

SYSTEMATIC REVIEW OF THE ETIOLOGY OF PROTEIN LOSS ENTEROPATHY

by

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STATEMENT BY AUTHOR


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Abstract

Background:

Patients born with a single ventricle physiology typically undergo three stages of palliative procedure. One of these, the Fontan procedure was developed 1971 by Dr. Francois Marie Fontan to connect the systemic venous return to the pulmonary arteries. Protein Losing Enteropathy (PLE) is the enteric loss of proteins, that present as a secondary comorbidity witnessed in patients post Fontan procedure.

Objective: This systematic review aims to assess the risk factors, etiology, and treatment of PLE following the Fontan procedure. *Methods:* I searched PubMed, Embase, PsycINFO, CINAHL, Web of Science, and Cochrane Library. Articles were selected based on mesh words: PLE, Fontan, Congenital Heart Defects (CHD), and Total Cavo-pulmonary Connection (TCPC).

Results: The risk factors associated with the development of PLE include: 1.) Chronic state of central venous hypertension, 2.) Single Ventricle (SV) Performance, 3.) Prolonged Cardiopulmonary Bypass Time and 4.) The type of SV anatomy. For the etiology of PLE there were three prevailing hypotheses which include autoimmune/inflammatory response, cellular structure breakdown, and elevated arterial pressures causing lymphatic dysfunction. Lastly, the treatment of PLE includes dietary modifications, Heparin, Budesonide, and IV of immunoglobulin.

Conclusion: The systematic review provides and assesses the clinical spectrum of CHD, specifically Single Ventricle defects, the Fontan Circulation, and PLE. The risk factors, etiology, and treatment of PLE provide a clinical framework for clinicians treating PLE patients post Fontan procedure.

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Introduction

Children who are born with a Single Ventricle (SV) physiology congenital heart defect (CHD) do not survive beyond infancy without surgical palliation called the Fontan procedure or a heart transplant. Once the procedure has been performed, these individuals are considered to have a “Fontan Circulation,” (Fraser, 2017). An unfortunate and often fatal comorbidity that can develop in these individuals secondary to the procedure is Protein Loss Enteropathy (PLE). Many improvements to the Fontan procedure, management, and surveillance for signs of protein loss and changes to the care of individuals with SV CHD have improved the outcomes for these individuals, but PLE continues to be a significant problem.

Therefore, this thesis focuses on the relationship between SV CHD, the Fontan procedure, and the emergence of PLE. In order to develop a better understanding of PLE following the Fontan procedure, I performed a systemic review of the available literature. The goal of this systemic review is to consolidate all clinical research related to etiology and management strategies for PLE in patients with Fontan Circulation and submit a plausible hypothesis to the etiology of the disorder. The hope is to provide a detailed summary of the scope of the research topic, advance clinical practices, and encourage further PLE-Fontan research efforts to improve patients’ outcomes.

Typical Heart Physiology

The heart has two major circuits within the circulatory pathway that subsequently work together in a closed circulatory system. The two pathways are the pulmonary pathway and the systemic pathway. The pulmonary pathway transports deoxygenated blood flow from the right ventricle to the lungs to be oxygenated, while the systemic pathway carries oxygenated blood to the rest of

the body. Blood returns from the systemic circulation through the venae cavae into the right atrium, passes thru the tricuspid valve (this valve has three leaflets) into the right ventricle (Sobonya, 2017). From the right ventricle blood is pumped through the pulmonary valve (which has three valves) and into the left and right main pulmonary arteries, next continuing to the lungs. From the lungs oxygenated blood enters the left atrium, passes thru the mitral valve (which has two leaflets) into the left ventricle. From the left ventricle blood passes through the aortic valve (also has three leaflets) to the rest of the body (Andrews, 2015). The heart's "pumping" and "relaxation" occurs in 2 stages, systole and diastole respectively, this is called a cardiac cycle. During systole, the ventricles in the heart contract, thus pushing the blood from the heart into the arteries. In contrast, during diastole, the heart muscle relaxes allowing the ventricles to fill and prepare for systole (Andrews, 2015).

Electrical System of the Heart

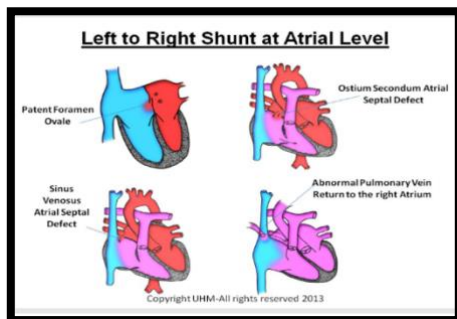
A major element of the cardiac conduction system is the Sinoatrial Node (SA) in the right atrium, which creates an electrical impulse that acts as the heart's pacemaker. Next the electrical impulse travels to the Atrioventricular Node (AV), located between the right and left ventricles. Lastly, the impulse moves to the His-Purkinje fibers, thus acting like a wire conducting an electrical signal into the left ventricle and the right ventricle (Wilchen, 2010).

Congenital Heart Defects

Congenital Heart Defects (CHD) are abnormalities that occur during fetal development in utero as early as the fifth week of pregnancy (Valenti, 2011). During this developmental period the fetus's spinal cord, heart, and organs begin to form. CHD is the most prevalent birth anomaly, with a reported worldwide frequency of 5 to 10 births per every 1000 (Campbell, 2001). Due to the advancement of medical management and surgical interventions, the population of CHD survivors is growing and since 2011, there are more adults with CHD in the US than infants born

each year with a CHD (Moodie, 2011). There are many different types of CHDs ranging from very small holes that do not interfere with function and may close on their own to severe structural defects. CHDs are divided into two main categories, non-cyanotic (left-to-right shunts) and cyanotic (right-to-left shunts). Left-to-right shunts are characterized by the shunting of the oxygenated blood from the systemic circulation to the pulmonary circulation (Soboya, R, 2017). As a result, the pulmonary circulation carries not only the blood that has entered the right atrium and the ventricle through the superior and inferior vena cava, but also the additional blood that enters through the atrial septal defects, ventricular septal defects, or a patent ductus arteriosus (See Figure: 1).

Figure 1. Left to Right Shunts.



Retrieved from: <http://www.universityhospitalmartinique.fr/about-CHD-Congenital%20Heart%20Diseases-Caribbean-Martinique-UHM>

Right to left shunting is the result of several different cardiovascular defects, including a failure of the pulmonary trunk and aorta to separate, incorrect locations of the vena cava, aorta, or the pulmonary trunk. Right to left shunts allow for the systemic circulation of deoxygenated blood, which leads to the development of the condition cyanosis, characterized by blue appearance of the skin and hypoxemia (Sobonya, R, 2017). An example of right-to-left shunting is seen in Tetralogy of Fallot, the most common cyanotic heart defect (Figure 2). Tetralogy of Fallot, is named for a combination of four cardiac findings including: 1.) An overriding aorta allowing blood from both ventricles to enter the aorta, 2.) Pulmonary stenosis, which is the narrowing of

the pulmonary valve and which increases right ventricular systolic pressure 3.) Right ventricle hypertrophy, the thickening of the muscle walls of the right ventricle in response to the increased pressure and 4.) Ventricular septal defect, a hole in the septum that divides the left and right ventricles.

Figure 2. Right to Left Shunt

Retrieved from: <https://www.slideshare.net/dinanathkumar/natural-history-of-right-to-left-shunts>

Single Ventricle

SV physiology is one of the most severe types of CHD. In patients with SV physiology, the heart only has one functional pumping chamber as opposed to two. Patients present as newborns with inadequate pulmonary blood flow or excessive pulmonary blood flow. There are different types of SV defects based on where the anomaly occurs and include: 1.) Double Inlet Left Ventricle (DILV); 2.) Hypoplastic Left Heart Syndrome (HLHS); 3.) Tricuspid/Mitral Atresia (TA), (MA); and 4.) Pulmonary atresia with an intact ventricular septum.

Double Inlet Left Ventricle

DILV occurs when the right atrium and the left atrium both feed into the left ventricle. In some cases, the right ventricle is very small or doesn't even exist. DILV is a rare single ventricle anomaly appearing in 5 in 100,000 newborns (d'Udekem, 2012). Infants born with DILV present with breathlessness, cyanosis, and have difficult gaining weight. In a multicenter analysis of 150 patients, patients with treated DILV had an overall survival of 88% at 1 month, 76% at 10 years of age, and a 20-year survival of about 70% (Tham, 2008).

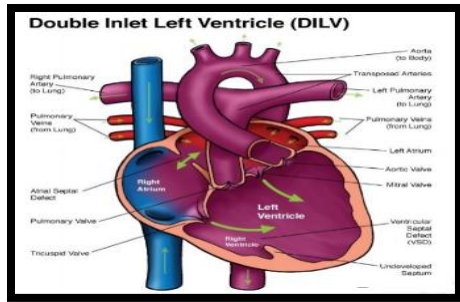


Figure 3. Double Inlet Left Ventricle

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Hypoplastic Left Heart Syndrome

HLHS involves hypoplasia, which means incomplete or under-development of tissue or organs.

In HLHS specifically, there is underdevelopment of the left ventricle, aorta, and mitral valve that do not grow to their full size and cause the organs in the body to also develop below their normal size. HLHS is fatal without treatment. The incidence of HLHS is about 1 out of every 4,000 babies born in the United States (Parker, 2006). In patients with HLHS, there is associated aortic arch hypoplasia, which typically requires three stages of palliative procedures. The first stage is the Norwood procedure. This first stage is an important procedure because the reconstruction of the aortic arch is required in order to provide unrestricted systemic blood flow. This method involves surgical connection of the divided main pulmonary artery to the reconstructed aortic arch. The next stages include the bi-directional Glenn procedure at 4 to 6 months, and the final stage is the Fontan procedure, typically performed between 2 and 4 years of age. Recent reports demonstrated that mortality rate after first-stage palliation of HLHS has improved dramatically, and that the five-year survival rate of 70% (Bove, 1998). The survival rate for one multi-center study reported a 76% one-year survival rate in patients who survived the second and third stages of the correction (Taylor, 2009).

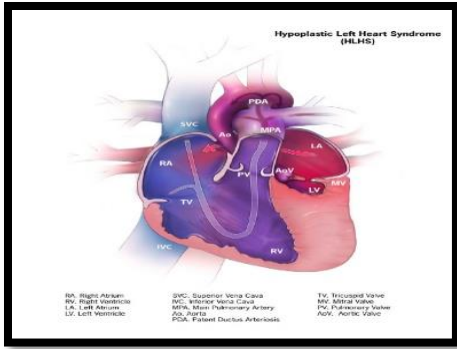


Figure 4. Hypoplastic Left Heart Syndrome

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Tricuspid Atresia (TA) – Right to Left Shunt

In patients with TA, the tricuspid valve located between the right atrium and right ventricle does not form and is atretic. This abnormality causes hypoplasia of the entire right ventricle. Due to the small size of the right ventricle, the heart cannot pump enough blood into the lungs, subsequently causing a lack of oxygen to the baby's organs and tissues (Fraser, 2017). TA has an estimated prevalence of 1 per 10,000 births (Hoffman, 2002). The postoperative mortality, in a single center study with TA reported an overall survival of 80% (Sittiwangkul, 2004).

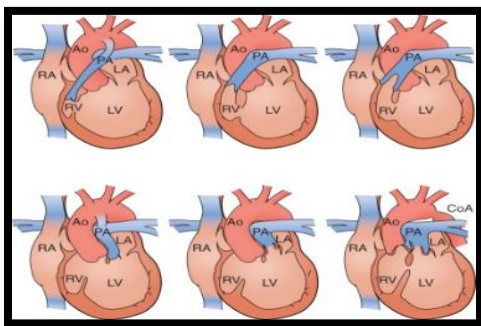


Figure 5. Different types of Tricuspid Atresia.

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[B9780323299879000588?scrollTo=%23s0285](https://www-clinicalkey-com.ezproxy2.library.arizona.edu/#!/content/book/3-s2.0-B9780323299879000588?scrollTo=%23s0285)

Congenital Mitral Artesia (CMA)

CMA is a rare congenital defect in the mitral valve located between the heart's left atrium and left ventricle. Several mitral valve anomalies can occur, and which include: thickening, arrested growth (hypoplasia), or fused valve flaps. These mitral values abnormalities can lead to mitral valve stenosis, which can cause blood flow to be reduced to the left ventricle (Bennasar, 2015). Mitral valve stenosis is the failure of the valve to open completely, the valve can be narrowed, or doesn't close completely. Stenosis causes regurgitation and reduced blood flow (Sobonya, 2017).

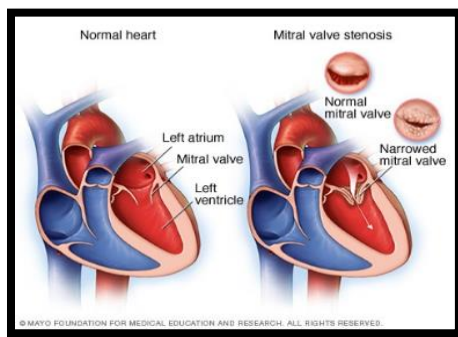


Figure 6. Mitral valve stenosis

Retrieved from <https://www.mayoclinic.org/diseases-conditions/congenital-mitral-valve-anomalies/cdc-20385802>

Pulmonary Atresia with Intact Ventricular septum (PAIVS)-Right to Left Shunting

In patients with PAIVS, the pulmonary valve is completely absent, blocking the flow of blood through the right ventricle out to the lungs for oxygenation. In some cases, the patent foramen ovale (PFO) may stay open to allow blood flow to the lungs (Fraser, 2017). The PFO is a small hole or opening inside the heart, which is located in between the right atrium and left atrium, this allows blood flow from the right atrium to the left atrium mixing deoxygenated blood with oxygenated blood. The PFO usually closes at some point after birth, however in some up to 25% of the population it may remain open (Fraser, 2017). PAIVS patients will almost have an anomaly in their tricuspid valve as well. According to Schneider in 2014, the reported incidence of PAIVS based on population studies with children born with CHD is approximately 4 to 8 per

100,000 live births and the surgical outcomes have improved with a five-year survival rate of 80%.

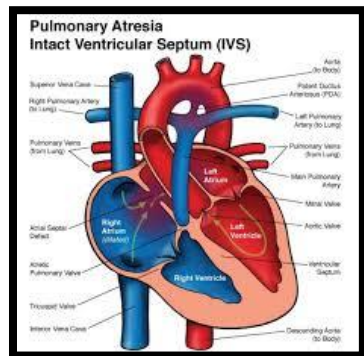


Figure 7. Pulmonary Atresia

https://www.google.com/search?q=pulmonary+atresia+with+intact+ventricular+septum&source=lnms&tbn=isch&sa=X&ved=0ahUKEwiCIYDC5f7aAhVms1QKHQ7NDxoQ_AUICigB&biw=1904&bih=989

CHD Etiology

A growing number of CHD (both left to right and right to left) are known to be associated with genetic mutations, inherited and some presumed to be sporadic (Fraser, 2017). The Notch signaling pathway (Figure 5), has also been implicated in CHD due to the pathway's involvement in the mechanism for cell growth and differentiation, which subsequently plays an important role in cardiac development. Notch elements differentiate the lateralization (the right and left sides) of the body plan. As such the directional looping of the heart tube can be impacted particularly in CHD pathology. Notch signaling is involved early in the formation of the endocardial cushions and continues to be active as they develop into the septa and valves. The pathway is also involved in the development of the ventricular wall and the connection of the outflow tract to the great vessels. Preuss, et, al., (2016) conducted whole exome sequencing in 182 individuals from 51 families affected with left to right shunting. In their cohort, they observed a mutational clustering in the extracellular NOTCH1 terminus in three families with 15 carriers. Notch1 is

also associated with calcification of the aortic valve, the third most common cause of heart disease in adults (Nissen, 2012). (See Figure 5). Nissen et al in 2012, also conclude that mutations in Jagged1 was also linked to Tetralogy of Fallot heart.

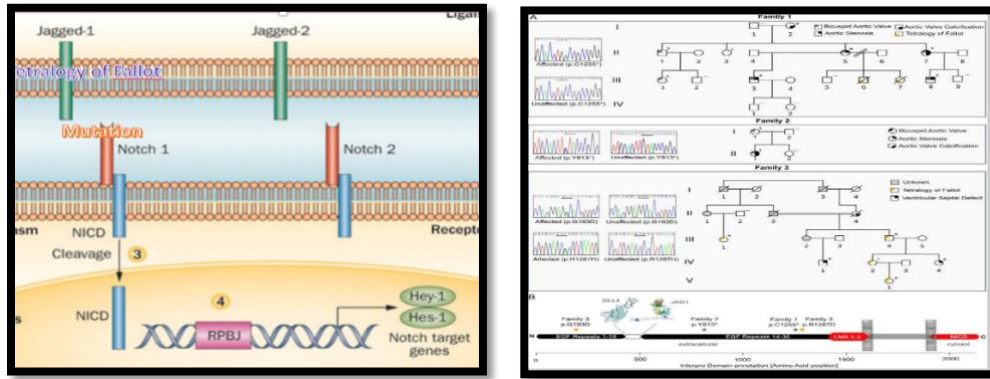


Figure 8. Notch signaling pathway (left) Modified by S. Damon and inheritance patterns of the NOTCH1 mutation (right).

The Notch pathway (Figure 8) is a simple signaling mechanism in which a ligand on one cell induces a series of proteolytic cleavage events in a Notch receptor on a contacting cell. These cleavage events release the Notch intracellular domain (NICD), which translocates to the nucleus to activate the transcription of Notch target genes together with CSL (CBF1/Suppressor of Hairless/LAG-1; also, known as RBPJ) and Mastermind-like protein (Anderson, 2014).

In Figure 8 (right), (A) Pedigrees of families harboring high impact NOTCH1 mutations.

Colors represent the different phenotype associations. The (+/-) symbols indicate mutation carrier status.

(B) Representation of the NOTCH1 locus. (Preuss, et, al., 2016). These figures are important because it displays how the mutation is expressed in families and connects the etiology of CHD to a cellular pathway.

Fontan Procedure

The Fontan procedure was developed in the 1971 by Dr. Francois Marie Fontan for surgical treatment of a tricuspid atresia (Jeong, 2007). This surgical procedure is currently performed to treat several complex SV CHD including double-inlet ventricle, hypo-plastic left heart

syndrome, tricuspid atresia, mitral atresia, and pulmonary atresia with intact ventricular septum. The first procedure, the Norwood reconstructs the aorta and connects the pulmonary artery to the aorta artery. The second stage is a bidirectional Glenn procedure, which involves the redirecting of the superior vena cava and connecting it to the right pulmonary artery. The last stage is called the Fontan completion, which involves the redirecting the blood from the inferior vena cava to the lungs. In this step, the oxygen-poor blood from the upper and lower body flows through the lungs without being pumped. This corrects hypoxia while allowing the single ventricle to be responsible for only supplying blood out to the body. Improved perioperative management and advanced surgical techniques have led to significantly reduced operative mortality associated with the Fontan procedure ($\leq 5\%$ compared with 15–30% in earlier decades). Post Fontan survival at 20 years is presently 85% (d'Udekem, 2007).

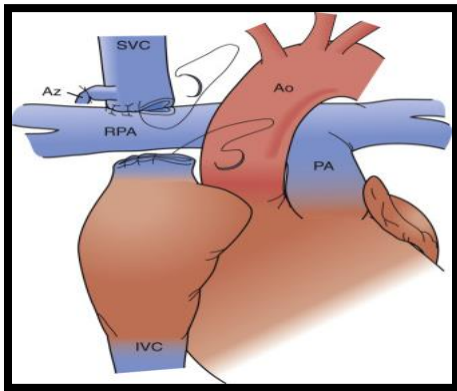


Figure 9. Bidirectional Glenn Shunt Procedure

Figure 9; The Bidirectional Glenn Shunt connects the superior vena cava to the right pulmonary artery, thus resulting in the deoxygenated blood returning from the head and upper body directly to the pulmonary artery for oxygenation by the lung.

Lateral Tunnel (LT)

There are two types of Fontan modifications: the Lateral Tunnel (LT), and the Extra-Cardiac Conduit (ECC). In the LT procedure, a Polytetrafluorethylene baffle is sewn inside the right atrium from the opening of the inferior vena cava to the pulmonary artery. The baffle may be fenestrated by punching a hole into the baffle (Jonas, 1997). The fenestration of the baffle is performed to allow the systemic venous pathway to decompress and enhance ventricular filling postoperatively (Jonas, 1997). The completion of Fontan using lateral tunnel procedure can be accomplished with approximately a 20-minute period of hypothermic circulatory arrest (Fiore, 2007).

Extra-Cardiac Conduit (ECC)

In the ECC, one end of a synthetic tube graft is connected to the inferior vena cava and the other end to the right pulmonary artery. First, the inferior vena cava is transected (incision is made) and the cardiac end is sewn. Next, a PTFE tube graft is connected to the inferior vena cava via a small suture. The tube is then cut to the appropriate length and then connected to the anterior surface of the pulmonary artery. The right atrial septum is sewn and closed (**See Figure 10**). Both procedures bring forth options to accommodate the patient's anatomy. This allows the systemic (deoxygenated blood) to be directly linked to the right pulmonary artery so the blood can be carried to the lungs to be oxygenated.

See Figure 10

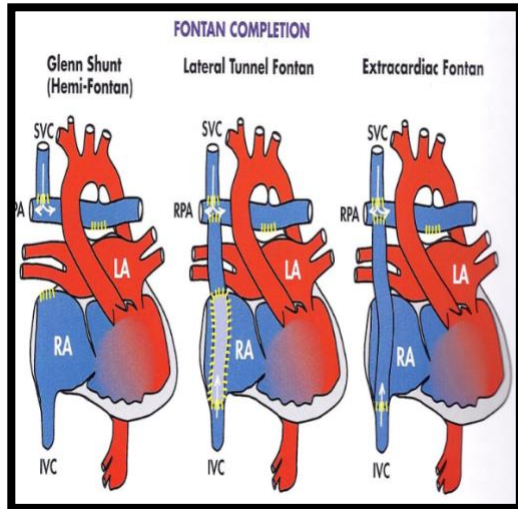


Figure 10. Fontan Completion connecting, Glenn Shunt, LT, And ECC. The Glenn connects the SVC to the right pulmonary artery. The LT, is an intra-atrial conduit that utilizes a direct sewn in baffle connecting the inferior vena cava the right pulmonary artery. The ECC, uses a non-valve PTFE tube conduit connecting the inferior vena cava to the right pulmonary artery.

Over the last two decades, the initial survivors of Fontan repairs have reached adulthood. Some older patients with Fontan circulation have active lives, however, many experience multiple complications. According to Fredenburg in 2011, complications of Fontan circulation include exercise intolerance, ventricular failure, right atrium dilatation and arrhythmia, systemic and hepatic venous hypertension, portal hypertension, coagulopathy, pulmonary arteriovenous malformation, and lymphatic dysfunction such as PLE.

Protein Loss Enteropathy

PLE, is an enteric loss of proteins, albumin (protein made in the liver), and immunoglobulins that leak into the intestinal mucosa. This presents as a comorbidity and is not restricted to patients with disease of cardiac etiology. In the cases of CHD, PLE has an incidence of occurrence of 5-15% in patients with a Fontan repair, with a mortality rate as high as 50% (Feldt,1996). PLE is a powerful predictor of morbidity in patients after a Fontan operation.

Patients with PLE present with the clinical picture of chronic diarrhea, ascites (buildup of fluid in the abdomen), and peripheral edema. The diagnosis of PLE is most commonly based on the determination of fecal alpha-1 antitrypsin clearance. Complications of PLE include; growth impediments due to loss of nutrients and an impaired immune system, from malnutrition and direct loss of immunoglobulins into the stool. -

Although more recent reports suggest that improved treatment has led to improved outcomes in some patients with PLE, there is still much to learn about this complex disease (John, 2011 and Veldtman, 2014). The disease process itself extends beyond simply serum protein loss into the gastrointestinal lumen and hypoalbuminemia, it also includes hematologic and immune abnormalities (Meadows, 2011). Impaired hemodynamics in the Fontan circulation contribute to the pathogenesis of PLE, but not all patients with Fontan physiology develop this disorder. The efficacy of steroids such as prednisone and budesonide in some patients suggests that an inflammatory process likely contributes to the disease (Mertens, 2011, John, 2011, and Veldtman, 2014). Nonetheless, the pathophysiology of this disease is multi-factorial and remains poorly understood.

Systemic Review Methods

Medline, Embase and Cochrane Library databases were search for any articles published from database inception through May 15, 2017. The searches were not limited by language. Two reviewers (SD and RS) screened all abstracts and full-text articles independently. Disagreement between reviewers at any stage was resolved through third party consensus discussion by the remaining authors. Figure: 11, provides details on the initial search criteria in Medline format and article selection using PRISMA Statement flow diagram criteria, (Moher, et al, 2009).

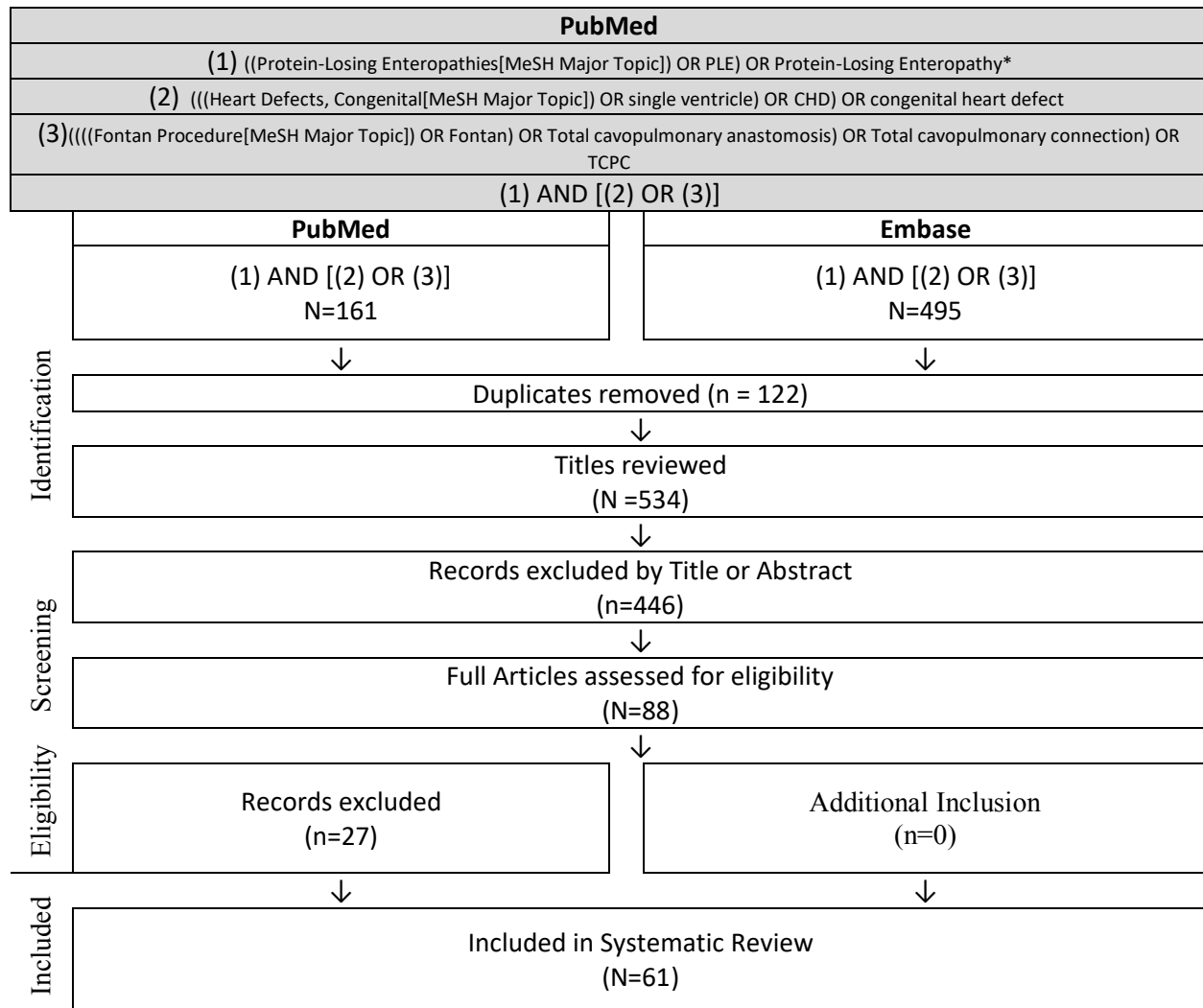


Figure 11. Search and Inclusion PRISMA criteria

Results-Risk factors for PLE

Low Cardiac Output and High Systemic Pressure

One of the most cited risk factors for PLE is a unique chronic state of central venous

hypertension, with diminishing cardiac output Rychik, (2014), Menon (2011), Ozawa,(2014).

Furthermore, the capacity to increase blood flow through the system during periods of increased demand is vulnerable. As this population ages, the state of chronic circulatory insufficiency exerts negative effects on organ system development and functionality. The direct connection of the superior and inferior vena cava to the pulmonary arteries results in systemic venous pressures in the range of 10 to 20 mmHg, 2 to 4 times that of normal. Because systemic venous return is

obligated to passively traverse the pulmonary vascular bed without the force generating benefits of a pump, there is diminished capacity to deliver a normal quantity of blood volume to fill the systemic left ventricle, thereby creating a state of ventricular preload deficiency (Rychik,2014).

SV Ventricle Performance

Patel, (2016), Rychik 2014 and Silvilairat, (2008) found that variables related to the SV malformation itself contribute to deterioration of the overall circulation, which may not be solely a consequence of the Fontan procedure. Relaxation properties of the ventricle are abnormal, leading to diastolic dysfunction, and further impairment to ventricular filling. Abnormal contractile mechanics in the malformed SV can potentially lead to alterations in systolic performance or development of atrioventricular valve regurgitation and PLE.

Prolonged Duration Cardiopulmonary Bypass Time

Power 2001 and Feldt, (1996), analyzed patients preoperatively, peri-operatively, and postoperatively, and concluded that patients with longer cardiopulmonary bypass time had an increase in left atrial pressure after the operation and had a longer hospital stay, which were both associated with the risk of developing PLE.

Type of SV anatomy

In patients with SV who have undergone Fontan operation, the survival rate is better in patients with a dominant left ventricle, than a dominant right ventricle, therefore, patients with Hypoplastic Left Heart Syndrome have the worse outcome. Also, the Driscoll (1992) and Collins' (2013) findings both concluded that patients treated for Tricuspid Artesia had a decreased incidence of developing PLE compared to others with SV anatomy.

Etiology

The precise pathogenesis for post-Fontan PLE is unknown, however the three prevailing hypotheses are an autoimmune/inflammatory response, cellular structure breakdown and elevated systemic venous pressure causing lymphatic dysfunction.

Immune and Inflammatory Markers

Patients who have undergone the Fontan procedure have elevated immune markers compared to controls (Bosci, 2007). Specifically, leukocytes, monocytes and neutrophils are markedly elevated overall post-Fontan (Bosci, 2007). These patients also exhibited elevated inflammatory markers (C3d, neopterin, IL-6) indicating the Fontan procedure may be eliciting an inflammatory response (Bosci, 2017). Those Fontan patients who have also developed PLE have a further suppression of serum immunoglobulins over controls and Fontan patients without PLE. Serum findings demonstrate a reduction in all IgG subgroups and are highest for IgG and IgA markers and similarly reduced anti-inflammatory cytokine IL-10, T cells and NK Cells (Bosci, 2007, Cheung 2004, & Magdo, 2015). T-cell involvement demonstrates a significant discrepancy in the number of CD4 cells produced resulting in a ratio reversal of CD4/CD8 T cells (Cheung 2004 & Magdo 2015). These immunological anomalies in PLE patients demonstrate a similar pattern to other autoimmune diseases like celiac disease and systemic lupus erythematosus.

Systemic Venous and Lymphatic Pressure

Lastly, abnormal vascular pressures are proposed to affect the lymphatic system in the intestines leading to PLE. Dori et al (2014) found a significantly increased thoracic duct diameter in patients with PLE, which is proposed to be the result of elevated venae cava pressure leading to the increase lymph production and subsequent thoracic duct dilation. The thoracic duct drains directly into the subclavians, therefore if pressure in the left internal jugular is elevated then the thoracic duct will dilate, causing the lymphatic pressure to go up, and then more leakage of proteins.

PLE patients are found to have lymphangiectasia, and tissue edema demonstrating a leakage of lymphatics (Dori, 2014). Lymphangiectasia is due to lymphatic obstruction due to increased pressure in lymphatics, which result in the leakage of lymph and proteins through the intestines.

These findings correlate with the reduction of CD8 and IgA, which is described in both PLE and intestinal lymphangiectasia.(Dori, 2014, Cheung 2004, & Magdo 2015) Others, however did not find any systemic elevated venous pressures in their study populations (Kim, 2001 & Meadows, 2008). Rychik et al., (2002) found tissue Doppler diastolic velocities were lower in all Fontan patients and those with PLE had higher systolic to diastolic velocities with an elevated mesenteric vascular resistance index.

Treatments

Treatment of PLE include daily dietary modifications, such as high-protein and low-fat diet along with medium-chain triglyceride supplementation, aspirin, anti-inflammatory medication, IVIG, and heparin.

Heparin

According to Rychik (2014) and Ryerson (2008) patients at high risk for PLE may benefit from low molecular weight heparin. Heparin improves forward flow in the Fontan circuit, which may lower central venous pressure and improve filling the ventricle with greater quantity of oxygenated blood, therefore improving oxygen delivery. Rychik (2014) also, hypothesized that heparin may stabilize the cell–matrix interactions at the capillary endothelium or at the intestinal mucosa to decrease the leakage of protein into the extravascular space or into the intestinal lumen, respectively. Alternatively, it may alter the cytoskeletal architecture in the perivascular space to help resorb protein into the lymphatics.

Budesonide

Turner, (2011), Schumacker, 2011, and Driscoll, (2011), all concluded that budesonide is a good treatment for PLE. Budesonide is a steroid, that works inside the intestine to reduce inflammation. Oral budesonide improved PLE symptom like edema and ascites; and increased

serum albumin levels. All authors noted that budesonide should be used with other established PLE interventions, such as diuretics, digoxin, albumin infusion, aspirin, and a high protein diet.

Intravenous Immunoglobulins

Zaupper et al, (2011), treated a small cohort of PLE patients post Fontan Circulation with high dose intravenous immunoglobulins. This was the only study found to administer 1g/kg of intravenous immunoglobulins for treatment periods ranging from 1 year to 5.3 years. During the IVIG treatments there were no serious infections, there was significant increase in plasma albumin, IGG levels, and resolution of edema. The only drawback to the results of this study was the small sample size of four patients.

Discussion

This report on risk factors, etiology, and treatment of PLE gives a consolidated examination of PLE. While conducting this clinical systematic review, I found it very interesting that that etiology of CHD and PLE are poorly understood.

After reading how the Notch pathway was implicated in the etiology of CHD based on DNA sequencing of affected family members with left to right shunting and that mutations in the Notch pathway were linked to Tetralogy of the Fallot and CHD, led me to consider cellular pathways and this permitted me to connect PLE to two cellular pathways.

Due to the hallmark characteristics of PLE, patients with post Fontan circulation, experience enhanced leakage of protein due to leaky and ulcerated intestinal mucosal lining. These symptoms led me to investigate and link the EGF/EGFR pathway to PLE. Epidermal Growth Factor (EGF) and Epidermal Growth Factor Receptor (EGFR) have been implicated in regulating cell proliferation, apoptosis, and differentiation of intestinal permeability and barrier function.

EGF has been identified as an effective intestinal regulator helping to protect the intestinal barrier (Damiano, et al., 2015). Dysfunction of the intestinal barrier is associated with increased gut permeability and development of other gastrointestinal diseases. From all the research gathered by conducting this clinical systematic review, I am able to submit a plausible etiology of how PLE affects the intestinal mucosa and a treatment strategy to manage the inflammation of patients with PLE.

Epidermal Growth Factors (EGF)-Plausible Etiology.

Epidermal Growth Factors (EGF) play a critical role in intestinal maintenance and I propose that EGF and EGFR plays an important protective role in Fontan patients who don't develop PLE. Therefore, I hypothesize that there is a down regulation in the EGF in the 5-15% of PLE patients post Fontan.

EGFR

EGF is mediated through binding to EGF receptor (EGFR) (Figure 12). EGFR is a transmembrane receptor of the ErbB receptor family, (ErbB1, ErbB2, ErbB3, and ErbB4). Binding to EGFR leads to the activation of EGFR and homo-dimerization/phosphorylation of transmembrane Receptor Tyrosine Kinase (RTK), and the next activation of four cell signaling cascades; 1.) Ras/Mitogen Activated Protein Kinase Ras/(MAPK), 2.) Phosphatidylinositol 3-kinase/AKT (PI3K/AKT), 3.) phospholipase C- γ /protein kinase C (PLC- γ /PKC), and 4.) JAK-STATS signal pathways, which promotes intestinal development, regulates tight junction proteins, reduces cell autophagy, and inhibits apoptosis by oxidative stress, and reduce the colonization of the intestinal epithelium by gastric pathogens (Xiaopeng, et al., 2016).

MAP Kinase Pathway

For the MAPK pathway, the interaction between EGFR and SHC/Grb2 result in the activation of SOS, thus activating the Ras, /Mitogen-Activated Protein Kinase (Ras/MAPK) by allowing

(GTPase RAS to bind to GTP). The activated Ras mediates Raf-1 then activates MEK, which in turn activates the ERKs (Erk-1 and Erk-2).

PLC- γ

PLC gamma is activated by the binding of phospholipase c-gamma and EGFR tyrosine kinase.

When activated the PLC gamma hydrolyses phosphatidyl inositol 4,5-biphosphate (PIP₂) to diacylglycerol(DAG)and inositol triphosphate (IP₃). IP₃ binds to the IP₃ receptor in the endoplasmic reticulum and the release of calcium ions. Calcium ions then bind and activate PKC.

JAK/STAT Pathway

The JAK/STAT pathway carries signals from the EGFR to the nucleus and activate transcription factors associated with immunity, cell proliferation, and apoptosis (programed cell death). The JAK/STAT pathway becomes triggered when the JAKs phosphorylate the tyrosine receptors, thereby creating a docking stations for STATs. Thereafter, STATs are recruited to their receptor, and also phosphorylated by JAKs. The activated STATS then translocate into the nucleus of the cell where they bind to a specific sequence of DNA and induce transcription. The JAKS are specifically activated by different receptors, thus they have distinct *in vivo* functions. JAK1 is activated by type II cytokine receptors. JAK2 transduces signals for cytokine receptors involved in hematopoiesis. JAK3 is mainly expressed in B and T lymphocytes, and TYK2 associates with other JAKS (Alves de Medeiros, et al., 2016).

PI3K/AKT Pathway/mTOR

PI3K, AKT, and mTOR are part of an intracellular signaling pathway that regulate the cell cycle. In this pathway, Phosphoinositide 3 Kinase (PI3K) is phosphorylated and activates AKT(Protein Kinase B). PI3K kinase is a member of a lipid family that is known to phosphorylate hydroxyl groups of inositol ring and generate PIP₃and PIP₂. Activated AKT interacts with these PIPs and phosphorylates them to regulate cell growth and cell survival.

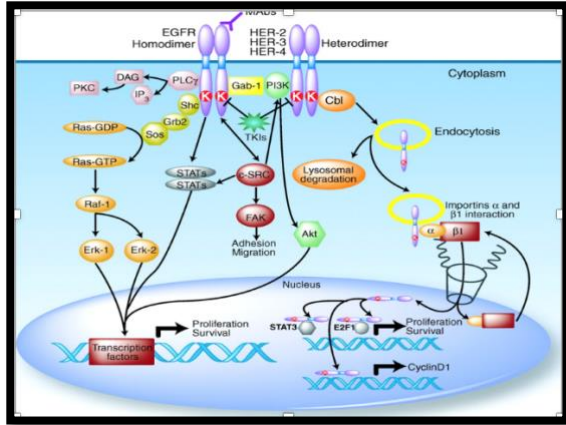


Figure 12. EGF/EGFR pathway.

EGF is a 53-amino acid peptide, established as a major trophic factor for the developing intestine (Rowland, 2013). The biological functions of EGF are mediated through binding to EGFR and inducing RTK autophosphorylation, thus subsequently activating various signal transduction pathways to regulate intestinal development, tight junction expressions, and mucins secretion which are important for the formation of intestinal barrier functions. (Dominquez, 2010).

EGF is normally found in fluids that immerse the developing intestine, including amniotic fluid, fetal urine, breast milk, bile, and saliva (Carpenter, 1980). EGF, functions as a gastrointestinal tract (GI) mucosal protective factor, which maintains epithelial cell homeostasis in the small intestines (Duh, 2000). EGF also promotes mucin production, therefore preventing bacterial infiltration and inflammation to occur on the intestinal barrier. Membrane bound mucins, including MUC12, MUC13, MUC15, MUC16, MUC17, and MUC20, are anchored into the apical membrane of epithelial cells and have transmembrane domains. MUC3 is expressed in in the small intestines, but also shows high level of crypt expression, therefore, suggestion a link between MUC3 expression and enterocyte differentiation. (Hollingsworth, 2006).

In a study conducted by Damiano (2015), they analyzed NOX and DUOX2, a Reactive Oxygen Species (ROS species) in EGF/EGFR signaling leading to mucin expression in human adenocarcinoma Caco-2 cells. Caco-2 cells were chosen because they undergo spontaneous differentiation in enterocytes. NOX and dual oxidase 1 and 2 (DUOX1, DUOX2), produce hydrogen peroxide, a ROS, a species have been found in thyroid, the respiratory epithelial cells, and intestinal tract (Damiano, 2015). Their results found that EGF modulates DUOX2 levels via the Mitogen Activated Protein Kinase signal MEK-ER1/2 and Protein Kinase C pathways and increased ROS levels, which induced MUC3 and MUC5AC mRNA expression. In conclusion, EGF acts as a key epithelial mucosa regulator to regulate intestinal permeability and intestinal barrier integrity through the following 2 ways: (1) inducing DUOX2 expression and ROS production to activate ERK1/2- PKC pathways, leading to the induction of Muc5AC and Muc3 expression and (2) via Muc5AC and Muc3 to reduce bacterial colonization and translocation.

To further support the plausible etiology that EGF and EGFR are protective factors for PLE, two separate studies found that EGF supplementation improves mucosal repair and regeneration in several conditions. A small study of humans treated with intravenous EGF demonstrated superior gastric ulcer healing with EGF as compared to anti-ulcer cetraxate hydrochloride treatment (Itoh, M., 1994). In the other study, oral EGF administration in patients with duodenal ulcer disease resulted in comparable success to treatment with cimetidine (Rowland, 2013). Human randomized controlled trials of EGF suppositories for patients with left-sided ulcerative colitis demonstrated EGF to be superior in healing compared to placebo regarding disease activity, sigmoidoscopic findings, and histologic grading of injury (Rowland, 2013). From these findings, I would suggest that EGF could also be used as a potential treatment for PLE.

Lastly, Rowland, (2013) has shown that that EGFR deletion was associated with embryonic lethality. Mice with EGFR deletion that do survive to delivery, die early in the neonatal period with a hemorrhagic enteritis (inflammation of the intestine). This known causal relationship between these entities and intestinal inflammation reaffirms that the EGF and EGFR pathway may be downregulated or mutated in patients with PLE.

Plausible Treatment for PLE

Due to the elevated immune markers, specifically leukocytes, reversed CD4/CD8 ratio, and neutrophils in PLE patients post Fontan allows me to submit a plausible treatment for treating PLE, JAK inhibitors. Inhibiting JAK1 and JAK2 this will disrupt the cytokine signaling cascade in the JAK/STAT pathway, therefore, decreasing STAT-3 mediated functions and suppressing lymphocytes, this is a plausible treatment for patients with PLE.

The JAK/STAT signaling pathway has been implicated due to its important role in the immune response. For example, it is involved in facilitating the binding of the interferon and interleukins to their specific transmembrane receptor. Hence, targeting the JAK/STAT pathway will initiate immunosuppression and anti-inflammatory responses, countering the immune and inflammation seen in patients with PLE.

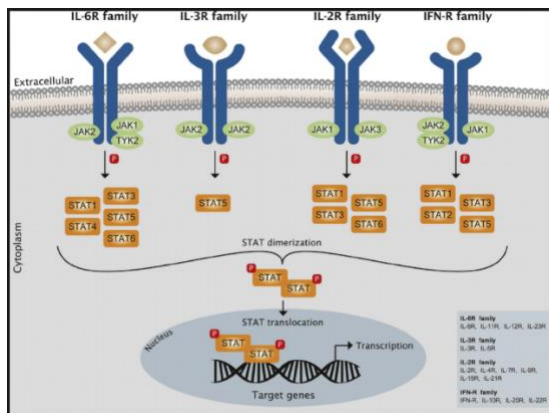


Figure 13. Typical non-mutated JAK/STAT family

Figure 13, shows a typical non-mutated JAK/STAT family which includes; 1.) JAK1, JAK2, JAK3, TYK2, and 2.) STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. The JAK/STAT pathway becomes activated when the JAKs phosphorylates the tyrosine receptors, thereby creating a docking stations for STATs. Thereafter, STATs are recruited to their receptor, and also phosphorylated by JAKs. The activated STATs then translocate into the nucleus of the cell where they bind to a specific sequence of DNA and induce transcription. The JAKs are specifically activated by different receptors, thus they have distinct in vivo functions. JAK1 is activated by type II cytokine receptors. JAK2 transduces signals for cytokine receptors involved in hematopoiesis. JAK3 is mainly expressed in B and T lymphocytes, and TYK2 associates with other JAKs (Alves de Medeiros, et al., 2016).

A recent study by Migita et al., (2103) demonstrated clinical proficiency of JAK inhibitors in Rheumatoid Arthritis. The study utilized JAK inhibitors CP-690,550 (inhibitor of JAK-3) and INCB028050 (inhibitor of JAK 1 and JAK2), which suppressed the activation of JAK 1, 2, and 3, which further inhibited the downstream effectors STAT 1, 3, and 5. The conclusions drawn from this study revealed that inhibiting JAKs 1 and JAKs 2 likely contributed to the treatment of RA in mice and in human cell lines.

Therefore, utilizing the JAK1 and JAK2 inhibitor INCB02850 as a promising treatment option or therapy for hypersensitivity reactions, subsequently, could be tested to treat and suppression immune activity with patients with PLE.

Conclusion

This systematic review provides a comprehensive assessment of the clinical disease spectrum of CHD, specifically SV, Fontan Circulation, and PLE. The risk factors, etiology, and treatment of PLE provides a framework for clinical guidelines and a basis for my proposal for a new treatment possibility.

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