FACULTY OF MEDICINE AND HEALTH SCIENCES

INVESTIGATION ON THE HEPATIC CONSEQUENCES OF A FONTAN CIRCULATION IN CHILDREN, WITH VALIDATION OF THE FIBROSCAN IN A PAEDIATRIC POPULATION

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Thank you all,
Fabian Hendricx & Hazel Van Overschelde

LIST OF ABBREVIATIONS

αFP	alfa-fetoprotein
ALT	alanine transaminase
AP	alkaline phosphatase
APC	atriopulmonary connection
APRI	aspartate transaminase-to-platelet ratio index
AST	aspartate transaminase
AUROC	area under the receiver operating characteristic
AV	atrioventricular
Bb	bilirubin
BCPA	bidirectional cavopulmonary anastomosis
CLD	chronic liver disease
CO	cardiac output
CT	computed tomography
CVP	central venous pressure
EDSL	end-diastolic sarcomere length
EF	ejection fraction
EDV	end-diastolic ventricular volume
ESV	end-systolic ventricle volume
FALD	Fontan associated liver disease
FIB-4	fibrosis-4
FU	follow-up
ɣGT	gamma-glutamyl transferase
GUH	Ghent University Hospital
HA	hepatic artery
HABR	hepatic arterial buffer response
HCC	hepatocellular carcinoma
HCT	hematocrit
HLHS	hypoplastic left heart syndrome
HV	hepatic veins
INR	international normalised ratio
IVC	inferior vena cava
IVCCI	inferior vena cava collapsibility index
LA	left atrium
LV	left ventricle
LS	liver stiffness
LSM	liver stiffness measurement
MRI	magnectic resonance imaging
NPV	negative predictive value
PA	pulmonary artery
PI	pulsatility index
PLE	protein losing enteropathy
PLT	platelet count
PPV	positive predictive value
PV	portal rein
PVR	pulmonary vascular resistance
Pro-BNP	pro-brain natriuretic peptide
RA	right atrium
RI	resistive index
RV	right ventricle
SMA	superior mesenteric artery
SVC	superior vena cava
SWE	shear wave elastography
TCPC	total cavopulmonary connection
TE	transient elastography
ULN	Upper Limit of Normal
US	Ultrasound
UVH	Univentricular Heart

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ABSTRACT

Background: Multiple studies have reported increased risk of hepatic dysfunction because of an initiated Fontan circulation in adults. However, literature concerning liver modifications already developing in adolescents or even children is limited.

Objective: Our study aimed to assess the prevalence and degree of liver fibrosis in paediatric Fontan patients through non-invasive examinations suitable for serial follow-up. Furthermore, we evaluated the applicability of transient elastography (TE) through FibroScan (Echosense, Paris, France) for diagnostic and prognostic purposes.

Methods: 35 Fontan patients (median age 11,8y; range 5,2 – 16,6y) of which 27 male, were gathered at Ghent University Hospital. Each patient was subjected to analysis of serological markers (such as aspartate transaminase, alanine transaminase, gamma-glutamyl transferase [ɣGT], alkaline phosphatase, bilirubin [Bb], alpha-fetoprotein (aFP) and platelet count [PLT]), fibrosis scores (APRI, FIB4 and Forns), ultrasound (US) and Doppler US examination of hepatic structures and blood vessels, and TE through FibroScan for assessment of liver stiffness. A substudy with a control population of 70 healthy children (median age 10,6; range 5,7 – 18,3y) was conducted in order to determine normal and age specific TE reference values.

Results: ɣGT, Bb, PLT, AST, ALT and aFP were increased in respectively 69%, 17%, 17%, 57%, 20% and 3% of the population. Liver morphology showed alterations in 11% of the children. A mean vena cava collapsibility index of only 13,7% (± 9%) was associated with hepatic congestion, portal vein mean and max flow velocities (respectively 19,9 cm/s and 14,2 cm/s) have shown to be indicative for portal hypertension, hepatic artery resistance index (HA RI) and the superior mesenteric artery resistance index (SMA RI) were both significantly inversely correlated with time post Fontan (respectively ($p < 0,05$; $r^2 = -0,369$ and $r^2 = -0,365$). In one child the HA RI and SMA RI were significantly lowered, indicating the onset of a failing Fontan. Liver stiffness was significantly increased in the Fontan group compared to the control population, respectively with a median (IQR) of 12,6 kPa (11,4 – 16,2) and 4,6 kPa (3,8 – 5,6). However, values have shown to be already increased shortly after TCPC completion and tend not to increase in correlation with time post Fontan.

Conclusion: In general tests were less frequently and less severely deviating from reference values in comparison to adult results, and were mostly correlated with congestion, instead of fibrosis, except for PLT and ɣGT. None of the tests performed have been able to confirm diagnosis of fibrosis. Clinical relevance and application of these tests lies in the combination of their values gathered repeatedly over time. In particular TE is an easy to learn and quick to use method. Although congestion is an important confounder, liver stiffness measurements may still be able to indicate a deteriorating hepatic situation, when increased values are evaluated individually over time. Although this may indicate that progression to liver fibrosis in

a paediatric Fontan setting is only minimal, a six-monthly follow-up with blood analysis and FibroScan, in addition with a 2-yearly US and Doppler US assessment, is advised.

SAMENVATTING

Achtergrond: In de literatuur werden de chronische gevolgen van een Fontan circulatie op de lever reeds beschreven in volwassen populaties. Studies die nagaan of leverstoornissen zich reeds bij adolescenten en/of kinderen instellen zijn echter beperkt.

Doelstelling: Onze studie richtte zich op het evalueren van de prevalentie en graad van fibrose bij pediatrische patiënten d.m.v. weinig invasieve testen die geschikt zijn voor seriële opvolging in een ambulante setting. Daarnaast werd ook de toepassing van transiënte elastografie (TE) o.b.v. FibroScan (Echosense, Parijs, Frankrijk) geëvalueerd voor diagnostische en prognostische doeleinden.

Methoden: 35 Fontan patiënten (mediane leeftijd 11,8 jaar; range 5,2 – 16,6 jaar), waarvan 27 jongens, werden gerekruteerd uit het Universitair Ziekenhuis Gent. Elke patiënt onderging analyse van bloedwaarden (waaronder alanine aminotransferase, aspartaat amino-transferase, gamma-glutamyltransferase [ɣGT], alkalisch fosfatase, bilirubine [Bb], alfafoetoproteïne [aFP] en bloedplaatjes [PLT]), fibrose scores (APRI, FIB-4 en Forns), echografie met Doppler van hepatische structuren en bloedvaten, en TE m.b.v. FibroScan om leverelasticiteit na te gaan. Een controlegroep van 70 gezonde kinderen (mediane leeftijd 10,6 jaar; range 5,7 – 18,3 jaar) werd opgericht, met als doel het bepalen van normale pediatrische TE referentiewaarden die momenteel ontbreken in de literatuur.

Resultaten: ɣGT, Bb, PLT, AST, ALT en aFP toonden verhoogde waarden in respectievelijk 69%, 17%, 17%, 57%, 20% en 3% van de populatie. Lever morfologie toonde verandering in zo'n 11%. Een gemiddelde vena cava inferior collapsibiliteits index van maar 13,7% (± 9%) werd geassocieerd met veneuze congestie. Gemiddelde en maximale vena porta stroomsnelheden (respectievelijk 19,9 cm/s en 14,2 cm/s) zou dan weer een indicatie voor portale hypertensie zijn. De a. hepatica en de a. mesenterica superior weerstands index (HA RI en SMA RI) waren beide significant omgekeerd gecorreleerd met de tijd sinds Fontan (respectievelijk $p < 0,05$; $r^2 = -0,369$ en $r^2 = -0,365$). Bij één kind werd zelfs een significant verlaagde HA RI en SMA RI gevonden, wat een eerste teken kan zijn van een 'failing Fontan'. Lever stijfheid was significant ($p<0,001$) gestegen in vergelijking met de controle populatie, respectievelijk met een mediaan (IQR) 4,6 kPa (3,8 – 5,6) en 12,6 kPa (11,4 – 16,2). Verhoogde waarden werden reeds bestudeerd kort na TCPC-initiatie, en deze correleren verder ook niet met Fontan interval.

Conclusie: Algemeen weken parameters minder frequent en minder sterk af van de referentiewaarden in vergelijking met volwassen populaties en werden deze ook vaker

gecorreleerd met congestie, eerder dan fibrose, met uitzondering van PLT en γGT. Geen enkel van de uitgevoerde testen kon lever fibrose in de Fontan kinderen diagnosticeren. Echter het longitudinaal opvolgen van een combinatie van parameters kan van diagnostische waarde zijn bij follow-up. In het bijzonder is TE een toegankelijke diagnostische methode. Congestie blijft een verstorende factor, doch kunnen leverelasticiteit metingen, wanneer individueel in de tijd geëvalueerd, helpen in het vroegtijdig herkennen van leverstoornissen. Ondanks de beperkte progressie naar leverfibrose bij kinderen, wordt 6-maandelijkse opvolging a.d.h.v. bloedafname en FibroScan, aangevuld met 2-jaarlijkse echografie en Doppler, geadviseerd.

1 INTRODUCTION

1.1 The Heart

1.1.1 Anatomy

The heart is a muscular organ which serves as a pump to provide blood to the entire body through the vascular system. Blood provision is essential for the delivery of O2 and nutrients and the removal of metabolic waste. The human heart is divided into four chambers: right atrium (RA), right ventricle (RV), left atrium (LA) and left ventricle (LV). RA and RV are often referred to as the “right heart”; LA and LV as the “left heart”. Blood from the inferior and superior vena cava (respectively IVC and SVC) drains into the RA. The pulmonary veins are drained into the LA. The pulmonary artery (PA) and aorta are attached to the right and left ventricle, respectively. Between each pair of atria and ventricles an atrioventricular (AV) valve is positioned: the tricuspid valve in the right heart and the mitral valve (bicuspid) in the left heart. The RV is separated from the PA by the pulmonary valve; the LV from the aorta by the aortic valve. The right and left heart are separated by the atrial and ventricular septum.(1)

1.1.2 Physiology

The pumping of the heart

The heart functions by generation of rhythmic and harmonised propagations of electrical pulses resulting in equal myocardial contractions (2). This provides a pressure gradient because of which the blood flows (1).

Throughout diastole, the AV valves are open and the pulmonary and aortic valves are closed. Blood flows into the heart, hereby filling the atria and the ventricles. The more blood runs into the ventricles, the more the AV valves close. The initial phase of ventricular filling occurs passively, providing about 70% of the end diastolic volume. At the end of diastole, an atrial contraction (atrial systole) occurs. Because of the atrial contraction, orifices of the venae cava and pulmonary veins are narrowed. The latter, in combination with the blood’s inertia, limitates regurgitation from the atria into the veins (3).

After closure of the AV valves, an isovolumetric ventricular contraction occurs. In this period, the ventricles contract relatively little but the ventricular pressure rises heavily until it exceeds the diastolic aortic pressure or pulmonary pressure. During this time the AV valves bulge into the atria because of the rising ventricular pressure, hereby augmenting atrial pressure. As soon as the aortic and pulmonary pressures are exceeded, the ejection phase occurs in which blood is ejected into the arteries. Ventricular pressure peaks and muscle contraction pulls down the AV valves hereby diminishing the atrial pressure. The high pressure and inertia of the blood flow provide an ejection of about 70-90ml per stroke. With a mean end-diastolic

ventricular volume (EDV) of 130ml, about 50ml of blood remains in the ventricle after the ejection phase, the end-systolic ventricular volume (ESV). The proportion of the EDV ejected, expressed in percent, is called the ejection fraction (EF) (3).

At the end of the systole, the velocity of the blood flowing to the periphery becomes higher than this of the ejected blood, thereby gradually diminishing ventricular pressures. The aortic and pulmonary valves close as soon as the ventricular pressure falls below the aortic pressure and the momentum of the ejected blood is overcome (i.e. protodiastole). Afterwards a period of isovolumetric relaxation occurs in which pressures continue to drop until atrial pressures exceed the ventricular ones and the AV valves open again (3).

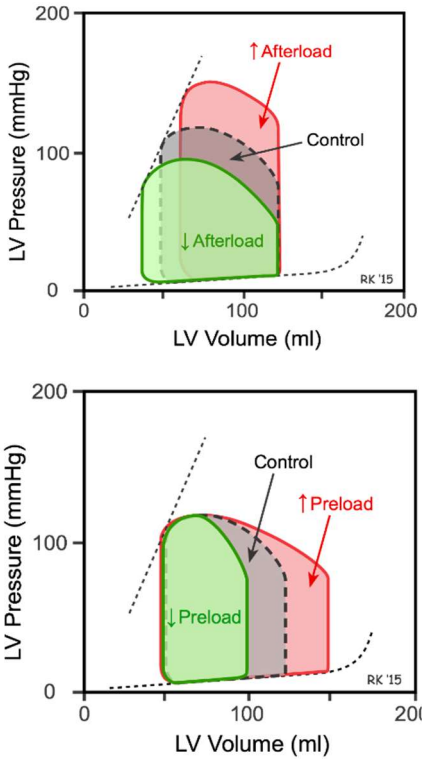

Figure 1 : the influence of afterload (a) and preload (b) on cardiac output (CO) (7).

The influence of afterload on the cardiac output (CO)

Afterload is defined as the vascular resistance which must be exceeded by the heart in order to eject blood. If afterload increases, more energy is needed to surpass those pressures and less energy can be provided to eject blood. Important causes of increased afterload are pulmonary and systemic hypertension (4) **(figure 1a)**.

The influence of preload on the CO

Preload is defined as the EDV volume. An increase in preload implies more stretching of the myocardium and augmented end-diastolic sarcomere length (EDSL). Based on the Frank-Starling mechanism, which states that the stroke volume of the heart increases in response to an increase in the volume of blood filling the heart (EDV), we can state that the force of contraction of cardiac muscle depends upon the preload. This regulation of contractility due to changes in muscle fibre length is called heterometric regulation (5, 6) **(figure 1b)**.

There are various factors affecting the EDV: diastolic ventricle compliance decrease, increasing importance of the atrial kick with higher heart rates and alterations in venous return. The latter can be positively influenced by an increase in total blood volume or constriction of the veins thereby reducing the size of the venous reservoirs. An increase in the normal negative intrathoracic pressure increases the pressure gradient along which blood flow to the heart, whereas a decrease impedes venous return (5, 6).

1.2 The liver and subdiaphragmatic venous circulation

The liver contains 2% of our body weight (ca. 1,3 - 1,8 kg). It has various functions, regarding regulation of the energy-metabolism by storage, the catabolism of lipids and glucose and filtering out drugs, toxins and excessive metabolites. It also plays a role in clotting mechanisms by the production of Prothrombin, Fibrinogen and Albumin. Production of bile helps the digestion and Kupffer cells play part in the immunologic mechanism against pathogens (8).

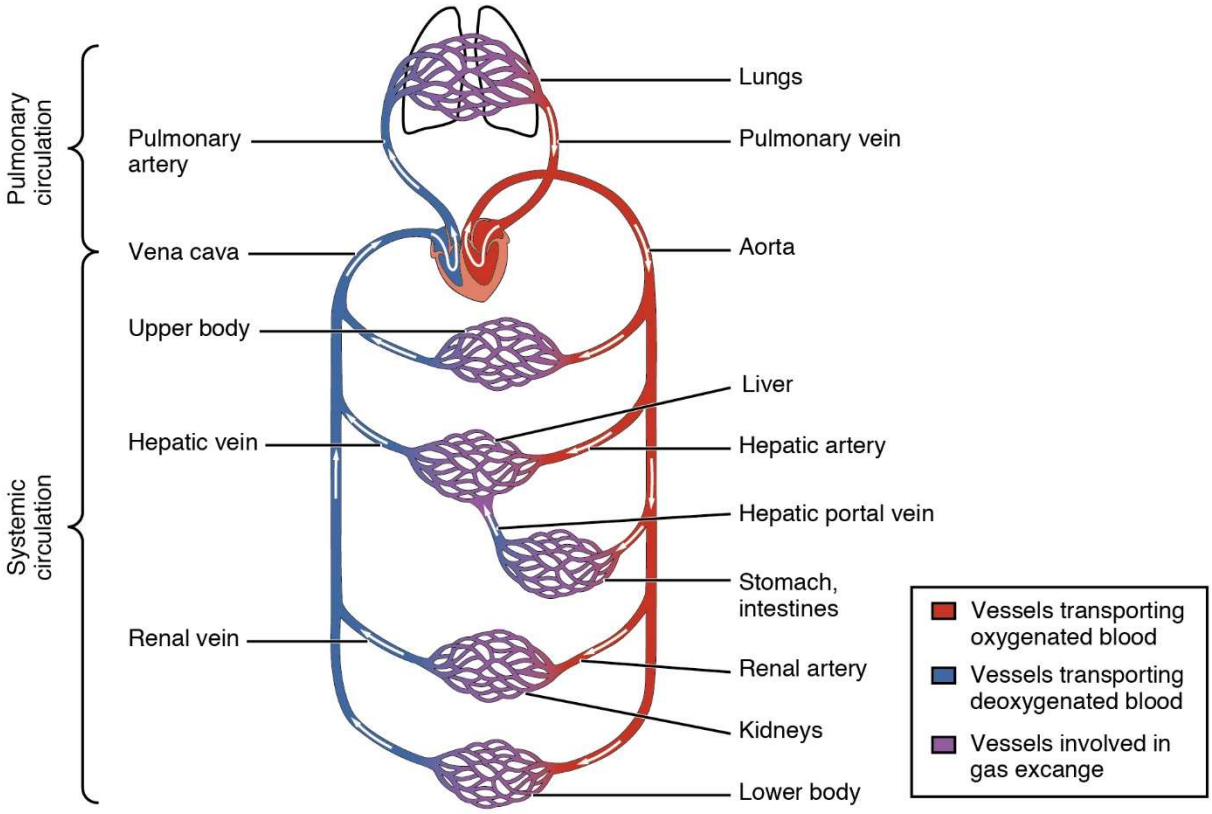

Figure 2: The subdiaphragmatic circulation, part of the systemic circulation (9).

1.2.1 Anatomy

The liver itself consists of a very large and unique circulation, managed in 2 (different) ways, resulting in 1.3 - 1.5 L/min blood passing through (approximately 25% of the resting CO). The first way of hepatic blood supply is by splanchnic or gastrointestinal circulation which consists of three main arteries branching from the aorta: the coeliac trunk, the superior and inferior mesenteric artery. They provide inflow of oxygenated blood into the intestines. After passage through specific abdominal vascular beds, which are in parallel circuits, de-oxygenated blood proceeds into the portal vein (PV), delivering a high inflow in the liver (70-75% of the liver flow rate) (10, 11). There, the blood will be divided over small sinusoids, where it circulates through the surrounding hepatocytes, starting from the periphery into the central vein. Even though the

blood from the PV is mainly de-oxygenated, it is the most important oxygen supply for the liver (12). Secondly, arterial blood reaches hepatic cells through the hepatic artery (HA) (derived from the coeliac trunk from the abdominal aorta, delivering the other 25% of the liver blood flow) (10, 11). Likewise, this artery will branch out into small sinusoidal capillaries and oxygenated blood will spread over the hepatic cells. Again, the central vein will function as drainage (12).

After passage through the hepatic tissue, the central veins will merge into three hepatic veins. Those veins will merge with venous blood vessels coming from the remaining subdiaphragmatic areas thereby forming the inferior vena cava (IVC). This results in the return of blood to the RA (12).

1.2.2 Physiology

The mean pressure in the HA is similar to the one in the aorta. On the other hand, PV pressure ranges from 6 to 10 mmHg. Further downstream the sinusoidal liver bed has a 2-4 mmHg higher pressure than the IVC (5mm Hg). Hepatic blood flow and pressures are regulated by multiple determinants.

Firstly, the hepatic arterial resistance determines the hepatic arterial flow. A myogenic constrictive response of the HA will occur if arterial pressure arises, for local blood flow to remain constant. This phenomenon called pressure-dependent autoregulation is, however, of minimal influence. In the PV, this mechanism is even more negligible, as it is mainly determined by the outflow from the intestinal vessels, that is the resistance across the latter. The intrahepatic portal venous flow thus follows a linear pressure-to-flow relationship: increased portal venous resistance equals low pressure gradients and vice versa 14).

As a second mechanism, the hepatic arterial buffer response (HABR) is active in the hepatic blood flow regulation. HABR has the ability to produce compensatory flow changes in the HA in response to changes in portal venous flow. This means, if portal blood flow reduces, the hepatic artery dilates and the other way around (13, 14).

1.3 The Univentricular Heart (UVH)

About 8-10% of all patients with a congenital heart disease have a single functional ventricle (13). The second ventricle, though always present, is rudimentary or underdeveloped (15). This ‘single’ ventricle has to maintain both the systemic and the pulmonary circulation (13). In a normal biventricular heart both circulations are in series and each circulation is supported by a ventricle whereas, in a univentricular heart (UVH), they are connected in parallel. Without arteriovenous blood shunts, this situation is incompatible with life (16). There are no intrauterine complications as AV shunting and parallel cardiac blood flow is physiologically

normal in this phase (open foramen ovale and ductus Botalli); Consequently, the functional ventricle can take over the function of the other defective ventricle. However, after birth a serial circulation arises where each ventricle has its own (different) function. If the systemic ventricle has an open connection with both PA and aorta, it will maintain the pulmonary as well as the systemic circulation. Proportional blood flow will depend upon the width of the orifices and resistance of the distal arterial bed. If the ventricle only connects to one of the arteries, the other artery will depend upon the ductus Botalli for its blood supply (15).

Such a circuit has two major, yet inevitable, disadvantages. First, chronic volume overload to the single ventricle, as it receives both pulmonary and systemic venous return. Chronic volume overload will eventually impair ventricular function. From the third decade onward, a progressive decline in function will occur due to congestive heart failure, with few survivors beyond the fourth decade. Secondly, arterial desaturation will occur due to complete mixture of systemic deoxygenated and pulmonary venous oxygenated blood (13, 15). What's more, because of the intracardiac right-left shunt a natural barrier for embolisms (thrombotic or infectious) disappears, thereby increasing the risk of stroke or brain abscesses (15).

Systemic left ventricle

Because of malformations such as pulmonary atresia or underdevelopment of the RV, pulmonary circulation will be compromised or absent thereby depending on the left-right shunt through the ductus Botalli. Once the ductus has closed (a couple of hours to days after birth), cyanosis will occur. Other causes of a univentricular LV are tricuspid atresia, Ebstein malformation, double inlet-LV and an unbalanced AV septal defect with left predominance (15).

Systemic right ventricle

A Hypoplastic Left Heart Syndrome (HLHS) is the most frequent congenital UVH disease. The LV is hypoplastic as are the aortic valve and aortic arch. This situation requires a left-right shunt from LA to RA. The systemic (functional) RV will send blood into the PA. A part of the blood proceeds to the pulmonary vasculature, the other part passes through the ductus Botalli (right-left shunt) into the aorta. When the ductus of Botalli closes, cyanosis and acidosis occur. Other causes of a univentricular RV are stenosis of the mitral valve and an unbalanced AV septal defect with right predominance (15).

Systemic RV versus LV

There are inherent differences between the architecture, AV valve characteristics and functional responses of the ventricle when the LV and RV of patients with HLHS, tricuspid atresia and double inlet LV are compared. However, little evidence exists to clinically

differentiate between those with a systemic RV or LV. A single RV is said to have no influence on the early outcomes of a Fontan population. However, a single RV has shown to have superior survival to patients with a systemic LV (14).

1.4 The Fontan circulation

1.4.1 The evolution of Fontan

In 1971, Francis Fontan and Eugene Baudet created a surgical procedure which provides a separated pulmonary and systemic circulation by bypassing the failing ventricle thereby diverting bi-caval venous return; all shunts on the venous, atrial, ventricular and arterial level are interrupted (17, 18). This has several hemodynamic consequences. Firstly, in such a construction, the energy to pump the blood through the lungs is provided by the postcapillary energy (in contrary to the ventricle in normal hearts) and thus depends on increased central venous pressure (CVP) (13, 19, 20). Secondly, CO no longer depends upon the heart contractility but upon the transpulmonary flow, itself mainly determined by pulmonary vascular resistance (PVR) (13).

In 1976, F. Fontan defined his recommendations for a successful Fontan operation as having a good CO at an acceptable systemic venous pressure. This means that after repair, left atrial pressure must be low (determined by good ventricular function) and that the transpulmonary gradient must be low (determined by the pulmonary vasculature) (15). Over the last 45 years, the Fontan circulation underwent several modifications (*see infra*). Cardiac requirements nowadays are: unobstructed ventricular inflow (no AV valve stenosis, no regurgitation), a reasonable ventricular function, an unobstructed outflow (no subaortic stenosis, no arterial hypertension and no coarctation). Pulmonary requirements include a non-restrictive connection from systemic veins with pulmonary arteries, good sized pulmonary arteries without distortion (at repair and later during growth), a well-developed distal vascular bed, (near) normal PVR <2,5 U/m2 and unobstructed pulmonary venous return. As soon as possible following birth, the pre-Fontan management must aim to reach these goals; some deviations are acceptable, however, often resulting in increased operative mortality and increased late morbidity and late mortality (13). Whereas only 70% survival was reported in the early years of Fontan, the survival rates for UVH disease with Fontan palliation have improved significantly over the past 20 years thanks to new surgical innovations (lateral tunnel and extracardiac conduit) and technological improvements. After stage 1 of the operation (neonatal stage) 90% survival is reported; 98% after stage 2 (bidirectional Glenn or hemi-Fontan) (21-23).

1.4.2 Anatomy

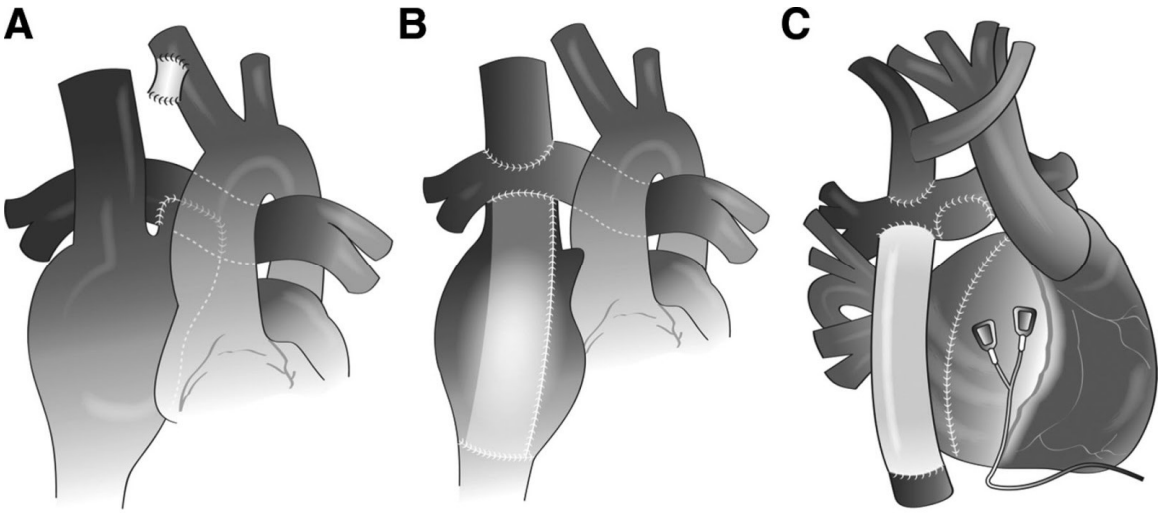

Figure 3: Anatomy of a Fontan circulation. Three types of Fontan circulations: a) Atriopulmonary anastomosis; b) Lateral Tunnel Conduit; c) Extracardiac Conduit (24).

Atriopulmonary anastomosis ***(figure 3, A)***

The Fontan operation consists of redirecting the bi-caval venous return directly into the pulmonary arterial circulation, bypassing the failing ventricle. From 1970 - 1990 the main procedure consisted of a direct valved conduit between the right atrial appendage and the PA where the SVC is left continuous with the atrium and the atrium acts as a functional pumping chamber (13).

Lateral tunnel conduit ***(Figure 3, B)***

As the bidirectional cavopulmonary anastomosis was becoming more popular, the atriopulmonary anastomosis was abandoned. From 1990 - 2000 a new way of creating a Fontan circulation was found by Marc de Leval: The 'lateral wall' total cavopulmonary connection (TCPC) (25). With this circuit, the SVC is anastomosed end-to-side immediately on to the right PA. A prosthetic patch is sewn into the RA thus diverting the blood from the IVC directly into the PA through an intra-atrial "passage" using the lateral wall of the RA and prosthetic material (26). Some patients with suboptimal PVR (10-25%) were given a small fenestration between the conduit and the pulmonary venous atrium, allowing a residual right-left shunt thereby limiting caval pressure and congestion, and increasing the preload of the systemic ventricle hereby augmenting the CO. Those advantages come at the expense of slight desaturation and thus cyanosis. The lateral tunnel conduit has growth potential because of which it can be used in children aged 1 year or older. However, it damages the atrial tissue increasing the risk of atrial arrhythmia (13).

Extracardiac conduit ***(Figure 3, C)***

Because of the important complications of the lateral tunnel (arrhythmias), the currently most popular Fontan design is the extracardiac conduit. In this circuit, the IVC is detached from the RA, connected to a prosthetic conduit, which is sewn onto the PA without passing through the RA, thus limiting the number of atrial sutures and as a result decreasing the number of arrhythmias. However, the extracardiac conduit has no growth potential and can therefore only be performed in children large enough to accept a graft adequate for an adult's IVC flow (13).

1.4.3 Fontan as a three-step process

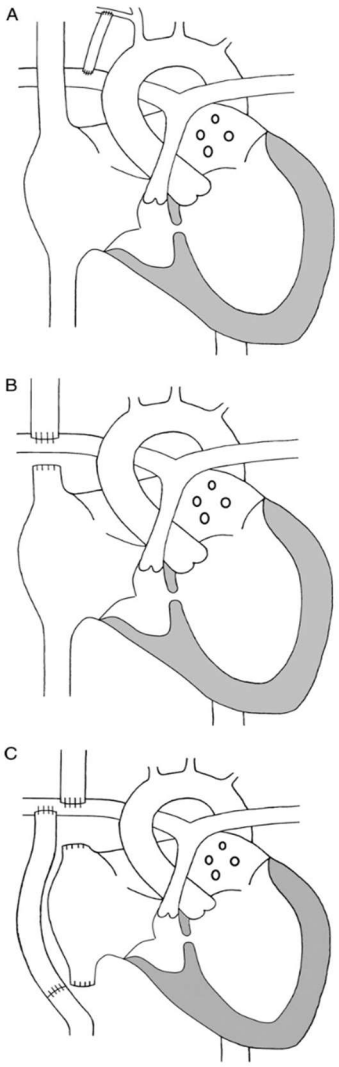

Figure 4: Fontan as a three step process (16).

a) Neonatal period;
b) superior cavopulmonary connection;
c) total cavopulmonary connection.

Neonatal period ***(figure 4, A)***

Because of a relatively high PVR in the neonatal period, an immediate Fontan operation is contra-indicated. To progressively adapt the body to the different haemodynamic conditions, a staged approach towards a Fontan completion is recommended (16). Another advantage of the staged approach is the better selection of Fontan patients and intermediate preparatory interventions (13). In the neonatal period, management is aimed at creating an unrestricted flow from the heart to the aorta, a balanced flow to the lungs and unrestricted return of blood to the ventricle (13). Once the ductus Botalli is closed, an alternative passage is required to provide sufficient pulmonary blood flow to maintain saturation. Yet, pulmonary blood flow must be kept low in order to limit PVR and ventricular overload (16). During this time, the heart experiences a chronic volume overload which is beneficial for the development of the pulmonary vasculature but can be devastating for the ventricular function if excessive (13). In patients with a systemic LV, this is acquired by placing a restrictive synthetic conduit between a major systemic artery (e.g. right subclavian artery) and a proximal PA (i.e. the Blalock-Taussig shunt). In HLHS the conduit can be placed between the RV and the left PA to provide a pulmonary conduit parallel to the systemic circulation (i.e. the Sano-shunt) (16).

Superior cavopulmonary connection ***(figure 4, B)***

After several months, when the PVR has come down (2-6 months according to Nayak et al. (16) or 4-12 months according to Gewillig et al. (13)), a bidirectional cavopulmonary connection (BCPC) is performed. The SVC is connected to the PA (or bilateral if bilateral SVC are present) (13) in order to perfuse both left and right lung (26). The previous restrictive systemic-pulmonary shunt is usually transsected (16). This intervention significantly decreases the volume overload of the ventricle to 1,5 times of normal (13). Secondly, this provides a low-pressure pulmonary blood flow (16). The ventricle will still receive deoxygenated blood from the IVC because of which peripheral saturation of about 80-85% will persist through complete mixing (13, 16). According to McElhinney et al. (27), after surgery, patients are at risk of producing arterio-venous shunts when no more blood flow from the liver is directed to the lungs (when no residual RV-PA connection persists).

Total cavopulmonary connection ***(figure 4, C)***

At the age of 2-5 years, depending on the centre preference and growth of pulmonary vasculature and symptoms (cyanosis at rest and during exercise), the final step of the procedure is performed: the connection of the IVC to the PA. This can be done either by an intracardiac or an extracardiac conduit; yet all result in an equally effective circulation (13, 16, 28).

Some patients with suboptimal PVR (10-25%) require **a fenestration** between the conduit and atrium thereby constructing a residual right-left shunt limiting caval pressure and increasing CO (by increasing EDV) at the expense of cyanosis (13, 16). Furthermore, after completion of the TCPC, patients tend to maintain a slight desaturation of about 90%. This may have multiple causes such as drainage of the coronary sinus into the LA, a residual atrial septum defect or fenestration, intrapulmonary arteriovenous fistulae, abnormal systemic venous drainage to the pulmonary venous atrium or incomplete occlusion of previous artificial shunts. This left-to-right shunting may, in time, result in volume overload and increase in PVR secondary to high regional pulmonary blood flow (13, 16).

Until today, the Fontan palliation remains the golden standard for patients with UVH (15), and represents 4,2% of all congenital heart surgeries performed in the United States (29).

1.5 Post-Fontan Physiology

This newly formed circulation aims at the normalisation of the oxygenation of blood and preservation of CO while reducing the volume overload on the single ventricle (14). Although pre-Fontan management goals are specified over the years (*see supra*), the Fontan circulation remains a palliative treatment for a UVH (30), as various negative effects will inevitably develop over time: preload insufficiency resulting in decreased CO, increased PVR, and systemic venous hypertension and congestion (31).

1.5.1 Cardiac output

As the main goal of a Fontan circulation is to maintain a sufficient CO, different variables will be looked into. **Preload** can be considered as the main determinant in controlling CO (31). As mentioned, in the UVH the systemic ventricle is occupied with both the systemic and pulmonary venous return, resulting in a preoperative volume overload up to 200% of normal for body surface area (BSA) (13, 25, 31, 32). However, chronic volume overload of this capacity will damage and remodel the single ventricle. Eccentric hypertrophy, dilatation, spherical configuration and hypocontractility will occur, leading to cardiac dysfunction (28, 30). The creation of a Fontan circuit will aim to reduce the preload of the single ventricle resulting in a preload of about 70% of normal BSA due to former cardiac remodelling (28, 32). Gewillig et al. (31) concludes that both **contractility** and **heart rate** play a negligible role in the control of CO. These variables will not lead to increases in CO, as they may make the ventricle squeeze harder/faster but, with the limited preload, will not result in an increase in ejection volume (14). **Afterload** on the other hand, is a consequence rather than a cause of decreased CO. As CO decreases, systemic arterial resistance will rise, in order to maintain blood pressure. If the afterload expands excessively, the described mechanism above is only intensified, leading to the rapid deterioration of the ventricle (31).

1.5.2 The systemic ventricle

Installing a Fontan circuit is thus necessary to prevent cardiac dysfunction and will initially stabilize the preload on the single ventricle (13, 28). The ventricle itself will postoperatively adapt to these physiological changes following the law of preservation of mass: due to the acute reduction in EDV, preserved shortening and constant wall mass, an increase in wall thickness can be observed (14). Abnormalities in early diastolic filling and early relaxation become the major issue. Though the ventricle is unloaded, diastolic dysfunction will establish in time, resulting in preload volume decrease and in CO reduction sequentially (14). If chronically, the ventricle may even enter a vicious circle of remodelling and reducing preload, with longstanding volume deprivation up to <70% of the ‘due’ preload (28). The ventricle

evolves from volume overloaded and dilated to overgrown and underfilled, which leads to the failing Fontan (28, 30, 31).

Preventing the enormous volume loading, preventing the excessive ventricular hypotrophy and accordingly preventing the major configurational changes discussed above, would all seem helpful in avoiding cardiac dysfunction and its complications (e.g. thromboembolism, arrhythmia) (14, 25). However, Gewillig et al. (30) mentions that excessive early unloading of the ventricle could lead to an even worse outcome for a Fontan patient, bearing in mind other variables of CO, such as PVR.

1.5.3 Pulmonary blood flow

Blood flow towards the lungs is generally facilitated by 3 components: low PVR, respiratory movements and high systemic venous pressure (which generates the energy for pulmonary perfusion). Developed pulmonary arteries and a full-grown pulmonary vascular bed are necessarily required for the maintenance of a low PVR, as this will control CO indirectly as well (30).

In a Fontan circulation due to absence of a hydraulic force, chronic pulsatile flow deprivation is present, influencing the development and functioning of the pulmonary vascular bed. Due to presence of a low velocity, laminar flow, vascular recruitment and lung growth will be impaired. Moreover, the endothelial function and nitric oxide (NO) release in the developed parts of the lung will be reduced, thereby all leading to an increase of the PVR (33). Hence, a Fontan circulation does not only miss a fairly working ventricle for maintaining pulmonary blood flow, it has a significantly less efficient pulmonary vascular system as the result of mechanical and cellular changes (33). Increases in PVR will lead to obstruction of systemic venous return through the pulmonary vascular bed, thereby not only decreasing cardiovascular output downstream but, to a significant extent and most importantly in our analysis, leading to venous congestion upstream as well (13, 28, 30).

With a limited ventricular filling range and, explained after, a limited possibility of systemic venous pressure to increase (only up to 20 mm Hg), the impedance of the pulmonary system becomes the leading determinant of the CO (13, 28). Ultimately, if a Fontan circuit is formed in a late stage, mild ventricular function, but an excellent PVR, will develop. On the contrary, early ventricular unloading leads to excellent cardiac function, though mildly increased PVR. The former should prevail as it induces the most beneficial CO (30).

1.5.4 Systemic venous circulation

Systemic venous return can no longer rely on a pre-pulmonary pump to reach the lungs and preserve CO. Instead, pulmonary blood flow depends on the presence of a continuous elevated pressure in the upstream venous circulation (33, 34). Ventricular insufficiency and increased PVR, both leading to a decreased preload as described above, will contribute in the obstruction of venous return (28).

The systemic venous system (*see normal physiology supra*) comprises the IVC, SVC and the subdiaphragmatic system with the splanchnic and hepatoportal venous circulation (10). In Fontan patients multiple authors have described increased pressures in the IVC (and SVC), preserving forward flow through the lungs (28, 35-37). The attained pressures are usually between 10 and 15 mmHg, up to three times normal (10). Pressures above 20 mmHg are rare and associated with poor outcomes (36). These increased caval pressures are transmitted through the hepatic vein to the portal system, leading to an increased wedge hepatic venous pressure (WHVP), that is, the hepatic sinusoidal pressure that reflects portal pressure (38, 39). The Hepatic Arterial Buffer Response (HABR) will try to compensate the decreased portal flow by dilating the HA, in order to guarantee adequate oxygen supply (40). However, if the HABR cannot compensate adequately, blood will pile up in the hepatoportal venous system, dilating and stressing sinusoids due to a situation of equalized flow and pressure between the PV and the HVs. Blood flow through the liver slows down, extending portal blood transit time. Together with an augmented hepatic blood volume, this results in splanchnic congestion and portal hypertension (41).

1.5.5 Influence of respiration, gravity, exercise, fenestration

Respiration

Inspiration creates a negative pressure, drawing blood into the chest cavity (35). The influence of respiration is particularly mediated by an increase in anterograde hepatic vein flow (36). Hsia et al. (42) showed that 20% and 30% of the venous return was respiratory dependent in APC and TCPC patients respectively, in comparison with 15% in the healthy population group.

Exercise

As there is a decreased circulatory output at rest, exercise only contributes to this situation. In a Fontan patient, there is no functional RV to increase and accelerate pulmonary blood flow. Furthermore, as pulmonary vascular reactivity is already limited or even absent due to NO reduction, this will lead to a restricted ability to boost CO during exercise (16, 28)

Gravity

Gravity has a significant negative effect on infradiaphragmatic venous return in Fontan patients when they are in upright position (36). Hsia et al. (42) mentions the adverse effect on IVC flow, with reduced antegrade and enhanced retrograde flows, more marked in APC than in TCPC patients.

Fenestration

Hsia et al. (43) suggests that fenestration results in a more efficient and less congested splanchnic circulation as venous pressures are restored.

1.5.6 Clinical presentation

Fontan biopsies show that the systemic venous congestion results in in massive sinusoidal dilatation, parenchymal atrophy and stimulation of the fibrotic response in the liver by sinusoidal collagen deposition (44). Moreover, via CT and MRI investigations altered liver perfusion patterns and arterialized nodules were seen (10, 45). Hepatomegaly but also splenomegaly, as a result from transmitted central venous hypertension, may be present as well. Portal hypertension can be found, though, the Hepatic Venous Pressure Gradient (HVPG) is not elevated, as the pressures in the IVC are equally high as the PV. Consequently, varices are uncommon but may develop due to the mechanism of marked generalized venous congestion. Ascites is reported in 2.5-10% of Fontan patients (45). In chronic situations, venous insufficiency in the lower limbs can be observed as well (46). Evolution from chronic passive congestion to hepatocellular carcinoma (HCC) has been documented before (47). Moreover, chronic hepatic venous congestion from cardiac failure may even lead to cardiac cirrhosis (48).

Kiesewetter et al. (44) showed that the degree of collagen deposition and fibrosis is related to the duration of a Fontan circulation, though details on the progression and detection of early hepatic fibrosis to irreversible cirrhosis are still unknown (49). Most importantly is that the level of liver dysfunction and symptoms are not correlated with the degree of fibrosis which makes detection and approach of hepatic changes in a Fontan setting a difficult challenge (16).

1.6 Other consequences of a Fontan circulation

The Fontan circulation is known to have multiple other consequences next to hepatic congestion. Arrhythmia occurs due to surgical damage to the sinoatrial node or its blood supply and innervation (13). As a consequence, a lower incidence is found for lateral tunnel and extracardiac Fontan types compared to atriopulmonary Fontan types (50). Those arrhythmias combined with atrial scarring, lowered CO, systemic venous hypertension and a decreased venous flow due to absence of a functioning RV, increase the risk of thromboembolisms (16, 50-52). Furthermore, the elevated CVP impedes the lymphatic drainage from the thoracic duct causing lymph oedema, pulmonary oedema, plastic bronchitis and protein losing enteropathy (PLE). The latter results in loss of albumin and immunoglobulins into the gut and has a poor prognosis of less than 20% of the patients surviving beyond 10 years (13, 16, 50).

1.7 Investigation and follow-up of Fontan patients

In the past, liver biopsy was the only existing method to diagnose liver fibrosis and thus the gold standard. However, it wasn't suitable to perform routinely because of its invasive character and low sensitivity. Less-invasive tests and protocols currently used to provide follow-up (FU) are ultrasound (US) and Doppler US, Magnetic Resonance Imaging (MRI), Shear Wave Elastography (SWE), blood analysis of serological markers and Computed Tomography (CT). In this study, a more recent diagnostic technique, still of limited use in a paediatric setting, will be validated: the FibroScan (Transient Elastography (TE)).

1.7.1 Transient Elastography

TE is a non-invasive method for the assessment of liver stiffness (LS) based upon ultrasounds but without image guidance. The main purpose is to detect the different stages of fibrosis with the aim of starting FU earlier on in the process and establishing priority for therapy. TE is also used for the FU itself and the assessment of therapy effectivity (53).

TE with the use of FibroScan has some major advantages such as its non-invasive character and excellent reproducibility (interclass correlation coefficient of 0,96) (53, 54). However, it has some disadvantages as well such as lack of image to determine the exact location of measurement, thereby following the possibility over overestimation of the LS through faulty position of the probe. Also, wrong use of probe sizes may over- or underestimate LS. Ascites and obesity make it unable to measure LS (correctly). Moreover, FibroScan is unable to measure inside big vessels or masses or to determine nonuniformity of the disease distribution.

TE has shown a strong correlation of LS with histological state of fibrosis in multiple studies and meta-analysis **(figure 5)** (55-58)**.** However, Fontan fibrosis scores have not been defined yet.

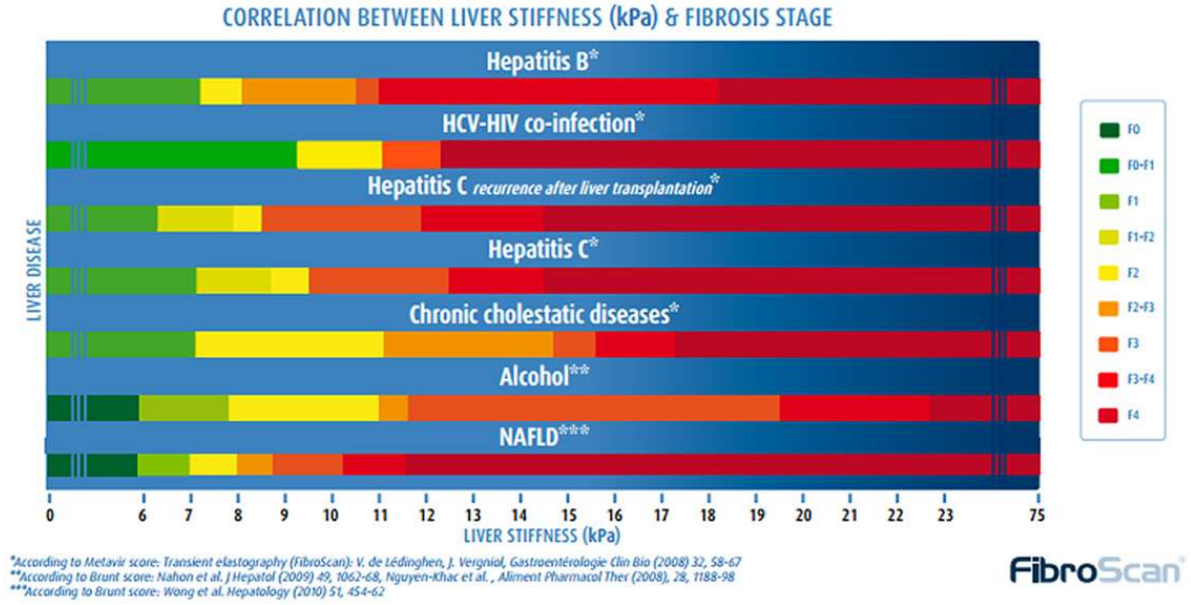

Figure 5: Correlation between liver stiffness (kPa) & fibrosis stage (59).

*** according to Metavir score**: Transient elastografphy (FibroScan); V. de lédinghen. J. verginol, gastroentérologie Clin Bio (2008) 32, 58-67
**** according to Brunt score**: Nahon et al. J Hepatol (2009) 49, 1062-68, Nguyen-Khac et al., aliment Pharmacol Ther (2008) 28, 1118-98
***** according to Brunt score**: Wong et al. hepatology (2010) 51, 454-62

2 OBEJCTIVE

The Fontan circulation, in children with a UVH disease, is the only effective therapy and necessary in terms of survival yet all but ideal. Several studies proved that in the long term, the abnormal physiological state of the circulatory system, leads to various consequences such as cardiac arrhythmia, protein losing enteropathy and thrombo-embolism. Furthermore, it leads to chronic systemic and splanchnic congestion increasing the risk of liver fibrosis and cirrhosis.

The hepatic long-term consequences of the Fontan circulation

The aim of our study is to examine whether there are (significant) signs of hepatic venous congestion, fibrosis or cirrhosis present in Fontan children aged 17 years or less and which parameters are helpful to detect these changes in an early stage. Therefore, the following investigations will be performed: serological markers analysis, urine laboratory markers analysis, FibroScan (TE) for hepatic stiffness assessments, US and Duplex US for morphological changes and flow patterns alterations. Furthermore, it looks into the link between the patient's age and interval since performance of the Fontan circulation versus the parameters for liver attainment / disease. We aim to provide longitudinal FU strategies for the evaluation of the progression of Fontan associated liver diseases (FALD).

The value of FibroScan in the assessment of liver fibrosis in children with Fontan circulation and its validation in a healthy paediatric population.

This study will also serve as an analysis of the FibroScan (TE). Our aim is to examine if the FibroScan is a valuable screening method for FU of liver deterioration in a paediatric Fontan setting. Additionally, we aim to describe reference values for liver stiffness / elasticity in a healthy paediatric population.

3 METHODS

3.1 Study Design

This study prospectively investigated liver modifications in Fontan patients born in 2001 or after, all registered at Ghent University Hospital (GUH), Ghent, Belgium. The study and technical protocols were approved by the Ethics Committee of GUH (B670201629625) **(addenda: attachment E)**. Informed consent was obtained from every accompanying parent.

This study was conducted by H. Van Overschelde and F. Hendricx (students master of medicine at Ghent University, prof. dr. K. François (head of cardiac surgery at GUH), dr K. Vandekerckhove (paediatric cardiologist at GUH) and dr. R. De Bruyne (paediatric gastroenterologist at GUH). All US examinations and Doppler US studies were performed by paediatric radiologist dr. C. Vande Walle (GUH).

This study took place as a continuation of the study "Hepatic changes in the Fontan circulation: identification of liver dysfunction and an attempt to streamline FU screening." (Ackerman T., François K.) also conducted at the GUH (60). The latter prospectively studied patients born before the year 2001 who had undergone a Fontan circulation, registered at GUH.

3.1.1 Substudy FibroScan

A substudy was conducted in order to define paediatric reference values for liver stiffness. Therefore, healthy controls born in 1999 or after were gathered, not in follow-up at GUH. Approval of the Ethics Committee of GUH (B670201629625) **(addenda: attachment E)** and informed consents of every accompanying parent were obtained as well. Further information will be explained at results – FibroScan (*see infra*).

3.2 Study population

A total of 50 minor Fontan patients were qualified to participate in the study. All patients were contacted by telephone. Some of them were also asked to participate by their own cardiologist at the six-monthly follow-up consultation. Informed consent was obtained from 35 patients (27 male, 8 female). Reasons of decline were inability to contact (3), inability to reach the hospital because they moved away (3), not being able to find a suitable moment to perform the liver US (8) or lack of interest (1). Blood analysis, US with Doppler US and TE – FibroScan - were performed on 35 patients. Characteristics of the study population were gathered at last follow-up and can be found in **table A**.

Table A: Characteristics Fontan group	
Total N	35
Male, percent	27 (77%)
Age, years (median, range) [a]	11,76 (5,20 – 16,58)
Length, cm (mean, SD)	141,0 (± 20,1)
Weight, kg (mean, SD)	35,99 (± 13)
BMI (mean, SD)	17,39 (± 2,35)
Age at Fontan, years (median, range) [a]	3,29 (2,17 – 6,98)
Time since Fontan, years (median, range) [a]	6,00 (1,17 – 13,83)
Type of Fontan - **Lateral Tunnel** - **Extra cardiac**	 7 24
Dominant Ventricle left	24
Fenestration - **Yes** - **No** - **Unknown**	 28 5 2
Patency fenestration - **Open** - **Closed** - **Unknown**	 10 11 14
Heart rate, bpm (mean, SD)	85 (± 16)
Systolic blood pressure, mmHg (mean, SD)	112 (± 21)
Diastolic blood pressure, mmHg (mean, SD)	66 (± 8)
Saturation, percent (mean, SD)	94 (± 3)
Ejection Fraction, percent (mean, SD)	45 (± 13)

Table A: Characteristics of the Fontan group gathered at last follow-up at Ghent University Hospital. Bpm = beats per minute; SD = standard deviation. [a] when variable distribution is significantly different from normal distribution for p<0,05 , median values were used.

3.3 Procedures

3.3.1 Laboratory Tests

A set of blood parameters were measured, enlisted in the blood analysis in **addenda: attachment C.** The hepatologic markers determined in this study are alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (ɣGT), alkaline phosphatase (AP), bilirubin (Bb), cholesterol and Apo-lipoprotein A1. Moreover, international normalized ratio (INR), partial prothrombin time (PPT), platelet count (PLT), total protein and albumin were measured to determine the metabolic function of the liver and presence of PLE. Pro-BNP was measured in function of cardiac evaluation. The laboratory estimation of liver fibrosis is based upon the APRI-score, the FIB-4 score and the Forns score.

APRI-Score

$$\text{APRI} = 100 \text{ x } \frac{\text{AST } \left(\frac{\text{IU}}{\text{L}}\right)}{\text{AST (ULN)} \left(\frac{\text{IU}}{\text{L}}\right) \text{ x PLT } \left(\frac{10^9}{\text{L}}\right)}$$

For this study we used a cut-off 1,5 to determine significant liver fibrosis F ≥ 2 (METAVIR) as suggested by Wai CT et al (61).

FIB-4-Score

$$\text{FIB} - 4 = \frac{\text{Age (y)} * \text{AST } (\frac{\text{IU}}{\text{L}})}{\text{PLT } \left(\frac{10^9}{\text{L}}\right) * \sqrt{\text{ALT}}(\frac{\text{IU}}{\text{L}})}$$

In continuity with the APRI score, we used a cut-off of 1,45 (METAVIR F2) to determine significant fibrosis (62).

Forns score

$$\text{Forns} = 7.811 - 3.131 \text{ x ln}[\text{PLT } \left(\frac{10^9}{\text{L}}\right)] + 0.781 \text{ x ln}[\text{ɣGT } \left(\frac{\text{IU}}{\text{L}}\right)] + 3.467 \text{ x ln}[\text{age(y)}] - 0.014 \text{ x } [\text{cholesterol } \left(\frac{\text{mg}}{\text{dL}}\right)] \text{ (63)}$$

Forns et al. concluded that a score > 4,2 has a sens. 94% and NPV of 96% (AUROC 0,86) for significant fibrosis (F2-F4). A score > 6,9 has a spec. 95% and PPV of 66%. The Forns score is thereby considered apt to exclude yet not to diagnose significant liver fibrosis. One should consider that the Forns score was designed to evaluate adult chronic hep C patients without (diagnosed) liver fibrosis (64).

3.3.2 Ultrasound (US) and Doppler US

In this study, General Electric Logiq S8 was used. All examinations were performed by the same examiner, Dr. C. Vande Walle (paediatric radiologist), to avoid inter-rater bias.

Protocol

All patients were instructed to fast for at least 12 hours before examination as all examinations took place in the morning. All measurements were made in supine position, in quiet respiration and at rest with no preceding physical activity.

Our protocol for US morphology and Doppler-US flow patterns can be found in **addenda: attachment D.**

Liver and spleen morphology

We performed a general anatomic evaluation of the liver, screening for irregularities such as nodularity, coarsened echotexture, ascites, collaterals and splenomegaly. However, US lacks a high sensitivity for diagnosis of liver fibrosis as liver morphology is not always altered in the early stages of cirrhosis. Liver and spleen size were evaluated respectively through US midclavicular liver diameter and splenic length.

Doppler US – flow patterns

US was combined with Doppler US. We examined the diameters of Inferior Vena Cava (IVC) and Portal Vein (PV) in in- and expiration together with the IVC collapsibility index (IVCCI). We also analysed the pulsatility ratio (PR) of the PV and Hepatic Vein (HV), based on min. and max. flow velocities. Damping Index (DI) was calculated as an alternative for PV Pulsatility Index (PI) (car. infra). Furthermore, we examined the Resistance index (RI) of the PV, Hepatic Artery (HA) and Superior Mesenteric Artery (SMA).

Formulas:

- Pulsatility ratio (PR) = min. peak velocity / max. peak velocity
- Pulsatility index (PI) = (peak systolic velocity - end diastolic velocity) / mean velocity over one cardiac cycle
- Resistance index (RI) = (max. peak velocity - min. peak velocity) / max. peak velocity
- IVCCI = 100 * ∆IVC diameter / IVC expiration diameter
- Dampening Index (DI) = min. antegrade flow velocity / max. antegrade flow velocity

3.3.3 Transient Elastography

In this study FibroScan (Echosens, Paris) was used to perform TE and compute hepatic pressure (kPa) regarding the assessment of liver fibrosis. All examinations were performed by the same 2 examiners, Fabian Hendricx & Hazel Van Overschelde.

Protocol

It is recommended to perform the examination after 4-6 hours of fasting as the fibrotic liver can produce falsely elevated measurements in a non-fasting state. The patient is examined in supine position with the right arm overhead. The probe is placed in one of the lower intercostal (IC) spaces (usually in the 9th - 11th IC space at the level of segment VII or VIII of the liver about 2cm below the Glisson capsule), which provide the best intercostal acoustic window. The probe should at all time be placed perpendicular to the liver to optimize refraction of the pulse. A different set of probes can be used depending on the build of the patient. The S probe (5.0 MHz) is used for small children, the median probe (3,5 MHz) for larger children,

adolescents and adults and the XL probe (2,5 MHz) for obese patients (53). In this study, the M-probe was used in most cases. Only in children in whom the intercostal space was too small for the M-probe, a small probe was used on S2 settings.

The analysed part of the liver is a cylinder about 6 cm deep. The computer shows a picture of the shear wave propagation which should be linear for a good set of data. The validity of a measurement is determined by the machine and if one of the measurements is invalid the machine will tell and not return data. A successful acquisition of data and thus a good set measurements is based upon three criteria: (a) IQR/med < 0,30, (b) there are at least 10 valid shots and (c) valid shots / total shots ≥ 60% (53).

3.4 Statistical Analysis

Analysis was performed with SPSS Statistics v. 24.0. Results were expressed as mean ± SD, or median (IQR) according to their distribution type. Significance was always defined by two-tailed $p < 0,05$. Kolmogorov-Smirnov and Shapiro-Wilk tests were combined to define normality within our variables. If both tests didn't agree, the result of the Shapiro-Wilk test was preferred over Kolmogorov-Smirnov as this test is more robust in smaller populations. Depending on normal distribution, correlation was calculated based upon the Pearson or Spearman correlation coefficient for normally and abnormally distributed parameters respectively. One-sample student-t test and One-sample Wilcoxon signed rank test were used to determine significant difference between a variable of our population and the normal values according to other sources. 2 sample Student t-tests or Mann-Whitney U tests were performed to compare means within a binominal split variable.

4 RESULTS

4.1 Laboratory markers

Results of the most relevant serological markers can be found in **table X (addenda: attachment A)**. Normal values were based upon GUH reference values. Paediatric age specific ranges were found for AST, ALT, AP and total Bb, based upon a review by H.A. Stirnadel-Farrant et al. who examined 5140 children (≤ 18y) from 24 stage II-IV clinical trials (65). Ranges for platelet count was based upon "Pediatric reference ranges (3rd ed.)" by Soldin et al. (66) **(table Y – addenda: attachment A)**

Median **ɣGT** was 30 U/L (21 - 50 U/L) and was increased in 24 (69 %) patients (max. 130 U/L). Yet no significant correlation was found with age nor Fontan interval. Mean **AST** was increased in 11 (31%) patients (max. 53 U/L). Mean **ALT** was increased in 4 (11%) patients (max. 42 U/L). For both AST and ALT, all children who exceeded the upper limit of normal (ULN) were ≤12y yet none of the patients exceeded the ULN more than twofold. **AP** was increased in 1 (3%) patient. Median **total Bb** was 0,8 mg/dL (0,6 – 0,1 mg/dL) with increased values in 6 (17%) patients. Direct Bb was increased in 7 (20%) and indirect in 6 (17%) patients. In literature, only normal paediatric ranges could be found, yet no means ± SD. It was therefore impossible to calculate z-scores for AST, ALT, AP or total Bb. As a result, we could not examine if there is any correlation with age or Fontan interval. Platelet count (**PLT**) was decreased in 6 (17%) patients with a minimum of 19 (10^9/L). Again, as no z-scores could be calculated.

Mean **pro-BNP** was 266,83 ± 463,55 pg/mL and exceeded the upper limit in 14 patients with a maximum of 1900 pg/mL. A gradual decline in value could be seen with increasing age, yet there was no significant correlation. However, we did find a significant difference for ɣGT ($p<0,05$) (**figure 6**) and SMA RI ($p<0,01$) (**figure 7**) between patients who showed normal (<125pg/mL) and increased levels.

Total protein was decreased in 3 (9%) patients and **albumin** was normal in all patients. IgM and IgG were normal in all patients and IgA was decreased in one (3%).

PTT and **INR** were only analysed in 22 patients whose results weren't influenced by the intake of anticoagulants. PTT with a mean of 84% (± 5,74%) was normal in all patients. INR (mean 1,1 ± 0,05) was increased in 10 patients (45%) with a maximum of only 1,19

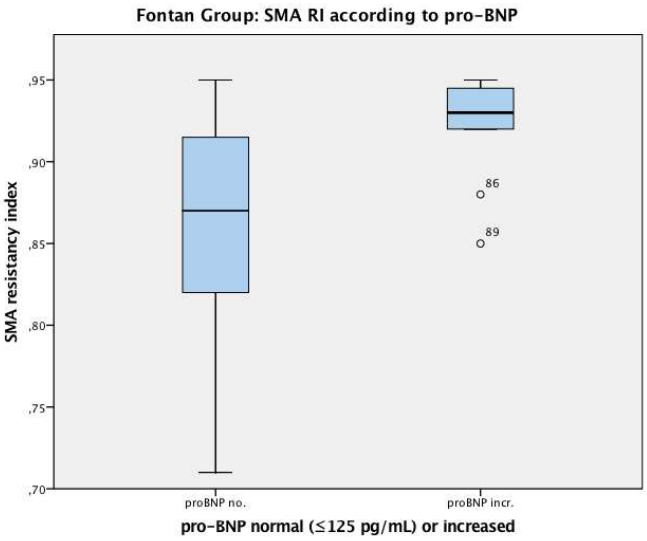

Figure 6: Fontan group - SMA RI according to Pro-BNP

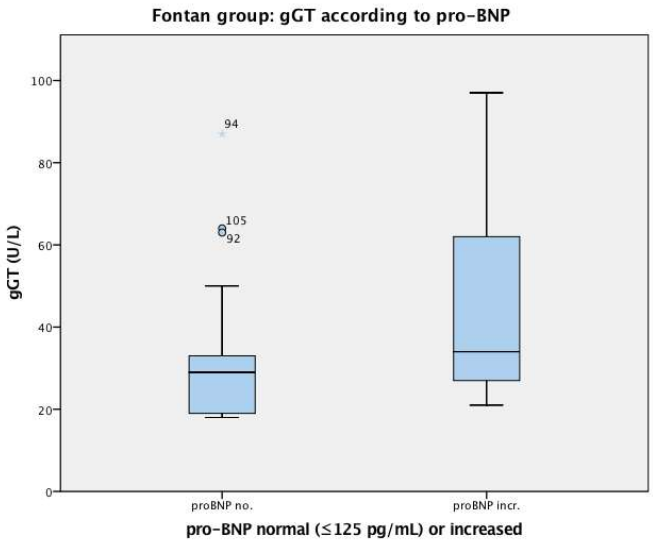

Figure 7: Fontan group - ɣGT according to Pro-BNP

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4.2 Fibrosis scores

Table B: fibrosis scores

Score	Mean (SD) Median (IQR)[a]	cut-off for F2 (F3-4)	Number above F2 (%)
APRI	0,51 (0,40 - 0,65)	≥ 1,5	1
FIB4	0,49 (0,13)	≥ 1,45 (≥ 3,25)	1 (1)
Forns	-0,18 (-1,44 - 1,95)	≥ 4,2 (≥ 6,9)	1 (1)

Table B: Fibrosis scores including APRI score, FIB-4 score and Forns score. SD = standard deviation, IQR = interquartile range, F2 = Fibrosis stage 2

[a] when variable distribution is significantly different from normal distribution for p<0,05 median values were used.

For all three fibrosis scores, there was only one patient who exceeded the cut-off value for METAVIR F2. This patient had an extremely low platelet count of 19*10^9/L which explains the fibrosis score results. However, these fibrosis scores are not validated in a paediatric setting nor a Fontan setting and can therefore not be relied upon.

4.3 US and doppler US

A total of 35 patients underwent US and doppler US of the liver and spleen **(table C).** Results of all variables in comparison with normal values can be found in **table Z (addenda: attachment B)**.

Table C: US and Doppler US results

	N	Mean (SD) Median (IQR) [a]	Range
IVC diameter inspiration (cm)	35	1,1 (1,0 - 1,3)	0,6 – 2,2
IVC diameter expiration (cm)	35	1,1 (1,0 - 1,3)	0,7 – 2,0
IVC diameter in/ex ratio	35	0,17 (± 0,11)	0,0 – 0,4
IVC Collapsibility Index (%)	35	13.7 (±9)	0,0 – 36,4
PV diameter inspiration (cm)	33	0,7 (0,5 – 0,8)	0,4 – 1,0
PV diameter expiration (cm)	33	0,6 (0,5 - 0,7)	0,4 – 0,9
PV mean diameter (cm)	33	0,65 (± 0,14)	0,40 – 0,90
PV min. flow velocity (cm/s)	35	8,3 (5,3 -12,7)	3,7 – 25,2
PV max. flow velocity (cm/s)	35	19,9 (17,1-28,1)	9,4 – 44,0
PV mean flow velocity (cm/s)	35	14,2 (11,9 – 20)	7,1 – 32,9
PV Pulsatility Ratio	35	0,42 (± 0,12)	0,22 – 0,71
PV Resistance Index	35	0,55 (± 0,11)	0,29 – 0,78
HA Resistance Index	35	0,70 (0,66 - 0,72)	0,41 – 0,79
SMA Resistance Index	31	0,90 (0,85 – 0,93)	0,71 – 0,95
HV min. flow velocity (cm/s)	35	-12,0 (-18 - -7)	-27,0 - -4,0
HV max. flow velocity (cm/s)	35	20,0 (16,0 - 25,0)	8,0 – 49,0
HV Pulsatility Ratio	35	-0,52 (-0,70 - -0,40)	-1,35 – 0,90
Dampening Index	30	0,45 (± 0,19)	0,0 – 0,74

Table C: Ultrasound and Doppler Ultrasound results. IVC = inferior vena cava, PV = portal vein, HA = hepatic artery, SMA = superior mesenteric artery, HV = hepatic vein; min. = minimal, max = maximal, SD = standard deviation, IQR = interquartile range [a] when variable distribution is significantly different from normal distribution for $p<0,05$, median values were used.

4.3.1 Liver and spleen morphology

Two (6%) patients showed signs of nodular structures in the liver. However, these structures were further unspecified. Two (6%) other patients showed a slightly irregular liver surface. None of the patients showed signs of ascites or collateral blood vessels.

In our study, the midclavicular diameter of the liver and splenic length was measured in 28 and 33 patients of which 9 (32%) and 5 (15%) showed increased values respectively. Patients with increased diameters were found at all ages, indicating that there is no relation between abnormal diameters and Fontan interval. There was no correlation with PTT nor INR and splenic length, or with bilirubin or other markers of hepatic function and liver size. However, splenic length did correlate with SMA RI ($p < 0,05$; $r^2 = -0,443$).

4.3.2 US Doppler

IVC diameters were increased in inspiration in 2 patients and normal in expiration. Nonetheless, a significantly (P<0,001) lowered mean IVCCI of 13.7% (± 9%) was found, with 22 patients (63%) showing IVCCI values below 17%, the lower limit of normal set by Kutty et al (67).

PV diameters were all within normal range. No patient showed a mean PV diameter of >1,3 cm (68). All patients showed a hepatopetal flow and mean flow velocities were decreased in 19 (54%) of the patients. Besides, the maximum flow velocity was decreased (< 15cm/s) in 6 (17%) patients according to normal values by Narkewicz et al. (69). Moreover, PV PR was significantly smaller ($p < 0,001$) than the normal adult reference. Significant correlation with the Fontan interval was only found for PV min. flow velocity ($p < 0,05$; $r^2 = 0,327$) and PV max. flow velocity ($p < 0,05$, $r^2 = 0,423$).

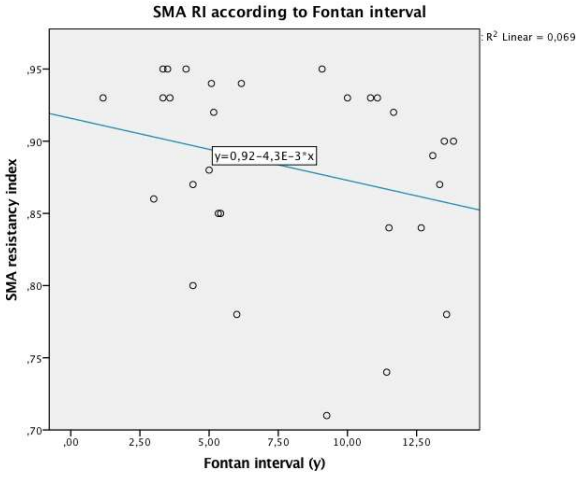

Figure 8: Fontan group - SMA RI according to Fontan interval

There was a significant decrease in **HA** RI with increasing age and Fontan interval ($p < 0,05$; $r^2 = -0,369$). 1 (3%) patient showed an absolute decreased HA RI of 0,41. The mean **SMA** RI showed significantly higher values ($p < 0,05$) compared to normal values. However, it also showed a significant inverse correlation with Fontan interval ($p = 0,05$; $r^2 = -0,356$) **(figure 8).** Furthermore, the SMA RI correlated significantly with the HA RI ($p < 0,05$; $r^2 = 0,416$).

33 patients were examined for **HV** blood flow patterns. 31 patients (94%) had a triphasic waveform pattern. 2 (6%) patients showed alternating biphasic and triphasic waveforms: 1 with bidirectional and 1 with alternating flow direction. Out of the other 31 patients, 2 showed unidirectional blood flow, 28 bidirectional and 1 alternating in flow direction. The HV PR showed no correlation with either age nor Fontan interval. Two patients showed a ratio smaller than 1, indicating inverted flow.

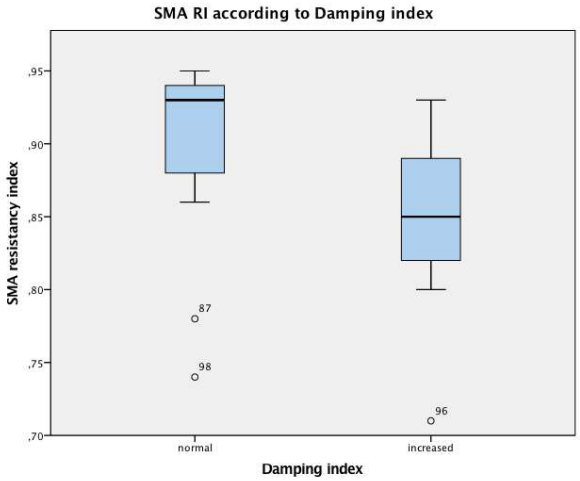

Figure 9: Fontan group - SMA RI according to DI

Damping Index (DI) was used as an alternative for the PV PI. Although not significant, DI showed a steady increase with older age or longer Fontan interval. 8 patients (23%) showed an increased DI (>0,60). A significant inverse correlation between the Damping index (DI) and HA RI ($p < 0,01$; $r^2 = -0,548$) was found. Yet no significant difference in HA RI could be seen between the patients exceeding the upper DI limit (>0,60) or not. SMA RI was also significantly inversely correlated ($p < 0,01$; $r^2 = -0,497$). Yet, SMA RI did differ significantly between patients with normal and increased DI ($p < 0,05$) **(figure 9)**.

4.4 Transient Elastography

4.4.1 Substudy: FibroScan in a healthy paediatric population

A substudy was set up with a control group of 73 children (38 male, 35 female), all aged 5 – 18 years (2012 – 1999). All children were healthy and were not in follow-up in GUH. A FibroScan was performed as well as a measurement of height and weight. No blood analysis nor US with Doppler US was performed in this group. Characteristics can be found in **table D**.

Table D: Characteristics Control group [a]

	5-7y [A]	8-9y [B]	10-11y [C]	12-14y [D]	15+y [E]	**Totaal**
N	21	8	25	13	6	**73**
Male	11	3	13	8	3	**38**
Median Age in years (Range)	7,17 (5,67 – 7,58)	9,67 (8,08 – 9,92)	10,42 (10,0 – 10,92)	13,92 (12,67 – 14,67)	17,42 (15,00 – 18,33)	**10,52 (5,67 – 18,33)**
Length in cm (mean SD)	125,7 (± 5,5)	134,6 (± 5,1)	140,5 (± 6,1)	164,3 (± 5,8)	174,3 (± 10,5)	**142,7 (± 17,3)**
Weight in kg (mean SD)	25,1 (± 3,7)	29,3 (± 4,2)	34,3 (± 5,1)	56,6 (± 11,8)	66,5 (± 8,8)	**38,0 (± 15,2)**
BMI percentile (mean SD)	15,82 (± 1,67)	16,70 (± 1,59)	17,61 (± 2,55)	20,92 (±4,14)	21,83 (± 0,73)	**17,93 (± 3,24)**

Table D: Characteristics of the control group. SD = standard deviation, IQR = interquartile range

[a] when variable distribution is significantly different from normal distribution for p<0,05 , median values were used.

4.4.2 Liver stiffness measurement

Liver stiffness measurement (LSM) results can be found in **table E.** In both the Fontan group as control group, 5 and 3 children were excluded due to the exclusion criteria respectively (*see supra*): IQR/med percentages above 30%.

Table E: Liver stiffness, kPa (FibroScan)

Age Categories		N	Median, kPa (IQR)	Range, kPa	*P – value*
5-7 years	Control	21	3,6 (3,4 – 4,6)	2,0 – 5,2	*P < 0,001*
	Fontan	8	15,0 (12,9 – 18,5)	6,6 - 25	
8-9 years	Control	8	4,1 (3,1 – 4,8)	2,8 – 5,6	*P < 0,01*
	Fontan	3	10,5 (8,2 – 13,7)	7,7 – 10,5	
10-11 years	Control	25	5,0 (4,1 – 5,6)	3,1 – 7,7	*P < 0,01*
	Fontan	3	11,9 (10,2 – 12,0)	9,7 - 12	
12-14 years	Control	10	5,8 (5,0 – 6,9)	4,1-8	*P < 0,001*
	Fontan	9	12,6 (11,6 – 21,0)	10,4 – 23,4	
≥15 years	Control	6	6,8 (5,9 – 9,1)	5,1-9,5	*P < 0,01*
	Fontan	7	13,1 (11,6 – 14,5)	8,9 – 25,7	
Total	**Control**	**70**	**4,6 (3,8 – 5,6)**	**2,0 – 9,5**	*P < 0,001*
	Fontan	**30**	**12,6 (11,4 – 16,2)**	**6,6 – 25,7**	

Table E: Liver stiffness assessment through FibroScan in kPa. kPa = kilopascal, D = standard deviation, IQR = interquartile range

[a] when variable distribution is significantly different from normal distribution for p<0,05 , median values were used.

A significantly different (p < 0,001) variable distribution was found for LSM between the Fontan group and control group, thereby showing a lower median LS in the control group **(Figure 10**). In the control group only 3 (4%) children exceeded the value of 8 kPa, whereas in the Fontan group only 2 (6%) were below this value. Moreover, if ranges per subcategory are compared, the maximum value of the control group never exceeded the minimum value of the Fontan children **(Table E).** Subsequently, when we compared values of control and Fontan group within a same age subcategory, a significant difference (p < 0,01) was present for every age group.

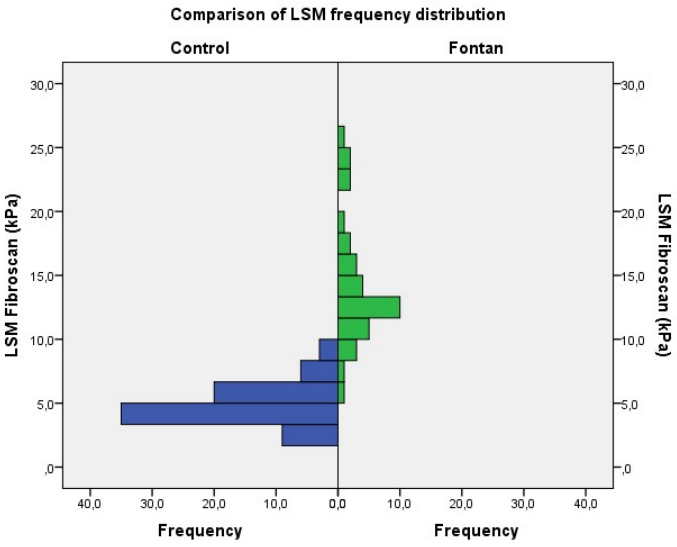

Figure 10: Comparison of LSM frequency distribution between the control group and Fontan group.

In the control group, a linear correlation could be found between LSM and age. The lowest LSM were seen in the youngest children and the highest LS in the oldest children ($r^2 = 0,633$; $p<0,001$) **(figure 11)**. In contrary, no such correlation could be found for the Fontan group between LSM and age **(Figure 12)**.

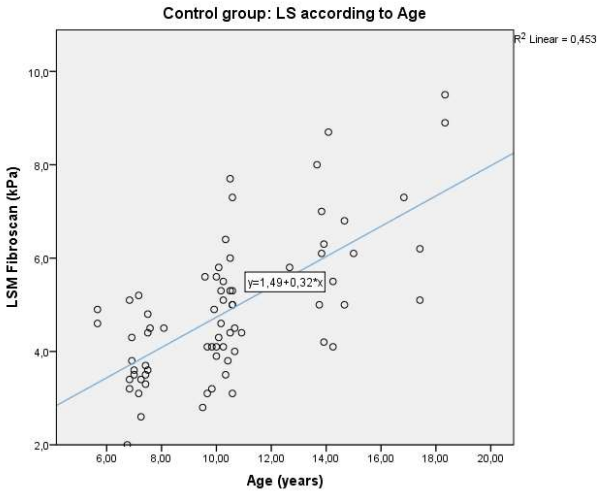

Figure 11: Control group - Liver Stiffness over Age

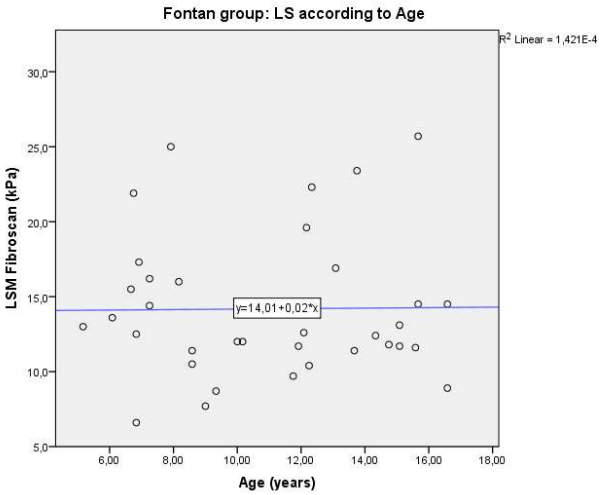

Figure 12: Fontan group – Liver Stiffness over Age

The control group showed strong correlations between LS and length ($p < 0,001$; $r^2 = 0,605$), weight ($p < 0,001$; $r^2 = 0,592$) and thus, BMI ($p < 0,001$; $r^2 = 0,437$), which was not present in the Fontan group.

5 DISCUSSION

5.1 Blood analysis

We aimed to determine certain serological markers which would allow us to detect increased risk of hepatic dysfunction early on. Assessment of serological parameters is inexpensive and easily accessible and gives a general view of the hepatobiliary functionality (70).

ɣGT was the most frequently increased parameter in our population (69%), as is confirmed by multiple studies with research on Fontan patients (63, 71-75). In an adult population, comparable frequencies of ɣGT were found (increased in 74%) (60). However, notice that both Kaulitz (74) and Schwartz et al. (76) showed a significant increase in ɣGT already shortly after TCPC completion in up to 80% of the patients, thereby concluding that ɣGT is elevated early on and does not tend to increase with longer Fontan interval. On the other hand, Shimizu et al. found that ɣGT was significantly increased between patients with and without liver cirrhosis ($p < 0,001$). A possible explanation of this clear increase in ɣGT may be found in the cholestatic pattern: the effect of hepatic congestion upon the vascular supply of the bile ducts results in damage to the bile epithelial cells thereby releasing ɣGT. Serologically this is marked by increased ɣGT in combination with increased AP and / or Bb (73, 74, 77). In this matter, the liver enzyme **AP** might help to discriminate between a hepatic or cholestatic pattern. In the latter, serum AP levels are more markedly increased, yet no specific cut-offs for discrimination were mentioned (45). Only 1 (3%) patient showed increased levels of AP, which is in line with the results of Ackerman et al. (7%). Moreover, a study by Kaulitz et al. (74) showed that there was no significant correlation of ɣGT with other signs of cholestasis such as AP and Bb in a Fontan setting, nor with parameters of hepatic function such as AST and ALT. Conclusively, this proves that the increase in ɣGT in our population is not due to cholestatic damage, nor clear hepatic dysfunction but hepatic congestion. On one hand, it is difficult to define a cut-off value for ɣGT to discriminate between hepatic congestion and fibrosis. On the other hand, ɣGT is frequently increased. Therefore, it is our opinion that ɣGT should be involved in FU. AP on itself is not relevant as a parameter for follow-up, but can be optionally requested if presence of cholestasis is considered.

In our study only 17% showed increased levels of total **Bb** and 20% increased direct Bb, which was clearly less than the results found in adults by Ackerman et al. (31 and 50% respectively) (60). Wu et al. has reported increased serum total bilirubin levels in up to 40% of the adult patients as well, usually below 3.0 mg/dL (78). Little specific information can be found about the cause of this hyperbilirubinemia. Possible explanations, next to the proposed cholestasis,

are hepatocellular dysfunction, obstruction of the hepatic veins or increased arterial stiffness (79), all of which might be consequences of hepatic congestion. Therefore, also Bb should be added in FU as it tends to increase over time, and may be a sign of hepatic dysfunction.

Although increased **AST** and **ALT** are often described in literature concerning hepatic damage, most studies agree that those increases are only seen in a minority of Fontan patients and only rarely outside the normal range or exceeding the ULN more than two- to threefold (71, 74, 75, 80). Our study found similar results. However, ALT was only increased in 11% of the patients (max 42 U/L), where in the study by Ackerman et al. (60) this was the case in 34% (max 89 U/L). There is no conclusive evidence on the relationship between elevated liver enzymes and the severity of hepatic damage (77, 80-83). However, an increase in serum aminotransferases levels may indicate the continuous small hepatocyte injury in Fontan patients or hepatic ischemia due to poor CO in decompensated patients (45). Nonetheless, none of the studies showed significant correlation with Fontan interval, nor did ours. So, whether evolution in ALT levels can be used as a parameter in follow-up for severity of hepatic congestion and / or fibrosis remains unclear, even more so in children.

PLT in our study was decreased in 17% of the patients. This is clearly less than the results of Ackerman et al. (60) who showed a decrease in 30%. Moreover, PLT count was strongly inversely correlated with the Fontan interval. In literature, platelet count has even shown to be inversely correlated to the degree of fibrosis determined by liver biopsy (84). Although PLT is clearly less affected in paediatric setting, FU approach should always include this parameter. As it decreases over time, and has shown its clinical relevance, it may be the first apparent sign of starting fibrosis.

The pathophysiological mechanisms of the consequences of hepatic venous congestion are similar to those of heart failure. Therefore, we also investigated **pro-BNP**. A steady decline in pro-BNP values with increasing age were seen, resulting in near-to-normal values at the age of 16, indicating that pro-BNP is no parameter of progression of hepatic congestion.

Furthermore, it is important to notice that **aFP** as well was within normal range for all patients. This was also the case in the study by Ackerman et al. (60) We can therefore say that it is safe not to perform routine follow-up of aFP. As aFP is a marker of HCC, it is however necessary to examine its evolution in patients who show strong deformations of hepatic morphology or nodularity upon US.

Furthermore, we would like to mention that **total serum protein and albumin** levels in our population were normal in almost all patients. These findings are supported by multiple studies which also indicate that protein synthesis and levels are normal within most patients (45, 60, 74, 75, 77). In general, 10-15% of adult Fontan patients show a decrease in one or both levels (45). However, none of our paediatric patients showed signs of progression of PLE, indicating that PLE is a problem mostly occurring in adulthood with longer Fontan intervals.

5.2 Liver imaging

In order to compare our results with reference values in absence of a control group, a literature study has been conducted. In **table Z (addenda: attachment B)** a summary of the results in comparison to the found reference values can be found.

5.2.1 Ultrasound - morphology

Splenomegaly can be seen as one of the results of elevated portal pressures. It can be the only evidence of portal hypertension though not necessarily present, as absence of splenomegaly does not rule out elevated portal pressures (85). In 5 children (14%) an absolute increase in **splenic length** was measured, however frequency of increased size was not related to age. It is therefore important to evaluate splenic size and its evolution in time for every patient individually.

Liver size on the other hand, was significantly increased in both children <10y ($p < 0,05$) and >10y ($p < 0,001$) compared to reference values mentioned by Amatya et al. (86) and showed 9 (31%) children with values exceeding the ULN. Liver size may be enlarged due to multiple reasons, though hepatic congestion is the most plausible notion. Parameters of fibrosis have not been correlated with this parameter yet.

Nodularity and morphology of the liver have been brought forward in the study by Ackerman et al. (60) as a clinical important element in the assessment of liver modifications. In our population, however only 11% of the children showed abnormalities in nodularity or liver surface, in comparison to 46% in the adult population. Although Ackerman's study showed no significant correlation with Fontan interval, this prognostic parameter should still be included in follow-up. It can indicate liver deterioration at any age and thus, starting follow-up already in a paediatric setting, might be able to mark which children should be further examined early on.

5.2.2 Doppler US – flow patterns

The IVC is a vessel which adapts its diameter according to total body fluid volume (87). Our values showing a subtle but significant increased **IVC inspiration** of 1,1 cm ($p < 0,001$) (67). Moreover, 2 children (6%) exceeded the upper limit of IVC inspiration paediatric diameter set by Kutty et al. (67). These increases in IVCin may thus be indicative for an increased volume status and / or venous congestion thereby dilating the vessel to the limits of its elasticity. However, the relation between diameter widening and its repercussion on the liver are of most clinical relevance. A significant correlation between IVC diameter and LS ($p < 0,05$; $r^2 = 0,35$;) was found by Yoo et al. (88), with IVC diameters ranging from 1,3 cm to 3 cm. In our population, no such correlation was present yet and IVC diameters were also particularly lower. Notice that our population was younger, and BMI was lower. If the IVC diameter is evaluated in comparison with LS over a period of time, it might have clinical value. However, further research should be conducted, before implementing this parameter in a FU approach.

Consecutively, the **IVCCI** (mean 13,7 ± 9%) showed significantly lower values in comparison with the reference values (30%) (67). As the IVCCI is broadly used in the assessment of congestive heart failure (89), the assumption can be made that comparable lowered IVCCIs are due to similar physiology, by name congestion. However, the adult population of Ackerman et al. (60) showed values with tendencies closer to normal (24%). Conclusively, although levels are severely deteriorated, the relation between IVCCI and liver modification remains unclear. Moreover, the adult Fontan values, although limited, tend to normalise over time, which make follow-up of the IVCCI unnecessary.

The **PV diameter** may be enlarged due to venous congestion in every Fontan circulation. If the adult upper normal limit of 1,3 cm is exceeded, this might be indicative for portal hypertension (68). Paediatric values were not found, however one can expect that when a child exceeds the adult upper limit, PPV of this cut-off value increases. Most importantly, none of our patients exceeded this value. Furthermore, specificity of diagnosis with mentioned cut-offs in an adult population is said to be only 40% (68). We therefore advise that until further research upon paediatric cut-off values for PV diameters is conducted, this parameter should not be implemented in a FU approach in children.

PV mean flow velocity may be reduced as high outflow resistance due to congestion and a low CO are present. The proposed paediatric normal values (31,0 ± 4,1 cm/s) by Gorka et al. (90) were significantly higher ($p < 0,001$) than our Fontan values (16,5 ± 6,8 cm/s). However, broadly used adult PV mean flow velocities range between 13 - 23 cm/s (85, 91). Zironi et al.

(92) considered mean flow velocities <15 cm/s as the best cut-off value for detection of portal hypertension (sens. 88%, spec. 96%). Nonetheless, as normal adult values are significantly lower than the paediatric ones, one might hypothesize that the cut-off value for portal hypertension in children lies higher. Based upon Zironi et al., 19 children (54%) in our population showed values correlated with portal hypertension. However, this might be an underestimation.

Also, the **PV max. flow velocity** can indicate portal hypertension if flow is beneath 15 cm/s (69). This cut-off would imply that only 6 children (17%) were exposed to portal hypertension, but clinical application of this parameter is less frequent than PV mean flow (85).
We would advise to introduce PV flow velocities in our follow-up approach as max. and mean PV flow are already severely restricted in respectively 17 and 54 % of the patients. However, further research upon normal paediatric values should be conducted primarily.

The **damping index (DI)** was calculated in order to assess the grade of flow dampening of the PV, which is caused by relative venous outflow obstruction e.g. fibrosis (93). 8 patients (23%) exceeded the cut-off value of 0,6 (max. 0,78) indicating portal hypertension (PPV 91.9%, NPV 58.1%) (93). Clinical relevance will be explained further down in correlation with SMA RI and HA RI (cfr. Infra).

Flow in the **HV** was investigated as well. In general, a triphasic waveform was seen (94, 95). however in the presence of a hepatic congestion, we expect Doppler US to show abnormalities such as less pulsatile flow or loss of the normal triphasic waveform e.g. hepatofugal (reversed) flow (95). In general, the repercussions of a cardiac disease on the liver are due to fibrosis: parenchymal changes with hypertrophy of the hepatocytes causes decreased compliance of the tissue thereby exerting a compressive effect on the HV due to the limited capacity of the liver capsule to stretch (96). In a Fontan circulation, hepatic congestion can contribute to this non-compliance/compressive situation as well. However only 2 patients (6%) had deviating **HV wave forms** (alternating triphasic-biphasic). None of these children presented with a monophasic waveform, although this has been found to be positively correlated with the presence of portal hypertension (sensitivity 74% specificity 95%) (94, 97).

The SMA is a high-resistance vessel at resting state inhibiting diastolic blood flow, as the distal vascular bed requires only intermittent or on-demand high blood supply (e.g. post prandial – as this influences resistance, all patients were examined in fasting state (91, 98)). In our Fontan setting, a significant increase ($p < 0,01$) in **SMA RI** could be found between our population (mean 0,88 ±0,06) and the adult reference value of 0,85 (99). Kutty et al. (17) found

comparable values of 0,89, significantly higher than (their) paediatric control values (0,84). In the adult Fontan study by Ackerman et al. (60) SMA RI showed increased values as well, however more subtle (0.86 ± 0.09).
These chronic elevated SMA RIs can be explained by the reduced CO as a result of the Fontan circulation. The superior mesenteric artery area tries to compensate loss of output by reducing blood flow in the distal vascular bed, in order to maintain flow levels in the vital organs (35). However, 1 patient (13 years) presented with an extremely (under 2,5 percentile) lowered SMA RI (0,7). This decreased value is of most clinical relevance as it may indicate a failing Fontan circulation: vasodilation sets in as a result of an inevitable situation of hyperaemia in the failing Fontan, reducing the SMA vascular resistance (100). Moreover, when Fontan interval increases, SMA RI is shown to decrease (r^2 = -0,356; p = 0,05). This correlation can thus explain why the mean SMA RI found in the adult Fontan population was slightly lowered in contrast with observed paediatric values (60).
Consequently, SMA RI can be put forward as an important US doppler parameter that should be assessed longitudinal to provide early information on the Fontan status. Notice, SMA Pulsatility Index (PI) remains more sensitive for detection of early impedance changes than SMA RI (101). Yet, in our study, only SMA RI was calculated.

Consecutively, the **HA RI** will exhibit the same tendencies. Values tend to decrease over time, by which older children and longer Fontan intervals showed lowest HA RI. The same patient with a significantly lowered SMA RI, showed a HA RI inferior to the lower limit of normal. It is thus advised to assess HA RI in addition to SMA RI.

Lastly, higher DI were correlated with lower SMA RI and HA RI, which confirms that when the failing Fontan sets in, portal hypertension can be additionally present. DI can accordingly be clinically relevant to assess the Fontan condition, however this parameter is not widely used and use of the PV PI is advised when possible.

5.3 Liver stiffness

FibroScan has been brought forward in the assessment of liver fibrosis as a non-invasive diagnostic technique. Measurements of LS by TE has shown to be a useful approach in the evaluation of liver fibrosis in multiple chronic liver diseases (CLD). Several studies have reported that LS showed a significant correlation with increasing degree of fibrosis according to the METAVIR score, in populations with CLD of different aetiologies (54, 102-105). More recent studies have started research upon the use of TE in Fontan patients (88, 106, 107).

Deorsola et al. (106) noticed a rise in TE values from 6,2 ± 1,5 kPa to 11,2 ± 4 kPa between preoperative LSM and 4 months after TCPC completion. This may indicate that shortly after the Fontan introduction LS is directly dependent upon intravenous pressures. Even in the absence of fibrosis increased LS measurements can already be observed, indicating that FibroScan on its own might not be suitable to differentiate between fibrosis and congestion.

In our population with a longer Fontan interval, LS remained as high, in some cases even more elevated. Yet the increasing line (5 kPa per 4 months) as described by Deorsola et al. (106) did not persist. Levels of LS remained significantly higher than the healthy control values in every age category. A median of 12,7 kPa, with 33 (94%) children above 8 kPa indicates that the liver is under more stressful circumstances throughout the whole childhood.

As liver biopsy is not performed, there is no 100% certainty of the meaning of the elevated LS. Whether there is presence of fibrosis or a continuing process of liver congestion, measurements should be looked at individually in addition with other investigations. In this study however, liver elasticity was only evaluated once. Based on the individual differences between children, and the rarity of defined fibrosis already being present at a young age, our values cannot be evaluated yet. Evaluation of measurements on a longitudinal scale are advised. Moreover, no agreement could be made whether LS correlated significantly with Fontan interval or not. Worse stages of fibrosis, determined by imaging, have been associated with longer Fontan interval (44, 49, 108), but both young age and small sample size may contribute to the disagreement in correlation in this study.

If FibroScan would be implemented in a standard follow-up protocol, frequent examination would be necessary for clinical relevance. The importance of the evaluation of values over time, cannot be emphasized enough. If values would progress excessively, without any correlation with age or time, we advise further diagnostic techniques (US, Doppler US and blood analysis) to be implemented to evaluate the underlying cause. These individual approaches can optimize the use of LSM in a clinical setting. Although TE was not able to discriminate between hepatic fibrosis and venous hepatic congestion in a Fontan setting in previous studies (88, 106, 107), specification of time-related change of liver modification by FibroScan, with US and blood analysis in addition, might still be a useful follow-up approach.

5.4 Fontan follow-up strategy

Although more studies are required to assess the clinical evidence of each of the tests performed, we feel that in every investigation there are parameters with potential in the assessment of developing liver fibrosis. However, the importance of serial follow-up cannot be emphasized enough.

Serological parameters (and derived existing fibrosis scores) have shown not to be able to diagnose current fibrosis in a paediatric population. Furthermore, all parameters have shown only small deviations from the reference values. Nonetheless, some parameters are worth evaluating because their evolution in time (including childhood and adulthood) might be an important indicator of developing fibrosis. Those parameters are γGT (as an indicator of hepatic congestion, differentiated by AP), PLT (as an indicator of liver fibrosis) and Bb (as an indicator of deteriorating liver functionality). AST should not be evaluated routinely because of the normal gradual decline with increasing age in children and its relatively lower liver specificity in comparison with ALT. ALT might be of use, though further research upon its clinical significance in the matter of fibrosis and correlation with the Fontan interval is required. aFP should only be investigated upon abnormal US morphological findings of the liver or results of other investigations clearly indicating developing fibrosis.

Regarding the US of liver and spleen, interpretation of absolute values is difficult due to the lack of paediatric reference values. Nonetheless, we ought it apt to perform US Doppler every two to three years in order to assess evolution. Parameters included should be liver and spleen morphology, since they are a very good indicators of liver deforming diseases such as fibrosis or HCC. Furthermore, PV flow velocity (mean and maximum), SMA RI and HA RI should definitely be included as they are indicators of the severity of hepatic congestion and have shown to be clearly aberrant from reference values. IVCCI is optional as it as a good indicator of the risk of hepatic congestion, however tends to normalize over time.

TE through FibroScan might be useful in FU, although values are already increased shortly after TCPC completion and tend not to increase over time. Moreover there is a current lack of ability to differentiate between hepatic congestion and fibrosis. Nonetheless, evaluation of the individual evolution of LS over time might indicate a deteriorating hepatic situation. Since FibroScan is very easy to use and does not take up a lot of time, we therefore advise to perform this investigation at the six-monthly FU with the new LS as a baseline value. However, one should remember that LS tends to increase with older age. Therefore, increases should always

be compared to normal increases. If LS increases too fast or too excessively, further investigation with US Doppler is advised.

5.5 Future prospects

Several studies have been conducted upon the hepatic consequences of Fontan patients. Both adult and paediatric populations have been examined, some studies including both. However, all studies have been cross-sectional at one point in time. Not a single longitudinal cohort study has been conduct so far. Furthermore, all studies, including ours, were based upon a relatively small population. Therefore, it is difficult to extrapolate findings of each study. Besides, those small populations lead to discrepancies in results, which also make it difficult to extrapolate findings of multiple studies through a review. We therefore advise to conduct further research upon hepatic parameters in larger populations and in a longitudinal follow-up setting.

In future research, a minimal set of parameters should be included. Serological parameters such as γGT, Bb, PLT and ALT have been shown to be related to hepatic deterioration in Fontan patients. Next to those parameters, although not investigated in our study, we also advise to perform evaluation of the clotting profile and hyaluronic acid, since they have also shown to be related to hepatic damage in Fontan patients according to literature. US and Doppler parameters such as liver and spleen morphology, IVCCI, PV flow velocities, HA RI and SMA RI should be evaluated as should the PV pulsatility index (although not investigated in our study). However, paediatric reference values of those US parameters are rare to non-existing. It is therefore recommended to perform future studies in the presence of a control group or to conduct separate research upon paediatric reference values.

TE through FibroScan, being non-invasive, time-efficient and easy to use, should be implemented in every future study upon liver fibrosis. Clear Fontan-interval specific cut-off values for LS should be determined as has been done for other CLDs. Furthermore, longitudinal FU studies should be conducted to assess the correlation of LS to degree of hepatic congestion and / or fibrosis.

In an ideal situation, a Fontan specific fibrosis score might be created based upon parameters mentioned above including the time post Fontan. This score might be able to combine results of different tests to differentiate between different stages of congestion to fibrosis or even cirrhosis. Further research on this hypothesis, remains to be conducted.

5.6 Limitations

As UVH diseases are rare, it is hard to gather a large population sample. Many discrepancies with literature might well be due to this relatively limited sample size. This should be kept in mind at all time when interpreting our results. Our UVH population only contained 8 girls (23%) which made it impossible to generalize results concerning differences in gender. In our group of healthy controls, all children were gathered with the inclusion criterium of being in good health with no hepatic diseases. However, no objective assessment of perfect health through laboratory tests or US was made.

US and Doppler US were always performed by the same radiologist, thereby eliminating interobserver variability. However, no control group was set up to evaluate laboratory markers and US and Doppler US results. As literature concerning normal age specific paediatric values, especially for US and Doppler US, are rare, clinical interpretation of our results was challenging. Moreover, studies specifying in the follow-up of a paediatric Fontan setting are limited as well.

TE is a very easy to learn and quick to use method yet acquisition of results might sometimes be disturbed due to patient build (especially if too small or obese) or excessive movement. We therefore had to exclude 5 out of 35 patients (IQR/med > 30%). Inappropriate use of adult M-probes on children however plays a role in the LSM as well. Goldschmidt et al. (109) proved that LS measurements were significantly higher with S1 compared to S2 and S2 compared to M-probes. It is therefore safe to say that the largest probe possible should be used at all time. FibroScan is specifically apt for lean patients with high degrees of fibrosis or cirrhosis, which limits the diagnostic value in young Fontan patients. As paediatric Fontan patients usually might have no to low grades of liver fibrosis, we therefore advise to limit the number of examiners. (54), (110)

Lastly, liver biopsy was not performed, therefore we could never assess the degree of fibrosis with a 100% certainty.

6 CONCLUSION

Serological parameters (ɣGT, Bb, PLT), US Doppler (liver and spleen morphology, PV flow velocities, HA and SMA RI) have shown their worth in the evaluation of hepatic congestion and liver fibrosis in a paediatric setting. So did TE through FibroScan as well: although LS increases early after TCPC completion and tends to stabilize over childhood, continuing and excessive increase might indicate further investigations.

However, even with a combination of all parameters, it remains hard to determine the current degree of fibrosis. The biggest asset of those investigations is in the combined results of their evolution in time. Nonetheless, although several parameters were already increased, frequency and severity of deviant values were clearly less compared to adults, indicating a smaller progression to liver fibrosis in children. We therefore recommend to continue frequent FU of Fontan patients as from the moment at which TCPC in completed. Serological parameters and FibroScan should be performed at the 6-monthly FU, while US Doppler should be performed every two to three years. Further research upon paediatric reference values of US Doppler and FibroScan should be conducted as should studies developing a Fontan specific fibrosis score.

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8 ADDENDA

8.1 Attachment A: Table X – Serological markers

Table X: Serological markers

Laboratory Marker	N	Mean (SD) Median (IQR) [a]	Min. – Max.	Normal Value	Number abnormal (%)
Htc (%)	35	42,9 (40,4 - 45,3)	35,7 – 52,7	M: 41 - 53 V: 36 - 46	14 (40%)
PLT (10^9/L)	35	217,29 (±13.98)	19 - 470	*See table y*	7 (20%)
WBC (10^9/L)	35	5,81 (4,82 - 7,41)	3,37 – 25	3.5 - 10	4 (11%)
Fe (µg/dL)	35	93 (74 - 107)	25 - 255	37 - 135	5 (14%)
Ferritin (µg/L)	35	55 (34- 70)	6 – 223	7 - 142	3 (9%)
Transferrin (g/L)	35	2.92 (±0,06)	2,33 – 3,74	2 - 3,6	1 (3%)
TIBC (µg/dL)	35	358,29 (±7,06)	286 – 459	246 - 442	1 (3%)
PTT (%)	23[b]	84 (80 - 86)	74 - 96	70 - 100	0 (0%)
INR	23[b]	1,1 (1,07 - 1,13)	1 – 1,19	*0,9 -1,1*	9 (45%)
Bilirubin (mg/dL)	35	0,8 (0,6 - 1)	0,3 – 4	*See table y*	6 (17%)
Direct Bb (mg/dL)	35	0,35 (±0,02)	0,14 – 0,64	≤ 0,45	7 (20%)
Indirect Bb (mg/dL)	35	0,43 (0,32 - 0,62)	0,10 – 3,58	0,1 - 0,8	6 (17%)
AST (U/L)	35	34,63 (±10,02)	14 – 53	*See table y*	12 (34%)
ALT (U/L)	35	25,29 (±8,03)	7 - 42	*See table y*	5 (14%)
ɣGT (U/L)	35	30 (21 - 50)	18 – 130	4 – 24	24 (69%)
Alkaline phosphatase (mg/L)	35	235,03 (±69,07)	63 – 371	*See table y*	1 (3%)
Total protein (g/L)	35	69,47 (±0,91)	55 – 81	63 – 79	4 (11%)
Albumin (mg/dL)	35	46,44 (±0,44)	40 – 51	35 - 52	0 (0%)
CRP (mg/L)	35	0,6 (0,6 - 0,7)	0,6 – 50,2	< 0,6	10 (29%)
Triglycerides (mg/dL)	35	57 (46 - 87)	34 – 272	30 - 101	7 (20%)
Apolipoprotein A1 (mg/dL)	35	139 (128 - 152)	64,3 – 181	110 - 205	1 (3%)
Cholesterol (mg/dL)	35	143,62 (±2,73)	84 – 180	121 - 203	11 (31%)
pro-BNP (pg/mL)	35	92 (49 - 229)	20 – 1900	≤ 125	14 (40%)
aFP (µg/L)	35	1,60 (1,27 - 2,58)	0,838 – 9,150	0 - 15	0 (0%)

Table X: Serological markers. Hct = Hematocrit, PLT = platelet count, WBC = white blood cell (count), TIBC = Total Iron Binding Capacity, PPT = Partial thromboplastin time, INR = internation normalized ratio, Bb = bilirubin, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ɣGT = gamma glutamyl transferase, CRP = C-reactive protein, Pro-BNP = Brain natriuretic peptide, aFP = alpha-fetoprotein.

[a] when variable distribution is significantly different from normal distribution for p<0,05 , median values were used.

[b] only patients who were not currently taking anticoagulants were included

8.1.1 Table Y - Normal paediatric values for AST, ALT, AP, Bb and PLT

Table Y: laboratory paediatric markers AST, ALT, AP, Bb and PLT					
Laboratory Markers	**BOYS**		**GIRLS**		**TOTAL**
	Normal values	**No° of abnormal**	**Normal values**	**No° of abnormal**	**No° of abnormal**
AST (U/L)[a]					**12**
5-8 y	20 – 43	8	19 – 41	0	8
9 – 11y	17 – 39	3	16 – 36	1	4
12 – 16y	15 – 40	0	13 – 34	0	0
≥ 16y	14 – 45	0	10 – 34	0	0
ALT (U/L) [a]					**5**
5-8 y	8 – 34	3	8 – 33	0	3
9 – 11y	8 – 38	1	3 – 30	1	2
12 – 16y	8 – 45	0	7 – 31	0	0
≥ 16y	8 – 63	0	6 – 3	0	0
AP (mg/L) [a]					**1**
5-8 y	147 – 728	0	143 – 869	0	0
9 – 11y	126 – 713	0	105 – 730	0	0
12 – 16y	97 – 571	0	70 – 466	1	1
≥ 16y	61 – 350	0	36 – 184	0	0
Bb (mg/dL) [a]					**6**
5-8 y	0,18 – 0,88	2	0,18 – 0,99	0	2
9 – 11y	0,23 – 1,05	1	0,18 – 1,28	0	1
12 – 16y	0,23 – 1,28	2	0,18 – 1,46	1	3
≥ 16y	0,29 – 1,57	0	1,18 – 1,52	0	0
PLT (10^9/L) [b]					**7**
2 – 6y	204 – 405	1	204 – 402	0	1
6 – 11y	194 – 364	3	183 – 369	0	3
12-16y	105 – 332	0	185 – 335	3	3

Table Y: laboratory marker result and specified reference values of AST, ALT, AP, Bb and PLT. No° = number; AST = aspartate aminotransferase, ALT = alanine aminotransferase, AP = alkaline phosphatase, Bb = bilirubin, PLT = platelet count.

[a] Stirnadel-Farrant HA, Galwey N, Bains C, Yancey C, Hunt CM. Children's liver chemistries vary with age and gender and require customized pediatric reference ranges. Regulatory Toxicology and Pharmacology. 2015;73(1):349-55.

[b] Soldin SJ, Brugnara C, Wong EC. Pediatric reference ranges: Amer. Assoc. for Clinical Chemistry; 2003.

8.2 Attachment B: table Z – US and Doppler US results

Table Z: US and Doppler US results in comparison with normal values						
Variable *(reference)*		**Normal value**	**N**	**Mean (SD) Median (IQR) [a]**	**Min. – Max.**	**Number of abnormal**
Splenic length (cm)			35	**9,59 (±2,23)**	**5,9 – 15,8**	**5**
Rosenberg et al., 1991 (111)	**<8y**	max. 9,5	10	7,8 (±1,4)	5,9 – 10,0	2
	<10y	max. 10	5	8,5 (±1,5)	7,2 – 10,4	1
	<12y	max. 11	2	9,70 (±2,07)	7,8 – 11,9	1
	<15y	max. 11,5	9	10,66 (±1,38)	8,8 – 12,9	2
	≥15y	max 12 (F) or 13 (M)	6	11,41 (±2,4)	8,6 – 15,8	1
midclavicular hepatic diameter (cm)			29	**11,40 (±1,60)**	**8,4 – 14,4**	**9**
Amatya et al., 2014 (86)	**<10y**	9,29 (± 0,91) Min. 8,0 – max.10,96	13	10,4 (±1,4)	8,4 – 13	4
	≥10	10,70 (± 1,11) Min. 8,30 – max. 13,30	16	12,5 (±1,3)	10,4 – 14,4	5
IVC inspiration (cm) *Kutty et.al, 2014 (67)*		0,89 Max. 1,92	35	1,1 (1,0 - 1,3)	0,6 – 2,2	2
IVC expiration (cm) *Kutty et.al, 2014 (67)*		1,21 Max. 2,44	35	1,1 (1,0 - 1,3)	0,7 – 2,0	0
IVCCI (%) *Kutty et.al, 2014 (67)*		30 Min. 17 – max 64	35	13.7 (±9)	0,0 – 36,4	22
PV diameter (cm)		**0,7 (± 0,19)** Min. 3,0 - Max. 1,12	34	**0,65 (± 0,14)**	**0,40 – 0,90**	0
Soyupak et al., 2010 (112)	**81-100cm**	0,641 (± 0,13)	1	0,70	0,70	
	101-120cm	0,755 (± 0,125)	5	0,53 (± 0,08)	0,45 – 0,65	
	121-140cm	0,805 (± 0,13)	10	0,61 (± 0,14)	0,40 – 0,85	
	> 141 cm	0,904 (± 0,12)	18	0,71 (± 0,13)	0,50 – 0,90	
PV max. flow velocity (cm/s) *Soyupak et al., 2010 (112)*		Min. 15	35	19,9 (17,1-28,1)	9,4 – 44,0	6
PV mean. Flow velocity (cm/s) *Chavhan et al., 2008(90, 91)Gorka et al., 1996 (87)*		18,0 / 31,0 Min. 15	35	14,2 (11,9 – 20)	7,1 – 32,9	19
PV pulsatility ratio *Gallix et al., 1997 (113)*		0,48 (± 0,31)	35	0,42 (± 0,12)	0,22 – 0,71	
HA resistance index *Chavhan et al., 2008 (91)*		0,62 – 0,74 Min. 0,55 – max. 0,81	35	0,70 (0,66 - 0,72)	0,41 – 0,79	1
SMA resistance index *Taourel et al., 1998 (99)*		0,85	31	0,90 (0,85 – 0,93)	0,71 – 0,95	
Damping Index *Kim MY et al., 2007 (93)*		<0,06	30	0,45 (± 0,19)	0,0 – 0,74	8

Table Z: Ultrasound and Doppler Ultrasound results of the Fontan children in comparison with normal values. IVC = inferior vena cava, IVCCI = inferior vena cava collapsibility index, PV = portal vein, HA = hepatic artery, SMA = superior mesenteric artery.

[a] when variable distribution is significantly different from normal distribution for p<0,05 , median values were used.

8.3 Attachment D: Blood analysis

UZ GENT

Invloed van fontancirculatie op de lever **PCARDIO**

T.a.v. Laboratorium voor Klinische Biologie
DE PINTELAAN 185 B-9000 GENT
Nr. 8-44700-73-383

Patientgegevens of adrema:

Datum afname : .....

Uur : .....

Aanvragende arts : Prof. Dr. K. François

R.I.Z.I.V. nr. : **1-44380-53-140**

Handtekening :

Aangevraagde testen

Batterijcode
SFON

Aanduiden of invullen wat past

O Visit =

E	Hematocriet	S	Glucose
E	Leucocyten	S	Creatinine
E	Thrombocyten	S	Ureum
E	WBC differentiatie	S	Bilirubine (totaal+dir)
		S	Totaal eiwit
E	Pro-BNP	S	Albumine
		S	CRP
S	Alfa-2-macroglobuline	S	AST / ALT
S	Apolipoproteïne A1	S	Alkalische fosfatase
S	IgA	S	GGT
S	IgM	S	LDH
S	IgG	S	CK
S	Ceruloplasmine	S	Cholesterol
S	Alfa-1-antitrypsine	S	Triglyceriden
S	Haptoglobine	S	Alfa-foetoproteïne
		S	Ijzer
S	ANF	S	Transferrine-TIBC
S	ASMA	S	Ferritine
S	AMA	S	Natrium
		S	Kalium
C	PTT/INR	S	Calcium
		S	Chloride
U	Urinestick	S	Bicarbonaat
U	Urinesediment		
		S	TSH
		S	HBsAg
		S	anti-HCV
		S	anti-HIV

Afname (<18j): 2 tubes EDTA (2mL) + 1 tube citraat + 2 tubes serum (met gel) (8mL) + 1 optrekspuit urine

Afname (>18j): 2 tubes EDTA + 1 tube citraat + 4 tubes serum (met gel) + 1 optrekspuit urine

Fact. Adres
SFON

DRAFT

8.4 Attachment D: Protocol US liver/Abdomen

Examination in **supine** position**.**

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 (= minimal flow velocity/maximal flow velocity)
   c. HA/SMA
      i. Resistance index (= peak systolic velocity – end diastolic velocity/ peak systolic velocity)
   d. HV
      i. Ratio of hepatic vein flow (=V2/V1)
         1. V1= peak velocity towards the heart
         2. V2= reverse flow towards the liver

8.5 Attachment E: Ethical Committee

Universitair Ziekenhuis Gent

UNIVERSITEIT GENT

Att: Commissie voor Medische Ethiek (UZP074)

IZ Cardiochirurgie
Kliniekgebouw K12 E - 5e verdieping
Prof. dr. Katrien FRANÇOIS
ALHIER

COMMISSIE VOOR MEDISCHE ETHIEK

Voorzitter:
Prof. Dr. D. Matthys
Secretaris:
Prof. Dr. J. Decruyenaere

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UW KENMERK	ONS KENMERK	DATUM	KOPIE
	2016/1107	09-dec-16	Zie "CC"

BETREFT Advies voor monocentrische studie met als titel:
De invloed van een fontancirculatie op de lever op kinderleeftijd.
Belgisch Registratienummer: B670201629625
Fase (Phase): NVT/NA

* Adviesaanvraagformulier dd. 9/09/2016 (volledig ontvangen dd. 26/09/2016)
* Begeleidende brief dd. 20/07/2016
* (Patiënten)informatie- en toestemmingsformulier
  Voor de ouders
  voor kinderen ouder dan 12jaar (aanpassingen 11.11.2016)
* Protocol
  Echo lever
  elastografie lever
* Diverse
  Aanvraagformulier bloedafname
* Antwoord onderzoekers
  Ontvangen via mail Hazel Van Overschelde dd. 8/12/2016 in antwoord op opmerkingen EC dd. 19/10/2016

Advies werd gevraagd door: Prof. dr. K. FRANÇOIS ; Hoofdonderzoeker

BOVENVERMELDE DOCUMENTEN WERDEN DOOR HET ETHISCH COMITÉ BEOORDEELD.
ER WERD EEN POSITIEF ADVIES GEGEVEN OVER DIT PROTOCOL OP 8/12/2016. INDIEN DE STUDIE NIET WORDT OPGESTART VOOR 8/12/2017, VERVALT HET ADVIES EN MOET HET PROJECT TERUG INGEDIEND WORDEN.
Voorafgaand het onderzoek te starten dient contact te worden genomen met Bimetra Clinics (09/332 05 00).

THE ABOVE MENTIONED DOCUMENTS HAVE BEEN REVIEWED BY THE ETHICS COMMITTEE.
A POSITIVE ADVICE WAS GIVEN FOR THIS PROTOCOL ON 8/12/2016. IN CASE THIS STUDY IS NOT STARTED BY 8/12/2017, THIS ADVICE WILL BE NO LONGER VALID AND THE PROJECT MUST BE RESUBMITTED.
Before initiating the study, please contact Bimetra Clinics (09/332 05 00).

DIT ADVIES WORDT OPGENOMEN IN HET VERSLAG VAN DE VERGADERING VAN HET ETHISCH COMITE VAN 20/12/2016
THIS ADVICE WILL APPEAR IN THE PROCEEDINGS OF THE MEETING OF THE ETHICS COMMITTEE OF 20/12/2016

° *Het Ethisch Comité werkt volgens 'ICH Good Clinical Practice' - regels*
° *Het Ethisch Comité beklemtoont dat een gunstig advies niet betekent dat het Comité de verantwoordelijkheid voor het onderzoek op zich neemt. Hieruit dient U er over te waken dat Uw mening als betrokken onderzoeker wordt weergegeven in publicaties, rapporten voor de overheid enz., die het resultaat zijn van dit onderzoek.*
° *In het kader van 'Good Clinical Practice' moet de mogelijkheid bestaan dat het farmaceutisch bedrijf en de autoriteiten inzage krijgen van de originele data. In dit verband dienen de onderzoekers ervoor te waken dat dit gebeurt zonder schending van de privacy van de proefpersonen.*
° *Het Ethisch Comité benadrukt dat het de promotor is die garant dient te staan voor de conformiteit van de anderstalige informatie- en toestemmingsformulieren met de Nederlandstalige documenten.*
° *Geen enkele onderzoeker betrokken bij deze studie is lid van het Ethisch Comité.*
° *Alle leden van het Ethisch Comité hebben dit project beoordeeld. (De ledenlijst is bijgevoegd)*

J.

Muriel Fouquet
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UW KENMERK	**ONS KENMERK**	**DATUM**	**KOPIE**
	2016/1107	09-dec-16	Zie "CC"

Vervolg blz. 2 van het adviesformulier betreffende project EC UZG 2016/1107

- *The Ethics Committee is organized and operates according to the 'ICH Good Clinical Practice' rules.*
- *The Ethics Committee stresses that approval of a study does not mean that the Committee accepts responsibility for it. Moreover, please keep in mind that your opinion as investigator is presented in the publications, reports to the government, etc., that are a result of this research.*
- *In the framework of 'Good Clinical Practice', the pharmaceutical company and the authorities have the right to inspect the original data. The investigators have to assure that the privacy of the subjects is respected.*
- *The Ethics Committee stresses that it is the responsibility of the promotor to guarantee the conformity of the non-Dutch informed consent forms with the Dutch documents.*
- *None of the investigators involved in this study is a member of the Ethics Committee.*
- *All members of the Ethics Committee have reviewed this project. (The list of the members is enclosed)*

Namens het Ethisch Comité / On behalf of the Ethics Committee

Prof. dr. D. MATTHYS
Voorzitter / Chairman

CC: De heer T. VERSCHOORE - UZ Gent - Bimetra Clinics
FAGG - Research & Development; Victor Hortaplein 40, postbus 40 1060 Brussel

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Universitair Ziekenhuis Gent

COMMISSIE VOOR MEDISCHE ETHIEK
Voorzitter:
Prof. Dr. D. Matthys
Secretaris:
Prof. Dr. J. Decruyenaere

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UW KENMERK	ONS KENMERK	DATUM	KOPIE
	2016/1107	07-jul-17	Zie "CC"

BETREFT

Advies Amendement voor studie met als titel:
De invloed van een fontancirculatie op de lever op kinderleeftijd.

Belgisch Registratienummer: B670201629625
Fase (Phase): NVT/NA

Geachte collega,

Wij ontvingen uw aanvraag op 5/07/2017 voor een amendement betreffende bovenvermelde studie. Het betreft:

- Begeleidende brief Amendement dd.16/06/2017
- Amendement
  Toevoegen controlegroep (enkel voor Fibroscan) van gezonde kinderen tussen 4 en 16 jaar
- Amendement op ICF
  - ICF ouders controlegroep (versie 1) dd 16/06/2017
  - ICF jongere + 12 jaar controlegroep (versie 1) dd 16/06/2017

Dit/deze document(en) werd(en) met bevoegdheidsdelegatie bekeken en goedgekeurd door de voorzitter van het Ethisch Comité 7/07/2017.
Hiervan wordt melding gemaakt in het verslag van de vergadering van het Ethisch Comité dd. 22/08/2017.
Het Ethisch Comité beklemtoont dat een gunstig advies niet betekent dat het Comité de verantwoordelijkheid voor het onderzoek op zich neemt.

Indien dit amendement een anderstalig patiënteninformatie en - toestemmingsformulier bevat, dient de promotor garant te staan voor de conformiteit met de nederlandstalige documenten.

Met collegiale groeten,

Prof. dr. D. MATTHYS
Voorzitter EC Gent

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Davina Verbeke
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UW KENMERK	**ONS KENMERK**	**DATUM**	**KOPIE**
	2016/1107	07-jul-17	Zie "CC"

Vervolg blz. 2 van amendement voor project Ethisch Comité UZGent 2016/1107

cc. FAGG - Research & Development; Victor Hortaplein 40, postbus 40 1060 Brussel
UZ Gent - Bimetra Clinics
UZ Gent - iZ Cardiochirurgie
UZ Gent - Pediatrie Pneumologie

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