoden:** 29 patiënten, mediane leeftijd 23.7 jaar (interkwartiel range: 20.5 – 27.2), werden prospectief onderzocht met echocardiografie, bloed analyse en beeldvorming van de lever. Het gemiddelde Fontan interval bedroeg 18.26 ± 4.98 jaar. Bloedanalyse omvatte de meting van thrombocyten, leverenzymen en a-foetoproteïne. APRI, FIB-4 en Forns indices werden berekend om de graad van fibrose te scoren. Daarnaast werd de levermorfologie bestudeerd.
Abstract

Aan de hand van echografie en werd er een Doppler onderzoek uitgevoerd van de venae hepaticae (HV), arteria hepatica (HA), vena porta (PV) en de arteria mesenterica superior (SMA). Op basis van de Doppler snelheidsmetingen werden weerstands- (RI) en pulsatilityindices (PI) berekend. Met behulp van shear wave elastography werd de lever stijfheid (LS) bepaald. Er werd gezocht naar correlaties tussen tijdsparameters (leeftijd bij operatie, leeftijd bij follow-up en het Fontan interval), serum merkers en beeldvorming.

Resultaten: Labo testen toonden abnormale waarden voor γGT, totaal bilirubine, direct bilirubine, ALT, thrombocyten en pro-BNP in 74%, 31%, 50%, 36%, 29% en 39% van de patiënten respectievelijk. APRI en FIB-4 waren niet in staat om lever fibrose aan te tonen. Forns index indiceerde een matige fibrose in 29% van de patiënten en deze parameter correleerde eveneens met Fontan interval (p=0.034). Echografie toonde morfologische leverafwijkingen in 46% van de gevallen. Nodulariteit van de leverrand werd gezien in 21% en nodulaire hyperplasie in 29%. Flowsnelheden in de bloedvaten en weerstands- en pulsatilityindices vielen voor de meeste patiënten binnen de normale range. Een trend richting lagere SMA RI bij toenemend Fontan interval werd geobserveerd (p=0.08). LS (gemiddelde 10.4 ± 3.7 kPa) was verhoogd in 96% van onze populatie. Hogere LS waarden correleerden significant met een langer Fontan interval (p=0.018), lagere SMA RI (p=0.008), grotere miltafmeting (p=0.025), lager albumine (p=0.015), lager totaal proteïne (p=0.002) en hoger AST (p=0.031).

Conclusie: (Jong-)volwassen Fontan patiënten vertonen matige tekens van leverdysfunctie. Het klinisch nut van serum parameters en serum fibrose scores in post-Fontan screening blijft onduidelijk in de afwezigheid van significante afwijkingen in leverenzymen en de afwezigheid van correlaties met morfologische veranderingen. Het hoge percentage van morfologische veranderingen ter hoogte van de lever bij patiënten met een Fontan palliatie ondersteunen het gebruik van echografie in follow-up. Dit is in contrast met de Doppler indices. Enkel voor SMA RI zijn er argumenten voor gebruik in follow-up screening. Gestegen LS werd gerapporteerd in bijna elke patiënt. Bovendien werd een stijging gezien in de tijd en met lagere SMA RI waarden. Maar, LS is in een Fontan populatie waarschijnlijk een overschatting als gevolg van levercongestie en validatie door sequentiële metingen lijkt noodzakelijk. Er werd een poging gedaan om een Fontan follow-up strategie voor te stellen startend 10 jaar na het aanleggen van de volledige Fontan circulatie. Screening bestaat hierbij minimaal uit meting van serum parameters (thrombocyten, γGT, totaal and direct bilirubine, serum transaminases) en Forns score. Daarnaast dient ook de lever en milt morfologie geëvalueerd te worden met behulp van echografie in combinatie met LS metingen.
1. Introduction

1.1. The normal heart

1.1.1. Anatomy

The human heart (Figure 1), surrounded by the pericardium, can be seen as the engine of the human body. By contraction of the myocardium (the actual muscle) it stows blood into a serial circulation consisting of the lung circulation for gas exchange and the peripheral circulation, providing the organs with oxygen and other nutrients. The normal human heart consists of four cavities: 2 atria and 2 ventricles whereas the atria and ventricles are separated by valves. The heart is divided in a ‘left’ and a ‘right’ heart by the interatrial and interventricular septa. Like the atria and the ventricle, both the lung and the peripheral circulation are separated from the ventricles by the pulmonary and aortic valve, respectively (1,2).

Figure 1: Anatomy of the human heart

1.1.2. Physiology

Blood must flow from the heart towards the end organs through the circulation. To do so a pressure gradient has to be created during the heart cycle, which will lead to movement of blood from high- to low-pressure areas. This cardiac cycle consists of two phases: a relaxation phase or diastole, in which blood is sucked in the heart so that the heart is filled, and a contraction phase or systole, wherein the heart ejects a certain volume of blood in both the
pulmonary and systemic circulation. The starting point of this cycle lies in the upper right atrium, the sinoatrial node, where an electrical signal is sent out on a regular basis. Via a conduction system this signal will activate the myocardial cells, resulting in a harmonized and hemodynamic efficient contraction of the myocardium(1,2).

When we focus on the circulation of the right heart, venous blood, low in oxygen, reaches the right atrium (RA) via the inferior (IVC) and superior vena cava (SVC). Multiple factors influence this flow towards the atrium and therefore preload. Cardiac function influences venous return in two ways, where residual pressure in the post-capillary venules and the suction of the RA during atrial systole stimulate forward venous flow. Together with muscular contraction in the lower extremities, respiration influences right heart flows. In expiration a cranial movement of the diaphragm occurs, consequently abdominal pressure decreases and flow from the lower extremities to the heart is stimulated. The inverse is true in the inspiratory phase, in which the diaphragm descents. However, intrathoracic pressure decrease stimulates forward flow from the upper extremities, head and neck. Gravity is a third important factor determining venous flow to the RA(1–3).

When the atrium contracts, blood is transported via the tricuspid valve to the right ventricle (RV). This volume is then ejected through the pulmonary valve during systole; the blood reaches the pulmonary circulation, a large vascular bed with low vascular resistance. Via diffusion over the alveolocapillary membrane blood will become oxygenated and CO₂ will be removed. Several factors influence pulmonary blood flow, whereas pulmonary vascular resistance (PVR) is a very important factor. In a healthy person this resistance is small, <3,125 wood-units(4). Secondly, a pressure gradient of 7 mmHg is responsible for propagation of blood through the lungs from the arterial to the venous side, determined by the difference of pulmonary artery (PA) pressure (25/10 mmHg, mean 15 mmHg) and left atrial pressure (8 mmHg). Driven by this pressure gradient oxygenated blood reaches the left atrium, and via the mitral valve the left, systemic ventricle where blood leaves the heart for the systemic circulation through the aortic valve into the aorta(1–3).

1.2. The normal subdiafragmatic (venous) circulation

1.2.1. Anatomy
The subdiafragmatic venous territory consists of two dynamically distinct circulations: the splanchnic and the systemic venous. The splanchnic venous circulation is constructed as a series of parallel circuits (Figure 2) with drainage of all intestinal, pancreatic and splenic
Introduction

blood through the added resistance of the liver via the portal vein (PV). Eventually this blood volume reaches the IVC after passage in the hepatic veins (HV). In the systemic venous circulation the IVC channels blood from the lower extremities, the kidneys and the pelvic organs. It is propagated towards the right heart by the same factors as described earlier.

**Figure 2:** Schematic representation of the anatomy of the splanchnic circulation(5).

1.2.2. Physiology

Blood supply to the liver differs from other organs by the fact that both venous and arterial pathways contribute to inflow via the PV and hepatic artery (HA), respectively. Approximately 25% of cardiac output (CO) is distributed to the liver(6). One-fourth of this flow supply to the hepatic circulation is oxygenated, arterial blood via the HA(7). The HA is subject to an arterial autoregulation and is responsible for all autoregulation of hepatic blood flow, leading to the hepatic arterial buffer response (HABR). This concept illustrates the observation of a reciprocal relationship between hepatic arterial flow and portal venous flow whereas increased HA flow can buffer a 30% to 60% decrease in portal inflow(8). The rest of hepatic blood inflow consists of venous efflux from all splanchnic organs, which is collected in the PV. In contrast to the HA, where HA resistance determines inflow(9), the liver is not capable of controlling portal blood flow, since hepatic venous resistance is low due to the unique hepatic vasculature, where blood reaches the microvascular unit of the acinus and exits via the hepatic venules flowing past only 20 hepatocytes approximately(6). The impossibility to control PV flow is illustrated by the fact that even maximal stimulation of the hepatic orthosympatic nerves does not alter splanchnic outflow in the PV, although portal venous pressure rises significantly(6). Portal blood flow however is dependent on the
mesenteric circulation and is driven through the liver across a pressure gradient between portal venous inflow and hepatic venous outflow, which under normal circumstances does not exceed 5 mmHg\(^5,8\). Interposition of the liver between splanchnic and systemic circulations has important hemodynamic consequences. As a result, splanchnic venous pressure is two or three times higher than that in the systemic inferior caval system\(^10\).

### 1.2.3. Influence of respiration

In the IVC and the HV, respiration exerts effects on blood flow, explained by intrathoracic pressure changes. During inspiration intrathoracic pressure drops, a change that is transmitted to other structures positioned in the thorax, eventually resulting in a flow increase towards the right heart. This phenomenon where inspiration provides additional energy to forward venous flow is called the cardiopulmonary interaction\(^11,12\). This, in a healthy population, is responsible for 15\% of the total IVC venous return\(^12\). Expiration leads to opposite changes on IVC and HV flow rates due to a fast increase of intrathoracic pressures\(^13,14\).

When effects of respiration are considered on the portal blood flow, an almost continuous flow is measured, except during inspiration\(^12,15\). In this phase of the respiratory cycle the diaphragm descents, thereby Moreno et al suggested that the portal venules and hepatic sinusoids are transiently compressed, resulting in a flow reduction\(^16\).

### 1.2.4. Influence of positional changes

Obviously hydrostatic forces influence intravascular pressures as well in the systemic venous as in the subdiaphragmatic venous circulation. However these gravitational changes are compensated for in the systemic venous circulation due to ventricular function\(^17\). Flow rates in the HV en PV on the other hand are significantly decreased in the upright position\(^18,19\). Brown et al even observed a flow reduction of 26\% in the PV in the upright position. This is possibly explained by a decrease of CO in standing persons, since the measured fall in CO is in line with the PV flow reduction\(^19\).

### 1.2.5. Influence of the cardiac cycle

HV and IVC flow profiles are cardiac dependent. A forward flow can be seen following ventricular contraction and with contraction of the atria a backward flow in both IVC and HV is observed. Normal PV flow profile is more influenced by respiration because flow is not in phase with the cardiac cycle due to liver interposition\(^12\).
1.2.6. **Normal US/Doppler flow patterns in the subdiaphragmatic venous circulation**

1.2.6.1. *Inferior vena cava (IVC)*

**Figure 3:** Normal IVC waveform. Note the similarity of this pattern with the HV pattern described in 1.2.6.2. Flow reversal during atrial systole is indicated by the blue arrow(20).

Doppler flow signals in the IVC reflect the contractions of the RA and therefore the pattern is pulsatile (Figure 3). Pulsatility increases when Doppler flow is measured closer to the RA. During atrial systole a flow reversal can be seen. Overall the pattern in the IVC is similar to the one observed in the HV(21).

1.2.6.2. *Hepatic vein (HV)*

**Figure 4:** Normal hepatic waveform with forward flow below horizontal line. Note the pulsatile flow pattern with a W configuration. TV: tricuspid valve(21).

Pulse wave Doppler imaging in the HV normally identifies a tephasic pattern generated by the cardiac cycle (Figure 4)(22). 1) A-wave: retrograde flow in late diastole; 2) S-wave: a large antegrade wave in early-mid systole; 3) D-wave: an antegrade wave in early-mid diastole. In some cases a fourth wave can be observed: V-wave: a small retrograde wave in late systole due to atrial overfilling(13). When the RA contracts flow from the liver to the heart decreases
(C), even resulting in a short period of flow reversal (C’). In the following phase the atrium is progressively filled, therefore HV flow is directed towards the right heart with gradually increasing velocity (R). However when RA volume increases flow out of the liver starts to slow down (R’). When the tricuspid valve opens, RA volume is passively transported into the RV. This in turn results in a second shorter phase of antegrade flow in the HV. Finally, the whole cycle is repeated(21).

1.2.6.3. Portal vein (PV)

Figure 5: Normal PV waveforms. A) PV waveform with moderate pulsatility. B) Normal PV waveform showing a continuous, hepatopetal monophasic flow(21).

Since the PV is not in direct communication with the right heart, because of the interposition of the hepatic sinusoids, cardiac activity does not lead to a pulsatile PV waveform as seen in the HV. As a result the normal PV Doppler signal is a continuous antegrade, hepatopetal flow, however some degree of pulsatility can be normal (Figure 5)(21).

1.2.6.4. Superior mesenteric artery (SMA)

Figure 6: Normal Doppler flow pattern of the SMA in a fasting state(21).
SMA shows mild turbulence waveform when measured near the arterial origin. However a more uniform flow is obtained when evaluation occurs more distally. In a fasting state, a high-resistance flow pattern can be seen, which is triphasic (Figure 6). During systole sharp peaks are measured. It is possible that a negative velocity at the beginning of the diastolic phase is observed. End-diastolic velocities in the SMA decline near zero. In a fasting state SMA resistance index (RI) is >0.7(21,23).

1.2.6.5. Hepatic artery (HA)

![Doppler flow pattern of the HA](image)

Figure 7: Normal Doppler flow pattern of the HA(24).

Hepatic arterial waveform has low-resistance flow characteristics, pulsatile with broad systolic peaks and gradual deceleration from systole to diastole (Figure 7). As in the other vessels, peak height reflects peak systolic velocity and end-diastolic velocity is measured at the lowest point of the flow pattern. Blood flow in the HA is hepatopetal and well maintained throughout the entire cardiac cycle. Normal HA RI ranges from 0.5-0.7(21,25).

1.3. Univentricular heart (UVH) disease

1.3.1. What is UVH?

Whereas the normal heart is defined by two circulations in series, driven by two ventricles this is not the case for several (complex) cardiac malformations that are characterised by the presence of only one functional ventricle, usually defined by the absence of an adequate atrioventricular valve or pumping chamber(26). These congenital cardiac defects with a functionally univentricular arrangement represent 9% of all congenital anomalies and all have
in common that only one of the chambers within the ventricular mass supports both the systemic and the pulmonary circuit(27).

1.3.2. Pathophysiology of the native UVH

In UVH disease the single well-developed chamber will have to support both the systemic and pulmonary circulation, not in a normal serial but in a parallel organisation. Such a circuit has two major disadvantages. Normally, the right heart pumps deoxygenated blood from the systemic circulation to the lungs where it becomes oxygenated via $O_2$-diffusion. Subsequently this oxygenated blood (pulmonary venous) is distributed in the systemic circulation by contraction of the left heart. Since systemic and pulmonary circulations are not separated in the ‘univentricular heart’ oxygenated and deoxygenated blood are mixed in the atria or the ventricles due to connections between these structures, resulting in chronic arterial desaturation. As the infant grows, cyanosis tends to become more pronounced(26).

The ventricle of a neonate with UVH receives both systemic and pulmonary venous return and thereby the ventricle is submitted to a volume overload(28). Starling mechanism tries to compensate for this overload, eventually leading to cardiac dilatation and eccentric hypertrophy(29). But when Starling mechanism fails, it leads to deterioration in myocardial mechanics, characterized by progressive development of a more spherical ventricular shape, increased wall stress and impaired ventricular function and contractility(30,31).

1.3.3. Clinical presentation

Two major factors will determine the clinical presentation of a patient with UVH disease: the nature of the ventriculoarterial connection and the degree of obstruction to pulmonary or systemic outflow(32). In general UVH can manifest itself in 2 ways: pulmonary overflow and obstruction of the outflow tract.

Pulmonary overflow

When both the pulmonary and the systemic ventricular outflow tract are free of obstruction symptoms of heart failure will arise as soon as the PVR drops in early infancy(27,32).

Outflow obstruction

The obstruction can be located at the level of the outflow valves, or a subvalvular narrowing is the cause of the obstruction. Third, a restrictive ventricular septum defect between the dominant and the rudimentary ventricle, from which a great artery arises, is the reason for obstruction.
1) Pulmonary outflow obstruction
Mild to moderate pulmonary stenosis may result in a balanced hemodynamic state, with minimal symptoms. Severe pulmonary stenosis results in minimal forward flow to the lungs and by consequence cyanosis is present. When the pulmonary outflow tract is atretic pulmonary blood flow is completely dependent on the persistence of the ductus arteriosus(27,32).

2) Systemic outflow obstruction
This type of obstruction will lead to the combination of low systemic blood flow and high pulmonary blood flow, with neonatal heart failure as a result. Prognosis for survival is low and will be even worse if an aortic coarctation is present. When obstruction to the systemic outflow is complete, as in hypoplastic left heart syndrome, systemic perfusion will be totally dependent on right to left ductal shunting(27,32).

1.3.4. Historical evolution in the treatment of UVH disease
In UVH disease construction of a biventricular circulation is impossible because the ‘non-dominant’ ventricle is too small to sustain CO(32). With no adequate surgical procedure available before the development of palliative procedures, most patients with single ventricle died within the first years of life(33). With the help of palliative procedures in the neonate, such as pulmonary artery banding or systemic-to-pulmonary artery shunt, most patients could be stabilized(34). Still, without further interventions most patients died during the second decade of life because of ventricular dysfunction, congestive cardiac failure or pulmonary hypertension due to cardiac volume overload and/or pressure overload of the lungs(30,34).

After stabilization of the patient in the neonatal period the goal of the next palliative procedure is twofold, reducing cyanosis and volume load to the ventricle. This is accomplished by creating a circulation in series rather than a parallel circulation. Fontan and Baudet in 1971 were the first to report on a new surgical approach where those two objectives were achieved, separating the systemic and pulmonary circulations by creating a right atrium-to-pulmonary artery valved connection (Figure 8)(35). In the first operation a homograft valve was placed in the atriopulmonary and cavo-atrial connection(26). So in a Fontan circulation the systemic venous return is connected to the pulmonary arteries without the interposition of an adequate ventricle. The idea behind this treatment strategy arose from experimental studies carried out in the 1940s regarding RA contribution to blood flow, RV bypass and its consequences on lung and venous pressures and effect of systemic venous pressure on pulmonary flow(36–38). Subsequent work demonstrated that a single ventricle could support
both circulations in series(39). Since its original description, the Fontan circuit has known several modifications. In these early series however, a very high incidence, reaching up to 40%, of late reoperations occurred. Hence, most of the older circuits are considered obsolete nowadays, with only a few patients still surviving on such circuits(26).

![Figure 8: Original Fontan operation(35).](image1)

In 1973 Kreutzer et al modified the original operation in reaction to concerns that arose regarding the use of valves in the Fontan circulation. As a result, the atriopulmonary connection (APC) was developed, where the circuit was built by a direct anastomosis between the RA and the PA without the interposition of valves in the circuit (Figure 9)(40).

![Figure 9: Atriopulmonary connection(40).](image2)

In 1988 de Leval et al reported the results of a hydrodynamic study, which was constructed to elucidate RA contribution in the APC(41). They concluded that turbulence and thereby augmented resistance to net forward flow was generated by pulsation of a valveless chamber in a simple continuous flow circuit. By flow visualization and measurements of energy losses in the second part of the study they emphasized that streamlining non-pulsatile circuits was important to improve the results of Fontan repair. Based on this knowledge a modified
approach to Fontan reconstruction was developed, the total cavopulmonary connection (TCPC). In the TCPC streamlining of the systemic venous flow towards both lungs is accomplished, while the pulmonary venous return and the coronary sinus drainage were left at the low pressure of the left atrium (LA)(41). Since the 1990s TCPC is considered the procedure of choice to obtain a Fontan circulation.

The caval veins, in the TCPC, are connected to the PA, thereby bypassing the right heart structures. The SVC is connected to the PA (bidirectional cavopulmonary anastomosis, BCPA). For connection of the IVC two surgical variants are available: the lateral tunnel and the extracardiac conduit. In the TCPC described by de Leval a lateral tunnel is created (Figure 10). This lateral tunnel provides a tubular path between the IVC and the PA, consisting of a prosthetic baffle and a portion of the lateral atrial wall(41).

![Lateral tunnel Fontan](image)

**Figure 10:** Total cavopulmonary connection with lateral tunnel. SVC: superior vena cava, IVC: inferior vena cava, PA: pulmonary artery(42).

In 1990 the extra cardiac conduit was introduced, which consists of a tube graft between the IVC and the PA bifurcation (Figure 11)(43).
The combination of improved hydrodynamics in the cavopulmonary connection(44), a reduced likelihood of atrial arrhythmias or sinus node dysfunction(45,46) have established a wider acceptance of this Fontan modification.

In some cases a fenestration (Figure 11), a connection between the intra-atrial baffle or the conduit and the pulmonary venous atrium of the TCPC, can be added both in the TCPC with lateral tunnel and with an extracardiac conduit. With such a fenestration a right-to-left shunt at the atrial level can be established, thereby maintaining the preload of the systemic ventricle and CO in conditions where pulmonary blood flow is limited(47). It has also been shown that perioperative morbidity and mortality of the Fontan operation is reduced by introduction of a fenestration, but a fenestration leads to a small reduction of systemic oxygenation(48,49).

The Fontan circulation has facilitated the survival of children with UVH disease. In the years since the original description of this surgery, improvements in postoperative care, surgical technique and staged approach to palliation have led to significantly increased survival.

1.3.5. Fontan physiology

1.3.5.1. The heart with a Fontan circulation

After completion of the Fontan circulation the pre-existing parallel circulation in the univentricular heart is converted into a serial circuit. However, the circulation in series in a Fontan patient differs from a normal circulation in 2 aspects. Systemic venous return is not powered by a high-energy hydraulic source and pulmonary flow occurs at a low energy state, in the absence of a pre-pulmonary pump, which normally adds forward energy to pulmonary flow(27,52). The goals of a Fontan circulation include normalisation of the oxygenation of blood, preservation of CO and reduction of the volume load on the single ventricle. But this
type of circulation also creates 3 majors disadvantages: increased central venous pressure (CVP), lack of preload reserve and limited/reduced CO.

**Cardiac output**

CO is influenced by heart rate and stroke volume (Figure 12). Thus raising CO can only be achieved by increasing heart rate or stroke volume through modification of contractility of the ventricle, afterload or preload.

![Figure 12: Positive and negative influencing factors of CO. PL: preload, INO: inotropism, AL: afterload, SA: stroke work, SV: stroke volume, HF: heart frequency, CO: cardiac output.](image)

**Afterload**

Increased afterload is inherent to Fontan physiology. This elevation can be explained by the fact that 3 resistors (the systemic vascular bed, the cavopulmonary connection pathway and the pulmonary vascular bed) are arranged in series(53).

**Preload**

Preload is probably the most important determinant for CO(54). It can be modified by a fenestration if present and the transpulmonary gradient (defined as the difference between the mean PA pressure and the LA pressure)(52). In a Fontan circulation there is a lack of a contractile subpulmonary ventricle. This means that for achieving an adequate gradient the mean pressure in the systemic veins must be higher than the LA pressure (Figure 13). The absence of a contractile subpulmonary ventricle also results in a more fixed preload and thereby to limited CO augmentation(52).

In a UVH, before any therapeutic intervention, the systemic ventricle is subjected to a volume overload of at least 200% of normal, since both the systemic and pulmonary circulation are connected to the single ventricle. The UVH in this volume overloaded phase is known to be dilated evolving to a more spherical configuration, hypertrophic (eccentric) and sometimes hypocontractile(30). After bidirectional anastomosis in the first stage of the pallation, the
preload is reduced to 150% (28). When palliation is completed preload reaches more or less normal values because the complete systemic venous return does not enter the heart anymore, but bypasses the heart.

Figure 13: Schematic representation of the normal cardiovascular circulation (Left) and Fontan circulation (Right). (Left) The pulmonary circulation (P) is connected in series with the systemic circulation (S). The RV maintains the RA pressure lower than the LA pressure, and provides enough energy for the blood to pass through the pulmonary resistance. (Right) Fontan circuit: the systemic veins are connected to the pulmonary artery (PA), without a subpulmonary ventricle or systemic atrium. In the absence of a fenestration, there is no admixture of systemic and pulmonary venous blood, but the systemic venous pressures are markedly elevated. Ao, aorta; CV, caval veins; LA, left atrium; LV, left ventricle; P, pulmonary circulation; PA, pulmonary artery; RV, right ventricle; S, systemic circulation; V, single ventricle (52).

The ventricle
Unloading of the single ventricle can lead to normalisation of ventricular function, but this is often incomplete (26). Positive effects of preload volume reduction on the ventricle can be seen such as reduction of ventricular volume and dimension. But a price has to be paid; indeed acute reduction in end-diastolic volume coincidentally results in a wall thickness increase leading to diastolic dysfunction (55). This can be explained by the law of preservation of mass, which predicts that given a marked reduction in ventricular preload, preserved shortening and constant wall mass, an increase in wall thickness must occur (54). The augmented wall thickness results in impaired early ventricular relaxation due to increased mass-to-volume ratio (55). After the initial phase, the volume unloading results in more favourable hemodynamics, as defined by Laplace, by reduction of ventricular wall stress and hypertrophy (27).

Together with worsening of ventricular compliance, several studies have reported that chronic diastolic dysfunction occurs, reducing the ability of the ventricle to fill (54,56–61). Diastolic dysfunction, characterized in a Fontan by increased diastolic stiffness and/or prolonged isovolumetric relaxation cannot be explained by hypertrophy alone because ventricular mass-to-volume ratio regresses during follow-up (FU) (56). Also diastolic abnormalities affect systemic venous pressure inducing an augmentation of this pressure. This observation
indicates that the RV, besides providing preload, also acts as a stabilizer of venous pressure in a normal circulation(59). Subsequently dysfunction may lead to a reduced CO and the Fontan patient hereby enters a vicious circle(26). In contrast to impaired diastolic function after TCPC construction, systolic function is relatively well preserved years after Fontan repair(58).

1.3.5.2. **Pulmonary blood flow**

Blood flow towards the lungs is facilitated by 3 factors: low PVR, respiratory movements and high systemic venous pressure (generating energy for pulmonary perfusion in absence of a hydraulic force).

**Pulmonary vascular resistance**

In Fontan patients PVR is a very important determinant of CO(52). It is desirable that resistance is as low as possible for a Fontan circulation to be successful(30,54,62). Volume overload in the neonatal period may help to improve PA growth and by expansion the pulmonary vascular bed but due to its detrimental effects at ventricular level, volume overload should not be present for too long(30). In a Fontan circulation the pulmonary blood flow occurs at low pressures. It is however non-pulsatile leading to an inadequate filling of the pulmonary vascular bed. Several studies showed that chronic deprivation of pulsatility in the pulmonary vascular bed may lead to endothelial dysfunction and consequently to decreased endothelial nitric oxide production, which under normal conditions is stimulated by shear stress. Together with less recruitment of the distal pulmonary vascular bed due to absence of systolic pressure rise(63), this results in elevated PVR(64,65).

**Respiratory movements**

Secondly, the Fontan pulmonary circulation is due to the lack of ventriculoarterial coupling largely dependent on external factors such as respiration and gravity(10). The importance of the cardiopulmonary interaction is emphasized in several studies(12,66,67). Inspiration acts as an additional energy source enhancing passive blood flow into the lungs via pulmonary arteries. It can be said that to some extent inspiration creates pulsatility. Flow in the SVC and IVC towards the lungs is synchronous with and increased by inspiration. Furthermore negative intrathoracic pressure as can be seen during inspiration enhances movement of blood from the systemic venous reservoir towards the lungs(66). Likewise, descent of the
diaphragm during inspiration and with compression of the liver will lead to a higher hepatic venous contribution in total venous return and eventually higher pulmonary blood flow\(^{(12)}\). In contrast, Valsalva manoeuver and other situations causing positive intrathoracic pressure e.g expiration, will lead to a marked attenuation of pulmonary blood flow, even flow reversal in IVC and reduced ventricular filling\(^{(66,68)}\). Approximately 30% of CO can be attributed directly to respiratory movements\(^{(54)}\).

Finally it also is important for venous flow towards the lungs that the connection between the PA and the caval veins is created in a way that maximal laminar flow and minimal energy loss is achieved. Otherwise an extra disadvantageous flow resistance is generated\(^{(52)}\).

\[1.3.5.3.\text{ Systemic venous and splanchnic blood flow}\]

As mentioned earlier the Fontan circulation represents a unique hemodynamical system, lacking a ventricular pump that propagates systemic venous return to the pulmonary arteries; pulmonary vascular resistance and systemic vascular resistance are run through sequentially. Systemic venous pressures are elevated in the Fontan circulation owing to different factors, but this elevation is necessary to maintain CO. First of all the higher venous pressure is necessary to generate the energy for pulmonary perfusion in absence of the subpulmonary pump. Secondly, due to the lack of this ventriculoarterial coupling external gravitational and hydrostatic forces exert greater influence on the peripheral venous system\(^{(10)}\). Where in a biventricular heart the RV also acts as a blood reservoir separated from the venous circulation by valves, in a Fontan circulation a greater blood column is piled up and thus higher venous pressures arise. By interposition of the cavopulmonary connection and the pulmonary vascular bed between the systemic venous system and the pulmonary venous atrium, resistance to venous return is increased\(^{(10)}\). As a consequence of an acutely elevated systemic venous pressure in the post-operative period pleural effusions (PE) can develop\(^{(10)}\). Chronic venous hypertension (mean pressure > 10 mm Hg)\(^{(69)}\) explains why, in Fontan patients, high prevalence of chronic venous insufficiency in the lower limbs is established\(^{(70)}\).

Besides the SVC and IVC, the systemic venous system also comprises the splanchnic system. Concerning splanchnic blood flow we have seen that the interposition of the hepatic resistance results in higher splanchnic pressures. This combined with the fact that in Fontan patients the pulmonary circulation is connected in series with the systemic venous return,
thereby creating an additional resistance, explains why these persons have a propensity to develop PE and protein-losing enteropathy (PLE)(10).

Augmented caval pressure is transmitted through the HV to the portal venous circulation, leading to increased wedge hepatic venous pressure (WHVP), which reflects portal pressure(71,72). Under normal circumstances, portal pressures initially are buffered from small changes in caval pressures by the intrahepatic vascular resistance at the sinusoidal level, whereas the elevated pressure is only partially transmitted(73). However, the more caval pressure exceeds the hepatic transmural pressure, the more liver sinusoids will dilate and will be recruited to become an open tube system, which allows synchronization of PV with HV flow, as well as pressure equalization(71). The higher the systemic venous pressures become, the lower the hepatic venous pressure gradient (HVPG; wedge hepatic venous pressure minus free hepatic vein pressure or pressure gradient between portal vein and hepatic veins) is(17). The main consequence of the phenomenon of pressure equalization is that centripetal portal blood flow through the liver slows down, thereby increasing portal blood transit time through the liver. Together with enlarged hepatic blood volume, owing to higher outflow resistance, this results in splanchnic congestion and portal hypertension(17). Fenestration of the Fontan conduit may lead to more favorable hemodynamics by limiting caval pressure and venous congestion(74).

Chronic elevation of systemic venous pressures causes HV outflow obstruction, which in turn affects hepatic sinusoids, resulting in dilatation and congestion(17,75). When acute sinusoidal congestion causes enzymatic dysfunction, chronicity is able to produce irreversible cellular damage due to a profibrotic effect, eventually leading to parenchymal necrosis and fibrosis, and more specific cardiac cirrhosis(17). This cardiac cirrhosis may have some similarities with the cardiac cirrhosis as a consequence of right heart problems. It is not exactly known how fibrosis due to cardiac pathology is established. There is evidence that fibrosis may develop independent of inflammation(76). Potential driving factors for fibrosis are repetitive mechanical stretch and compression of sinusoids and other cells due to passive congestion(76). With disease progression hepatic remodeling becomes more apparent along with increasing hepatic sinusoidal pressures, resulting in further elevation in WHVP(75). So both sinusoidal and postsinusoidal portal hypertension may occur with prolonged Fontan physiology. In several studies liver congestion and the extent of hepatic fibrosis were correlated with elevated systemic venous pressures and the time since the Fontan circulation was created(76,77). Furthermore hepatic dysfunction is probably also evoked by relatively low CO. As a result of diminished CO hepatic blood supply is chronically reduced, which
may compromise the HABR. HA is not able to sustain liver blood supply, therefore the liver receives less oxygen inducing a state of hypoxia, which can lead to fibrosis(78).

Since HVPG is diminished or sometimes even absent other factors become important for blood propagation. Hsia et al showed that hepatic blood flow is highly dependent on respiration in Fontan patients, where HV forward flow is mostly driven during inspiration. This also means that Fontan patients are more susceptible to defects in respiratory mechanics(12), for instance diaphragm paralysis. Normal inspiration dependent decrease in portal forward flow is absent in Fontan patients, suggesting decreased hepatic compressibility due to congested sinusoids. So forward flow in the PV occurs during the same phase of respiration as in the HV, because the congested sinusoids can be compared to a fluid-filled column that is always open(17).

Besides respiration the splanchnic venous flow is also affected by gravity. In supine position, where effect of gravity on venous return is eliminated, the rate of blood flow is higher than in the upright position(79). In Fontan patients gravity has more pronounced effects on venous return than in a healthy subject, presumably because of the absence of a ventricular input(12). It decreases net forward flow in the IVC and increases retrograde flow as well in APC as in TCPC patients(12). Hsia et al also observed that HV flow was more severely reduced in the Fontan circulation (40% vs 20% in controls) as a result of gravitational forces(12). In the same study no adverse effect of gravity on PV flow could be found in the functionally well Fontan patients, this was not the case in functionally poor patients(17). Regarding hydrostatic influences higher systemic venous pressures result in poorer splanchnic outflow. Under normal circumstances there are autoregulatory mechanisms playing a role in orthostasis to balance for increased outflow resistance. However, in a failing or high IVC pressure Fontan circulation this autoregulation is overruled, leading to further decreased splanchnic flow and increased venous pooling(17).

Concerning the cardiac influence on subdiaphragmatic flow patterns a difference has to be made between APC and TCPC (Figure 14, Figure 15). Equal to controls subjects, in APC patients antegrade and retrograde IVC and HV flow patterns remain cardiac dependent, meaning that on Doppler an antegrade flow after ventricular contraction and a backward flow following atrial contraction can be seen. In TCPC patients this is not the case, because atrial work is excluded from the venous circulation in this Fontan type(12). For the same reason
retrograde flow in IVC and HV and therefore hepatic venous regurgitation is reduced in a TCPC, compared to APC, which possibly may be protective for the liver (12,80). Venous flow in the PV, due to liver interposition, is not in phase with the cardiac cycle.

**Figure 14:** Pulsed-wave Doppler recordings with simultaneous respiratory and ECG monitoring from PV in a control subject, a patient with APC and a patient with TCPC (12).

**Figure 15:** (A) Pulsed-wave Doppler recordings with simultaneous respiratory and ECG monitoring from IVC in a control subject, a patient with APC and a patient with TCPC. (B) Pulsed-wave Doppler recordings with simultaneous respiratory and ECG monitoring from HV in a control subject, a patient with APC and a patient with TCPC. The upper line represents the electrocardiogram. The second, thin, white line shows spontaneous respiration, with upward (up arrows) and downward (down arrow) deflections indicating the onset of inspiration and expiration, respectively. Flow below the zero reference line is antegrade towards the heart; above zero is retrograde. Note reversal of flow with atrial contractions (oblique arrows) (12).
1.4. Current Fontan approach

Since the approach as described in the PhD thesis of prof. dr. K. Francois reflects best the current surgical management of patients with UVH at the University Hospital Ghent, a summary of this approach is reported below (27).

Since the PVR remains raised for several weeks, it is impossible to create a Fontan circulation in the neonatal period. Achievement of 3 objectives is essential in neonatal management: 1) unrestricted return of blood to the ventricle, 2) unrestricted flow from the ventricle through the aorta to the systemic circulation and 3) a well balanced limited flow to the lungs to prevent high pulmonary pressures and to preserve the function of the pulmonary vascular bed. During this time, the heart is still submitted to a chronic volume overload. When these goals are not achieved naturally, several surgical techniques are available to ensure optimal survival chances for the neonate (26). It would take us too far from the actual dissertation to explain all surgical possibilities in every circumstance.

At the age of 3-6 months, when the PVR has fallen to normal levels, the BCPA is constructed, where the SVC is connected (bilateral if present) to the PA. It has been shown that the intermediate BCPA step reduces morbidity and mortality at the Fontan completion (50, 51).

As SVC flow in young children comprises of more than 50% of the total systemic venous return, this shunt leads to a considerable reduction of the volume load to the single ventricle. As a consequence the mechanical efficiency of the single ventricle is improved. Oxygen saturation is maintained around 80-85%, since the desaturated blood from the IVC still passes through the heart. After this stage most children improve and remain well for some years, until they become more physically active and desaturation gradually becomes more severe, particularly during exercise (27).

At the age of 2-4 years, depending on patient size and weight, the physical ability to walk and the degree of cyanosis (at rest and during exercise), the TCPC is performed either by an extracardiac conduit or a lateral tunnel construction. A lateral tunnel Fontan can be performed at younger age since this circuit has growth potential (26). A fenestration can be electively added in case of e.g. decreased ventricular function, AV valve incompetence or raised PVR (27). In a later stage, when the body is adapted to the new hemodynamic condition, the fenestration can be closed, but this is still a topic of discussion since a fenestration possibly has advantageous effects on late complications, such as liver pathology (74).
1.5. Late Fontan complications

1.5.1. Arrhythmias
Due to scarring after atriotomy, atrial wall distension and hypertrophy, Fontan patients are predisposed to develop atrial arrhythmia, with increasing incidence when the patient ages(26,46). Arrhythmias are observed in all surgical approaches, but incidence of arrhythmia in the Fontan population varies widely, mostly explained by differences in length of FU(81,82). Although, it must be said that in consecutive studies the APC showed the highest incidences. Fenestration may prevent the late onset of tachyarrhythmia(83).

Whatever arrhythmia may occur in a Fontan patient, it is of utmost importance to gain control over this disturbance in rhythm for maintaining good hemodynamics and ventricular function and to prevent thromboembolism(84).

1.5.2. Thromboembolic events
Thromboembolism is a significant contributor to late morbidity and mortality after Fontan surgery. Several risk factors for development of a thromboembolic event are present in a Fontan patient, most of them explained by the unique Fontan physiology, rather than the type of Fontan connection. At first it was thought that obvious factors due to elevated systemic venous pressures were responsible for elevated thromboembolic risk, such as venous valve dysfunction, inflammatory microcirculation and chronic venous insufficiency (inducing stasis), as well as low CO(70). Nowadays it cannot be ignored that liver dysfunction caused by congestion implies higher incidence of abnormal coagulation profile(17). But not only the liver is held responsible for coagulation factor deficiencies since they may also be related to enteric losses(84). As stated before, arrhythmias impose an important risk factor for thrombus formation, together with progressive ventricular dysfunction. It is safe to state that Fontan patients have a lifelong risk of thromboembolic events(84).

1.5.3. Systemic venous and splanchnic hypertension
See 1.3.5.3 for pathophysiology and consequences of this complication.
2. Purpose of the study

Since the conception of the Fontan operation surgeons were able to reduce postoperative complications through better operative and postoperative care, resulting in better early survival(85). However, the hemodynamic properties of the Fontan circulation can cause various complications in the long term. Consequently, focus from short-term outcome shifted towards late morbidity and mortality, including hepatic complications in long-term survivors. In several studies it has been suggested that chronic venous hypertension and splanchnic congestion with a reduced CO and late ventricular dysfunction combined with longstanding hypoxia preceding the Fontan operation predisposes to hepatic dysfunction(17,58,76,77). When acute hepatic sinusoidal congestion may induce temporary enzymatic dysfunction, there is increasing evidence that effect of chronic liver congestion is more severe, producing diffuse irreversible parenchymal necrosis and fibrosis with progression to more severe liver pathologies(86). Spectrum of Fontan associated liver disease (FALD) may vary from abnormal liver function tests to fibrosis; even cirrhosis, hepatocellular carcinoma (HCC) and liver failure have been described(77,87–90). This is a major concern in the growing patient population with palliated UVH disease. In several studies the possible impact of liver abnormalities on late morbidity and mortality has been described(91,92). The prevalence and severity of liver disease in Fontan patients has not been extensively studied, but in recent literature liver pathology in Fontan patients is more and more subject of attention and it seems to be more common than previously thought(93). In patients treated for UVH disease in Ghent University Hospital no such research was performed yet. Therefore this study will try to assess impact of Fontan physiology on the liver in a group of adolescent and adult patients that underwent Fontan completion. First of all liver function, assessed by laboratory tests and ultrasound, in Fontan patients will be researched and compared with normal values. Concerning laboratory tests serologic correlates of hepatic pathology, including known markers of fibrosis will be evaluated in our study group. Subsequently, liver imaging will be performed for visualisation of morphology and flow patterns, followed by evaluation of liver stiffness (LS). Then, results from these investigations will be correlated to the time-interval since Fontan completion to examine whether or not liver disease in our Fontan population is time related. Results will also be related to cardiac function and atrio-ventricular valve regurgitation. Finally all results will be implemented in an attempt to work out a Fontan FU
strategy for screening and early detection of structural and functional liver abnormalities in patients with palliated UVH disease.
3. Methods

3.1. Study Design & Patients

To investigate the influence of a Fontan circulation on the liver, all patients, born before the year 2001, who previously had undergone Fontan completion at Ghent University Hospital, were prospectively studied. The Ethics Committee of Ghent University Hospital (B670201525115) approved the study protocol and informed consent was obtained from all subjects. The study was carried out in a period from August 2015 to October 2016.

Most Fontan patients are followed up approximately every 6 months at the cardiology department in Ghent University Hospital. When a patient consulted the cardiologist in the study period, patients were asked to enter the study. Informed consent was obtained on each patient. For patients that were in FU by a cardiologist in another hospital, the treating physician was contacted and he/she was informed about the purpose of the study. When this physician gave his/her approval for contacting his/her patient, information was given to the patient by telephone.

A total of 48 patients qualified for inclusion. However in our study informed consent was obtained from 29 patients. 19 patients were not included in the study because they refused to give their consent (13), were lost-to-follow up (3) or were dead (3). After patients assented to participation they were contacted individually to make an appointment for liver imaging and blood sampling.

The study protocol was fully completed by 23 patients. The patient characteristics are summarized in Table 1. There was a female predominance. All but one of our patients had a TCPC configuration at the time of the study. Whereas in 2 patients originally an APC procedure was performed, the APC was converted into a TCPC in 1 patient due to severe atrial arrhythmia. 8 of 29 patients (23%) received a TCPC with an extracardiac conduit; in the other 20 patients a lateral tunnel was constructed to complete the Fontan operation. All of our patients had negative viral hepatitis serology, which is important since viral hepatitis can be a confounding factor in the measurements of liver fibrosis and liver stiffness.
Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male number, %)</td>
<td>9 (31)</td>
</tr>
<tr>
<td>Age, years (median, IQR)</td>
<td>23.7 (20.5-27.2)</td>
</tr>
<tr>
<td>Age at operation, years (median, IQR)</td>
<td>5.0 (3.5-8.5)</td>
</tr>
<tr>
<td>Fontan interval, years (median, IQR)</td>
<td>19.7 (14.5-21.4)</td>
</tr>
<tr>
<td>Dominant ventricle</td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>17</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>9</td>
</tr>
<tr>
<td>Missing</td>
<td>3</td>
</tr>
<tr>
<td>Type of intervention</td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>1\textsuperscript{1}</td>
</tr>
<tr>
<td>TCPC</td>
<td>28</td>
</tr>
<tr>
<td>Extracardiac</td>
<td>8</td>
</tr>
<tr>
<td>Lateral tunnel</td>
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<tr>
<td>Fenestration</td>
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<tr>
<td>Yes</td>
<td>25</td>
</tr>
<tr>
<td>Open</td>
<td>11</td>
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<td>6</td>
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<tr>
<td>Unknown</td>
<td>8</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
</tr>
</tbody>
</table>

\textsuperscript{1}In 1 patient initially APC (atriopulmonary connection) was performed, but conversion to TCPC (total cavopulmonary anastomosis) was necessary. IQR: interquartile range.

3.2. Procedures

3.2.1. Laboratory tests

A list of all laboratory tests performed can be found in addendum 1. Some parameters were used to calculate liver fibrosis scores. In our study we applied 3 scoring systems on our study population: APRI test, FIB-4 and Forns score. Serum liver fibrosis markers are extensively studied in chronic viral hepatitis\(^{94–98}\). It is, as a consequence, predominantly in this group that serum fibrosis markers are considered useful. These non-invasive tests may be etiology-dependent and it is unclear if they can also be used as markers for liver fibrosis and cirrhosis due to venous congestion\(^{99}\). Kiesewetter et al reported changes in hepatological markers in Fontan patients, but no useful markers for evaluating congestive liver fibrosis have been identified so far\(^{77,100}\).

In a study conducted by Baek et al, Forns index and APRI among other fibrosis markers were evaluated as fibrosis markers in a Fontan population. They concluded that the Forns index had the highest area under the ROC curve (AUROC) \(^{0.786, 95\%\text{ confidence interval (CI) 0.61-0.95}}\) and therefore was the best predictor for the presence of Fontan hepatopathy. They also
Methods

suggested that APRI was a reliable method for predicting liver histology, though it should be noted that they measured an AUROC of 0.563, which is not accurate enough to support clinical decision-making(101).

The following scoring systems were applied in our study:

**APRI (aspartate aminotransferase-to-platelet ratio index)**

\[
\text{APRI} = \left(\frac{\text{AST}}{\text{ULN}}\right) \times \frac{100}{\text{platelet count}}
\]

With ULN= upper limit of normal, AST (IU/L) and platelet count (10^9/L)(102).

AUROC for predicting significant fibrosis and cirrhosis were 0.83 (95% CI, 0.78-0.88) and 0.90 (95% CI, 0.86-0.94) respectively, reflecting good accuracy. AUC of 1 is characteristic for an ideal test, whereas value of 0.5 indicates a test with no diagnostic value. Two cut-off values, <0.5 (sensitivity 91%) and >1.5 (specificity), were used for exclusion or prediction of significant fibrosis (F2-F4), respectively(103). Positive (PPV) and negative predictive value (NPV) for prediction of significant fibrosis of an APRI score >1.50 (corresponding with METAVIR F2 or more) were 91% and 65%(103). For APRI <0.5 PPV and NPV were 64% and 90%, respectively. APRI <1 has NPV of almost 100% for the presence of cirrhosis. This fibrosis scoring system was developed by Wai et al to predict fibrosis and cirrhosis in a population with chronic hepatitis C(102).

**FIB-4 (fibrosis-4)**

\[
\text{FIB-4} = \frac{\text{Age} \times \text{AST}}{\text{platelet count} \times \sqrt{\text{ALT}}}
\]

With age (years), AST (IU/L), platelet count (10^9/L) and ALT (IU/L)(104).

Based on the AUROC, 2 cut off points, <1.45 (NPV 90%) and > 3.25 (specificity 97%, PPV 65%) were obtained for exclusion and prediction of (advanced) fibrosis. Values <1.45 and >3.25 indicate F0-F1 and F2-F4, respectively(104). Originally FIB4 was developed by Sterling et al as a non-invasive fibrosis marker for use in HCV/HIV co-infection(104).
Methods

Forns score

\[ \text{Forns score} = 7.811 - 3.131 \times \ln(\text{platelet count}) + 0.781 \times \ln(\gamma GT) + 3.467 \times \ln(\text{age}) - 0.014 \times \text{cholesterol} \]

With platelet count \((10^3/\mu\text{l})\), \(\gamma\text{GT (IU/L)}\), age (years), cholesterol (mg/dL)(97).

AUROC for detection of significant fibrosis was 0.81. Based on AUROC Forns et al recommended that values <4.21 (NPV 96%) were best in excluding significant fibrosis (F2-F4), so in our study this cut-off value was also used(97). Furthermore a value >6.9 (PPV 66%) was used as cut-off value for presence of significant fibrosis, as suggested by Forns et al. Scores between these two cut-offs are indicative for a moderate degree of fibrosis. Reflected by PPV and NPV, Forns index is good for excluding significant fibrosis, rather than for detection of significant fibrosis. Initially this scoring system was developed for staging hepatic fibrosis in chronic hepatitis C(97).

Pro-brain natriuretic peptide (pro-BNP) was measured since Levin et al stated that it could be used as a marker of heart failure(105). Specifically for a Fontan population it was demonstrated that this serum marker correlated with atrioventricular valve insufficiency (AVVI)(106) and was associated with risk of Fontan failure and death, independent of age and Fontan type(107).

3.2.2. Liver imaging

As mentioned before, progressive hepatic failure is more and more recognized in literature and so early identification of changes in the liver status are of great importance in the Fontan population. One method for the assessment of hepatic fibrosis or cirrhosis is liver biopsy (LB). However, we did not choose to use LB in our study because biopsy is invasive, it is not an ideal method when patients have to be examined repeatedly, has low sensitivity for the early stages of fibrosis(108), and lesions may be inhomogeneous throughout the liver in a Fontan patients(109–111). Since non-invasive imaging techniques in literature are widely evaluated for hepatic fibrosis these were our methods of choice(112–118). Standard ultrasound (US) and shear wave elastography (SWE) were combined to compensate for the limitation of US to detect fibrosis(7).
3.2.2.1. Liver ultrasonography and Doppler flow patterns

The Philips IU22 US-SWE system with a C5-1 transducer was used. Before liver imaging was performed patients were instructed to fast for a minimum of 4 hours to exclude the effect of the post-prandial state on flow measurements. First a general evaluation of liver structure and spleen was carried out (see addendum 2 for imaging protocol). Second, diameters in both in- and expiration were measured during apnoea in the IVC and PV and eventually pulsed-wave Doppler recordings in the IVC, PV, HA, SMA and HV were made with the patient in supine position during apnoea. For each vessel, colour flow mapping was used as guidance to determine the correct sampling site, at the centre of the colour signal. SMA flow was measured in the main trunk nearby its origin in all patients but one and portal flow in the main portal trunk before division into its branches in most patients. For HV and IVC recordings were made at less standardised positions. For liver structure as well as for vascular measurements a digital copy was made into PACS (picture archiving and communication system) for off-line analysis.

When RA pressure is normal IVC diameter on abdominal US is less than 20mm and collapses more than 50% with inspiration. This collapsibility index is measured as followed: 

\[(\text{IVC}_{\text{expiration}} \text{ diameter} - \text{IVC}_{\text{inspiration}})/\text{IVC}_{\text{expiration}} \text{ diameter}\] (119). Correlations between CVP and IVC characteristics have been made, with collapsibility index decrease and diameter increases when CVP increases(119). We considered PV diameter over 13mm to be abnormal, since this cut-off for normal PV diameter has been intimated(120). Nevertheless PV diameter is not sufficient for diagnosing portal hypertension. As for PV diameter, diagnosis of portal hypertension is hard to make when relying on PV velocities. Where very low velocities (<13 mmHg) are indicative, velocities within normal range do not exclude portal hypertension(21). Under normal circumstances PV flow is hepatopetal and with minimal pulsatility, but when CVP is high hepatofugal flow direction and pulsatility increase can be seen(121).

In normal individuals, it was shown that PV pulsatility correlated with CVP and New York Heart Association functional class (NYHA)(122,123). PV pulsatility can be quantified in two ways, using the pulsatility index (PI) and the pulsatility ratio (PR).

\[
\text{PI} = (\text{maximum velocity} - \text{minimum velocity})/\text{maximum velocity}
\]

\[
\text{PR} = \text{minimum velocity}/\text{maximum velocity}
\]

Using PV PI it should be noted that increased pulsatility would result in a value closer to 1.
When PV PR is used the opposite is true, where increased pulsatility leads to PR decrease. Rengo et al demonstrated that PR was inversely correlated with RA pressure(124). This can be explained as followed: with increasing CVP and the resulting hepatic outflow obstruction, IVC and HV diameter increase gradually, thereby buffering the effect of elevated CVP on the PV. When maximal dilation occurs CVP can transduce increased pulsatility to the PV. However sinusoids also connect the HA to the PV and therefore are a possible cause of pulsatility increase, e.g arteriovenous shunting as seen in cirrhosis(25). Furthermore PV PR and PI are also seen as signs of liver congestion(125,126).

Arterial resistance indices (RI) can be calculated using the following formula:

\[ \text{RI} = \frac{\text{maximum velocity} - \text{minimum velocity}}{\text{maximum velocity}} \]

We considered HA RI <0.7 as normal(86). In patients with inflammatory liver disease HA RI has been associated with degree of liver fibrosis(127), however it should be taken into account that it is less likely that FALD has a major inflammatory cause. Furthermore HA RI is probably the best measure of resistance of flow through the hepatic vasculature(128). Since SMA is, like the HA, a low-resistance artery the same cut-off value is used, however in a fasting status normal SMA RI is >0.7(25). Mori et al suggested that SMA flow pattern, including peak velocity and RI, was indicative for major events (death, transplant) due to Fontan failure. In this situation significant RI decrease and peak velocity increase can be seen(129).

### 3.2.2.2. Shear wave elastography

Following the US, examination of LS with SWE was performed (Philips IU22 US-SWE system). With this technique validated in viral hepatitis liver tissue stiffness can be measured(114,117,118).

SWE relies on the generation of shear waves inside the liver, near the region of interest (ROI) in the liver parenchyma, by using radiation force from a focused ultrasound beam. The shear waves are lateral waves, with a motion perpendicular to the direction of the force that has generated them. They travel slowly (between 1 and 10 m/s) and are rapidly attenuated by tissue. The propagation velocity of the shear waves correlates with the elasticity of tissue and is displayed in kilopascal (kPa). The obtained values increase also with increasing stiffness of the liver parenchyma(130).
To correctly read the results, it should be kept in mind that elastography assesses liver elasticity that could be modified by factors other than fibrosis, such as edema, inflammation, extrahepatic cholestasis, and congestion. In fact these factors may lead to overestimation of liver fibrosis, thus the results obtained should always be interpreted in clinical settings. LS value assessed with SWE in healthy individuals is <4 kPa.

SWE was performed with the patient in supine position with the right arm in full abduction. In this position access to the right hypochondrium is increased via enlargement of the intercostal space. The examination was performed in the parenchyma of the right liver lobe through the intercostal space. A ROI, free from large blood vessels, in the liver parenchyma was chosen. During every measurement the patient was asked to hold his/her breath for several seconds to minimalize the influence of liver movement. The measurement depth did not exceed 50mm, but was at least 15 to 20mm beneath the Glisson capsule. This cycle was repeated until 10 successful measurements were obtained from every patient, and the values were averaged.

One of the advantages of SWE compared to other techniques is that it can be performed together with US and Doppler evaluation since it is part of the US system. In a large prospective study conducted by Ferraioli et al it was shown that SWE was more accurate for detection of significant fibrosis compared to transient elastography (TE), which was another argument for the use of SWE in our study. Even the combination of TE and serum fibrosis score did not yield a significant advantage over SWE. This was proven in a study conducted by Lannerstedt et al where FIB4 and TE were combined in the detection of liver fibrosis. They showed that this combination enhances sensitivity, but only in advanced fibrosis. The same analysis was made after exclusion of patients with clinically significant cirrhosis and in this study group the FIB4/TE combination only resulted in identification of one more patient with advanced fibrosis. So the effect of combination on sensitivity was only marginal and no advantage of the use of TE instead of SWE was present, since we did not expect our patients to have clinically obvious cirrhosis. With TE relatively low volumes of the liver parenchyma are explored, which is possibly another disadvantage in Fontan patients where a patchy distribution of fibrosis is usually described. In detection of early stages of fibrosis Osaki et al observed that acoustic radiation force impulse (ARFI) was insensitive, therefore limiting the use of this technique as a screening method in an asymptomatic Fontan population. Furthermore it has been shown that SWE is relatively...
Methods

Insensitive to respiratory motion artefacts (137) and inter- and intraobserver variability is limited (135, 138). Taking the (dis)advantages of the aforementioned techniques into account we preferred to use SWE. However it should be noted that in one study TE, SWE and ARFI had similar diagnostic accuracy (139).

![Figure 16: SWE of the liver performed with the Philips system in the right liver lobe through intercostal access.](130)

### 3.2.3. Evaluation of cardiac function

In the diagnosis and assessment of congenital heart disease echocardiography has become the primary imaging tool (140). After construction of the Fontan circulation several problems can occur. These can be classified in 3 major categories 1) obstruction of surgically altered venous and arterial channels for blood flow, 2) development of new, disadvantageous flow patterns (including AVVI) and 3) deterioration of myocardial function. To assure these manifestations will be detected in the long-term after Fontan repair, a full echocardiographic examination is necessary (141). Whereas with echocardiography systolic and diastolic ventricular function, two dimensional anatomic findings and Doppler flow patterns can be assessed (141).

A standard clinical examination of the heart and lungs is performed twice a year for every Fontan patient during follow-up by a cardiologist specialized in congenital heart disease. On each visit an echocardiography was carried out with a GE Vivid 7 with M3S transducer in order to evaluate ventricular function and haemodynamic status of our Fontan patients.
Methods

US protocol encompasses the following parameters:
- Ventricular dimensions (end-diastolic/end-systolic diameters) and evaluation of systolic and diastolic ventricular function
- Atrioventricular valve function (AVVI)
- Ventriculo-arterial valve function
- Conduit (spontaneous contrast, thrombi, fenestration) and pulmonary branches
- IVC diameter and variability
- Doppler of IVC/SVC

Three electrodes (left/right fossa iliaca and 2\textsuperscript{nd} intercostal space right) were placed for simultaneous ECG-recording with the US. With the patient in left lateral position the probe was placed parasternally for visualisation of the outflow tracts and the ventricular morphology. End diastolic and end systolic dimension was measured in 2D M-mode. Pulsed Doppler of the ventricular outflow tract was executed next for the evaluation of outflow valve function. Afterwards the transducer was placed in the inframammary fold for apical view to evaluate functionality of the atrioventricular valves with pulsed Doppler. With the patient in supine position diameter of the IVC was measured with pulsed-Doppler of HV and IVC. In conclusion SVC flow was evaluated with pulsed Doppler.

3.3. Statistical analysis

Continuous variables were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. However because sample size in this study is quite small we also evaluated normality visually via symmetry of the histograms of each variable. Only when histograms showed too much asymmetry we decided to report the concerned variable as median plus 25\textsuperscript{th}-75\textsuperscript{th} percentile. To explore a linear correlation between two variables we used the Pearson correlation coefficient $r$ for normally distributed parameters and Spearman correlation coefficient $r_s$ for non-parametric distribution. For comparing a continuous, normally distributed variable between two independent groups the unpaired student t-test was used. The non-parametric Mann-Whitney U-test was used for comparison of variables between two groups when normality of the continuous variable was not observed. Chi-square test was chosen when two categorical variables were compared and one-way ANOVA when a categorical variable was compared to a continuous variable. Statistical significance was set at two-tails $p<0.05$. Testing was performed using SPSS 24 statistical software.
4. Results

4.1. Functional status and Fontan complications

The NYHA classification was determined at the last FU in the outpatient clinic. The majority of our patients can be grouped in class 1 (Table 2). On ECG atrial flutter was documented in 2 and atrial fibrillation in 1 patient.

Sinus node dysfunction was present in 2 patients, 1 patient displayed non-sustained ventricular tachycardia and in another ventricular and supraventricular extrasystoles were seen. Four individuals were treated in the past with a pacemaker implantation for one of the following reasons: atrial flutter (1 patient), sinus node dysfunction (2 patients) and 3rd degree atrioventricular block (1 patient). Saturation ($S_o_2$) (mean 93.8 ± 4.06) was subnormal (<95%) in 13 patients (48%) and correlated significantly with hematocrit (HCT) values (p= 0.001), explained by hypoxia induced secondary polycythemia. Based on $S_o_2$ measurement, cyanosis (saturation value <92%) was observed in 6 patients, four of them had were in NYHA class 1, one in NYHA class 2 and one in NYHA class 3. In table 2 you can find current medication, specifically for cardiac indications. It should be noted that patients could receive one or more of the displayed classes of drugs.

<table>
<thead>
<tr>
<th>Table 2: Status at last FU</th>
<th>NYHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1/2/3</td>
<td>23/4/1</td>
</tr>
<tr>
<td>Rhythm</td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>25</td>
</tr>
<tr>
<td>PM</td>
<td>4</td>
</tr>
<tr>
<td>AAI/VVI/DDD</td>
<td>1/1/2</td>
</tr>
<tr>
<td>Saturation (mean ± SD, %)</td>
<td>93.8 (4.1)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
</tr>
<tr>
<td>Anticoagulants</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>15</td>
</tr>
<tr>
<td>VKA</td>
<td>7</td>
</tr>
<tr>
<td>LMWH</td>
<td>2</td>
</tr>
<tr>
<td>NOAC</td>
<td>2</td>
</tr>
<tr>
<td>ACE-I</td>
<td>7</td>
</tr>
<tr>
<td>Diuretics</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3: Cardiac function at last FU</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular function</td>
<td></td>
</tr>
<tr>
<td>Normal/Depressed</td>
<td>23/6</td>
</tr>
<tr>
<td>VEDD (mm)(N=26)(median, IQR)</td>
<td>55 (46-63)</td>
</tr>
<tr>
<td>VESD (mm)(N=19)(median, IQR)</td>
<td>43 (33-46)</td>
</tr>
<tr>
<td>AVVI (N=28)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
</tr>
<tr>
<td>Grade 1</td>
<td>15</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6</td>
</tr>
<tr>
<td>Grade 3</td>
<td>1</td>
</tr>
<tr>
<td>Conduit (N=26)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous contrast</td>
<td>11</td>
</tr>
<tr>
<td>Thrombi</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Status at last FU of patient functionality, heart rhythm, saturation and drug intake. SR: sinus rhythm, PM: pacemaker, AAI/VVI/DDD: PM setting, VKA: vitamin K antagonists, LMWH: low molecular weight heparine, NOAC: new oral anticoagulantia; ACI-I: angiotensin-converting enzyme inhibitor.
4.2. Echocardiography at last FU

Echocardiography showed a depressed systemic ventricular function in 6 patients, 23 patients had AVVI (Table 3). In all patients with reduced ventricular function, a certain degree of AVVI was noted. AVVI correlated significantly with age at FU and Fontan interval (p=0.003 and p=0.012, respectively). However it is difficult to evaluate fenestration patency, we observed at least a minimal flow over the right-to-left shunt in 11 of 25 (44%) patients were a fenestrated TCPC was initially constructed (Table 1), obviously leading to some desaturation. The presence of a fenestration did not correlate with any parameter of liver function.

4.3. Laboratory tests

Results of the most relevant lab tests can be found in addendum 3. Elevated HCT values were seen in 15 patients out of 28 (54%). Platelet count was too low in 8 patients (29%). Clotting profile was extended through measurement of international normalised ratio (INR) in patients that were not taking vitamin K antagonists. In 10 out of 21 (48%) patients INR, was slightly higher than normal. Nor platelet count, nor INR correlated with observed morphology changes. However none of our patients are currently known to have PLE, total protein was deviant in 3 patients (13%), but only in 2 of the cases (8%) protein concentration was abnormally low. Total bilirubin differed from normal in 9 patients (31%), however 12 patients (50%) showed elevated direct bilirubin. In 5 patients (21%) indirect bilirubin was higher than normal. Liver transaminases were mildly elevated; aspartate transaminase (AST) in 2 patients (7%) and alanine transaminase (ALT) in 10 patients (36%), with maximum values of 42 and 89 U/l, respectively. Of all blood parameters, gamma-glutamyltransferase (γGT) was the only parameter that was consistently elevated in the majority of the patients (21 of 28 patients, 75%). None of these parameters could be significantly correlated with patient age, age at operation, duration of FU or ventricular dominance.

Evaluation of pro-BNP, a marker of heart failure, showed higher values compared to normal in 11 patients (39%). Highest value was 2780 pg/mL in a patient that presented with heart failure in our clinic at the moment of liver assessment. No correlation could be observed between pro-BNP and the degree of AVVI.

Furthermore it is important to state that alfa-fetoprotein (αFP) did not reach abnormal levels in any of our patients.
Table 4: Obtained values of serum fibrosis scores expressed as median with interquartile range (IQR), with cut-off value for excluding significant fibrosis and abnormal percentages in our population. \(^*\)Patients with fibrosis scores correlating with at least F2 fibrosis were considered abnormal.

Concerning the fibrosis scores (APRI, Forns and FIB-4)(Table 4), a Forns score below 4.21 could be seen in 20 (71%) of our patients, thereby excluding significant fibrosis. The highest Forns score was 5.78, which is still below the aforementioned cut-off value of 6.9 for significant fibrosis. However, moderate degree of liver fibrosis could be calculated in 29%.

APRI score \(>1.5\) was absent in all of our patients. APRI could exclude significant fibrosis \((<0.5)\) in nearly 70% of the patients. FIB-4 was also calculated. As in APRI, it was also impossible with this fibrosis scoring system to detect significant fibrosis \((>3.25)\) in our study group. Moreover in all but one patient significant fibrosis could be excluded. Forns score correlated with Fontan interval \((p= 0.034)\).

Figure 17: Correlation between Forns score and Fontan interval.

4.4. Liver imaging and LS

In 23 out of 24 patients examined with liver imaging the complete imaging protocol could be performed.

4.4.1. Morphology

Of the 24 Fontan patients that underwent liver US, abnormal morphological findings (Table 5) were observed in 11 (46%). Based on the combination of US findings ascites and aberrant
liver parenchyma we suggested the presence of liver cirrhosis (LC) in 2 patients (8%). Irregularity of the liver surface was reported in 5 (21%), hepatic nodules were observed in 7 (29%) and ascites could be seen in 3 (13%) patients. Only in 2 (8%) patients these anomalies were all present. In 2 patients focal nodular hyperplasia (FNH)-like lesions were confirmed with additional MRI investigation. In 1 patient we found parenchymal nodules on US that were suggestive for FHN, hamartoma or hemangioma.

<table>
<thead>
<tr>
<th>Table 5: US findings</th>
<th>Abnormal (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenchymal hyper-echoic nodules</td>
<td>7 (29%)</td>
</tr>
<tr>
<td>FNH-like lesion</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Coarse echotexture</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Aberrant liver surface contour</td>
<td>5 (21%)</td>
</tr>
<tr>
<td>Signs of portal hypertension</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly*</td>
<td>9 (41%)</td>
</tr>
<tr>
<td>Ascites</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Collaterals</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 5: Liver morphologic alterations and signs of portal hypertension assessed with US. *Diagnosis of asplenia was made in 2 patients.

Splenic size (Table 5) was also measured, excluding 2 patients with asplenia. In 9 (31%) a splenic diameter ≥12cm was measured, correlating with splenomegaly(142). When we compared the occurrence of morphological anomalies with spleen size, it became clear that in 4 of 7 (57%) patients with one of these morphological aberrations splenomegaly was present. However in the other 5 patients with splenomegaly no deviant morphological characteristics could be observed. No correlation could be obtained between spleen size and thrombocyte count.

In table 6 vein diameters and ratios are displayed. In 6 patients IVC diameters greater than 20mm were observed in in- and/or expiration, suggesting increased CVP. In 21 patients (88%) IVC collapsibility index was less than 50%, even more respiratory variation in IVC diameter was (nearly) absent (<10%) in 7. No correlations could be obtained with time-related, serum or imaging parameters. PV diameter was deviant in 1 patient, 9 patients showed lack of respiratory variation in the PV. However when mean PV in-/expiratory diameter ratio (0.96 ± 0.23) is considered almost every patient shows a significant decrease in respiratory variability. PV in-/expiratory ratio correlated significantly with Fontan interval (p=0.044). With longer Fontan interval PV respiratory variability becomes smaller. Furthermore LS correlated with PV in-/expiratory diameter ratio (p=0.049). Higher LS occurred in patients with loss of PV respiratory variability.
4.4.2. Doppler

<table>
<thead>
<tr>
<th>Table 6: Doppler findings</th>
<th>Mean (SD)</th>
<th>N</th>
<th>Mean (SD)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC diameter inspiration</td>
<td>16.8 ± 4.8</td>
<td>24</td>
<td>PV in/ex ratio</td>
<td>0.96 ± 0.23</td>
</tr>
<tr>
<td>IVC diameter expiration</td>
<td>14.9 ± 4.3</td>
<td>24</td>
<td>PV PR</td>
<td>0.63 ± 0.18</td>
</tr>
<tr>
<td>IVC in/ex ratio</td>
<td>0.9 ± 0.24</td>
<td>24</td>
<td>PV PI</td>
<td>0.37 ± 0.18</td>
</tr>
<tr>
<td>IVC collapsibility index</td>
<td>0.24 ± 0.18</td>
<td>24</td>
<td>HA RI</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>PV diameter inspiration</td>
<td>8.5 ± 2.9</td>
<td>24</td>
<td>SMA RI</td>
<td>0.87 ± 0.09</td>
</tr>
<tr>
<td>PV diameter expiration</td>
<td>8.0 ± 2.9</td>
<td>25</td>
<td>LS</td>
<td>10.4 ± 3.68</td>
</tr>
</tbody>
</table>

Table 6: Results of Doppler examination of hepatic vasculature.

All patients had hepatopetal PV flow with a median maximum flow of 21.7 (IQR: 19.1-27.2) cm/s. In 2 patients maximum PV velocity was less than 13 cm/s. PV PI varied from 0.10 to 0.70, with 19 patients (79%) displaying PI less than 0.5, indicating that pulsatility is not increased in our population. No significant correlation could be shown between biochemical tests and Doppler parameters of the PV. Lower PV max velocity correlated with higher direct bilirubin (p=0.041). PV flow measurement did not differ significantly between patients with and without structural abnormalities.

Mean HA RI is 0.67 ± 0.12, reflecting values within normal range, nevertheless 9 (38%) patients HA RI higher than 0.7 was calculated. SMA RI was slightly increased. When Doppler indices were correlated with demographic and serum parameters, we could obtain a correlation between HA RI and αFP (p=0.029) and ALT (p=0.03). Lower SMA RI correlated with lower LDH (p=0.011), lower total protein (p=0.004), higher spleen size (p=0.025), lower PV PI (p=0.044) and higher LS (p=0.008). Also, SMA RI tended to correlate with Fontan interval (p= 0.08).

4.4.3. Liver stiffness

Measurement of liver stiffness (LS) using SWE was performed in 23 patients. LS (mean 10.4 ± 3.7 kPa) was abnormally increased in all, but one patient (96%). We found no significant correlation between LS and serum fibrosis markers. However higher LS was related to longer time interval since the Fontan operation (p=0,018), lower AST (p=0,031), lower albumin (p=0,015), lower total protein (p= 0,002), larger spleen size (p=0,025) and lower SMA RI (p=0,008).
Results

**Figure 18:** Scatterplot illustrating the evolution of LS after Fontan completion.

**Figure 19:** Scatterplot illustrating that SMA RI decreases with increasing LS.
5. Discussion

5.1. Serum tests

In the first part of this study we aimed to assess the prevalence of liver (function) abnormalities in an adult Fontan cohort utilizing a variety of laboratory parameters, serum biomarkers of liver fibrosis.

In our patient population abnormal $\gamma$GT levels (74%) were most commonly seen. In literature there are consistent reports on persistent elevation of this serum parameter during mid- and long-term follow-up of patients with palliation of UVH disease(86,143–148). Even more, a rise of $\gamma$GT shortly after Fontan completion can already be seen when compared to the pre-Fontan value(149). We could not find a correlation between $\gamma$GT and patient age, Fontan interval at group level, however in an individual there might possibly be a link between $\gamma$GT levels and time. Few studies are conducted in a Fontan population to assess evolution in time of serum parameters in a Fontan population, and interpretation of the evolution may possibly be of more value. This was illustrated by Kaulitz et al; measurement of $\gamma$GT at two different moments separated from each other in a minimal time interval of 4 years revealed a significant increase in $\gamma$GT levels in more than 80% of patients(143). It is considered that the mechanisms resulting in $\gamma$GT increase may be hepatic congestion(150,151) and damage to bile canaliculi due to increased pressure in the hepatic sinusoids or ischemia(152). In a chronic heart failure (CHF) population Poelz et al documented that $\gamma$GT serum levels were related to disease severity and also provided prognostic information(153). Basic pathological principles are similar in CHF and UVH disease and therefore $\gamma$GT can be an important serum marker in the FU strategy due to these associations seen in CHF. And however $\gamma$GT did not correlate significantly with LS and presence of morphological abnormalities in our study, other studies did find an association with liver fibrosis, which can be used as an extra argument for the use of this serum marker in FU(154,155).

Elevation of $\gamma$GT can also be seen in the context of a cholestatic pattern. Besides increased $\gamma$GT value, this pattern includes augmented serum levels of total bilirubin (mostly direct bilirubin) and alkaline phosphatase (AP) and is typically seen in Fontan patients in a mild form(77,156). AP was aberrant in only 7% of the cases. Here it also concerns a diminutive increase. However, AP can be helpful in distinguishing hyperbilirubinemia induced by chronic hepatic congestion from that of biliary obstruction, as in the latter AP elevation is
more pronounced(157). The absence of significant AF increases might suggest that bilirubin elevation is caused by intrinsic liver pathology and not by biliary obstruction in a Fontan population. Mild elevation in total bilirubin (30%) on the other hand was one of the most common laboratory findings in our patients with UVH. The indirect fraction is reported in literature as being the predominant factor of bilirubin elevation, possibly reflecting a certain degree of hemolysis(158). No arguments for hemolysis were present in our study (no anemia, normal haptoglobin). Moreover direct bilirubin (40%) was deviant in more cases than indirect bilirubin (19%), which we think reflects changes due to FALD more accurately because of its association with liver congestion. Secondly, in two patients Gilbert syndrome was established. After exclusion of these patients indirect bilirubin was aberrant in only 10%, which strengthens our opinion that FALD results rather in direct bilirubin increase than indirect bilirubin.

Raised serum aminotransferases were reported not only in our study. Typically ALT and AST derangements are not greater than two or three times the upper limit of normal, yet normal values for serum aminotransferases are also frequently described. The absence of or the minimal elevation is possibly due to a lack of significant inflammation and cell death in a Fontan population as was demonstrated on LB(77,86,145–148,156).

Low platelet count was observed in 30%. Schwartz et al showed that platelet count correlated inversely with the degree of fibrosis, illustrating the potential of platelet count as a marker of portal fibrosis(159). We did not perform LB, but with SWE no correlation between LS and platelet count could be obtained. No significant result was seen when platelet count was compared between the group with and without morphologic aberrations. But still Ohuchi et al state that reduction in platelet count indicates fibrotic change(160). In our population it was not possible to compare platelet count between patients with and without a functional spleen, since only 2 patients were known to have asplenia. Lindsay et al compared platelet counts in Fontan patients with and without a functional spleen and measured lower platelet counts in the first group(144), thus thrombocytopenia may be the result of hypersplenism secondary to portal hypertension(161) and decreased thrombopoetin synthesis by the liver with bone marrow suppression(162). On the other hand in Fontan patients Rychik et al demonstrated that platelet counts are often normal despite significant portal hypertension(7).

αFP was normal in all our patients and correlation with Fontan interval and LS was absent. Higher αFP correlated with higher HA RI and it is possible that increase of αFP, as AH RI increase, is a late manifestation of liver disease. As a result we suggest that regular screening of this serum parameter is not necessary. Only when US is suggestive of cirrhotic changes or
FNH-like lesions are present, αFP combined with US at regular basis should be included in the FU screening.

As liver complications are becoming more and more manifest, clinicians started to investigate possible serum markers for the non-invasive assessment of liver fibrosis and cirrhosis, rather than performing a liver biopsy in a Fontan patient, which is often asymptomatic. In our study we used Forns index, APRI and FIB-4 as non-invasive markers of liver fibrosis. All of these scoring systems were developed for assessment of liver fibrosis in the context of viral hepatitis, and are only validated in these populations. In a Fontan population literature about serum fibrosis markers is sparse(101,163). Using FIB-4 and APRI significant fibrosis could be excluded in all patients but one, where FIB-4 value was higher than the cut-off value for exclusion. Only Forns score was able to detect a moderate degree of fibrosis in 29%. In a study conducted by Shimizu et al Forns index was used for evaluation of cirrhosis. For a similar Fontan interval as our patient population, they found higher Forns scores (mean value of 11.5 for patients with LC and 6.8 without LC on CT scan, respectively). They also detected a higher incidence of LC (54%) through imaging compared to our study(163).

Only Forns correlated significantly with Fontan interval. Elevation over time of γGT (included in the Forns calculator) is seen with worsening of ventricular function in patients with right heart failure(153). Increasing RA pressure seems to correlate with higher γGT values(164). For platelets decrease over time can be explained by aggravation of portal hypertension and its secondary effects on the spleen.

It has to be said that in a Fontan population it is however not unlikely that FIB-4 and APRI scores are relatively low. Both scoring systems include serum aminotransferases, which tend to be normal or only mildly elevated in Fontan patients, since congestive hepatopathy occurs with little inflammation. Forns formula on the other hand encompasses two serum parameters, which are most commonly deviant in Fontan patients (γGT and platelets). Furthermore, serum fibrosis scores did not correlate with LS and no statistical significant difference in fibrosis marker values was obtained in patients with aberrant US compared to normal US.

Only Forns and APRI were investigated in a Fontan population and Forns was considered by Baek et al to be the best predictor of the presence of Fontan hepatopathy. This is in line with our findings. Baek et al also suggested that APRI was a reliable method for predicting liver histology(101). We doubt this statement and our findings are supported by another study that found similar values for APRI in a Fontan population (0.4 ± 0.19)(165). All of the above
suggests that FU in time of Forns score is more useful in a Fontan population, than does repeated measurements of FIB-4 and APRI.

In recent studies other biomarkers such as Fibrotest and hyaluronic acid were investigated as potential non-invasive serum markers for liver fibrosis. They too have shown promise for staging of liver fibrosis in other liver diseases, but here also lack of studies in Fontan patients remains an issue. Potential of Fibrotest was tested in 3 studies, and neither of them was able to detect a correlation with liver dysfunction(112,166,167). Fibrotest nor hyaluronic acid were able to differentiate Fontan patients with evolving or established cirrhosis and those without cirrhosis(166).

Finally, on serum markers it was suggested in prior studies that serum hepatic markers are no perfect screening tools for hepatic fibrosis in Fontan patients, because lower hepatic blood flow post-Fontan surgery may lead to lower values for serum parameters of liver function(77,86,159,168). Though low sensitivity still remains an issue, meaning they are not reliable enough to allow for staging of liver fibrosis or detect intermediate fibrosis, they have the potential to exclude patients with significant liver disease, monitor functional aberrance and reduce the need for LB. However they may be helpful in monitoring liver disease progression as the incidence of liver complications increases with longer Fontan interval and correlations between Fontan interval and Forns and other serum parameters have been shown. However, if these markers are used in clinical setting to guide FU, it should be noted that careful interpretation of these scores is necessary, especially since they are not validated in patients with congestive hepatopathy(100,154,155).

5.2. Liver imaging

5.2.1. Morphology

In 11 patients (46%) we were able to detect liver morphological aberrations, but the findings were not correlated with biochemical or hemodynamic parameters. Kiesewetter et al supported this observation, as they too did not demonstrate a correlation between markers of liver function and observed morphological changes(77). It should be noted nonetheless that US has limited capability in determining fibrosis, especially in early stages, possibly explaining these observations(7). But due to high frequency of morphologic liver alterations sequential assessment of liver structure is advised. Johnson et al described a correlation between morphologic changes and time since Fontan completion. However Fontan interval in our population did not differ significantly between the patients with normal liver morphology.
and those with anomalies of liver structure(111). More astonishing is that in both shortest and longest Fontan intervals abnormalities were detected via liver US. This suggests that the etiology of liver abnormalities is more complex than just the duration of the new circulation and thus congestion. Pre- and post-Fontan insults may play a significant role(169). Hypoxia, episodes of congestive heart failure, cardiovascular collaps, low-output state, systemic-pulmonary artery shunt, pulmonary artery banding and the impact of major surgery all contribute to perioperative liver insult(7). Also, patients with higher venous pressures were more likely to develop structural changes(158,170).

So far 2 patients underwent further liver imaging via MRI, because of the presence of a big nodule in the liver parenchyma. In both patients it became clear that these hypervascular lesions were suggestive for FNH-like lesions. These are reported in several studies in relatively high frequency (up to 30%) in Fontan patients(77,90,171). Bryant et al confirmed in patients with arterialized nodules what was suggested on MRI via post-mortem histology, namely that the underlying pathology is most suspect for FNH(171). The exact etiology of these nodules is not fully understood, notwithstanding it is hypothesized that these regenerative nodules represent arterialisation of hepatic blood supply. Arterialisation via the HABR is a direct consequence of elevated CVP, because this deprives the liver parenchyma of portal blood flow. Bryant et al illustrated the role of venous pressure by showing that patients with higher RA pressures had a higher incidence of hypervascular nodules(171). This highlights the potential importance of vascular pathophysiology underlying FALD. In a failing Fontan heart, more rapid damage may occur, especially if hepatic venous pressures increase(158). Further atrophy is provoked by diminished flow, followed by compensatory nodular hyperplasia in areas receiving increased arterial flow(17,75,77,171). In Budd-Chiari syndrome, right heart failure and other causes of hepatic venous outflow obstruction similar nodules can be seen(172–174). FNH classically develops in previously undamaged livers, though regenerative and dysplastic nodules are associated with underlying liver disease, most commonly cirrhosis(175). Imaging and histological characteristics of FNH-like nodules are identical in both cirrhotic and non-cirrhotic livers(176). In chronic liver disease, such as can occur in Fontan patients, progression of regenerative to dysplastic nodule to HCC is a potential pathway, however the malignant potential of FNH-like lesions is uncertain(90). The meaning of coarse echotexture is unsure, but it may implicate early fibrosis or hepatic injury(149).

Ascites was observed in 3 patients (10%). Detection of ascites can be seen as a sign of a decompensated circulatory state and might be of use as a prognostic marker in Fontan
patients(177). In 2 patients the preliminary diagnosis of cirrhosis was made based on US findings of ascites and liver parenchymal aberrances, even without significant elevation of serum fibrosis markers. In one patient ascites was present without other morphological abnormalities. The heart is the likely cause in this situation, since LS values were only moderately elevated.

**5.2.2. Duplex**

5.2.2.1. *Portal vein*

Doppler US is commonly used to assess for portal hypertension in patients with normal cardiac anatomy. In Fontan patients however the utility of this technique is unclear. Liver disease in Fontan patients is determined by high resistance to hepatic venous outflow and likely explains changes in flow patterns, histology and liver stiffness(135). As a consequence of high hepatic afterload and the resulting hepatic congestion, dilated PV is a frequent finding in UVH disease(135). Despite, only one patient displayed a PV diameter >13mm. Furthermore due to high outflow resistance and low CO, we also expect lower PV flow velocities compared to normal. Bearing in mind that normal PV flow velocities can range up to 39 cm/s, median maximal flow velocity of 21.7 cm/s suggests lower flow velocities in Fontan PV(178). However it should be noted that low flow velocities are not sufficient for the diagnosis of portal hypertension(21) and PV flow velocities are not that deviant in our study when keeping in mind that normal PV velocities ranging from 16 to 39 cm/s are described(178,179).

The study conducted by Kutty et al is one of the few published studies on infradiafragmatic venous Doppler flow in the Fontan circulation(135). When comparing pulsatility measures, it can be seen that in our population pulsatility is less pronounced (PV PR: 0.53 vs 0.64). Pulsatility increases with increasing CVP concomitantly IVC compliance is reduced(124). Nevertheless we could not detect a correlation between PV PI/PR and IVC collapsibility index. FU of PV pulsatility indices may possibly represent a useful, non-invasive method to evaluate disease progression in Fontan patients, because of its reflection of CVP, which is associated with decreased prognosis. However, in screening for asymptomatic FALD the role of this parameter is limited.

Inferred from the fact that elevated CVP is inherent to Fontan physiology, we have to take into account that some degree of portal hypertension is present. As liver function and degree of fibrosis are correlated poorly, Guha et al also showed a resembling divergent relationship
between portal hypertension and hepatic fibrosis in Fontan patients(180). Recognising the possibility of portal hypertension and its complications is necessary in long-term Fontan FU. However PV diameters and flow velocities are not sufficient enough to diagnose portal hypertension, only via invasive measurement portal hypertension can be defined in a Fontan population and therefore this is not part of our screening protocol(91).

5.2.2.2. Hepatic artery

Mean HA RI was $0.67 \pm 0.12$, which is within (high)-normal ranges for a low-resistance vessel (0.5-0.7). However according to HABR-theory lower values were expected, since portal blood flow is decreased in a Fontan circulation. This paradoxical result might be explained by an observation made by Burchell et al; they stated that abnormally elevated sinusoidal pressure, which is inherent to Fontan physiology, initiates a feedback loop where hepatic venous outflow obstruction leads to constriction of the hepatic arterioles, ultimately increasing hepatic arterial resistance(181). Parallel to this theory and to our findings Kutty et al suggested that a fibrotic liver presents high resistance to vascular inflow, both arterial and venous(135). Secondly, increased HA RI has been shown when LC is present, however this is a late manifestation and morphologic alterations should be present on US(182). Due to relatively normal results in our study and the absence of correlations between HA RI and morphologic alterations, it is possible that increasing values only present itself in advanced stages of liver disease, e.g. cirrhosis. Therefore the potential of HA RI in screening of Fontan patients to detect early fibrotic changes seems limited.

5.2.2.3. Superior mesenteric artery

In a Fontan circulation CO is known to be declined. Consequently mesenteric circulation may be compromised, which may be compensated for by an increase in SMA resistance to shift blood flow from the gut to more vital organs(85). In our study population mean SMA RI (0.86 $\pm$ 0.09) was slightly higher than normal, reflecting this theory. Only in 1 patient RI (0.65) was significantly decreased, suggestive for failing Fontan. In this situation an uncompensated hyperemic state sets in with vasodilatation, thereby reducing vascular resistance(183). Unsurprisingly, this significant decrease occurred in a patient with the most evident hepatic abnormalities (highest LS value, surface nodularity, ascites, highest spleen size). Thus SMA RI may be useful to detect Fontan failure. Together with the observed trend towards lower
SMA RI with increasing Fontan interval (p=0.08), serial evaluation of this parameter in FU screening can potentially provide useful prognostic information.

5.3. Liver stiffness

Although studies that have used SWE in patients with Fontan circulation are limited, we chose to use SWE to evaluate congestive hepatopathy, as it is an easy to use non-invasive method. Our results do not unequivocally support that this non-invasive method can be used to estimate global hepatic status. This is based on the facts that no correlation was obtained between LS and most frequently abnormal serum parameters and serum fibrosis markers. Plus LS value was not significantly different between patients with and without morphological alterations. On the other hand LS correlated with AST, albumin, total protein, spleen size, SMA RI and Fontan interval. The fact that LS seems to increase with longer Fontan interval indicates that serial LS measurements in a Fontan population are useful in screening for FALD. And for the assessment of liver fibrosis Guibal et al advocate that SWE is an accurate and reproducible non-invasive method, especially in the diagnosis of significant fibrosis in varying liver pathologies (non-alcoholic steatohepatitis, chronic hepatitis B or C infection, alcoholic liver disease...)(118). Moreover, they showed that LS gauged with SWE is well correlated to LB using METAVIR score. Discrepancies between serum fibrosis markers and overall high LS measurement might be explained by the fact that serum parameters tend to become abnormal when liver function is severely compromised, in contrast to fibrosis which can be present even before Fontan completion and further develops as time since Fontan completion progresses(169). Guha et al were the first to describe this uncoupling of liver structure and function(180). They also speculated that the topography of fibrosis in Fontan livers has permitted relative preservation of hepatocyte integrity, particularly since inflammation is generally absent. On the other hand a second and possibly more important cause of this discrepancy is the factor of liver congestion in FALD. It is established that early after Fontan completion liver congestion is already present, therefore LS value is an overestimation to start with(184). Moreover Millonig et al reported that elevated CVP concomitant with impaired hepatic venous drainage can mimic severe fibrotic changes(133). In Fontan patients it is important to differentiate liver fibrosis from hepatic congestion in the assessment of hepatic complications. However hepatic congestion is an established confounding factor in determining liver disease using SWE(133). Because LS values reflect congestion, they can provide predictive information for hepatopathy after the Fontan operation(165). This can be illustrated by the following example: early after Fontan
Discussion

completion high LS value might indicate severe hepatic congestion due to inappropriate Fontan circulation rather than fibrosis(184,185). But high and changing LS values in a chronic stable Fontan circulation are more likely to reflect progression of liver fibrosis(165). It is important for future FU to determine reliable values that reflect these changes and to improve our understanding of how these fibrotic changes influence hepatic function.

Ferraioli et al measured LS value <4.0 kPa in healthy individuals, in all our Fontan patients nevertheless values were remarkably higher (mean 10.4 ± 3.7 kPa)(130). In one patient a near normal value of 4.5 kPa was determined, this can be explained by the fact that this patient had the shortest Fontan interval as Fontan circulation was completed only 6.8 years ago. Compared to other studies LS values in our study were relatively low. Kutty et al demonstrated higher hepatic stiffness (15.6 kPa) and Byung et al reported on LS values of 21.1 ± 8 kPa assessed with TE(135,165). Both studies were performed in younger Fontan populations: 13.8 ± 6 years and 18.2 ± 7.6 respectively versus 24.1 ± 5.12 years in our study. Most recently Chen et al showed values of 18.6 kPa in a population with Fontan interval of 9.6 years(186) and Agnoletti et al reported a median value of 17.3 kPa(187).

When LS value is interpreted, it should be considered that cut-off values used to diagnose fibrosis might differ according to the underlying etiology. In patients with chronic hepatitis C biopsy matched cut-offs were set at >6.2, >7.6, >10 >15.6 kPa for F1, F2, F3 and F4 respectively(117). Not only in our study, but also in studies conducted by Byung et al and Kutty et al different ranges of LS values are seen in Fontan patients, possibly due to congestion, than those for patients with chronic hepatitis.(135,165). The majority of Fontan patients in our study displayed LS values >10 kPa, often without US based characteristics of severe fibrosis or cirrhosis. In the observation of Byung et al, abnormal US findings were hardly seen in patients with LS < 20 kPa(165). Periodic measurements can possibly help to discriminate between progressive decline and LS values in the context of fluctuating hemodynamic states.

5.4. Liver biopsy

Clinical and laboratory parameters are not accurate enough and due to the lack of validation of non-invasive methods (serum fibrosis scores, LS measurements) in the assessment of fibrosis in a Fontan population, LB is currently still considered the gold standard.

Biopsy findings in a Fontan liver consist of dilated sinusoids, reflecting elevated CVP and chronic passive congestion(110). With a median Fontan interval of 16.9 years Schwartz et al
identified some degree of both portal and sinusoidal fibrosis in all patients with failing Fontan, a phenomenon that is confirmed in other studies.(87,109,110,159,161). Pathophysiology of fibrosis is thought to share characteristics with typical cardiac hepatopathy, although portal-based fibrosis suggests involvement of additional factors.(7,159). Moreover progression of both types of fibrosis is described.(159,169). Despite the fact that a certain degree of fibrosis is present in almost every Fontan patient, the degree of fibrotic changes is poorly associated with clinical characteristics and outcomes(109).

Although LB is the gold standard for staging of liver fibrosis, certain factors should be taken into account when choosing LB over non-invasive methods in Fontan follow-up management. First of all LB is an invasive method, leading to patient discomfort(188). Furthermore (bleeding) complications might occur, particularly in Fontan patients, who frequently are under anticoagulant therapy or display coagulation factor abnormalities. These are important issues, especially in a population where progression of liver fibrosis should be monitored at regular basis. Secondly, it was noticed that pattern of fibrosis on histopathology was patchy, with areas of cardiac cirrhosis and areas of relatively normal parenchyma(109–111). Additionally, LB samples are smaller than the ROI measured with elastography(189). Both are stressing the potential for sampling error. Thirdly, scoring systems were designed to evaluate fibrosis in chronic hepatitis(7), revealing the need for cardiac hepatopathy specific scoring systems to assess significance of observed fibrosis in a Fontan population. LB has low sensitivity for early stages of fibrosis, which is disadvantageous for screening in a Fontan population(190). Finally, one should wonder whether staging of liver fibrosis is linked to a therapeutic response. Since it became clear that in Fontan livers a structural and functional uncoupling occurs, you can impugn the value of LB. Does staging through LB have any clinical implications except for situations where fibrosis or cirrhosis is associated with liver failure? Is LB justified in an asymptomatic Fontan patient where presence of a certain degree of fibrosis is assured, but where liver function often seems to be preserved? We think that as long as liver function is acceptable LB should not be carried out, as contribution of the result of biopsy to therapeutic strategy is possibly not significant.

**5.5. Fontan follow-up strategy**

However serum parameters in most patients remain normal in most Fontan patients until late stages of hepatic dysfunction, we suggest that liver parameters should be determined regularly, because of the simplicity of this screening method, e.g. once a year at the time of
cardiology consultation and because serial evaluation and interpretation of several parameters will deliver more information to the clinician. We suggest that preferably γGT, bilirubin (total and direct), albumin, aminotransferases and thrombocytes are measured. Careful monitoring of αFP, plus US, should start when imaging is suggestive for advanced fibrosis/cirrhosis or when FNH-like lesions are detected. In extension to serum measurements, we primarily suggest that Forns index is calculated, since this scoring system shows potential as a screening tool in a Fontan population.

Histological and morphological changes may occur with only mildly abnormal liver serology and in the absence of clinical symptoms, therefore routine clinical protocol should consist of several screening modalities, including imaging tools, preferably US. In addition to revealing evidence of congestive hepatopathy or cirrhosis, it is helpful for ruling out other causes of liver injury such as thrombosis and for screening for HCC in patients with established cirrhosis. US protocol should at least consist of measurement of liver and spleen size, assessment of liver surface nodularity, coarse echotexture and presence of nodules in the liver parenchyma, ascites and collaterals. With reference to recommendations made by Kaulitz et al, we suggest further investigation of liver function and morphology by MRI in 2 specific circumstances: liver surface nodularity and/or increased parenchymal nodular echogenicity, regardless of Fontan interval(143). The role of Doppler in the Fontan liver screening process seems limited, but if Doppler is easily combined with US, we propose measurement of SMA RI. Since SWE and US can be combined in one examination and LS seems to increase over time, we suggest an evaluation of LS at the same time of liver US. Moreover liver imaging should always be combined with cardiac evaluation through imaging. The role of routine LB in post-Fontan management is limited, nevertheless in patients with non-cardiac risk factors for hepatic injury, such as viral hepatitis and toxic/metabolic disease, it may be helpful(158).

In general we advise that full screening protocol (including serology) is carried out in every asymptomatic Fontan patient with Fontan interval of 10 years or sooner in patients were clinical symptoms and/or serology is indicative for structural deterioration, to search for underlying liver disease using imaging methods(7,143,158). Further FU should then be guided by the results of each patient individually. In patients with normal liver status or with mild signs of structural and/or functional liver alterations time until next screening can be longer, for example every 2 or 3 years. However in patients with significant abnormalities we recommend an annual assessment of liver status.
5.6. Future prospects

Due to the rarity of UVH disease, small study populations are used when research is conducted in a Fontan population. This might partially explain some of the variations that are seen in literature. To draw more uniform conclusions and for better understanding of Fontan pathophysiology and pathology however, it would be beneficial to study large Fontan cohorts. Another potential profit of studying large cohorts is its effect on methodology, which would be more homonymous. Organising multicentre studies thus would seem like a valid option for the future.

One of the most important topics of future research is the validation of serum fibrosis markers and elastography in a population with congestive hepatopathy. In an ideal scenario both fibrosis markers and elastography should be correlated with US analysis of liver morphology, cardiac catheterization and LB. Only then true meaning and clinical use might become clear. Besides, this may also improve our understanding in pathophysiological mechanisms of Fontan liver disease. Which in turn will make it possible to adapt therapeutic measures.

We are still left with several questions, but longitudinal FU possibly can give answers to our questions. However serum fibrosis markers were deviant only in a minority of our patients and therefore give limited information, it is possible that progression of these scoring systems in time will help in our diagnostic and treatment strategy. The opposite can be true also. When fibrosis markers remain normal in most patients while liver disease becomes more apparent, search for other serologic screening modalities would be indicated. Furthermore SWE is a very recent technique and few studies are available, especially in a Fontan population. Here periodic examinations would possibly allow us to differentiate between a continuous decline and fluctuating hemodynamic states.

At the moment the same study has started in our pediatric Fontan population. This might give us more insights in the evolution of these non-invasive modalities, the relationship with patient age, Fontan interval and liver aberrances and the potential of serum fibrosis markers and SWE in periodic screening of Fontan patients. Even more value of our measurements might become clearer.

5.7. Limitations

Sample size in our study is limited, combined with the lack of studies that observe progression of liver status in time it is hard to make unequivocal decisions. Since LB was not performed, no correlations could be made with histology. Therefore it is hard to draw definite
conclusions regarding the clinical use of biomarkers. Our hemodynamic data of HV are incomplete, which made it hard to find correlations with other parameters, to observe HV changes in the Fontan circulation and to include HV parameters in the screening protocol. Considering the difference of our SWE values and those observed in other studies we are aware of the possibility that LS measurements might not truly reflect fibrotic changes in our population. Finally we have to admit that data collection of vessel diameters and flows was not made at standardised positions.
6. Conclusion

We found that abnormalities of serum parameters are frequent in an adult Fontan population with predominantly a mild cholestatic pattern and low thrombocyte count. Of the calculated serum fibrosis scores only Forns was able to indicate a moderate degree of liver fibrosis in 29%. However Forns score seems to increase with time, thereby highlighting the potential usefulness of this fibrosis marker in longitudinal FU. APRI and FIB-4 on the other hand were unindicative for liver fibrosis and therefore seem to have limited value in the Fontan screening process. High percentage of the study subjects displayed morphologic abnormalities of the liver, which supports the use of liver US in periodic FU of patients with UVH disease. However correlations with biochemical parameters, serum fibrosis scores and LS could not be obtained. Liver Doppler indices in contrast remain normal in most patients, but may become aberrant when cardiac function deteriorates and/or late manifestations of liver dysfunction occur, e.g. cirrhosis. LS, assessed with SWE, was abnormally elevated in all patients and increases with time and higher SMA resistance. Nevertheless, LS increase, partially explained by liver congestion, seems to overestimate the grade of fibrosis arguing for the need of validation through sequential measurements.

The clinical impact of (structural) hepatic abnormalities in Fontan patients remains largely unknown. None of the individual screening methods seems able to reflect true global liver status, thus it seems appropriate that diagnosis of liver fibrosis (and cirrhosis) is not based on serum markers only, but that these markers are interpreted in line with results of multiple fibrosis tests, including imaging modalities.

Due to the fact that routine biochemical tests (including serum fibrosis scores) may not reflect morphologic alterations and the degree of fibrosis and that correlations between LS and morphological abnormalities are inconsistent, screening for liver disease in an asymptomatic patients is not self-evident. However we suggest start of liver screening in UVH disease 10 years after Fontan completion. Screening minimally encompasses the following tests: measurement of serum parameters (thrombocyte count, γGT, total and direct bilirubin, serum transaminases) and Forns score and assessment of liver and spleen morphology with US in combination with LS measurement.
Reference list

27. Francois K. The surgical palliation of the functionally univentricular heart: early and late outcomes. Ghent University; 2012.
31. Sluysmans T, Sanders PS, Van Der Velde M, Matitiaux A, Parness IA, Spevak PJ, et al. Natural history and patterns of recovery of contractile function in single left ventricle after Fontan operation.
References


References


References


130. Kutty SS, Peng Q, Danford DA, Fletcher SE, Perry D, Talmon GA, et al. Increased hepatic stiffness as


185. Chen B, Schreiber RA, Human D, Potts JE, Gutman OR. Assessment of Liver Stiffness in Pediatric


Addendum

Addendum 1: List of laboratory tests carried out during the study

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<th>Aangevraagde testen</th>
<th>Batterijcode</th>
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<td>O Thrombocyten</td>
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<td>O WBC differentiatie</td>
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<td>O Ceruloplasmine</td>
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<td>O Alfa-1-antitrypsine</td>
<td>O Albumine</td>
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<td>O IJzer</td>
<td>O CRP</td>
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<tr>
<td>O Transferrine-TIBC</td>
<td>O AST / ALT</td>
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<td>O Ferritine</td>
<td>O Alkalische fosfatase</td>
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<td>O GGT</td>
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<td>O LDH</td>
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<td>O anti-HCV</td>
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<td>O AMA</td>
<td>O Kalium</td>
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<td>O Pro-BNP</td>
<td>O Calcium</td>
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<td>O Urinestick</td>
<td>O Chloride</td>
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<td>O Urinesediment</td>
<td>O Bicarbonaat</td>
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<tr>
<td>Afname: 2 tubes EDTA(paars) + 1 tube citraat (blauw) + 4 tubes gestold (rood) + 1 potje urine</td>
<td>SFON</td>
</tr>
</tbody>
</table>
Addendum 2: US liver/abdomen protocol

1. Structure liver parenchyma + spleen
   - Nodularity
   - Contour
   - Ascites
   - Collaterals
   - Dimensions of the spleen

2. Vascular
   a. IVC
      i. Diameter changes between in- and expiration
   b. PV
      i. Diameter changes between in- and expiration
      ii. Pulsatility ratio
      iii. Pulsatility index
   c. HA/SMA
      i. Resistance index
   d. HV
      i. Ratio of hepatic vein flow (=V2/V1)
         1. V1= peak velocity towards the heart
         2. V2= reverse flow towards the liver
**Addendum 3:** Most frequent deviant lab tests with normal value, mean and frequency of abnormality

<table>
<thead>
<tr>
<th>Laboratory Tests</th>
<th>Normal Value</th>
<th>Number of Patients Tested</th>
<th>Mean (SD)</th>
<th>Number Abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>M: 39-49.7 F: 35.8-43.7</td>
<td>28</td>
<td>46.11 (4.70)</td>
<td>15 (54)</td>
</tr>
<tr>
<td>Platelets (10^3/μl)</td>
<td>M: 149-319 F: 171-374</td>
<td>28</td>
<td>209.0 (62.88)</td>
<td>8 (29)</td>
</tr>
<tr>
<td>PTT (%)</td>
<td>70-120</td>
<td>23</td>
<td>74.74 (26.91)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>INRa</td>
<td>0.9-1.1</td>
<td>21</td>
<td>1.11 (0.19)</td>
<td>10 (48)</td>
</tr>
<tr>
<td>Total bilirubin (mmol/L)</td>
<td>≤ 0.45</td>
<td>24</td>
<td>0.46 (0.15)</td>
<td>12 (50)</td>
</tr>
<tr>
<td>Direct bilirubin (mmol/L)</td>
<td>0.1-0.8</td>
<td>24</td>
<td>0.61 (0.50)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Indirect bilirubin (mmol/L)</td>
<td>64-83</td>
<td>24</td>
<td>73.58 (6.06)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>35-52</td>
<td>28</td>
<td>48.07 (4.65)</td>
<td>4 (14)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>M: 0-31 F: 0-31</td>
<td>28</td>
<td>26.43 (6.35)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>M: 7-40 F: 7-31</td>
<td>28</td>
<td>33.57 (16.11)</td>
<td>10 (36)</td>
</tr>
<tr>
<td>Gamma glutamyl transpeptidase (IU/L)</td>
<td>M: &lt;64 F:&lt;36</td>
<td>28</td>
<td>68.25 (39.10)</td>
<td>21 (75)</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>30-120</td>
<td>28</td>
<td>83.29 (24.01)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>&lt;190</td>
<td>28</td>
<td>160.82 (34.19)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Apolipoprotein A1 (mg/dl)</td>
<td>M: 110-205 F: 125-215</td>
<td>28</td>
<td>145.52 (23.55)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>α1-antitrypsin (g/l)</td>
<td>0.9-2</td>
<td>27</td>
<td>1.67 (0.44)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>α-fetoprotein (mg/l)</td>
<td>0-15</td>
<td>28</td>
<td>3.15 (1.21)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pro-brain natriuretic peptide (pg/mL)</td>
<td>≤ 125</td>
<td>28</td>
<td>243.64 (528.60)</td>
<td>11 (39)</td>
</tr>
</tbody>
</table>

*aExclusion of patients under anticoagulation therapy*