UNIVERSITA' DEGLI STUDI DI VERONA

DEPARTMENT OF
SURGICAL, ODONTOSTOMATOLOGICAL AND MATERNAL-INFANTILE SCIENCES

GRADUATE SCHOOL IN
LIFE AND HEALTH SCIENCES

DOCTORAL PROGRAM IN
CARDIOVASCULAR SCIENCE

Cycle XXXIII
Year 2017

LIVER STIFFNESS EVALUATION USING ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY IN PEDIATRIC AND ADULT PATIENTS WITH CONGENITAL HEART DISEASE

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ABSTRACT

Background: Hepatic complications are common in patients with congenital heart disease as a consequence of the primary cardiac defect or as a result of surgical palliation (e.g. Fontan procedure). Liver involvement represents a significant challenge and an adequate hepatic surveillance is fundamental. Liver biopsy represents the gold standard for diagnosis and staging of hepatic fibrosis but it's an invasive procedure not suitable for a routine setting. Acoustic radiation force impulse (ARFI) elastography is a recently developed technique that allows to assess hepatic stiffness in a non-invasive and reproducible way. The usefulness of ARFI imaging has been described in adult Fontan patients but only few studies have been reported in the pediatric Fontan population and no one in CHD others than Fontan.

Aim: The aims of this study were to assess liver stiffness, using ElastPQ™ acoustic radiation force impulse elastography, in pediatric and adult patients with CHD, to compare liver stiffness values with healthy controls and to analyze possible associations between ARFI values and clinical, biochemical, cardiac and hepatic parameters.

Materials and methods: Pediatric and adult patients that underwent heart surgery for CHD and were followed at the Cardiology Unit of the "Azienda Ospedaliera Universitaria Integrata" of Verona between October 2018 and October 2020 were prospectively enrolled. Controls subjects without any liver or cardiac disease matched for age and sex to the case group were also included. The latest laboratory tests and echocardiogram available were collected. Liver ultrasound and ARFI measurement of liver stiffness were performed by a specifically trained single expert radiologist using the Philips Healtcare® ultrasound with ElastPQ™ software.

Results: A total of 50 subjects were enrolled for the study: 20 Fontan patients (13 males, median age at ARFI 8.4 years), 13 non-Fontan (9 males, median age at ARFI 4.8 years) and 17 controls (6 males, median age at ARFI 10 years). The median values of ARFI elastography were significantly higher in patients with CHDs (Fontan and non-Fontan patients) compared to control subjects ($p<0.01$). Patients with morphological right ventricle overload showed significantly higher results ($p=0.02$). The cut-off of 5.7 kPa at elastography was used to discriminate between normal liver and liver with signs of congestion or fibrosis. All controls subjects showed ARFI values <5.7 kPa whereas only 25% of Fontan patients and 46% of non-Fontan were below that threshold. Liver stiffness values were positively correlated with time from surgery and age at liver evaluation ($p<0.01$). The number of platelets and white blood cells were inversely related to liver stiffness measurements ($p=0.04$ and $p=0.05$ respectively). The AST to platelet ratio index positively correlated with ARFI elastography results ($p<0.03$). No significant correlations between ARFI results and other biochemical or cardiac parameters were found.

Conclusions: Our data showed that the median values of liver stiffness measured with ElastPQ™ pSWE were significantly higher in patients with CHDs compared to control subjects and, in particular, in those with morphological right ventricle overload. Liver stiffness values were also correlated with time from surgery and age at liver evaluation. The number of platelets and white blood cells were inversely related to liver stiffness measurements supporting the need of a screening for portal hypertension and splenomegaly in these patients. The AST to platelet ratio index was also correlated to ARFI elastography results suggesting that liver stiffness may reflect the evolution of liver fibrosis. In conclusion, our study demonstrated, for the first time in literature, that acoustic radiation force impulse elastography (pSWE) with ElastPQ™ software can be a useful tool to assess liver stiffness in patients with Fontan circulation and other congenital heart disease.

CHAPTER ONE: SCIENTIFIC PREMISES

1. CONGENITAL HEART DISEASE: TWO VENTRICLE AND SINGLE VENTRICLE PHYSIOLOGY

1.1 INTRODUCTION

A congenital heart disease (CHD) is defined as a defect in the structure of the heart or great vessels that is present at birth. It is the most common type of birth defect, with an overall prevalence of approximately 1% [1]. Signs and symptoms of CHD depend on the specific type of defect and can vary from none to life-threatening. Critical CHD, is defined as requiring surgery or catheter-based intervention in the first year of life [2]. Although many newborns with critical CHD are symptomatic and identified soon after birth, others are not immediately diagnosed and the risk of morbidity and mortality increases when there is a delay in diagnosis [3].

To date, thanks to medical, surgical, and technological evolution over the past decades, more than 90% of individuals with CHD survive into adulthood [4]. Since patients with CHD can now present at advanced ages, including the elderly, the term grown-up CHD (GUCH) has been replaced with the term adult CHD (ACHD). Special healthcare organization and training programs are required to meet the needs of this patient population [5].

According to clinical presentation CHD may be classified into acyanotic and cyanotic heart disease (Table I). The most common CHD will be described in the next sections of the manuscript.

1.2 ACYANOTIC CONGENITAL HEART DISEASE

1.2.1 Atrial septal defects

Atrial septal defects (ASDs) comprise 7% to 10% of CHD [6]. Based on their embryological origin, they can be classified as secundum ASDs (the most common type, due to a deficiency in the central part of the septum primum), primum ASDs (due to deficient proliferation of the endocardial cushion) and sinus venosus ASDs (defects near the superior or inferior vena cava. The superior sinus venosus defect might include also partial anomalous drainage of one or more pulmonary veins). Shunting of blood from left to right at the atrial level leads to increased diastolic blood volume in the RV, which causes right-sided chamber dilation.

ASDs are typically discovered when a murmur is heard at a regular 4- to 6- month infant well-child visit. The murmur heard is produced by the increased antegrade flow trough the pulmonary valve thus causing a relative pulmonary stenosis. Patients with ASDs are usually asymptomatic but may have some fatigue. They are followed up with Doppler echocardiography to monitor to monitor the degree of right heart dilatation, which, if present, indicates hemodynamic significance.

Patients with hemodynamically significant ASDs are referred for intervention around 3 to 4 years of age. Rarely ASDs have to be closed earlier. Based on defect size and anatomy, secundum ASDs can be repaired surgically by using a patch or a primary suture closure or in the cardiac catheterization laboratory by using an ASD closure device [7].

1.2.2 Ventricular septal defects

Ventricular septal defects (VSDs) are the most common CHD lesions and are present in 50% to 60% of all children with CHD [6]. VSDs develop from defective formation of the interventricular septum and are classified on the basis of their location in the septum. Perimembranous VSDs (deficiency in a fibrous part of the septum at the base of the heart) are the most common type, constituting

about 80% of all VSDs, followed by doubly committed juxta-arterial (deficiency in the infundibular septum, between the pulmonary and aortic valves), muscular (in the trabecular part of the septum) and inlet (deficiency in the septum inferior to the perimembranous region and above the level of cordal attachments of the atrioventricular valves).

A newborn with a VSD may not initially have a murmur; however, as the pulmonary resistance decreases with age, a pansystolic murmur can be heard. A child with a hemodynamically significant VSD presents with features of pulmonary overcirculation and congestive heart failure (CHF). Chest radiography may show cardiomegaly with pulmonary vascular congestion.

Children with VSDs should be regularly followed up by a pediatric cardiologist. Children with CHF symptoms may require diuretic therapy and adequate caloric intake. Surgical patch repair of the VSD is performed either in a symptomatic infant or in a toddler with enlargement of the left atrium or ventricle. Some VSDs (e.g. muscular VSDs) can be closed with devices in the cardiac catheterization laboratory [7].

1.2.3 Atrioventricular septal defects

Atrioventricular septal defects (AVSDs), constitute 5% of CHD and is more frequent in subjects with Down syndrome [6]. AVSDs arise from defects in the proliferation and fusion of the endocardial cushions.

Patients with AVSD typically have a systolic ejection or holosystolic murmur from the VSD component. These children may also present with features of CHF and failure to thrive.

Patients with AVSD typically require surgical intervention around 4 to 6 months of age or earlier if they have severe CHF or failure to thrive [7].

1.2.4 Patent ductus arteriosus

An arterial duct is a normal fetal connection between the aorta and the pulmonary artery that is present in all newborns. It closes functionally within 24

hours and anatomically within 3 to 4 weeks in most patients. It may remain patent longer in some patients, especially infants born prematurely.

The examination of patients with PDA varies with their age. In a newborn with higher pulmonary resistance and pressures, the PDA may not shunt much blood and may not be audible. In an older infant, however, a systolic or continuous murmur of blood shunting left to right can be detected during the entire cardiac cycle. The left-to-right shunting caused by the PDA may also lead to features of CHF. Chest X-rays illustrate features of pulmonary overcirculation and cardiac enlargement. Echocardiograms can demonstrate left atrium (LA) and left ventricle (LV) enlargement, characteristics of flow, and anatomy of the PDA.

Ibuprofen and indomethacin are usually used to medically close PDA in premature infants. A symptomatic or hemodynamically significant PDA is an indication for intervention, with either device occlusion in the interventional catheterization laboratory or surgical ligation. Both procedures have similarly high success rates and a complication rate of less than 1% [7].

1.2.5 Aortic and pulmonic valve stenosis

Aortic stenosis constitutes 5% to 8% of all CHD, and pulmonary stenosis constitutes about 8% to 10% of all CHD [6]. The stenosis is categorized as critical if the blood flow through the respective valve is insufficient and requires additional contribution from the PDA. These valves undergo aberrant tissue resorption during in utero development, which leads to thickened dysplastic valves that do not open or close normally. Hence, they create obstruction to blood flow and may also be regurgitant. Aortic stenosis may be associated with a bicuspid or even a unicuspid aortic valve, as well as other levels of obstruction in the left-sided circulation, including coarctation of the aorta (CoA), subaortic obstruction, and mitral stenosis.

Patients present with an ejection systolic murmur that is loudest over the respective valvar region and may also have an ejection click (if stenosis is moderate or mild). The electrocardiogram (ECG) may show ventricular hypertrophy of the RV in cases of pulmonic stenosis and of the LV in cases of

aortic stenosis. Doppler echocardiograms help estimate the velocity of blood flow across the valve; the narrower the valve area, the faster the flow velocity. This flow velocity is then used to estimate the pressure gradient across the stenotic valve [7].

Patients with critical aortic stenosis or pulmonic stenosis meet the indication to undergo a catheter-based balloon valvuloplasty procedure in the newborn period to ameliorate ductal dependence [8]. For noncritical stenosis, the time of intervention is determined by the presence of peak gradient of ≥50 mmHg obtained across the aortic valve or ≥40 mm Hg across the pulmonary valve at echocardiography or during cardiac catheterization. Patients with aortic stenosis may undergo a surgical valvuloplasty, depending on the valve anatomy, annulus size, and experience and preference of the personnel at the center [7].

1.2.6 Coarctation of the aorta and interruption of the aorta

CoA is a narrowing at the isthmus of the arch of the aorta, constituting 5% to 8% of CHD [6]. Interrupted aortic arch (IAA) is the most extreme end of this spectrum, with a discontinuation of the arch and distal continuation through a PDA past the point of interruption.

Severe and critical CoA and IAA may be detected at the time of newborn screening with a lower saturation level at the postductal site or via clinical suspicion on the basis of poor pedal or femoral pulses at the first newborn visit to the pediatrician. These patients may present in extremis at 2 to 3 weeks of age, in shock, with feeble pulses, lethargy, poor feeding, decreased urine output, and metabolic acidosis. Mild CoA may be detected on the basis of a gradient of more than 20 mmHg between the upper and lower extremity blood pressures during infancy or later on in life. A systolic murmur that is loudest over the back may be heard. CoA may also be found in young adolescents undergoing workup for hypertension. There may not be a clinically significant blood pressure gradient in older patients with CoA because they develop collateral vessels over time that supply blood distal to the narrowing. [7].

Newborns with severe or critical CoA and IAA are dependent on prostaglandin infusion to keep the PDA open until the time of surgical repair. Some children (particularly older children) are candidates for intervention in the cardiac catheterization laboratory, with balloon angioplasty and stent placement for the coarctation [9].

1.3 CYANOTIC CONGENITAL HEART DISEASE

1.3.1 Tetralogy of Fallot

Tetralogy of Fallot (ToF) is the most common cyanotic CHD, accounting for 5% of all CHD [6]. It develops from anterior malalignment of the interventricular septum, which leads to a VSD, as well as overriding of the VSD by the aorta. There is a narrowing of the pulmonary outflow tract due to the septal deviation and this causes RV outflow obstruction and consequent RV hypertrophy.

Patients with ToF have an ejection systolic murmur heard over the pulmonic area, indicating pulmonic stenosis. Higher saturations indicate less RV outflow obstruction. Patients who do not receive a diagnosis prenatally may present for the first time as children having a hypercyanotic spell in a period of agitation, fever, or other concurrent illness. Agitation and crying increase pulmonary vascular resistance, while also increasing the heart rate. Owing to a subsequently shorter diastolic period, ventricular filling is less, which adds to the obstruction to the RV outflow from its hypertrophic muscle bundles [7].

The time of presentation and intervention for ToF is determined by the degree of RV outflow obstruction and the limitation of pulmonary blood flow. These clinical indicators are the oxygen saturation levels and the development of hypercyanotic spells. The echocardiographic indicators are the pressure gradients observed across the RV outflow tract. Children with saturations less than 80% or those having hypercyanotic spells are scheduled for surgery. Surgical management might include an initial palliation via a creation of a modified Blalock-Taussig

(mBT) shunt to increase oxygen saturation; this is then followed by complete repair at the age of 4 to 6 months. Complete repair of the heart comprises closure of the VSD, as well as resection of the RV obstruction, resulting in normal saturations [7]. Even after complete ToF repair, patients require lifelong cardiology follow-up, since their pulmonary valve may become more regurgitant, which can lead to RV dilation. Patients may also develop arrhythmias because of the RV dilation and/or hypertrophy and scarring with an increase in risk of sudden cardiac death [10]. Cardiac magnetic resonance imaging is used to estimate the volume and the function of the RV and LV as well as the pulmonary valve function, the anatomy of the right ventricular outflow tract, of the main pulmonary artery (MPA) and of the branch of the PAs. Alongside other imaging and functional assessment is used to timing re-intervention on the pulmonary valve and the best modality (surgical versus percutaneous). Complete repair of ToF ensures that these children have normal saturations [7].

1.3.2 Transposition of the great arteries

Transposition of the great arteries is the second most common cyanotic CHD, accounting for about 2% of all CHD [6]. It is the most common cyanotic heart disease manifesting in the first week after birth. There is ventriculo-arterial discordance, with the aorta arising from the RV and the pulmonary artery arising from the LV. Hence, the systemic and pulmonary circulations are in parallel, with systemic venous (deoxygenated) blood returning to the right atrium, the RV and going out the aorta again. There is mixing of blood at the atrial level through a patent foramen ovale or an ASD or at the ventricular level through a VSD (35% to 40% of transposition of the great arteries).

Newborns with transposition of the great arteries present with cyanosis within the first 12 hours after birth and are not responsive to oxygen or mechanical ventilation. The presence of a VSD may delay presentation. There is usually no murmur at examination [7].

Once the diagnosis is established, these patients require reparative surgery to switch the great vessels to the appropriate ventricles, known as the arterial

switch procedure [11]. They may need initiation of prostaglandin E1 until the time of surgery. Prostaglandin allows the PDA to remain open, which shunts blood from the aorta into the pulmonary circulation. This increases the amount of blood returning to the left atrium and mixing at the atrial level, as long as the foramen is open. Most patients undergo a catheter-based procedure called balloon atrial septostomy to help create or enlarge the ASD to allow more mixing while awaiting surgery. After repair, these patients require regular cardiology follow-up for life [7].

1.3.3 Truncus arteriosus

Truncus arteriosus accounts for 2% to 5% of CHD and manifests early in the neonatal period [6]. It develops from lack of formation of the aorticopulmonary septum; hence, the common truncal outflow does not divide into an aorta and a main pulmonary artery. There is always an associated VSD, and there is a common truncal valve. About one-third of the patients with truncus arteriosus have DiGeorge or velocardiofacial syndrome [7].

Patients with truncus arteriosus present within the first 48 hours after birth with symptoms of profound pulmonary overcirculation. On examination, they have a systolic murmur. Their general physical examination may also demonstrate features of DiGeorge or velocardiofacial syndrome, including a small mouth (micrognathia), cleft lip and/or palate, and flat cheek bones (malar flattening) [12].

Symptoms of pulmonary overcirculation may be managed with diuresis and fluid restriction. These children undergo surgical repair in the first 2 weeks after birth. The truncal outflow and the truncal valve are committed to the aorta, and the branch pulmonary arteries are committed to a conduit placed from the RV to the pulmonary artery. These children should have normal saturations after repair. They continue to require close cardiology follow-up over the years [7].

1.3.4 Total and partial anomalous pulmonary venous drainage

Abnormal return of the pulmonary veins to the systemic veins or the right atrium comprises about 1% of CHD [7]. Anomalous pulmonary venous return is

defined as partial anomalous pulmonary venous drainage (PAPVD) when at least 1 pulmonary vein returns to the left atrium and total anomalous pulmonary venous drainage (TAPVD) when none of the pulmonary veins return to the left atrium. TAPVD can be further classified on the basis of the site of return of the anomalous drainage to the systemic veins: supracardiac (into the superior vena caval system), intracardiac (into the coronary sinus or right atrium), or infracardiac (into the inferior vena cava or hepatic venous system). Blood returning from the pulmonary veins to the right side of the heart causes right atrial hypertension, with right-to-left shunting across a patent foramen ovale and/or an ASD. In TAPVD, all the venous return to the heart returns to and mixes in the right atrium before shunting across the interatrial septum or coursing across the tricuspid valve. Hence, the saturations in each of the cardiac chambers are the same.

The number of veins returning anomalously and the degree of obstruction determine the rate and severity of the manifestation. Physiologically, TAPVD can be categorized as (i) obstructed, in which case the pulmonary venous return is impeded, which can lead to pulmonary venous hypertension and pulmonary edema, or (ii) unobstructed, in which case the anomalous drainage causes cyanosis due to mixing after return to the right side of the heart but does not cause respiratory distress in the first days after birth. The newborn with obstructed TAPVD presents with respiratory distress and cyanosis within 12 to 24 hours after birth, with a classic chest radiographic finding of (a) “whiteout” of the lung fields due to backing up of blood flow prior to the site of the obstruction of pulmonary vein drainage and (b) a small heart on chest radiographs. The respiratory distress does not respond to oxygen and may potentially be worsened by starting prostaglandin as more blood starts shunting to the pulmonary circulation at the ductal level, to the point of pulmonary edema or lung bleeding. PAPVD (which may or may not be associated with an ASD) typically manifests later in life in childhood or adolescence with signs similar to those of an ASD, with an ejection systolic murmur that is loudest over the pulmonic region owing to the increased

blood returning to the right side of the heart. ECG shows right atrium and RV enlargement, and Doppler echocardiographic findings confirm the diagnosis [7].

Obstructed TAPVD constitutes about one third of the cases and is a surgical emergency [13]. Children with unobstructed TAPVD can be followed up by a cardiologist for a brief time as outpatients with a definite plan for early surgical repair, since obstruction can develop over time and manifests similarly with respiratory distress. After repair of TAPVD, patients continue to be closely monitored as outpatients, since pulmonary venous obstruction can recur, with reported rates around 15% [14]. Recurrent pulmonary venous obstruction manifests as respiratory distress, tachypnea, and wheezing that is not responsive to bronchodilation. These patients require chest radiography and echocardiographic evaluation and, if stable, elective surgical repair [7].

1.3.5 Hypoplastic left heart syndrome, tricuspid atresia, and single-ventricle palliation

Hypoplastic left heart syndrome (HLHS) is the fourth most common form of cyanotic CHD, comprising severe stenosis or atresia of the mitral and aortic valves [6]. Owing to decreased flow from the left atrium into the LV, as well as from the LV into the aorta, the LV is hypoplastic, and there is associated hypoplasia of the ascending aorta, as well. This lesion is PDA dependent for systemic circulation, as the descending aorta continues from the PDA insertion, and the coronary arteries, innominate arteries, left common carotid arteries, and left subclavian arteries are supplied primarily by retrograde flow from the PDA. Prostaglandin infusion is required to maintain PDA patency. A corresponding lesion on the right side of the heart is a hypoplastic RV in the setting of severe tricuspid stenosis or atresia and severe pulmonary stenosis or atresia. The absence of two normal-sized ventricles, the absence of a normal or repairable atrioventricular connection (in the form of severe stenosis or atresia of the mitral or tricuspid valves), the absence of a repairable outflow tract in some situations (severe stenosis or atresia of the pulmonary or aortic valve), or any combination of these conditions indicates that patients should undergo single ventricle

palliation. These lesions are dependent on a PDA maintained by prostaglandin to ensure cardiac output into both systemic and pulmonary circulations.

The first stage of surgical palliation is to help stabilize both cardiac outputs, either by surgically constructing a “neoaorta” by using the main pulmonary artery tissue to supplement the ascending aorta (the Norwood procedure), creating a mBT-shunt (or RV-to–pulmonary artery conduit) to supply blood flow to the pulmonary arteries, and creating an ASD. The second stage, known as the Glenn procedure, involves anastamosing the superior vena cava to the pulmonary arterial system to direct part of the deoxygenated venous blood directly to the lungs. This also allows take-down of the mBT-shunt, as there is consistent pulmonary blood flow through the Glenn connection. The final step of this palliation (the Fontan procedure) is to connect the inferior vena cava (IVC) to the pulmonary arterial system as well, to allow all the deoxygenated blood to go straight to the lungs without mixing in the heart first (Figure 1). The Fontan connection may be fenestrated in some cases, creating a connection between the IVC–pulmonary arterial anastomosis and the pulmonary venous atrium. This may be especially useful as a “pop-off” for blood passively returning from the IVC to the pulmonary circulation in cases of high pulmonary vascular resistance.

The presentation of single-ventricle lesions depends on the adequacy of cardiac output. Systemic cardiac output is dependent on the ductal blood flow. In patients with HLHS, when the duct starts to close, the infant begins to have signs of poor systemic perfusion with feeble pulses, poor urine output and shock, in addition to pulmonary overcirculation, seen as tachypnea and respiratory distress. Chest radiographs will show pulmonary vascular congestion, in addition to possible cardiomegaly due to right ventriculomegaly (as a result of the RV effectively handling systemic as well as pulmonary cardiac output).

The first stage surgical intervention is performed within the first 2 weeks after birth. Saturations after the first stage palliation can vary from 80% up to 95%, depending on the amount of pulmonary blood flow. A continuous or pansystolic murmur is easy to appreciate in a child with a mBT-shunt, and a systolic ejection murmur is heard with a RV-to–pulmonary artery conduit. As

these children grow older and bigger, the relative size of their mBT-shunt limits the amount of pulmonary blood flow for their body surface area, and their saturations start to drift down into the 70% range. These patients are very closely followed up until they undergo the Glenn procedure. The period between the Norwood and Glenn procedures (the “interstage period”) is a particularly vulnerable time. The Glenn procedure is typically performed around 4 to 6 months of age. By this age, the pulmonary vascular resistance decreases to the physiological minimum, and the branch pulmonary arteries grow adequately, so that a stable and durable superior vena cava–pulmonary anastomosis can be created. After the Glenn procedure, the saturations are in the 80% to 90% range, and these patients generally do not have any murmurs. After the Glenn procedure, as the children grow older, their oxygen saturations trend downward, as the proportion of their cardiac output from the lower part of their body that returns through the IVC increases relative to the proportion from the upper body and the head that returns through the superior vena cava. These patients are followed up until their final Fontan palliation at around 2 to 4 years of age, depending on trends of saturation. After the Fontan palliation, the patients are expected to be fully saturated in the case of a non-fenestrated Fontan procedure or around 90% to 95% saturated in the case of a fenestrated Fontan procedure. These children are still at risk of developing right sided heart failure or systemic ventricular heart failure because they have a single ventricle driving their entire circulation. Hence, accurate assessment and documentation of history of CHF symptoms, such as tachypnea, poor exercise tolerance, decreasing appetite, diarrhea, chronic cough, or pedal edema, are important for these patients during their pediatric clinic visits [7].

Table I. Classification of CHD according to clinical presentation.

Acyanotic congenital heart disease
Atrial septal defects
Ventricular septal defects
Atrioventricular septal defects
Patent ductus arteriosus
Aortic and pulmonic valve stenosis
Coarctation of the aorta and interruption of the aorta
Cyanotic congenital heart disease
Tetralogy of Fallot
Transposition of the great arteries
Truncus arteriosus
Total and partial anomalous pulmonary venous drainage
Hypoplastic left heart syndrome, tricuspid atresia, and single-ventricle palliation

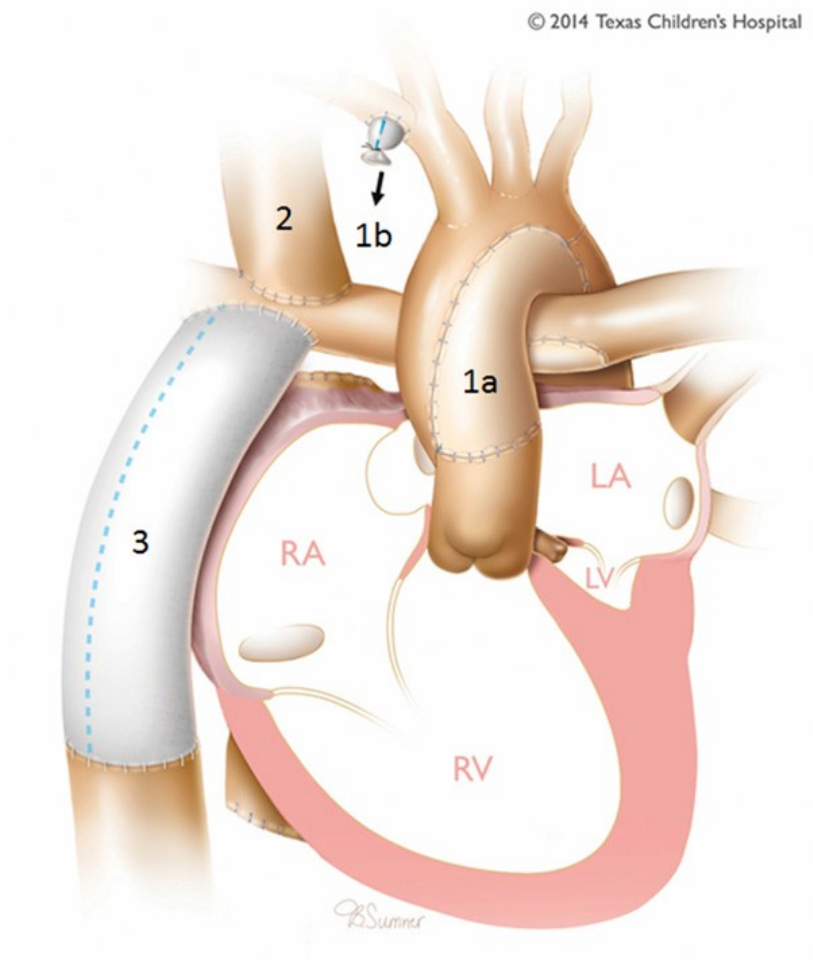

Figure 1. Diagram shows the shunts and sites of anastomosis for single ventricle palliation in hypoplastic left heart syndrome. 1a) indicates the reconstructed neoaorta by using the hypoplastic ascending aorta, as well as the normal main pulmonary artery, to ensure stable systemic outflow. 1b) indicates the site of the modified Blalock-Taussig shunt to direct blood flow from the right subclavian artery into the pulmonary circulation. 2) indicates the Glenn procedure that involves the creation of an anastomosis between the superior vena cava and the right pulmonary artery. 3) indicates the Fontan anastomosis. This disconnects the remaining deoxygenated blood coming in through the inferior vena cava and redirects it through the Fontan conduit to the pulmonary circulation. This final step eliminates any mixing and completely separates the oxygenated and deoxygenated blood, ensuring normal saturations [7].

2. FONTAN CIRCULATION

2.1 INTRODUCTION

The Fontan procedure, initially described in patients with tricuspid atresia, is the most common procedure in patients with single-ventricle physiology. The term ‘univentricular heart’ summarizes a variety of malformations where either the right ventricle or the left ventricle is missing or, if present, is hypoplastic, and thus not amenable for biventricular repair (Table II) [15]. In patients with single-ventricle physiology, intracardiac mixing of blood leads to arterial hypoxemia with excessive volume load on a single ventricle. Hence, separation of the pulmonary and systemic circulation is desirable. The Fontan operation allows systemic venous return to the pulmonary arteries bypassing the right ventricle [16].

Surgery consists of the separation of the systemic and pulmonary venous returns without a subpulmonary ventricle and restores them to being ‘in series’. The Fontan operation is accomplished by creating a direct cavopulmonary (sometimes in a staged manner) anastomosis. In the first stage (i.e., superior caval-pulmonary anastomosis or bidirectional Glenn procedure), the superior vena cava is connected to the pulmonary arteries. Eventually, the inferior vena cava is also connected to the pulmonary arteries completing the circulation (Fontan completion). Currently, a cavopulmonary anastomosis is achieved by the use of an intra-atrial tunnel or patch or by utilizing an extracardiac conduit to connect the vena cava to the pulmonary arteries (Figure 2) [16].

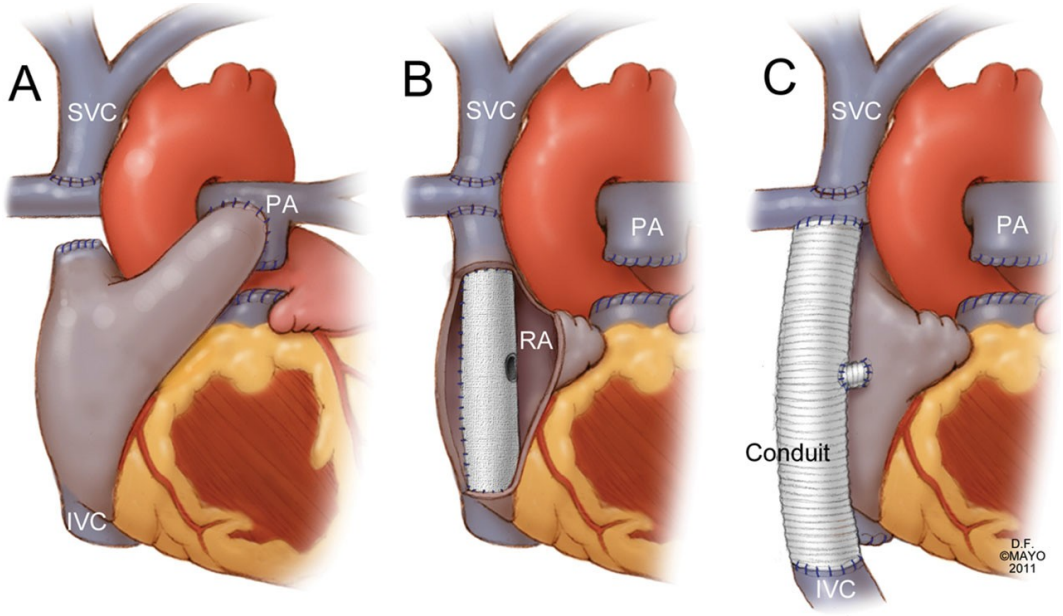

Figure 2. (A) Anastomosis between the superior vena cava and the right pulmonary artery (bidirectional cavopulmonary shunt). Blood from the inferior vena cava is baffled to the lungs by means of a patch to the pulmonary artery. The main pulmonary artery is ligated to avoid competitive flow from the ventricle. (B) Fontan with an intra-atrial conduit baffling blood from the inferior vena cava through the right atrium to the pulmonary artery. A bidirectional cavopulmonary shunt carries blood from the superior vena cava to the pulmonary artery. (C) Extracardiac Fontan: cavopulmonary extracardiac conduit from the inferior vena cava to right pulmonary artery. The superior vena cava is anastomosed as a bidirectional cavopulmonary anastomosis. IVC, inferior vena cava; PA, pulmonary artery; RA, right atrium; SVC, superior vena cava.

2.2 CLINICAL PRESENTATION AND NATURAL HISTORY

The lack of a subpulmonary ventricle results in chronic systemic venous hypertension, markedly altered pulmonary hemodynamics, and a chronically 'preload-deprived' ventricle. A number of important problems have emerged during long-term follow-up. After the Fontan operation, the majority of patients do well during childhood and adolescence, although exercise capacity is reduced when measured objectively. However, clinical complications may develop subsequently, with a progressive decline in exercise performance and heart failure, cyanosis, chronic venous insufficiency, and development of important arrhythmias [17]. Although 10-year survival may approach 90%, it should be appreciated that a premature decline in cardiovascular performance, with reduced survival, is inevitable even in the best Fontan patients. Important hemodynamic issues contributing to late Fontan failure include a progressive decline in systemic ventricular function, atrioventricular (AV) valve regurgitation, a rise in pulmonary vascular resistance (PVR), atrial enlargement, pulmonary venous obstruction, and the consequences of chronic systemic venous hypertension including hepatic congestion and dysfunction [18]. Protein-losing enteropathy (PLE) is a rare but important complication and results in peripheral edema, pleural effusions, and ascites. It can be diagnosed by documentation of low serum albumin and elevated alfa-1-antitrypsin levels in the stool. It is associated with a very poor prognosis and available treatments are of limited effectiveness [19].

2.3 DIAGNOSTIC WORK-UP

Clinical examination plays a major role and includes careful evaluation with regard to any changes in auscultation findings or blood pressure or development of signs of heart failure. Clinical findings include commonly mild, non-pulsatile jugular venous distension. However, significant jugular venous distension and hepatomegaly, raise suspicion of Fontan obstruction or ventricular

failure. ECG frequently shows junctional rhythm or atrial arrhythmias. Pleural effusion on chest X-ray raises suspicion of PLE.

Echocardiography is the first-line diagnostic tool, providing information on ventricular and valve function as well as patency of the Fontan circuit. Annual blood tests should include hematology, serum albumin, and liver and renal function.

Cardiac magnetic resonance (CMR) and computed tomography (CT) are particularly helpful for evaluation of the Fontan pathway, collaterals and pulmonary veins, and differential pulmonary flow.

As liver dysfunction and fibrosis, cirrhosis, and hepatocellular carcinoma are typical complications, regular liver imaging (ultrasound, computed tomography, magnetic resonance) and laboratory assessment should be performed.

Cardiac catheterization should be performed at a low threshold in cases of unexplained edema, exercise deterioration, new-onset arrhythmia, cyanosis, and hemoptysis [5].

2.4 FOLLOW-UP

As a result of these many complex issues, the care of Fontan patients is one of the major challenges for practitioners, and they should all be followed in specialized centers, usually at least annually, and include echocardiography, ECG, blood, and exercise testing. Intervals for CMR should be decided on an individual basis. Yearly follow-up hepatic assessments including liver ultrasound (and possibly elastography) and alpha-fetoprotein measurement should be considered in these patients [5].

Table II. CHD with single-ventricle physiology

CHD with single-ventricle physiology
Tricuspid atresia
Hypoplastic right heart syndrome variants, e.g. pulmonary atresia with intact ventricular septum variants
Hypoplastic left heart syndrome variants, including mitral atresia
Double-inlet LV
Double-inlet RV
Extreme forms of unbalanced complete AV septal defects
Single ventricle with undefined morphology.

Table III. Major types of CHD defects associated with hepatic dysfunction.

Right-sided failure
Single-ventricle physiology after Fontan procedure (e.g., tricuspid atresia)
d-Transposition of the great arteries after atrial switch repair (Mustard, Senning)
Eisenmenger syndrome
Repaired tetralogy of Fallot with pulmonary regurgitation
Ebstein's anomaly
Pulmonary stenosis/pulmonary regurgitation
Secundum atrial septal defect with pulmonary stenosis or pulmonary hypertension
Partial atrioventricular septal defect with tricuspid regurgitation and/or pulmonary hypertension
Left-sided failure
Left ventricular outflow tract obstruction/coarctation of the aorta
Repaired complete atrioventricular septal defect
Ventricular septal defect

3. HEPATIC INVOLVEMENT IN CONGENITAL HEART DISEASE

3.1 INTRODUCTION

Nowadays, approximately 85% of patients with CHD survive into adulthood as a result of successful reparative surgery [20]. Currently, the estimated number of adults with CHD in the United States ranges from 650,000 to 1.3 million, and it is expected this number will increase by approximately 5% every year [21]. Hepatic complications are common in patients with CHD either resulting from the primary cardiac defect or from palliative surgical procedures performed in infancy or childhood, or from transfusion or drug-related hepatitis [16].

Although, there are several known associations between primary liver disease and CHD defects (e.g., Abernethy malformation, Alagille syndrome and mitochondrial fatty acid oxidation disorders), hepatic disease as a result of CHD is more common than cardiac disease associated with liver disease. Several CHD defects may lead to either left or right ventricular failure (Table III). In these cases, hepatic dysfunction may ensue as a result of the primary cardiac defect or as a result of surgical palliation, especially in patients with Fontan operation [16].

Congestion and low cardiac output are the main causes of liver disease secondary to congenital heart disease and, for this reason, the term ‘cardiac hepatopathy’ reflects the pathophysiology of liver disease secondary to congenital heart disease. The Fontan procedure is an operation for children with complex congenital heart malformations and generally achieves prolonged survival in association with good quality of life. However, an increased risk to develop hepatocellular carcinoma (HCC) is reported after Fontan palliation. The first case of HCC following Fontan palliation was reported in 2005 [22]. The median age at diagnosis of HCC is 29 years. These findings suggest that routine surveillance for cardiac hepatopathy is required for children as well as young adults. However, HCC can occur also in patients suffering from congenital heart disease (TGA and ToF) without undergoing the Fontan operation [23;24;25]. In fact, severe and prolonged hypoxia and the decline of blood flow to liver could damage liver tissue and, for

this reason, also patients suffering from HLHS, TGA, ToF and Eisenmenger syndrome require routine examination for cardiac hepatopathy [26]. The predictors of cardiac hepatopathy have not yet been identified. It is difficult to predict precisely who will suffer from HCC among children with congenital heart disease. Advanced hepatic fibrosis is presumed to be associated with HCC [27].

Because liver biopsy is an invasive examination, non-invasive methods are preferable as a diagnostic tool. Serological markers, hepatic fibrosis indices, US, CT, and MRI are suitable for use in examinations to detect cardiac hepatopathy, but such examinations are not always able to detect this condition. Elastography seems to be a promising tool to assess liver congestion and fibrosis but its usefulness in clinical practice for detecting cardiac hepatopathy has not been yet determined. In order to clarify the interaction between the liver and congenital heart disease, there is an urgent need for standardization of the methods for evaluating cardiac hepatopathy. Routine surveillance of cardiac hepatopathy could contribute to an improvement of prognosis in patients suffering from HCC. Close cooperation among hepatologists, cardiologists and pathologists is needed to resolve the problems in patients with cardiac hepatopathy [27].

3.2 PATHOPHYSIOLOGY

The mechanisms leading to hepatic dysfunction may be multifactorial. As an example, hepatic dysfunction may result from a combination of passive venous congestion of the liver and hypoxia, with the latter being driven by the CHD or concomitant pulmonary disease. Volume overload and low cardiac output may lead to both congestive hepatopathy and hepatic ischemia [16].

Several factors may interact to lead to end-stage liver disease. For example, patients with underlying liver disease (e.g., viral hepatitis, alcohol, or obesity) may be more susceptible to liver injury as a result of decreased functional mass [28]. In addition, the presence of cardiac disease and subsequent passive congestion may itself predispose the liver to hepatic injury [29]. Over time, cardiac

cirrhosis (i.e., central vein to central vein bridging fibrosis and nodule formation) may develop and result in portal hypertension (PH) with ascites and varices [16].

Specifically, the mechanisms by which a CHD can induce hepatic injury are as follows:

- *Passive venous congestion of the liver with central venous hypertension*.

Right ventricular failure is a consequence of several defects and is reflected by hepatic zone 3 sinusoidal dilation and hemorrhagic necrosis in the liver. Zone 3 necrosis may also be caused by ischemia. As an example, CHD may be associated with elevated right atrial pressure resulting from left-to-right shunting through a septal defect with secondary pulmonary hypertension, univentricular physiology (e.g., tricuspid atresia), and with a failing systemic ventricle, which is a morphologic right ventricle. Restrictive physiology in the right ventricle (e.g., with repaired atrial septal defect and tetralogy of Fallot) also contributes to passive congestion. Narrowing of the venous pathway to the lungs (e.g., Fontan operation) or in the inferior vena cava (after atrial baffle procedures for d-transposition of the great arteries) may contribute to hepatic venous congestion. The most common biochemical abnormalities in passive venous congestion of the liver are elevated indirect bilirubin and prolonged international normalized ratio (INR) with minimal elevations of the aminotransferases [16].

- *Low-output cardiac failure and hypoxemia*

Patients with CHD are susceptible to ischemic hepatitis because right heart failure elevates hepatic sinusoidal pressure and reduces portal inflow. This results in increased sensitivity to any decrease in hepatic artery flow, resulting from a decrease in cardiac output (e.g., caused by concurrent arrhythmias or hypotension). For example, left ventricular outflow tract obstruction/coarctation of the aorta is associated with hypoperfusion and, in some clinical situations, may lead to hepatic ischemia [30]. Chronic hepatic ischemia may also lead to hepatic fibrosis [31].

Central fibrosis (centrilobular and sinusoidal fibrosis) and sinusoidal dilatation are the main features of the postmortem liver histology in cardiac hepatopathy [32]. The centrilobular area is vulnerable to hypoxia due to passive

congestion and low cardiac output. The fibrous septa formation arises from the central vein [33]. This finding is in contrast to chronic viral hepatitis, in which hepatic fibrosis begins from the portal area. Hypoperfusion and hypoxia caused by low cardiac output contribute more to liver injury than passive congestion in infants with congenital heart disease [34].

Severe hepatic fibrosis can lead to an increased risk of HCC also in patients with CHD [24]. Thus, the quantification of hepatic fibrosis is indispensable for predicting the development of HCC in this population. Dai et al. developed a central zone-based scoring system for the evaluation of hepatic fibrosis in patients with cardiac hepatopathy [35]. This novel scoring system showed that the right atrial pressure was significantly higher in patients with portal fibrosis than in patients with no fibrosis [36].

3.3 FONTAN ASSOCIATED LIVER DISEASE (FALD)

The Fontan procedure is the most common procedure in patients with single-ventricle physiology (e.g., tricuspid atresia) or when biventricular repair is not feasible (e.g., double- inlet left ventricle and hypoplastic left heart) [15]. Although the short-term prognosis is excellent in patients with Fontan circulation, arrhythmia, thromboembolism, protein enteropathy, heart failure and liver dysfunction (Fontan-associated liver disease: FALD) could influence the long-term prognosis [37].

As a consequence of surgical palliation, significant liver disease can develop as a result of the interplay of central venous hypertension/passive congestion and hypoxia resulting from left ventricular dysfunction. Development of significant hepatic injury after a Fontan procedure is multifactorial. The determinants of cardiac output are central venous pressure, pulmonary vascular resistance, and systemic ventricular end-diastolic pressure. Over time, a ‘‘failure of Fontan physiology’’ is common. Failure of the Fontan circuit may result from elevated pulmonary vascular resistance, pulmonary thrombi, narrowing and

scarring in the Fontan pathway or pulmonary arteries, and failure of the systemic ventricle, which results in elevated pressure in the pulmonary venous atrium. Chronic elevation of central venous pressure is common, and reduced cardiac output from the functioning single ventricle is frequent, particularly as diastolic and systolic dysfunction ensues. The former results from the absence of a subpulmonic pump [38]. There is impaired coupling between the ventricles and the arterial system with late ventricular dysfunction [39]. Atrial arrhythmias may contribute to this decline with relative hypotension and desaturation. The development of pulmonary veno-venous collaterals as pressure ‘‘pop-offs’’ are not uncommon in the adult population and further contribute to hypoxemia. Pulmonary arteriovenous malformations, most often observed after a classic Glenn procedure, also contribute to hypoxemia. Chronic hypoxia resulting from a depressed cardiac output, in addition to the aforementioned changes, may also lead to hepatic injury [38;40].

On histology, hepatic sinusoidal fibrosis (within the space of Disse in a pericellular manner) and marked sinusoidal dilatation are universal, extending from zone 3 toward the portal tract. Sinusoidal dilation is associated with higher right atrial pressures, similar to that observed in patients with cardiac cirrhosis. In contrast to cardiac failure, the extent of dilation as well as fibrosis is more severe in patients with Fontan circulation [38;40]. After a failed Fontan procedure (at least in patients with cavopulmonary anastomosis), the back pressure on the liver is usually continuous (i.e., nonpulsatile), as opposed to the back pressure secondary to problems such as tricuspid regurgitation (i.e., pulsatile). This continuous high venous pressure may explain why liver dysfunction is frequent after a Fontan procedure. Several studies showed that both centrilobular and portal fibrosis were present in 80%–100% of liver specimens from Fontan patients [36;41]. Sinusoidal dilatation is also a common finding; it has been observed in ≥90% of liver specimens from Fontan patients. By contrast, necrosis and inflammation are uncommon in the liver histology of patients with Fontan circulation [36].

Fibrosis in cardiac cirrhosis or after Fontan palliation may develop independent of inflammation and, potentially, driven by repetitive mechanical

stretch and compression of sinusoid and other resident cells as a result of passive congestion [42]. This, along with hypoxia driven by a low cardiac output, may lead to significant structural and function alteration of the liver parenchyma. During the neonatal period, hypoxia and shock due to congenital heart disease cause liver injuries. At the same time, the liver tissue suffers perioperative insults from multiple staged operations, such as ischemic reperfusion injury or drug injury. Furthermore, liver injury including hepatic fibrosis could be present before the Fontan [43]. In return for a reduction of intra-cardiac mixing and an increase in arterial oxygen saturation, Fontan palliation results in an elevation of CVP and low cardiac output [44]. The elevated CVP increases the hepatic vein pressure, which in turn leads to a decrease in the portal vein inflow. This combination (decreased portal inflow plus a diminished portal circulation due to low cardiac output) results in the massive reduction of portal blood flow. When the reduction of portal inflow occurs, the hepatic arterial buffer response (i.e. autoregulation of hepatic blood flow) increases the hepatic blood inflow derived from the hepatic artery (so-called 'arterialization') and can compensate for the reduction of portal flow (Figure 3) [44].

If the hepatic arterial buffer response is insufficient, the hepatic perfusion becomes low, and eventually the liver tissue suffers from ischemic injury [45]. Additionally, the elevation of CVP causes the dilatation of sinusoids and congestion. The dilatation of sinusoids stimulates the satellite cells by stretching and compression, and the stimulated satellite cells initiate a fibrotic reaction [40]. The congestion leads to the propagation of thrombosis and an accumulation of deoxygenated blood in the liver [46]. These circumstances also contribute to the development of hepatic fibrosis. FALD is multifactorial and attributable to an elevation of CVP, low cardiac output, and pre-Fontan injury [44].

Hepatic complications of a failed Fontan are, in part, related to the duration of follow-up. As compared to a duration of less than 5 years, the odds of hepatic complications for a post-Fontan duration of 11-15 years is 4.4 and 9.0 for a duration of 16-20 years [47]. Furthermore, the extent of hepatic fibrosis on pathological specimens is strongly correlated with elevated hepatic venous

pressures, low cardiac index, and ventricular function [38]. Hepatic dysfunction correlates best with a low cardiac index and ventricular function. Progression to cirrhosis may even be observed within 10 years of the initial Fontan surgery [48]. The majority of hepatic complications are incidentally discovered. In patients with Fontan circulation followed for a median of 12 years, elevated liver function tests, coagulopathy, hepatomegaly, cirrhosis, and hepatic masses are recognized. Portal hypertension (PH) with gastroesophageal varices may develop, resulting in increased risk of gastrointestinal hemorrhage. Hepatocellular carcinoma (HCC) may also develop [49].

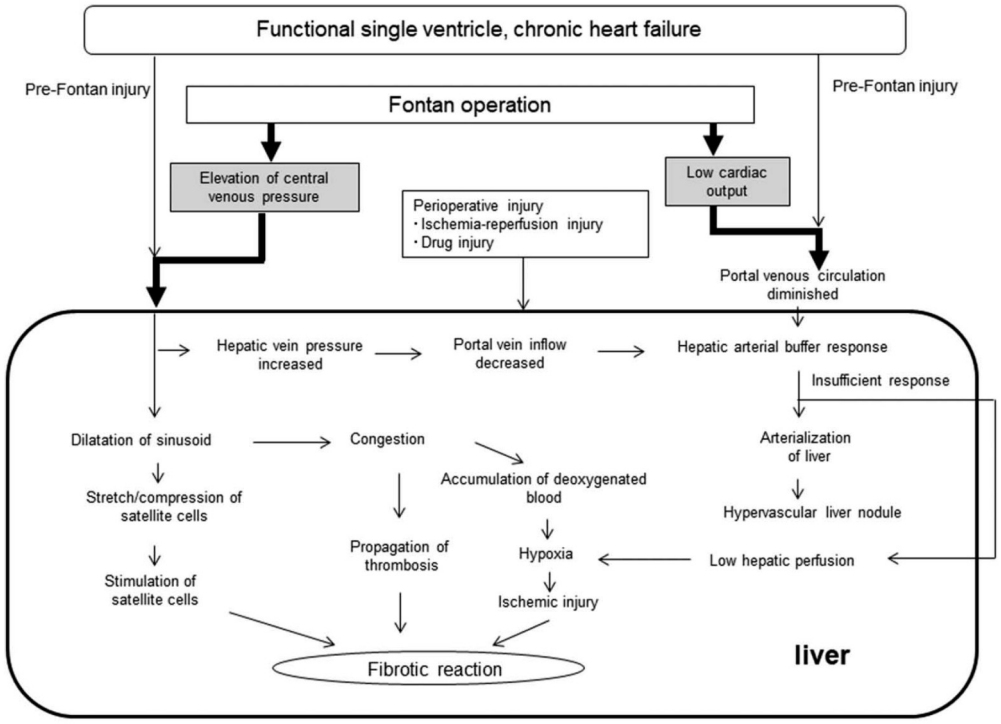

Figure 3. Pathogenesis of cardiac hepatopathy [27].

3.4 CLINICAL PRESENTATION

Hepatic disease may be caused by an acute or a chronic cardiac dysfunction. The clinical presentation on an acute injury may be indistinguishable from a primary liver disease. A marked elevation in transaminase levels is characteristic of ischemic hepatitis. The increase of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels after cardiac surgery occurs particularly in the setting of right-sided heart failure. Extreme elevations of ALT, AST, and lactate dehydrogenase correlate negatively with postoperative survival [50]. ALT levels are correlated highly with right atrial pressure, free hepatic venous pressure (FHVP), and wedge hepatic venous pressure (WHVP), but not the hepatic venous pressure gradient (HVPG) or cardiac index. Total bilirubin correlates better with HVPG [31]. Acute cardiac dysfunction is more likely to be associated with jaundice and encephalopathy, as compared to chronic cardiac dysfunction [31]. However, in persons with chronic cardiac dysfunction, a correlation of biochemical parameters with hepatic pressures is not present [16].

The development of ascites is driven by cardiac status (i.e., right heart failure), dysfunction of surgical palliation, including constrictive pericarditis, or narrowing of the Fontan circulation or protein-losing enteropathy, sinusoidal hypertension resulting from either coexisting liver disease (e.g., viral hepatitis) or cardiac cirrhosis, or portal vein thrombosis (PVT). Measurement of the serum ascitic albumin gradient and total protein on evaluation of the ascitic fluid may help differentiate between a cardiac or liver etiology for ascites [51]. Often, measurement of HVPG and a transjugular liver biopsy is needed to determine whether the cause of ascites is cardiac, hepatic, or combined. Cannulation of the portal vein provides the most accurate assessment of portal pressures [31].

3.5 DIAGNOSIS

3.5.1 Biochemical tests.

Liver function test and coagulation abnormalities (especially protein C deficiency), particularly in patients with Fontan circulation, are common [52]. The approach to abnormal liver function tests is similar to other patients with liver disease. However, there are salient features that may be unique to patients with CHD. Besides primary liver disease, elevated tests may be the result of cardiac dysfunction (univentricular or biventricular failure and pulmonary hypertension), related to medical treatment (transfusion-related viral hepatitis, antiarrhythmic drug toxicity, and transfusion-related iron overload) or surgical palliative correction (e.g., stenosed Fontan conduit, constrictive pericarditis and hepatic vein thrombosis). The pattern of elevation may guide diagnostic testing. A hepatocellular pattern with elevation in aminotransferases results from low flow states and hepatic ischemia. Passive congestion is associated with isolated hyperbilirubinemia and elevated prothrombin time. In symptomatic patients with cholestatic jaundice, ischemic cholangiopathy and pigment stones should be considered. Serum albumin, unless accompanied by protein-losing enteropathy, is preserved until the onset of decompensated cirrhosis [16]. The alpha-fetoprotein (AFP) value is useful for surveillance for HCC [27].

3.5.2 Hepatic fibrosis index

The METAVIR score, initially developed in patients with hepatitis C, is a system used to assess the extent of inflammation and fibrosis in liver biopsies. The grade indicates the degree of inflammation (A0= no activity; A1= mild activity; A2= moderate activity; A3= severe activity) while the stage represents the amount of fibrosis (F= no fibrosis; F1= portal fibrosis without septa; F2=

portal fibrosis with few septa; F3= numerous septa without chirrosis; F4= chirrosis) [53].

The Forns index and the aspartate aminotransferase (AST)-to-platelet ratio index (APRI), as noninvasive blood markers for hepatic fibrosis, have been used to predict the severity of hepatic fibrosis in chronic hepatitis C. APRI values < 0.5 are correlated with the absence of significant fibrosis, >0.5 indicates some liver damage, >0.7 correlates with hepatic fibrosis and >1.0 correlates with cirrhosis [54;55]. There is no study in patients with liver specimen-confirmed cardiac hepatopathy that shows a significant association between liver histology and the hepatic fibrosis index. Both the Forns index and the APRI have been significantly correlated with the time elapsed since the Fontan operation [47;56].

Studies in which cirrhosis was diagnosed by CT reported that both the Forns index and the APRI were significantly different between patients with cirrhosis and non-cirrhosis in Fontan populations [47]. Because there is little evidence that hepatic fibrosis indices are a useful predictor of hepatic fibrosis in cardiac hepatopathy, the advantage of noninvasive blood tests is limited at the moment [27].

3.5.3 The Model for End-Stage Liver Disease excluding the INR (MELD-XI) and the varices, ascites, splenomegaly, and thrombocytopenia (VSAT) scores

The Model for End-Stage Liver Disease (MELD) was developed as a disease severity index for patients with end-stage liver disease and is based on the values of creatinine, bilirubin, sodium and INR [57]. The The MELD score is a good predictor of short-term mortality in liver cirrhosis. However, because the MELD score includes the INR, it cannot measure the mortality risk in patients treated with anticoagulants. To eliminate the influence of anticoagulation therapy, a MELD score excluding the INR (MELD-XI) was introduced for the assessment of short-term survival in cirrhosis [58]. The study of Agnoletti *et al.* showed that the MELD-XI score correlated significantly with the time elapsed since the Fontan

operation [59]. One study in pediatric patients with specimen-confirmed cardiac hepatopathy indicated a significant correlation in Fontan patients between the MELD-XI score and hepatic fibrosis [60]. These findings suggest that the MELD-XI score has the potential to become a useful maker for the detection of cardiac hepatopathy.

The VAST (varices, ascites, splenomegaly, and thrombocytopenia) score was developed to evaluate the severity of liver disease in Fontan patients. The VAST score is based on four signs of portal hypertension and a 4-point system (each sign has one point). A higher VAST score has been significantly correlated with major adverse events such as heart failure and sudden death in Fontan patients [61]. Liver stiffness showed a significant positive correlation with the VAST score in Fontan patients [62]. In addition, a higher VAST score has been associated with both CVP and pulmonary capillary wedge pressure [63].

3.5.4 Conventional abdominal ultrasonography (US)

Because abdominal US is a widely available, noninvasive procedure with no risk of radiation, it is useful for the routine examination of cardiac hepatopathy. Homogeneity, echogenicity, surface nodularity of the liver, hepatic mass/focal lesion, ascites, collateral vasculature, the portal/splenic vein diameter, and spleen size should also be evaluated in patients suspected of having cardiac hepatopathy [64]. There is a correlation in chronic hepatitis between US signs (surface nodularity, heterogeneous parenchymal echotexture, caudate lobe hypertrophy) and the late stage of hepatic fibrosis [65]. Heterogeneous parenchymal echotexture and surface nodularity are associated with the time elapsed since the Fontan operation. The liver histology of hyperechogenic lesions remains unclear. A hyperechogenic lesion might indicate the early stage of hepatic fibrosis [64].

3.5.5 Abdominal computed tomography (CT) and magnetic resonance imaging (MRI)

From 19% to 67% of the Fontan patients may present cirrhosis at CT and MRI scan. A heterogeneous contrast enhancement (the so-called 'reticular pattern'), which is a diffuse patchy enhancement during the portal phase reflect advanced hepatic fibrosis [38]. However, heterogeneous contrast enhancement is frequently detected on CT and MRI in patients who underwent the Fontan operation and these findings were not always correlated with the histological findings [66].

In addition to their use in the evaluation of hepatic fibrosis, CT and MRI play a crucial role differentiating HCC from hypervascular nodules in the Fontan population. Hypervascular nodules, defined as intense vascular blushes observed during arterial phase imaging, are observed in patients with high Fontan venous pressures [38]. Selected postmortem pathology reveals that these nodules are focal nodular hyperplasia (FNH) [22]. On the other hand, early arterial enhancement with rapid washout during the portal venous phase is considered to be highly suspicious for HCC [67]. The presence of hypervascular nodules should be critically evaluated, especially in patients with cirrhosis. There have been increasing reports of HCC in Fontan patients with cardiac hepatopathy and correlates with the duration of the Fontan circuit. In contrast to FNH, HCC may be associated with an elevated alpha-fetoprotein (AFP). The incidence of HCC in patients with CHD is likely to increase in the future, because patients survive longer [22]. When the nodules are large or multiple or atypical, serial imaging combined with the measurement of AFP in the blood should be performed. In the presence of cirrhosis, serial monitoring is with AFP and imaging every 6 months, with biopsy when imaging is not diagnostic [68]. Although the quality of evidence is low, 1- to 2-cm indeterminate nodules in liver cirrhosis could be closely followed by repeated CT or MRI without biopsy [69]. According to the European Association for the Study of the Liver clinical practice guidelines, a biopsy is recommended when an FNH-suspected nodule is >3 cm [70].

Because the risk of cirrhosis increases with duration of Fontan circulation, it may be reasonable to start HCC surveillance at 10 years after Fontan completion or earlier, if there is imaging or clinical evidence of cirrhosis [16].

3.5.6 Elastography

Elastography is a noninvasive imaging tool for the assessment of hepatic fibrosis. Ultrasound-based and magnetic resonance-based elastography are commercially available. Ultrasound elastography can be divided into three types: TE, point shear-wave elastography (pSWE), and two-dimensional SWE (2-D SWE) [71]. The diagnostic accuracy of the FibroScan system for hepatic fibrosis has been validated in chronic viral hepatitis and nonalcoholic fatty liver disease. However, evidence of the utility of ultrasound-based elastography for cardiac hepatopathy remains insufficient [72]. Because ultrasound-based elastography cannot differentiate blood from fibrosis, congestion influences the liver stiffness assessed by elastography. Thus, ultrasound-based elastography is prone to the overestimation of liver fibrosis in patients with congestion [73].

Magnetic resonance-based elastography (MRE) of the liver is currently the most accurate noninvasive technique for the evaluation of liver fibrosis in chronic hepatitis [74;75]. Compared to ultrasound-based elastography, MRE has several advantages. For example, MRE can evaluate a larger volume of liver and reduce the sampling variability. MRE is also highly reproducible and available for patients with obesity or ascites. It has also been reported that liver stiffness measured by MRE showed a significant positive association with the time elapsed since the Fontan operation [76]. However, liver congestion, steatosis, and iron overload could affect the liver stiffness measured by MRE [77]. Furthermore, the use of magnetic resonance may be limited by the presence of cardiac pacemakers and in children [16].

3.5.7 Hemodynamics

The hemodynamic predictors of hepatic fibrosis have been evaluated in Fontan patients. Two studies enrolling patients with specimen-confirmed cardiac hepatopathy showed positive results; one showed that high atrial pressure was significantly associated with portal fibrosis [35]. The authors of the other study reported that there was a positive correlation between inferior vena cava (IVC) pressure and congestive hepatic fibrosis [36]. Although a specimen-based assessment was not performed, several hemodynamic parameters showed a significant association with hepatic fibrosis. Hepatic vein pressure was significantly higher in Fontan patients with cirrhosis compared to those without cirrhosis [38].

A low cardiac index and reduced heart rate were significantly related to liver dysfunction in a Fontan patient series [49]. The CVP was a significant predictor of liver stiffness measured by MRE in a Fontan population [78]. However, the hemodynamic predictors of hepatic fibrosis show conflicting results in Fontan patients.

3.6 TREATMENT

There is no specific medical treatment for cardiac hepatopathy. Although the Fontan circulation is defined by the elevation of central venous pressure and low cardiac output, chronically elevated pulmonary resistance is also present in Fontan patients. The elevated pulmonary resistance causes an increase of end-diastolic pressure, which worsens systemic venous congestion and further reduces cardiac output [79]. Therefore, it has been expected that pulmonary vasodilator therapy (phosphodiesterase type-5 inhibitor, endothelin receptor antagonist, prostanoid) would be effective for the treatment of heart failure in Fontan patients [80]. Although the effectiveness of pulmonary vasodilator therapy for FALD is unknown, a review article suggests that pulmonary vasodilator therapy may be

initiated in Fontan patients having extended hepatic fibrosis with the elevation of central venous pressure [44].

Before cardiac hepatopathy reaches an advanced stage, patients should be considered for heart transplantation [44]. Combined heart and liver transplantation is an uncommon procedure in failing Fontan patients [81]. However, if extended hepatic fibrosis is accompanied by HCC or portal hypertension, combined heart and liver transplantation is considered [82].

Management of complications of PH (e.g., ascites, PVT, or variceal bleeding) follows standard guidelines [83]. However, there are certain differences in the management of PH in CHD patients. Transjugular intrahepatic portosystemic shunt is not recommended in the presence of high right-sided pressures, given the risk for shunt dysfunction as well as the potential for abrupt increase in preload. Second, gastric variceal bleeding is harder to manage, because variceal obliteration with tissue adhesive may be associated with a risk of systemic emboli, given the presence of potential right-to-left intracardiac shunts. Finally, one may need to consider ruling out the presence of varices before the initiation of anticoagulation in CHD patients with cirrhosis [84].

For all these reasons, patients with CHD increasingly require the expertise of a hepatologist to manage the hepatic disease resulting from CHD and to address issues related to cardiac surgery and organ transplantation [16].

3.7 SURVEILLANCE AND PROGNOSIS

Liver cirrhosis and HCC are frequent in patients with Fontan palliation but may also occur in subjects without Fontan circulation. Congenital heart disease with a functional single ventricle or which falls into the category of chronic heart failure should be the primary target of the surveillance for cardiac hepatopathy. In particular, any of the following may be considered a functional single ventricle: tricuspid or mitral atresia, a double-inlet left or right ventricle, hypoplastic left heart syndrome (HLHS), a double-outlet right ventricle, Ebstein's anomaly,

complete atrioventricular septal defect, and pulmonary atresia with an intact septum [85].

Considering that cases of HCC were diagnosed at 20 years of age in patients without Fontan palliation (TGA, ToF), these patients should be examined in their late teens and, therefore, routine examinations for cardiac hepatopathy should be started at 15 years of age in this population [24;27]. Compared to patients without Fontan palliation, those with Fontan palliation need an earlier routine examination for cardiac hepatopathy. Although several studies demonstrated that hepatic fibrosis is associated with the time elapsed since the Fontan operation, a variety of different time ranges have been recommended for the initiation of screening examinations for cardiac hepatopathy [47;86]. Considering that the shortest interval of time between the Fontan operation and the diagnosis of HCC was only 2 years [87], it is thus difficult to determine the appropriate time elapsed since a Fontan operation for the initiation of surveillance in a Fontan population. If possible, blood testing and US imaging should be performed every year after a Fontan operation. It is also possible that the severity of hepatic fibrosis before the Fontan operation can predict the hepatic complications and development of HCC after the operation [88]. Therefore, an abdominal imaging study using US, computed tomography (CT) or magnetic resonance imaging (MRI) should be performed before a Fontan operation [27].

Based on all these considerations, children with a functional single ventricle or chronic heart failure are candidates for the examination of liver disease (Figure 4) [27]. In children who have not undergone a Fontan operation, it's recommend that routine examinations be initiated at 15 years of age. The pre-Fontan evaluation of liver disease is indispensable, and a pre-Fontan evaluation could be useful for the prediction of severe hepatic fibrosis after a Fontan operation. Blood examinations (white blood cell count, hemoglobin, platelet count, AST, ALT, total protein, albumin, total bilirubin, direct bilirubin, GGT, creatinine, cholesterol, INR, AFP, and hyaluronic acid) and conventional US are useful tools in pre-Fontan surveillance in addition to routine examinations [27].

Regardless of the time elapsed since the Fontan operation, when a child has undergone a Fontan operation, it is preferable that routine examinations be initiated at 7–8 years of age. When children undergo a Fontan operation after 7–8 years of age, routine examinations should be initiated 1 year after the Fontan operation. Based on the current evidence, an ideal interval for the routine examination appears to be 6–12 months [27]. Blood tests combined with abdominal US should be performed as a routine examination. The Forns index, APRI and MELD-XI could be helpful to identify cardiac hepatopathy. If a blood test or US suggests the presence of advanced hepatic fibrosis, cirrhosis or a hypervascular nodule, CT or MRI should be performed [27]. Because the availability is limited, elastography is optional.

The indications for a liver biopsy for cardiac hepatopathy are a matter of controversy. When cirrhosis is confirmed by CT or MRI, the follow-up should be continued according to the American Association for the Study of Liver Diseases guidelines regarding the surveillance, diagnosis, and treatment of HCC occurring in the setting of adults with cirrhosis [89]. Even if cirrhosis is not confirmed, hypervascular nodules should be repeatedly evaluated by serial imaging examinations (US, CT, MRI) combined with the measurement of AFP. When cirrhosis is suspected, upper gastrointestinal endoscopy is needed for variceal assessment. When neither CT nor MRI provides a differential diagnosis of hepatic tumor, a liver biopsy is recommended [90].

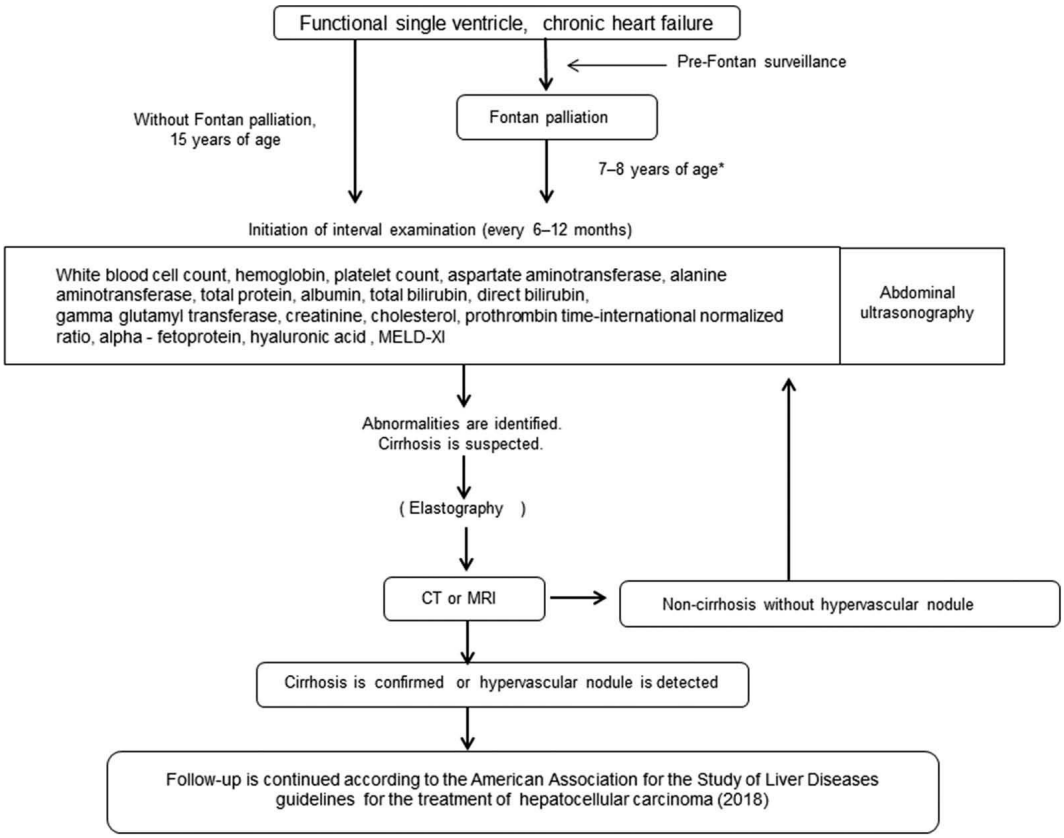

Figure 4. Proposed follow-up for cardiac hepatopathy in children with congenital heart disease [27].

4. NON-INVASIVE TECHNIQUES FOR LIVER STIFFNESS ASSESSMENT

4.1 INTRODUCTION

The Fontan procedure has become the standard of care for single ventricle (SV) CHD patients. The Fontan operation diverts hepatic and inferior vena cava blood from the right atrium to the pulmonary arteries resulting in decreased preload reserve, lower cardiac output, elevated systemic vascular resistance, abnormal ventricular-vascular coupling, and elevated central venous pressure [44]. Despite the short-term operative outcomes have improved over the last decades, the long-term complications of the Fontan operation are becoming increasingly recognized. The liver is at substantial long-term risk in patients with Fontan physiology with hepatic fibrosis as a significant complication in adult patients [91]. Increased hepatic venous pressures and lower oxygen delivery secondary to diminished cardiac output across the stages of palliation are proposed etiologies for liver disease in this population [16], in fact, children and adolescents status-post Fontan may develop hepatic fibrosis and cirrhosis [44].

The gold standard for diagnosing and staging liver fibrosis is a liver biopsy. Hepatic disease in Fontan patients is characterized by centrilobular fibrosis surrounding the central vein and extending along the sinusoids with little inflammatory activity [38]. The extent of fibrosis is postulated to relate to an increase in hepatic venous pressure, low cardiac index, and decreased ventricular function [16]. Liver biopsy is an invasive procedure with potential complications of bleeding and severe pain. Furthermore, sampling error is an intrinsic problem due to the small sample size in a heterogeneous process. Inter-observer variability also limits diagnostic consistency [92].

In order to minimize the risks associated with liver biopsy non-invasive methods are being explored to replace or help select appropriate patients for biopsy [93]. Ultrasound elastography is a noninvasive modality used to assess hepatic fibrosis by measuring tissue stiffness. There are four techniques currently

available including transient elastography (TE), acoustic radiation force impulse elastography (ARFI), 2D-shear wave elastography (2D-SWE) and magnetic resonance (MR) elastography (Figure 5) [94]. Currently, children with SV physiology are not routinely assessed for the development of liver disease, in part due to absence of proven, low-risk screening methods. Therefore, hepatic fibrosis in Fontan survivors is often not recognized until it is in advanced stages [95].

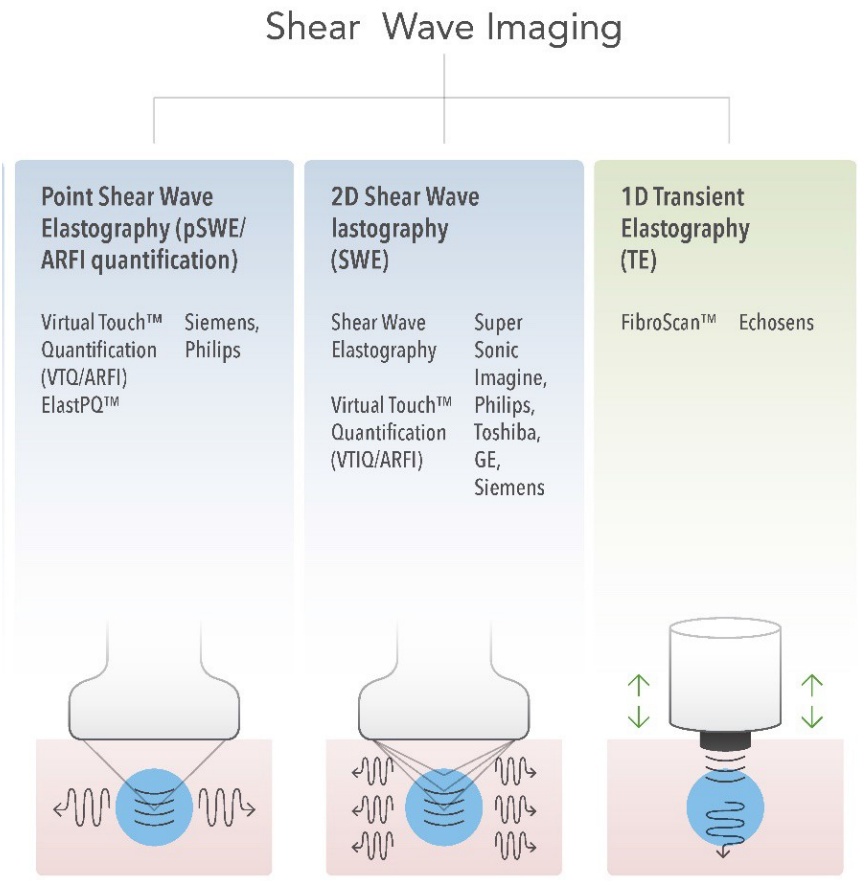

Figure 5. The 3 available ultrasound-based elastography techniques: point SWE/ARFI (VTQ™ Siemens software or ElastPQ™ Philips software), 2D SWE and TE.

4.2 TRANSIENT ELASTOGRAPHY (TE)

Transient elastography is performed with the FibroScan device (e.g. Echosens, Paris, France) which incorporates a 5-MHz ultrasound transducer probe mounted on the axis of a vibrator. The vibrator generates a completely painless vibration (with a frequency of 50 Hz and amplitude of 2 mm), which leads to an elastic shear wave propagating through the skin and the subcutaneous tissue to the liver. The shear wave velocity (expressed in kiloPascals-kPa) is directly related to the stiffness of the tissue [96].

Since in TE excitations are applied at the skin surface, it is limited by patient obesity, narrow intercostal spaces and the presence of ascites [97]. The equipment does not provide B-mode images, which can limit selection of an appropriate sampling area. These factors contribute to a high rate of unreliable results (approximately 16%) with TE [98].

4.3 ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY (ARFI)

Acoustic radiation force impulse (ARFI) elastography is an ultrasound-based technique integrated into a conventional ultrasound machine and used to measure liver stiffness through measurement of shear wave speed (SWS) in a region of interest (ROI), taking care not to include large vascular or biliary structures [99]. It evaluates deep tissue stiffness by generating qualitative and quantitative velocity values measured in meters per second (m/s) and/or in kiloPascals (kPa) through an acoustic pulse generated by the ultrasound probe. The amplitude of the SWS is inversely proportional to the tissue elasticity or stiffness, thus the propagation speed increases with worsening fibrosis [100]. Shear wave elastography uses ARFI to “push” tissue, causing it to move by a few micrometers. This movement generates a transverse (shear) wave that moves more slowly in soft tissue and more rapidly in tissue that is stiffer. This technique provides also a large color-coded map that allow to assess changes in tissues

stiffness in real time. The color-coded map reflects the quality across the stiffness value map. This assists the user in obtaining measurements from the areas with the highest shear wave quality. Every pixel in the ROI is assigned a confidence value from 0 to 100 and a corresponding color between red and green. Low values (red) indicate that the stiffness value for a given pixel is less reliable. High values (green) indicated that the stiffness value for a given pixel is more reliable (Figure 6). Several factors can lower the confidence value: tissue areas with blood flow (vessels), low echogenicity (such as the gallbladder) or with large tissue motion (as with no breath pause).

When compared to TE, shear wave elastography has been found to show similar accuracy but with several advantages. First of all, unlike TE, ARFI can be performed on a conventional ultrasound machine using a standard ultrasound probe [101]. Secondly, the operator can use the US and the color map to directly visualize the liver and select a uniform area of parenchyma without large vessels or dilated bile ducts. Finally, unlike TE where the shear waves are produced by excitation at the body surface, point share wave elastography (pSWE) produces shear waves which originate locally inside the liver, making pSWE less affected by ascites and obesity allowing measurements also in these conditions [102]. Currently, the most used ARFI elastography devices, approved in children and adults, are Siemens-ACUSON with Virtual Touch Quantification (VTQ™) software and Philips with ElastPQ™ software. Both these two pSWE techniques have very good feasibility for the non-invasive liver fibrosis assessment and a good performance for predicting the presence of liver pathology. However, liver stiffness values obtained by ElastPQ™ technique are significant lower than those obtained by VTQ™ elastography [103].

The usefulness of ARFI imaging has been recently described in adult Fontan patients but only few studies have been reported in the pediatric Fontan population [104].

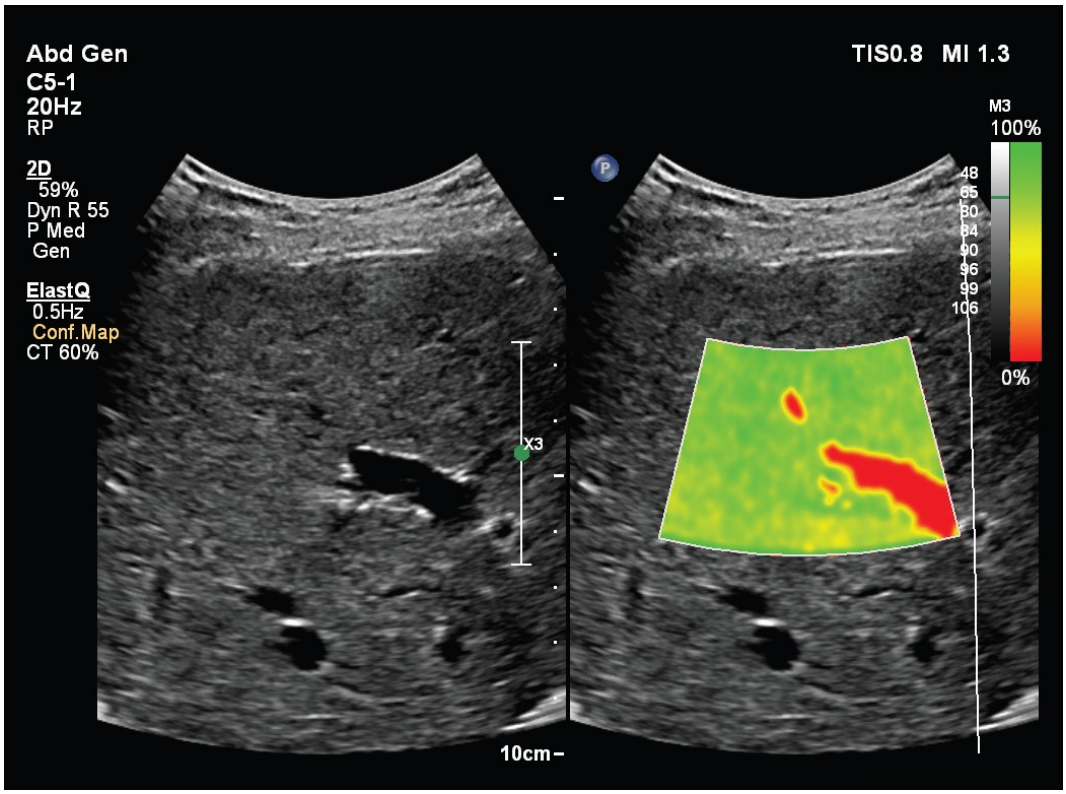

Figure 6. Color-coded map reflecting changes in tissues stiffness in real time. Red area indicates that the stiffness value for a given section is less reliable. Green area indicates that the stiffness value for a given section is more reliable.

4.4 MAGNETIC RESONANCE ELASTOGRAPHY (MRE)

Magnetic resonance elastography (MRE) is a magnetic resonance imaging-based method for quantitatively imaging the stiffness of the hepatic parenchyma [105]. The technique provides quantitative maps of tissue stiffness over large regions of the liver, whereas transient ultrasound-based techniques provide localized spot measurements at limited depth in the liver in areas where there is an acoustic window. MRE is much less operator dependent than ultrasound-based techniques. Therefore, MRE can be readily included in standard abdominal MRI protocols which can provide a comprehensive evaluation of the liver, including assessment of fat content, presence of focal disease, and of complications of chronic liver disease such as varices [106].

The tissues with shear waves of longer wavelengths are represented as areas of higher stiffness as compared to those with shorter wavelengths. Hence, MRE is a three step technique: 1) generating mechanical waves in tissue; 2) imaging the waves with a special MRI sequence, and 3) processing the wave information to generate elastograms, which are images that quantitatively depict tissue stiffness in units of kiloPascals (kPa), and may be displayed in a gray scale or with a color scale. Liver stiffness is typically assessed by drawing regions of interest (ROI's) in the elastograms. Large vessels, the gall bladder fossa, and any areas affected by cardiac and vascular artifacts should be excluded [106].

Normal liver parenchyma has shear stiffness values less than 3 kPa. Hepatic fibrosis can be diagnosed with high sensitivity and specificity if the hepatic stiffness is above this value. MRE has also been shown to be useful for differentiating between various stages of fibrosis. Liver stiffness increases incrementally with histological stage of fibrosis [107].

While the presence of liver fibrosis appears to be consistently associated with increased hepatic parenchymal stiffness, the reverse is not always true. Studies with transient elastography have shown that acute inflammation of liver without presence of fibrosis can cause increased hepatic stiffness [108]. Similar observations are emerging with MRE and, in particular, marked elevation of MRE-assessed hepatic stiffness without biopsy evidence of fibrosis has been reported in patients with acute hepatitis [106].

MRE technique is well tolerated by most patients and can be incorporated into a standard liver MRI study with minimal effect on exam time. MRE is not affected by obesity, ascites or bowel interposition between liver and anterior abdominal wall, all of which may limit the application of TE [106].

Regarding the limitations of MRE, this technique requires cooperation by patients who have to remain still and hold the breath when needed. Therefore, MRE results scarcely applicable routinely in pediatric setting as sedation or general anesthesia are necessary. Furthermore, in patients with moderate to severe iron overload due to hemochromatosis or hemosiderosis, the hepatic MRI signal

may be so low that waves cannot be adequately visualized. Lastly, it might be not applicable in subjects with pacemakers [109].

CHAPTER TWO: SCIENTIFIC STUDY

1. RATIONALE AND AIMS OF THE STUDY

Nowadays, approximately 85% of patients with CHD survive into adulthood as a result of successful reparative surgery [20]. Hepatic complications are common in patients with CHD either resulting from the primary cardiac defect or from palliative surgical procedures performed in infancy or childhood, or from transfusion or drug-related hepatitis [16].

In fact, several CHD defects may lead to either left or right ventricular failure (Table III). Congestion and low cardiac output are the main causes of liver disease secondary to congenital heart disease. The term 'cardiac hepatopathy' reflects the pathophysiology of hepatic injury secondary to CHD. In these cases, hepatic dysfunction may ensue as a result of the primary cardiac defect or as a result of surgical palliation, especially in patients with Fontan operation [16].

The Fontan operation, a palliative surgery for children with single functional ventricle CHD, consists in the connection of the systemic venous return from the superior and inferior vena cava to the pulmonary arteries without the interposition of the morphologic right ventricle and its propulsion. The resulting new physiology is named Fontan circulation [110]. This new hemodynamic condition, despite the good short-term outcomes, determines several long-term complications involving also other organs. The liver, in particular, is mainly affected and presents a spectrum of ailments ranging from congestion to fibrosis and even cirrhosis and hepatocellular carcinoma (HCC). This condition is known as Fontan-associated liver disease (FALD) and is the consequence of a combination of passive venous congestion and decreased cardiac output [111]. Considering the increasing survival of subjects who underwent Fontan palliation, FALD represents a significant challenge in the management of this patients who may develop severe hepatic complications leading also to heart transplant or combined heart-liver transplantation [16]. Liver fibrosis appears to be inevitable in

Fontan patients and directly related to time from surgery, therefore, an adequate hepatic surveillance is fundamental [86].

However, liver fibrosis and HCC can occur also in patients suffering from others congenital heart disease (TGA and ToF) without undergoing the Fontan operation [23;24;25]. In fact, severe and prolonged hypoxia and the decline of blood flow to liver could damage liver tissue and, for this reason, also patients suffering from TGA, ToF and Eisenmenger syndrome require routine examination for cardiac hepatopathy [26]. The predictors of cardiac hepatopathy have not yet been identified. It is difficult to predict precisely who will suffer from HCC among children with congenital heart disease [27].

Liver biopsy represents the gold standard for diagnosis and staging of hepatic fibrosis. However, it is an invasive procedure that often requires sedation and may cause several complications and, therefore, is not suitable for a routine setting [92]. Furthermore, biopsy allows the evaluation of the fibrosis but is unable to identify the early stages of cardiac hepatopathy in which the hepatic congestion predominates [44]. The liver, in fact, is wrapped in a distensible but non-elastic capsule and, thus, hepatic stiffness is not determined only by fibrosis but also by liver congestion due to the increase in central venous pressure (CVP) [112].

For these reasons, in order to avoid invasive procedure but, at the same time, to ensure an adequate follow-up of these patients, non-invasive techniques to assess liver stiffness and fibrosis were recently been considered [113]. Serological markers, hepatic fibrosis indices, ultrasound, computed tomography and magnetic resonance are suitable for use in CHD patients but are not always able to detect early stages of cardiac hepatopathy.

In order to clarify the interaction between the liver and congenital heart disease, there is an urgent need for standardization of the methods for evaluating cardiac hepatopathy. Routine surveillance of cardiac hepatopathy could contribute to an improvement of prognosis in patients suffering from liver fibrosis and HCC. Therefore, a close cooperation among hepatologists, cardiologists, pediatricians and pathologists is essential to prevent, identify and effectively address hepatic problems in patients with cardiac hepatopathy [27].

Recently, it has been shown that non-invasive assessment of hepatic stiffness can be carried out during an ultrasound evaluation of the liver using the acoustic radiation force impulse (ARFI) elastography. This software, installed in modern ultrasound systems, evaluates the speed of propagation of a shear wave in the tissues allowing to establish its stiffness and generating reproducible and comparable values [99].

The usefulness of ARFI imaging has been recently described in adult Fontan patients but only few studies have been reported in the pediatric Fontan population and no one in CHD others than Fontan [104].

Therefore, the aims of this study are:

- to prospectively assess liver stiffness, using ElastPQ™ acoustic radiation force impulse elastography, in pediatric and adult patients with congenital heart disease and to compare liver stiffness values with healthy controls;
- to analyze possible associations between ARFI values and clinical, biochemical, cardiac and hepatic parameters.

2. MATERIALS AND METHODS

2.1 PATIENTS AND CLINICAL FEATURES

In the study were enrolled pediatric and adult patients that underwent heart surgery for congenital heart disease and were followed at the Cardiology Unit of the "Azienda Ospedaliera Universitaria Integrata" of Verona between October 2018 and October 2020. Subjects with known liver disease not related to the cardiac defect were excluded.

Furthermore, were included as controls, subjects without any liver or cardiac disease, matched for age and sex to the case group.

2.2 CLINICAL FEATURES

For each patients enrolled, clinical and surgical data were registered alongside with age, sex, weight, height, body mass index (BMI), blood pressure and oxygen saturation. Ongoing therapy was also reported.

For control subjects, instead, only age and sex at time of ARFI examination were considered.

2.3 LABORATORY TESTS

For each patient, the latest laboratory tests available were reviewed and the following parameters were considered:

- full blood count with formula (red blood cells, platelets and white blood cells);
- hemoglobin and hematocrit;
- mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW);
- albumin;
- alanine aminotransferase (ALT);
- aspartate aminotransferase (AST);
- total bilirubin;
- gamma-glutamyl transpeptidase (GGT);
- creatinine;
- clotting profile (PT: prothrombin time; aPTT: activated partial thromboplastin time);
- troponin;
- C-reactive protein (CRP).

Furthermore, AST to platelet ratio index (APRI) was calculated. According to the literature, the following cut-off were used: APRI values < 0.5 were correlated with absence of fibrosis; values >0.5 indicated some liver damage;

values >0.7 were correlated with hepatic fibrosis and values >1.0 with cirrhosis [114].

2.4 ECHOCARDIOGRAPHY

The most recent echocardiogram (performed within 1 year of the ARFI study) was reviewed by a single investigator. Ventricular function and diameters, atrioventricular valve regurgitation or stenosis, inferior vena cava (IVC) diameter and Fontan circuit function (if present) were recorded. Furthermore, for each patient, a qualitative evaluation on the overall outcome of the surgical correction was performed.

2.5 ARFI ELASTOGRAPHY AND ULTRASOUND OF THE LIVER

Liver ultrasound and ARFI measurement of liver stiffness were performed by a specifically trained single expert radiologist using the Philips Healtcare® ultrasound system with a convex broadband probe and the ElastPQ™ software.

ARFI imaging acquisition were obtained at the same time of the ultrasound of the liver. The patient was placed in the supine position with arms maximally abducted. If possible, breath-holding for shear wave speed acquisition was performed. The liver was visualized through the intercostal or subcostal space, with longitudinal probe placement preferred. ARFI measurements were obtained at an area of homogeneous echotexture devoid of large vessels or other liver structures. The region of interest (ROI) was identified using a color-coded map that allow to assess changes in tissues stiffness in real time and assists the user in obtaining measurements from the areas with the highest shear wave quality avoiding large vessels or dilated bile ducts. Successive acquisitions of SWS were obtained at varying locations, expressed as kPa and the mean value was calculated.

Cut-off values for the distinct stages of fibrosis (based on the METAVIR score [53]) were adopted according to those proposed by Ferraioli et al validated in adult patients with chronic hepatitis C. The different stages of fibrosis were: F0-1 (no to mild fibrosis) <5.7 kPa; F2 (significant fibrosis) =5.7 kPa; F (severe fibrosis) ≥ 5.8 kPa; F4 (chirrosis) ≥7.2 kPa [115].

Conventional liver ultrasound using the same device was also performed, at the same time, to evaluate the size and parenchymal structure of the liver. The hepatic findings were, then, classified as normal or abnormal.

2.6 STATISTICAL ANALYSIS

Data were summarized in groups. Continuous variables were summarized using descriptive statistics, including number of observations, median and interquartile range [IQR 25% – 75%]. Categorical values were summarized using the number of observations and percentages.

Statistical comparisons of differences between treatment groups for continuous variables were made using Wilcoxon-Mann-Whitney or Kruskal Wallis tests. Post hoc pairwise comparisons were made using Bonferroni correction. Statistical comparisons of differences between treatment groups for categorical variables were made using Chi-Square test. Spearman's rank correlation coefficient was used to assess correlations between continuous variables.

All statistical tests were performed using two-sided 5% significance levels, unless otherwise specified.

Statistical analyses were performed using R software, version 4.0.2 [116].

2.7 ETHICS COMMITTEE

The current cross-sectional, single-center, observational study was reviewed and approved by the Ethics Committee of the “Azienda Ospedaliera Universitaria Integrata” of Verona in September 2018.

This research study was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent was obtained from all participants and their guardians before the enrollment.

3. RESULTS

3.1 PATIENTS AND CONTROL SUBJECTS

A total of 50 subjects were enrolled for the study; 33 patients with congenital heart disease and 17 subjects without cardiac or liver diseases as controls. Patients with CHD were then divided into two subgroups: patients who underwent Fontan procedure and subjects who underwent surgery other than Fontan operation.

The Fontan group was composed by 20 patients (60,6% of patients), thirteen males (65%) and seven females (45%). The median age at completion of Fontan circulation was 3.1 years [interquartile range (IQR) 2.9 – 3.62] whereas the median age at ARFI elastography was 8.4 years [IQR 3.2 – 20.5]. In this group, 9 (45%) subjects underwent surgery for tricuspid atresia (TA), 3 (15%) for hypoplastic left heart syndrome (HLHS), 3 (15%) for double inlet left ventricle (DILV), 2 (10%) for pulmonary atresia (PA), 2 (10%) for double outlet right ventricle (DORV) and 1 (5%) for congenitally corrected transposition of the great arteries (ccTGA) with ventricular septal defect and pulmonary stenosis.

In the non-Fontan group were enrolled 13 patients (39,4% of patients), nine males (69%) and 4 females (31%). The median age at surgery was 4.8 years [IQR 0.7 – 6.1] while the median age at ARFI elastography was 4.8 years [IQR 0.8 – 6]. In this group were included 3 (23%) patients with atrial septal defects, 2 (15%) with tetralogy of Fallot, 2 (15%) with aortic valve defects, 3 (23%) with pulmonary valve defects, one with ventricular septal defect, one with coarctation of the aorta and one with Shone complex syndrome.

The control group was composed by 17 subjects, six males (35%) and eleven females (65%). The median age at ARFI elastography was 10 years [IQR 0.7 – 20].

The median value of oxygen saturation was significantly lower in the Fontan group compared to non-Fontan patients (94% [89 – 96] vs 98% [97 – 99]; $p<0.01$). On the other hand, the differences of age at ARFI elastography and sex distribution between the Fontan group, the non-Fontan group and controls were statistically non-significant.

The specific CHD and the main clinical characteristics of Fontan and non-Fontan patients are summarized in Tables IV and V.

Table IV. Congenital heart defects in Fontan and non-Fontan patients. CcTGA: congenitally corrected transposition of the great arteries.

	Fontan (n=20) number (%)	**Non-Fontan** (n=13) number (%)
Tricuspid atresia	9 (45)	
Double inlet left ventricle	3 (15)	
Hypoplastic left heart syndrome	3 (15)	
Pulmonary atresia	2 (10)	
Double outlet right ventricle	2 (10)	
ccTGA	1 (5)	
Atrial septal defects		3 (23)
Ventricular septal defects		1 (7,7)
Tetralogy of Fallot		2 (15)
Aortic valve defects		2 (15)
Pulmonary valve defects		3 (23)
Shone complex syndrome		1 (7,7)

Table V. Clinical characteristics of Fontan and non-Fontan patients

	Fontan (n=20) median [IQR] or number (%)	**Non-Fontan** (n=13) median [IQR] or number (%)	**P**
Sex males (%)	13 (65%)	9 (69%)	p=0.13
BMI Kg/m^2	15.9 [14.3 - 20.4]	15.9 [14.1 - 16.8]	p=0.48
Age at surgery years	3.1 [2.9 - 3.6]	4.8 [0.7 - 6.1]	p=0.37
Age at ARFI years	8.4 [3.2 - 20.5]	4.8 [0.8 - 6]	p=0.48
Oxygen saturation %	94 [89 - 96]	98 [97 - 99]	**p<0.01**
Systolic blood pressure mmHg	100 [90 - 111]	105 [92 - 106]	p=0.98
Diastolic blood pressure mmHg	60 [56 - 64]	62 [57 - 71]	p=0.29

3.2 LABORATORY TESTS

Laboratory tests of Fontan and non-Fontan patients were collected. The median value of hemoglobin and hematocrit as well as the number of red blood cells were significantly higher in the Fontan group compared to non-Fontan subjects ($p<0.01$; $p<0.01$ and $p=0.02$ respectively).

The median number of platelets was lower in patients that underwent Fontan procedure compared to the non-Fontan group but the difference resulted non-significant (217 x10^9/L [145 – 384] vs 331 x10^9/L [257 – 488]; p=0.07).

The median value of AST and ALT was within the normal range in both groups. However, the median level of ALT was significantly higher in the Fontan group (26 U/L [19 – 37] vs 16 U/L [14 – 19]; p=0.01).

Although with a normal median value, creatinine also was significantly higher in the Fontan group compared to non-Fontan patients (0.36 mg/dL [0.29 – 0.72] vs 0.27 mg/dL [0.22 – 0.3]; p=0.01).

Furthermore, the AST to platelet ratio index (APRI) was calculated. The median value of APRI resulted 0.38 [0.18 – 0.45] in the Fontan group and 0.27 [0.17 – 0.38] in the non-Fontan group. This difference was statistically non-significant (p=0.28).

All laboratory tests of Fontan and non-Fontan patients are summarized in Table VI.

Table VI (part 1). Laboratory tests of Fontan and non-Fontan patients

	Fontan (n=20) median [IQR] or number (%)	**Non-Fontan** (n=13) median [IQR] or number (%)	**P**
Hemoglobin g/dL	13.6 [11.5 - 14.5]	10.3 [10.2 - 11.5]	**p<0.01**
Hematocrit %	42.3 [35.9 - 44.7]	32.4 [30.6 - 38]	p<**0.01**
Red blood cells 10^12/L	4.89 [4.29 - 5.36]	3.79 [3.72 - 4.6]	p=**0.02**
MCV fL	87.8 [80.8 - 89.5]	83 [74.9 - 84.7]	p=**0.04**
MCH pg	27.4 [26.2 - 29.0]	27.3 [25.5 - 28.0]	p=0.28
MCHC g/L	322 [302 - 332]	324 [306 - 333]	p=0.96
RDW %	14.6 [13.4 - 15.4]	14.4 [13.2 - 15.8]	p=0.92
Platelets 10^9/L	217 [145 - 384]	331 [257 - 488]	p=0.07
White blood cells 10^9/L	7.88 [5.29 - 10.35]	8.60 [7.10 - 13.43]	p=0.12
Albumin g/L	37.6 [33.2 - 43.3]	40.4 [38.2 - 41.0]	p=0.24

Table VI (part 2). Laboratory tests of Fontan and non-Fontan patients

	Fontan (n=20) median [IQR] or number (%)	**Non-Fontan** (n=13) median [IQR] or number (%)	**P**
Total proteins g/L	64.7 [47.1 - 75.8]	72.0 [69.3 - 75.0]	p=0.27
AST U/L	29.5 [26.4 - 39.6]	33.0 [30.0 - 45.1]	p=0.23
ALT U/L	26 [19 - 37]	16 [14 - 19]	**p=0.01**
APRI ratio	0.38 [0.18 - 0.45]	0.27 [0.17 - 0.38]	p=0.28
GGT U/L	28 [17 - 56]	23 [19 - 30]	p=0.47
Total bilirubin mg/dL	0.4 [0.21 - 1.08]	0.4 [0.2 - 2.1]	p=0.86
Creatinine mg/dL	0.36 [0.29 - 0.72]	0.27 [0.22 - 0.3]	**p=0.01**
PT INR	1.73 [1.19 - 2.25]	1.36 [1.26 - 1.59]	p=0.29
aPTT ratio	1.01 [0.99 - 1.12]	1.02 [0.94 - 1.10]	p=0.80
CRP mg/L	4.5 [1.4 - 36.8]	33 [23.8 - 47.5]	p=0.11
Troponin ng/L	36 [9 - 491]	132 [47 - 678]	p=0.37

3.3 ECHOCARDIOGRAPHY

The function of the main ventricle was evaluated on the basis of the ejection fraction (EF). Twenty (60%) subjects had an EF >50 % (12 Fontan patients and 8 non-Fontan), twelve (36%) presented with an EF between 20 and 50% (7 Fontan patients and 5 non-Fontan) whereas only in one (4%) Fontan child an EF <20% was observed.

The main ventricle was slightly dilated in 18 (55%) subjects (10 Fontan and 8 non-Fontan) while 15 (45%; 10 Fontan and 5 non-Fontan) presented with normal ventricular volume. Severe ventricular dilation was not observed in any patient.

Twenty three (70%) subjects had some degree of atrioventricular valve regurgitation or stenosis. Of them, 14 (61%) belonged to the Fontan group whereas 9 (39%) to the non-Fontan group.

The inferior vena cava (IVC) was dilated in 6 patients (18%; 5 Fontan and 1 non-Fontan).

In Fontan patients with extracardiac conduit (19 patients) the patency of the conduit was studied and resulted normal in all of them.

Based on the echocardiographic parameters, the overall outcome of the surgical correction was defined as good in 16 patients (48%; 7 Fontan and 9 non-Fontan) and intermediate in 17 subjects (52%; 13 Fontan and 4 non-Fontan). In none the final result of the correction was considered poor.

Furthermore, in the Fontan group, patients were further divided according to the severity of the cardiac prognosis based on the original cardiac defect. Subjects that underwent Fontan palliation for tricuspid atresia were considered with a good prognosis (9 patients, 45%); those operated for HLHS were assigned to the poor prognosis subgroup (3 patients, 15%) and children with DILV or other CHD were considered with intermediate prognosis (8 subjects, 40%).

Finally, patients with CHD were divided in two subgroups according to the presence or absence of morphological right ventricle overload/failure. In eleven subjects (33%) a certain degree of morphological right ventricle failure was detected, whereas 22 (66%) presented a normal right heart function.

3.4 ARFI ELASTOGRAPHY AND ULTRASOUND OF THE LIVER

The median value of ARFI elastography were significantly higher in patients with CHDs (Fontan and non-Fontan patients) compared to control

subjects ($p<0.01$) (Figure 7). In particular, the median value of ARFI elastography was 8.61 kPa [5.49 – 11.9] in the Fontan group compared to 5.72 kPa [4.99 – 6.79] in the non-Fontan group. The difference between these two groups of patients was statistically non-significant ($p=0.24$). On the other hand, the median value of ARFI elastography in the control group was 4.51 kPa [4.02 – 4.82], significantly lower than Fontan and non-Fontan group ($p<0.01$). In particular, the difference between Fontan group and controls and between non-Fontan group and controls were statistically significant with a $p<0.001$ for both associations.

According to the cut-off suggested by Ferraioli et al. [115], in the Fontan group 5 (25%) patients showed values <5.7 kPa that indicates no to mild fibrosis at METAVIR score, while 15 (75%) had values ≥5.8 kPa and are classified as F3 (3 patients; 15%) or F4 (12 patients; 60%). In the non-Fontan group 6 (46%) subjects presented with no to mild fibrosis, one (7%) with F≥2 stage, 4 (32%) with F≥3 stage and only 2 (15%) with F4. In the control group, all subjects showed ARFI values <5.7 kPa suggestive for no to mild fibrosis (Table VII).

Based on echocardiographic parameters, patients were divided in several subgroups and ARFI results were compared in the various subgroups.

ARFI median value was 5.37 kPa [4.87 – 6.59] in patients with absent right heart/main ventricle failure, whereas 8.61 kPa [5.81 – 12.14] in those with right heart/main ventricle overload/failure ($p=0.02$).

Considering the function of the main ventricle, ARFI score was 6.62 kPa [5.48 – 10.01] in those with an EF>50%, 5.85 kPa [4.65 – 8.61] in patients with an EF between 20 and 50% and 12.9 kPa [12.9 – 12.9] in those with an EF<20% ($p=0.35$).

ARFI median score was 7.66 kPa [5.11 – 11.73] in patients with atrioventricular valve regurgitation or stenosis while 6.23 kPa [5.17 – 7.20] in those without valvular disfunction ($p=0.36$).

Subject with dilation of the main ventricle showed an ARFI median value of 6.30 kPa [4.81 – 9.00] whereas in those without dilation the value was 7.07 kPa [5.37 – 10.41] ($p=0.32$).

ARFI results were higher in patients with dilation of the IVC compared to those without dilation (8.75 kPa [7.51 – 10.30] vs 6.28 kPa [4.87 – 9.79]; p=0.15).

ARFI median value was 6.11 kPa [5.26 – 7.41] in patients classified as a good prognosis on the basis of the overall outcome of the surgical correction while 8.63 kPa [4.88 – 12.35] in those with intermediate prognosis (p=0.27).

In Fontan patients, divided according to the prognosis, median ARFI values resulted 10.0 kPa [5.9 – 14.2] in the good prognosis group, 8.61 kPa [4.50 – 10.69] in the group with intermediate prognosis and 6.47 kPa [4.34 – 8.41] in the one with poor prognosis (p=0.25).

ARFI median values in patients divided on the basis of the cardiac parameters are summarized in Table VIII.

At conventional liver ultrasound, liver abnormalities were detected in 15 subjects (45%). Thirteen (86%) of them were Fontan patients. Abnormal findings included alteration of the normal hepatic echotexture, irregular liver margins and hepatomegaly. Only in one adult patient that underwent Fontan palliation the ultrasound was suggestive for cirrhosis. There were no focal liver lesions in any of them.

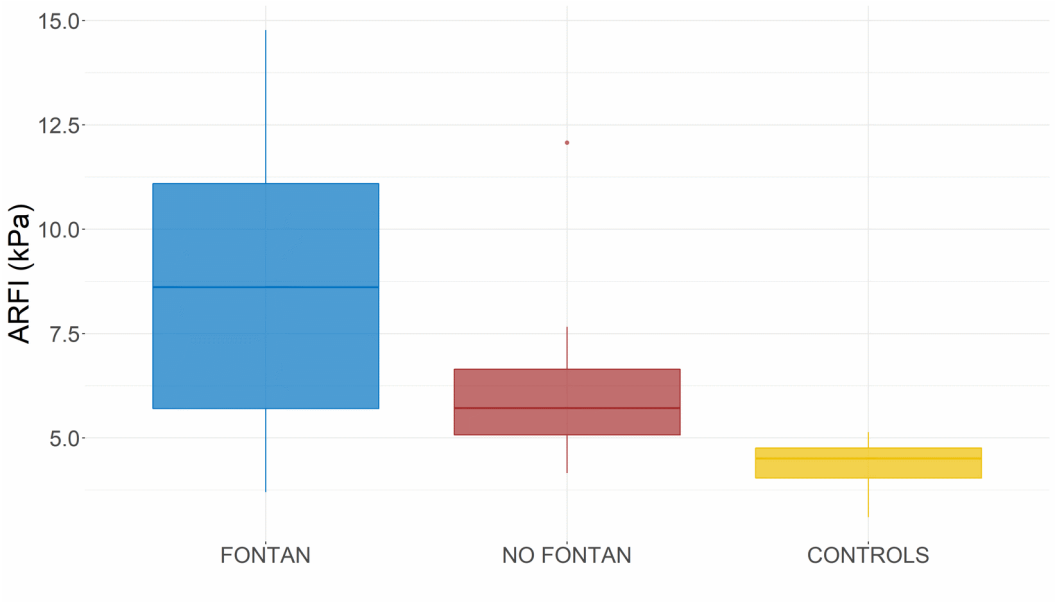

Figure 7. Median value of ARFI elastography in Fontan, non-Fontan and control group ($p<0.01$).

Table VII. Classification of subjects according to METAVIR stage based on ARFI values.

METAVIR stage ARFI cut-off	**Fontan** (n=20) number (%)	**Non- Fontan** (n=13) number (%)	**Controls** (n=17) number (%)	**Total** (n=50) number (%)
F 0-1 <5.7 kPa	5 (25.0)	6 (46.2)	17 (100.0)	28 (56.0)
F 2 ≥5.7 kPa	0 (0.0)	1 (7.7)	0 (0.0)	1 (2.0)
F 3 ≥5.8 kPa	3 (15.0)	4 (30.8)	0 (0.0)	7 (14.0)
F 4 ≥7.2 kPa	12 (60.0)	2 (15.4)	0 (0.0)	14 (28.0)

Table VIII. ARFI median values in patients divided on the basis of the cardiac parameters.

	Number of patients	**ARFI value kPa** median [IQR]	**P**
Right heart/main ventricle failure			**p=0.02**
- Absent	22	5.37 [4.87 - 6.59]	
- Present	11	8.61 [5.81 - 12.14]	
Main ventricular function			p=0.35
- EF>50%	20	6.62 [5.48 - 10.01]	
- EF 20-50%	12	5.85 [4.65 - 8.61]	
- EF<20%	1	12.9 [12.9 - 12.9]	
Atrioventricular valve disfunction			p=0.36
- Present	23	7.66 [5.11 - 11.73]	
- Absent	10	6.23 [5.17 - 7.20]	
Dilation of the main ventricle			p=0.32
- Mild	18	6.30 [4.81 - 9.00];	
- Absent	15	7.07 [5.37 - 10.41]	
Inferior vena cava dilation			p=0.15
- Present	6	8.75 [7.51 - 10.30]	
- Absent	27	6.28 [4.87 - 9.79]	
Overall outcome of surgical correction			p=0.27
- Intermediate	17	8.63 [4.88 - 12.35]	
- Good	16	6.11 [5.26 - 7.41]	

3.5 CORRELATIONS

As expected, the median value of hemoglobin negatively correlated with the percentage of oxygen saturation (rho= -0.4; p<0.02).

Platelets count inversely correlated with age at study (rho=-0.56; p<0.01) and time from cardiac surgery (rho=-0.39; p=0.03).

ARFI results positively correlated with AST to platelet ratio index (APRI) (rho=0.37; p<0.03) (Figure 8).

ARFI median score was also positively associated with time from surgery (rho=0.47; p<0.01) and age of measurements acquisition (rho=0.52; p<0.01).

Platelet count and number of white blood cells were inversely correlated with ARFI values (rho=-0.36; p=0.04 and rho=-0.35; p=0.05 respectively) (Figure 9 and 10), while creatinine was positively correlated with ARFI (rho=0.47; p=0.01).

No significant correlations between ARFI results and other biochemical or cardiac parameters were found.

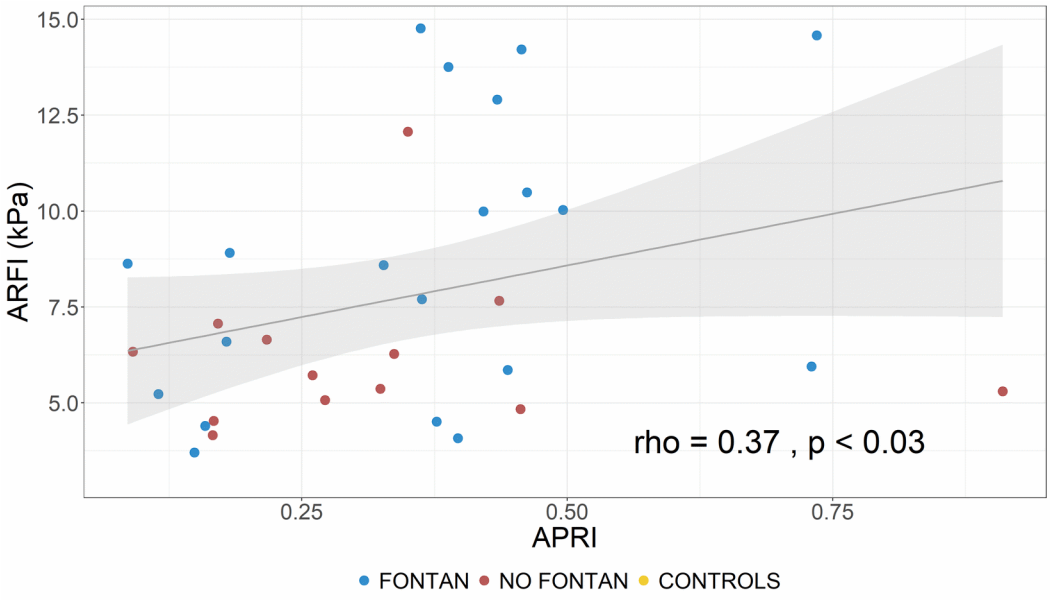

Figure 8. Positive correlation between ARFI values and AST to platelet ratio index (APRI)

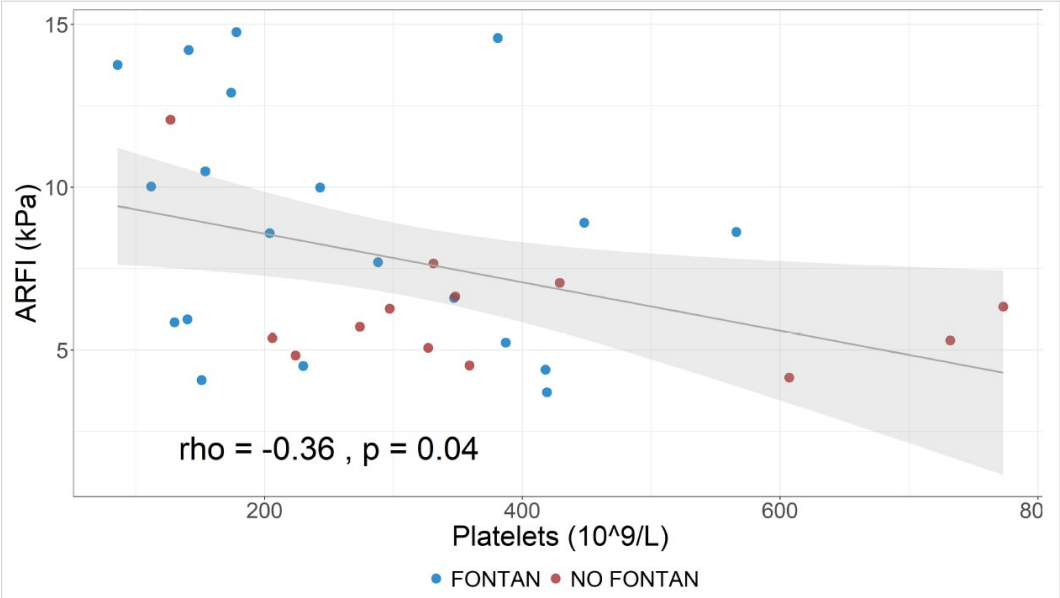

Figure 9. Negative correlation between ARFI values and platelet count

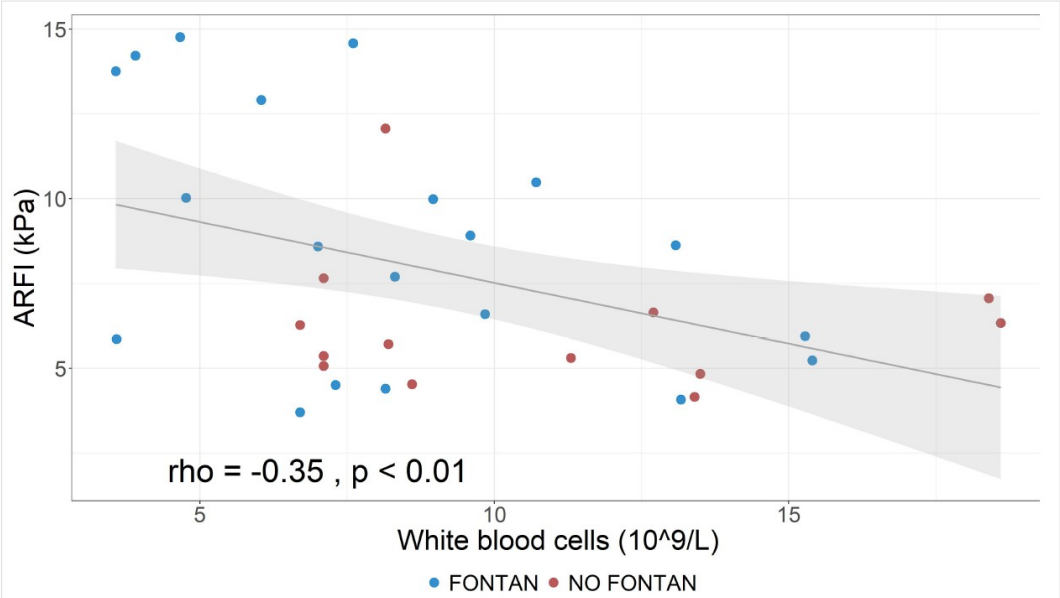

Figure 10. Negative correlation between ARFI values and white blood cells count

4. DISCUSSION

To the best of our knowledge, this is the first study that use acoustic radiation force impulse elastography (pSWE) with ElastPQ™ software to assess liver stiffness (LS) in patients with congenital heart disease.

Our data demonstrates that the median values of LS measured with ElastPQ™ pSWE are significantly higher in patients with CHDs compared to control subjects ($p<0.01$). In particular, the Fontan group showed the highest median value (8.61 kPa [5.49 – 11.90]) followed by the non-Fontan group (5.72 kPa [4.99 – 6.79]), whereas the median value in the control group was 4.51 [4.02 – 4.82]. This result confirms what previously reported in literature for Fontan patients using VTQ™ pSWE and transient elastography (TE) [94;95;104;117] and suggests that ARFI with ElastPQ™ software can be a useful tool to assess liver stiffness in patients with Fontan circulation and other CHDs.

In fact, the increase in liver stiffness in CHDs is the result of a combination of hepatic congestion and liver fibrosis. These two factors, induced by central venous hypertension and low cardiac output, may be the consequence of the primary cardiac defect or the result of surgical palliation, as in Fontan operation. The persistent hepatic congestion alongside with the hypoxia due to low cardiac output may lead to a progressive damage of the liver parenchyma inducing fibrosis and increasing the risk of HCC [16;24]. The gold standard for central venous pressure measurement is cardiac catheterization, whereas liver biopsy is the reference method for fibrosis assessment [44;118]. Both these techniques are invasive and may cause several complications and, for this reason, are not applicable in a routine follow-up. On the other hand, ARFI elastography is a non-invasive, rapid and reproducible method for liver stiffness measurement [95;102].

Ferraioli et al., using liver biopsy as gold standards, defined the optimal cut-off values for ElastPQ™ pSWE for each METAVIR stage of liver fibrosis [115]. In our sample, according to these cut-off, only 5 (25%) patients of the Fontan group showed values <5.7 kPa that indicates no to mild fibrosis while 15 (75%)

had values ≥5.8 kPa and are classified as F3 (3 patients; 15%) or F4 (12 patients; 60%). Better values were found in the non-Fontan group where 6 (46%) subjects presented with no to mild fibrosis, one (7%) with F≥2 stage, 4 (32%) with F≥3 stage and only 2 (15%) with F4. Finally, in the control group, all subjects showed ARFI values <5.7 kPa suggestive of no to mild fibrosis. These findings suggest again a significant involvement of the liver in patients with CHDs and with Fontan circulation in particular compared to healthy controls. Despite elastography itself is not able to distinguish between hepatic congestion and fibrosis, it may be a useful tool to follow-up these patients to decide if and when invasive investigations are necessary [117]. Recently, in particular, the Society of Radiologists in Ultrasound Liver Elastography suggested that each patient becomes his own control using the percentage of LS changes to estimates the progression of the disease [119].

This suggestion found also a support in our data that shows a positive correlation between ARFI values, measured with ElastPQ™ pSWE, and time from surgery (rho=0.47; p<0.01) and between ARFI values and age at measurements acquisition (rho=0.52; p<0.01). These results indicate that liver stiffness tend to increase over time probably because of the prolonged and persistent liver exposure to congestive and hypoxic insults induced by the congenital heart defect or by the palliative surgery. Therefore, ElastPQ™ pSWE may be a useful tool to monitor the evolving of the LS over time.

Considering echocardiographic parameters, our data shows that ARFI values were higher in patients with right heart/main ventricle overload/failure compared to those without this finding (p=0.02). This result seems to confirm that, as reported in the literature, liver stiffness is, at least in part, influenced by liver congestion secondary to increased central venous pressure [118;120]. No significant associations with other echocardiographic parameters were found in our study.

Furthermore, we demonstrated an inverse correlation between liver stiffness and platelet count (rho=-0.36; P=0.04). This result confirms what previously reported by Rathgeber et al. using transient elastography [117]. The decreased platelet count may be the consequence of splenic sequestration due to

splenomegaly that may indirectly indicate the progression of liver disease [95;117;121]. In fact, in our sample, platelet count was inversely correlated with time from surgery (rho=-0.39; p=0.03) and was lower in Fontan patients, in which the hemodynamic impact on the liver is probably greater, than in non-Fontan subjects.

Moreover, for the first time in literature, our study described an inverse correlation between the number of white blood cells (WBC) and LS measured with ElastPQ™ pSWE (rho=-0.35; p=0.05). As reported by Alsaied et al., one third of patients with Fontan may presented with low WBC count and lymphopenia. Its etiology is unknown but an association with portal hypertension and splenomegaly was described [122]. The correlation with increased LS found in our sample, resulting from a certain degree of hepatic congestion, seems to support this hypothesis. Further studies are needed to confirm this correlation.

According to Alsaied et al. a low WBC count and a decreased platelet number may be associated, and both related, to splenomegaly, resulting from an increased central venous pressure and portal hypertension. Therefore, authors suggest to perform a screening for portal hypertension in Fontan patients with lymphopenia and/or low platelet count considering that portal hypertension is associated with increased mortality in this population [61;122].

Finally, a positive correlation between LS values and AST to platelet ratio (APRI) was found in our study (rho=0.37; p=0.03). As the APRI has been proposed as a noninvasive tool for liver fibrosis assessment, this result supports the hypothesis that LS may reflect the evolution of liver disease [114;117].

4.1 Limits of the study

Although this is the first cross-sectional observational study evaluating ARFI (pSWE) with ElastPQ™ software as a tool to assess liver stiffness in patients with congenital heart disease, the sample size was small and limited the statistical power when patients were aggregated into different groups for statistical analysis.

Furthermore, the ARFI technique and cut-offs have not been validated specifically in patients with CHD.

Finally, our laboratory and imaging findings were not confirmed with liver biopsy or invasive measurement of central venous pressure, the gold standards for assessing liver fibrosis and CVP.

5 CONCLUSIONS

In conclusion, our study demonstrated, for the first time in the literature, that acoustic radiation force impulse elastography (pSWE) with ElastPQ™ software can be a useful tool to assess liver stiffness in patients with Fontan circulation and other congenital heart disease.

Our data showed that the median values of LS measured with ElastPQ™ pSWE were significantly higher in patients with CHDs compared to control subjects and, in particular, in those with right heart/main ventricle overload. Furthermore, LS values were correlated with time from surgery and age at liver evaluation. In our study, using a cut-off of 5.7 kPa, it was possible to discriminate between normal liver and liver with signs of congestion or fibrosis. The number of platelets and white blood cells were inversely related to liver stiffness measurements supporting the need of a screening for portal hypertension and splenomegaly in these patients. The AST to platelet ratio index was also correlated to ARFI elastography results suggesting that liver stiffness may reflect the evolution of liver fibrosis.

Considering that hepatic complications are common and potentially severe in patients with Fontan circulation and other congenital heart disease, liver stiffness assessment with ElastPQ™ elastography should be considered part of the routine follow-up of these patients. Low threshold for further investigations should be maintained in patients with elevated ARFI values (>5.7 kPa); in particular if associated with low levels of platelets and white blood cells or with high AST to platelet ratio index.

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