Fluid accumulation and transcapillary fluid balance in children with congenital heart defect

Evaluation of colloid osmotic pressures assessed by the wick method

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List of Papers

1. Guthe HJ, Indrebo M, Nedrebo T, Norgard G, Wiig H, Berg A. Interstitial fluid colloid osmotic pressure in healthy children. PLoS One. 2015;10(4):e0122779.

2. Indrebo M, Berg A, Holmstrom H, Seem E, Guthe HJ, Wiig H, Norgard G. Fluid accumulation after closure of atrial septal defects: the role of colloid osmotic pressure. Interact CardioVasc Thorac Surg. 2018;26(2):307-12.

3. Indrebo M, Berg A, Holmstrom H, Seem E, Guthe HJT, Wiig H, Norgard G. Fluid accumulation in the staged Fontan procedure: the impact of colloid osmotic pressures. Interact CardioVasc Thorac Surg 2019; 28(4):510-517.

Abbreviations

ASD	Atrial Septal Defect
BCPC	Bidirectional Cavopulmonary Connection
CFC	Capillary filtration coefficient
CHD	Congenital Heart Defect
COP	Colloid Osmotic Pressure
COPi	Interstitial Colloid Osmotic Pressure
COPp	Plasma Colloid Osmotic Pressure
CPB	Cardiopulmonary Bypass
CVP	Central venous pressure
CUF	Conventional ultrafiltration
ECV	Extracellular Volume
HTx	Heart transplant
ICV	Intracellular Volume
IF	Interstitial fluid
MAP	Mean arterial pressure
MUF	Modified ultrafiltration
PAP	Pulmonary artery pressure
PE	Pericardial effusion
PLE	Protein-losing enteropathy
SIRS	Systemic inflammatory response
TBW	Total Body Water
TCPC	Total Cavopulmonary Connection

Background for the thesis

The management of univentricular type of congenital heart defects has evolved during the last 50 years and the Fontan operation is now the current standard of care. The Fontan procedure has created survival for thousands of children with a univentricular heart (1). The procedure is not an anatomical correction, but " a physiological pulmonary blood flow restoration independent of a subpulmonary ventricle "(2). This allows for oxygenation at near normal levels but at the cost of a chronic state of systemic venous congestion and decreased cardiac output. Despite the now excellent survival after Fontan surgery, the hemodynamic compromise in Fontan circulation causes increased morbidity (3). Postoperatively low cardiac output and fluid accumulation with edema formation, impaired respiratory function and prolonged pleural effusion are of concern. The continuously elevated central venous pressure (CVP) and impaired cardiac output, the hallmark of Fontan circulation, may induce a cascade of pathophysiological consequences, including liver failure, renal failure, fluid accumulation and protein-losing enteropathy (PLE), named the failing Fontan (4). The circulatory changes will act on the transcapillary fluid exchange with elevated intravascular hydrostatic pressure and fluid extravasation. There has been an extensive research to find risk factors for developing fluid accumulation and the failing Fontan but to the best of my knowledge there are no published studies on transcapillary fluid exchange in these patients.

Fluid accumulation with edema formation is common after surgery for congenital heart defects in children. This accumulation may compromise cellular function due to increased diffusion distance for oxygen and nutrients. The fluid shift may be due to the use of cardiopulmonary bypass (CPB) or other unknown factors. Fluid accumulation and edema formation in children during surgery for congenital heart defects has been studied (5, 6). A few studies have evaluated the colloid osmotic pressure (COP) in plasma, one of the determinants of transcapillary fluid exchange, but none of these have evaluated interstitial COP (7-10). To determine the flux of fluid, it is the difference in plasma and interstitial COP (ΔCOP) that is of interest. To the best of my knowledge interstitial COP has not been reported in pediatric cardiac surgery studies. In order to evaluate ΔCOP in a complex heart defect we needed to first evaluate these changes in a simple heart defect.

Atrial septal defect (ASD) is a common congenital cardiac malformation (11). It is classified as a non- severe or simple defect. The open-heart surgery era started with an ASD closure in 1952 and closure of ASD was one of the first to be done using a cardiopulmonary bypass machine (12). Although surgical closure of the ASD is considered safe with very low mortality rate, one in four develops complications with fluid accumulation as the most common (13). Interventional closure with device is now the most common procedure for ASD closure. The procedure is done without the use of CPB and is safe with few complications (14). Having two procedures, with and without the use of CPB, gave the opportunity to compare the contribution of CPB on transcapillary fluid exchange in these patients.

Normal and specific pathological values of COP measured in plasma and interstitial fluids are to some degree known for adults (15-19). There was, however, an information gap in the pediatric population. Some data existed on plasma COP in healthy and sick newborn and infants (20, 21) In order to evaluate transcapillary fluid balance in children with congenital heart defect, it was necessary to assess data on normal values of plasma and interstitial COP in healthy children. The development of these forces as a function of age in children was also of interest.

Introduction

Congenital heart defects

Congenital heart defects (CHD) are the most common birth defects worldwide with an incidence of 0.6-1.4 % of live-birth (11, 22). Despite improvements in survival over the last 50 years following advances in diagnostic and treatment, CHD still remains an important cause of death amongst children (23, 24). Improved survivals among live-born children are contributed to the reduced operative mortality (25, 26). The prevalence of CHD has increased during the last decades, mainly as a consequence of improved diagnostic rather than an actual change in prevalence. Increasing use of prenatal diagnostic may change the number of terminations of pregnancies with CHDs. As a consequence, the improved prenatal and later diagnostic have changed the prevalence of CHD with prenatal terminations of fetus with severe CHD and the possibilities to find small and insignificant defects. Despite the reduced operative mortality still the burden of significant morbidity is of concern (27, 28). In the two last decades approximately 300 surgeries a year has been performed for congenital heart defects in Norway (25).

The CHDs can be classified according to the diagnosis hierarchy as complex or simple CHD, table 1(25). The defects can also be classified into cyanotic or non-cyanotic defects or classified into left-to-right or right-to-left shunts were the first will cause increased pulmonary flow and the latter a cyanotic heart defect. According to this an ASD will be classified as a simple, acyanotic, left to right shunt defect, whereas univentricular hearts will be classified as a complex, cyanotic, right to left shunt defect.

Complex	**Simple**
1.Univentricular hearts (UVH)	9. Ventricular septal defect (VSD)
2.Truncus arteriosus communis (TAC)	10. Coarctation of the aorta (CoA)
3.Interrupted or hypoplastic aortic arch (I/HAA)	11.Aortic stenosis (AS)
4.Transposition of the great arteries (TGA)	12.Pulmonary stenosis (PS)
5.Atrioventricular septal defects (AVSD)	13. Mitral valve defect (MV)
6.Totally anomalous pulmonary venous drainage (TAPVD)	14.Partially anomalous pulmonary venous drainage (PAPVD)
7.Pulmonary atresia (PA)	15. Atrial septal defect (ASD)
8.Tetralogy of Fallot (TOF)	16. Patent ductus arteriosus
	17. Other (MISC)

Table.1 Diagnosis Hierarchy

Atrial Septal Defects

ASD is one of the common congenital cardiac malformations with an incidence of 0.3-0.9 per 1000 live births which is nearly 10% of all congenital heart diseases (11, 29, 30). ASD is

considered a relatively benign cardiac disease but will, if left untreated, cause significant morbidity and mortality. The symptoms are often not present in childhood and ASD is often diagnosed after an echocardiogram evaluating a heart murmur, even though some children report a slightly less stamina than peers. To prevent volume overload of the right atrium and ventricle, causing symptoms of atrial arrhythmias, pulmonary hypertension and heart failure, closure of the defect is preferably done during childhood. Routine closure of ASD in childhood is justified by the low-risk, curative closure (31). ASD can be closed either by closure with device during interventional catheterization or by surgical closure.

Historically, surgical closure of an ASD was the standard of care. The first experimental surgical closure of ASD was attempted in 1939 at Columbia Presbyterian Hospital. The first clinical operation for ASD was reported from Toronto by Gordon Murray in 1948 (12). In 1952, F. John Lewis operated on a 5-year-old with ASD under general hypothermia and this is considered the beginning of the open- heart surgery era (12, 32). John Gibbon in Philadelphia performed the world's first successful closure of ASD using a cardiopulmonary bypass machine, which is now the standard procedure (12). The surgical technique now consists of approximating the edges of the defect in small ASD or using native pericardium or synthetic patch in larger ASD to close the defect. Surgical closure of the ASD is considered safe and has a very low mortality rate. One in four develops complications requiring further management and corresponding longer length of stay (13).

In 1974, Kings and Mills demonstrated the feasibility of closing ASD using a device. Amplatz developed, in 1997, a self-expanding prosthesis wire mesh with two round disks and a connecting short waist which was FDA approved in 2001. The Helex occluder was FDA approved in 2006. A study of safety of the Amplatzer device found overall complication rate for the device group to be 7.2% and 24% for the surgical group with mortality of 0% for both groups (14). Interventional closure with device is now the most common procedure and is safe with few complications.

The choice between these two procedures is made according to anatomical considerations. ASD are classified into ostium primum, ostium secundum, sinus venosus and coronary sinus type and only the ostium secundum type, which consists of nearly 60 %, is amendable to device closure, whereas all can be surgically repaired. In order to do an interventional device closure, the ratio of septal rim and septal defect must be favourable.

Complications like pericardial effusions (PE) and post-pericardiotomy syndrome are more likely to occur after ASD closure than other cardiac surgeries (33). Some studies show postoperative PE up to 52% in patients undergoing surgical repair of ASD (13). The ASD surgery is generally considered as a low-risk procedure, but pericardial effusion is one of the risk factors for early and late mortality. PE can occur late in the postoperative course, up to 4-6 weeks after surgery.

Univentricular hearts

Univentricular heart is an entity of several morphological complex heart defects creating a functional single ventricle. The functional ventricle may be morphological left ventricle as in Tricuspid atresia or morphological right ventricle as in Hypoplastic left heart syndrome. The prevalence of univentricular hearts is estimated to be 1 per 3000 live birth (34). This prevalence depends on several factors and varies between different countries. The quality of prenatal care and diagnostics, the legislation as well as the acceptance of termination of pregnancies with fetuses diagnosed with complex heart defects will affect the incidence in live birth. There will sometimes be doubt whether the defect can be surgically corrected into a four-chamber repair or is best palliated as a functional single ventricle. This will again influence the prevalence of single ventricle. During the last five years, ~ 10 children a year have been requiring surgery for a univentricular heart defect in Norway.

Fontan surgery

In 1971 Fontan and Baudet published the first description of “a corrective procedure for tricuspid atresia, which completely suppresses the blood mixing” (2). This was the first successful attempt to reroute the systemic venous return directly to the pulmonary arteries as a palliation for a single ventricle. In the previous decades there had been several attempts to bypass the right ventricle but was only successfully achieved in animal experiments. Still this provided the groundwork for the operation now known as the Fontan procedure (35). The initial operation involved creation of a Glenn anastomosis, anastomosis of the right atrium to the pulmonary artery and closure of any interatrial communications. Valves were placed into the right atrium to pulmonary artery connection and the inferior vena cava (36). The operation bears the name of Francis Fontan (1929-2018), but important contributions made by Kreutzer and others have refined this procedure into the now preferred procedure for all single ventricles. The technique has evolved, and progress includes elimination of the implanted valves, staging of the procedure with the first stage being a bidirectional superior vena cava anastomosis, creation of an intra atrial tunnel, and later, lateral tunnel and extracardiac tunnel modifications. The staged procedure with the use of an extracardiac conduit with or without a fenestration is now the preferred procedure. The numerous surgical advances, along with advances in intra- and postoperative care including anesthesia management, perfusion strategies and surveillance have transformed this once high-risk operation into a procedure with early mortality reported to be 0%-5.5% and still improving (37, 38). Survival for patients undergoing Fontan procedure in the current era is excellent, but patients still remain at risk for increased morbidity.

The postoperative fluid balance derangement contributes to this increased morbidity. Postoperatively low cardiac output and fluid accumulation with edema formation, impaired respiratory function and prolonged pleural effusion are major determinant of the postoperative morbidity and length-of-stay. Prolonged peri-operative course has been associated with

decreased long-term survival and the development of late complications and eventually the development of a failing Fontan (3, 38).

The ongoing improvement in both selections of patients, surgical procedure and perioperative strategies such as anesthesia management and perfusion strategies make it difficult to assess the contribution of one single factor for the improvement in mortality and morbidity. The single most important factor is therefore found to be the era of surgery.

At the same time as the improvement has lowered the mortality and a proper selection of patients has been an advantage for a good result, the Fontan palliation has been applied to a wider range of anatomic subgroups. Initial the Fontan procedure was designed for Tricuspid atresia but now applies for almost all single ventricles. Factors found to be predictive for good long time survival and outcome include a staged procedure and the creation of an extracardiac conduit, whereas factors associated with decreased survival has been operation in the early era, before 1991, longer cardiopulmonary bypass time, atrioventricular valve (AV) regurgitation, elevated pressure in the Fontan pathway and left atrial pressure, increased pulmonary vascular resistance (PVR), prolonged chest tube drainage, post-operative ventricular arrhythmias, renal insufficiency, and the development of protein-losing enteropathy (PLE) (3, 37, 39-43). Left ventricular morphology of the systemic ventricle has in general been viewed as beneficial for long-term survival but in some studies the outcome was good regardless of ventricular morphology (44, 45).

Long term survival for Fontan patients is reported to be from 90 to 95 % at 5 years and 74-94% at 10 years (34, 40, 46, 47)

The early mortality after Fontan operations in the current era is low, nevertheless, unfavorable post-Fontan outcome with prolonged inotropic support, prolonged mechanical ventilation, renal failure and prolonged pleural drainage are still signs of unsatisfactory early outcome. Long term outcome is associated with peri- and postoperative complications such as cross clamp and cardiopulmonary bypass time and longstanding pleural effusions. Therefore, defining factors associated with prolonged recovery may be essential to predict long-term outcomes. Factors found to be predictive of postoperative outcome include preoperative mean pulmonary artery pressure (PAP), younger age, heterotaxy syndrome, morphologic right systemic ventricle, pulmonary artery distortion, non-fenestration, and longer bypass time (48). Other authors have described common AV valve anatomic variants, high postoperative atrial pressure, longer bypass and aortic cross clamp times, and preoperative palliation to be risk factors for Fontan failure. Risk of prolonged pleural drainage has been described in patients with lower preoperative oxygen saturation, higher preoperative PAP, longer bypass times, diastolic dysfunction, and postoperative infections. Salvin found increased pulmonary vascular resistance, CPB time, lactate and postoperative central venous pressure to be associated with prolonged recovery where need for greater volume resuscitation during 24 hours after surgery was the single most important factor (49). Also prolonged mechanical ventilation has been associated with prolonged pleural effusion (43).

The awareness of how crucial an optimal hemodynamic flow is for the outcome of Fontan procedure has improved the care with timing of an early correction of any obstruction in the Fontan pathway and systemic circulation. Also, attention to veno-venous collaterals causing lowering of systemic oxygen saturation has been of benefit.

Arrhythmias remain a significant issue after the Fontan operation. The arrhythmias are predominately supraventricular tachycardia. Pundi et al found arrhythmias to be present in 29% ten years after Fontan completion while only 24% had freedom from arrhythmias after 30 years. Atrial flutter and fibrillation was dominating and only 5 % was ventricular tachycardia (50). The change in surgical technique with the development of an extracardiac conduit has prevented the atrial scaring and the atrial dilatation. Thus, we can expect a reduction in the long-term occurrence of arrhythmias caused by dilatation and scaring. The arrhythmias in the Fontan patients are now more caused by the original congenital heart defect.

The Fontan procedure is a palliative procedure. However, a univentricular circulation does not reproduce biventricular physiology and has been termed paradoxical in that systemic venous hypertension occurs simultaneously with pulmonary arterial hypotension. Some have questioned whether the Fontan circulation is a success or failure (51) and even called it a failed strategy (52). The hallmark of Fontan circulation- elevated venous pressure and reduced cardiac output is responsible for the cascade of pathophysiology changes now referred to as the failing Fontan. Failing Fontan is the main cause for long time morbidity included PLE and liver cirrhosis.

PLE is defined as the abnormal loss of serum proteins into the lumen of the gastrointestinal tract. PLE following Fontan operation was first described by Crupi et al. (53) The prevalence of PLE in Fontan patients is reported to be 3.7 to 11%. (40, 54, 55) Survival rate after the diagnosis of PLE in current era is reported to be 88% and 72 % at 5 and 10 years, respectively (56). The risk of developing PLE has been associated with postoperative longstanding pleural effusions, elevated pre-operative mean PAP and post-bypass atrial pressure. The cause of PLE is still not clear, but the elevated venous pressure in combination with the altered cardiac output and lymphatic involvement has been suspected. The same mechanisms are proposed as responsible for the development of liver fibrosis and cirrhosis now seen in some of these patients.

The final treatment for the failing Fontan will be a heart transplant (HTx). Patients with failing Fontan circulation are at high risk for complication after HTx because of multiple prior operations, chronic inflammation, hepatic dysfunction, reduced renal function, coagulopathy and PLE. Studies in HTx patients after previous Fontan have found the mean time from Fontan completion to transplant to be from 7-13 years and a survival rate of 77-80% at 1 year and 45-69% at 10 years (57, 58).

Cardiopulmonary Bypass

The success of the modern cardiac surgery was possible due to the development and refining of the CPB. In the 1940s cardiac surgery were limited to extracardiac procedures (59). The main challenge in the development of CPB was the creation of an oxygenator. In 1951, intracardiac repair in a child were done using CPB with a rotating disc oxygenator and in 1953 a closure of an ASD was a success using CPB with a film oxygenator. In 1955, Lillehei et al. performed a ventricular septal defect repair in a one-year old child using cross-circulation with the patient's father as the functional oxygenator in the CPB circuit (60-62). Further development included the use of bubble and membrane oxygenators in the next two decades. The mortality rate for infants and neonates were high but improved markedly with the development of deep hypothermic circulatory arrest in the 1970s. Improvements in CPB have caused a significant reduction in morbidity in cardiac surgery. One of the improvements has been to reduce the size of perfusion circuit for use in the pediatric population. The current efforts is to develop strategies to reduce the hemodilution and the systemic inflammatory response (SIRS) seen in these patients (63).

The CPB circuit must be primed with fluid, and the optimal composition of the priming fluid is under debate. The hematocrit value, the viscosity, electrolytes contents and colloid contents are all important. The CPB circuit can be primed with crystalloids, colloids, or blood products. There are controversies regarding the priming constituents. Blood-less priming has the advantage of potentially avoiding transfusion-related complications, however, low hematocrit will reduce oxygen delivery. Hemodilution is inevitable and is more pronounced in children due to the relatively larger ratio of circuit to body size. A weight-specific bypass circuit minimizes prime volumes and hemodilution and miniaturized circuits are one of the advances responsible for better outcome in infants and children after CPB. In addition, it will reduce exposure of the patient's blood to artificial surfaces, thought to be a factor in the development of the immune response seen.
To create the energy needed for blood flow through the circuit a roller pump is the most common in use in the pediatric population. Oxygenators perform the gas-exchange functions of the lung in the CPB circuit. Gas exchange occurs through a microporous membrane at atmospheric pressure. The use of ultrafiltrators to concentrate blood and remove excessive fluid from the circuit is common during pediatric surgery. Conventional ultrafiltration (CUF) removes extra fluid during bypass by directing blood actively or passively through the filtrator. The modified ultrafiltration (MUF) filtrate both the circuit volume and the patient's blood after weaning from CPB, and thus are able to create a higher hemoconcentration, but at the same time, it is more time consuming with longer bypass time for the patients. The CUF hemofiltration is almost always in use in pediatric bypass, but the benefit of adding MUF is still debated although many authors claim its superiority (64-66).

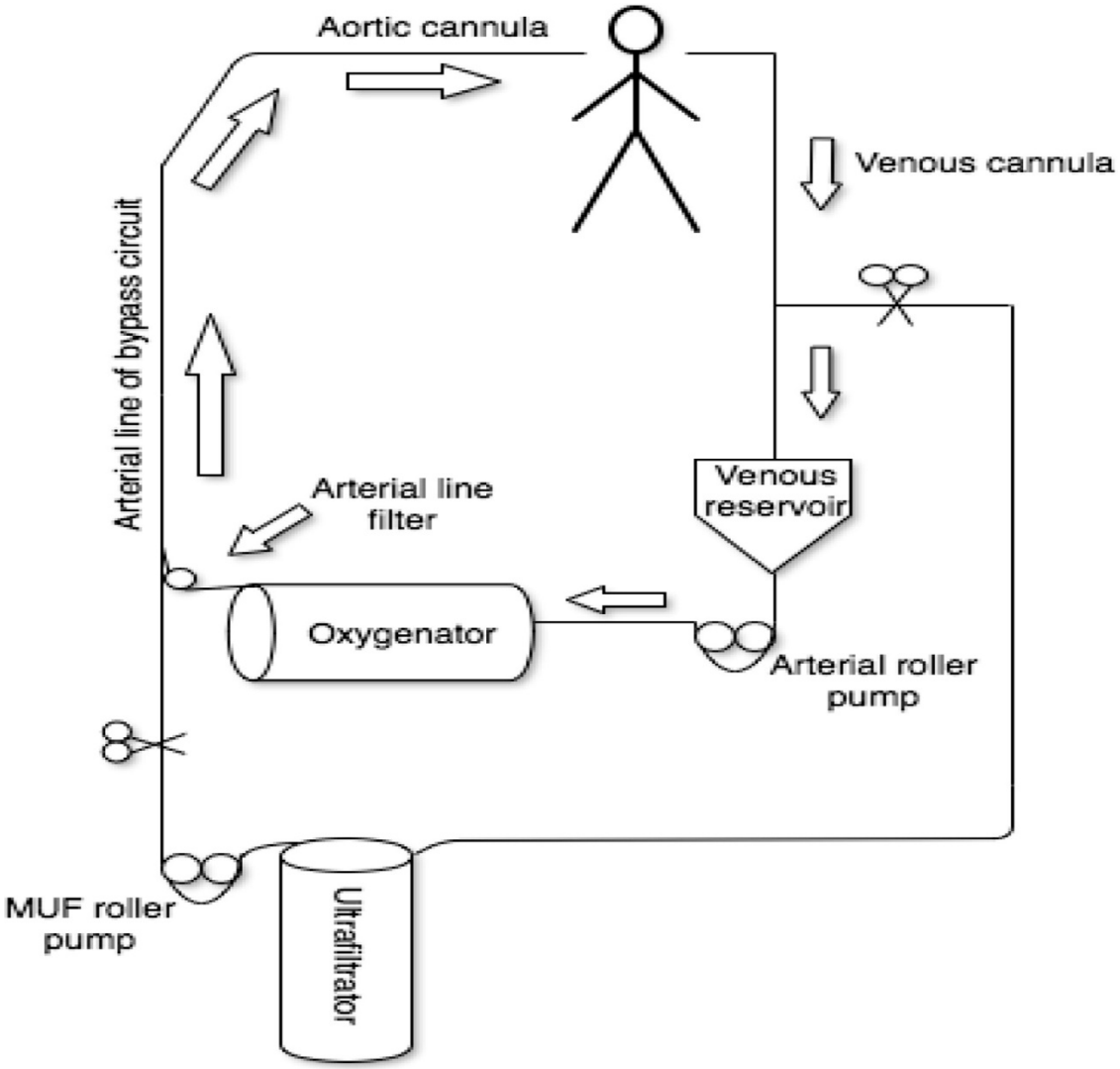


Fig.1 Schematic of cardiopulmonary bypass circuit (Reprinted from Whiting et al with permission from Elsevier (63))

Hypothermia is used in complex intracardiac procedure to reduce the metabolic demand and oxygen consumption, especially in the brain. In surgery for simple cardiac defect, however, normothermic CPB has been shown to be as safe as hypothermic CPB (67). In a study by Taylor et al moderate hypothermia was shown to decrease cerebral blood flow autoregulation during CPB (68). However, neurodevelopmental disorders after the use of CPB and hypothermia are still of concern (69-71). To what degree it is the altered cerebral flow or cyanosis due to the cardiac lesion, or surgery with hypothermic CPB that are the main contributor to the neurodevelopmental disorder seen has yet to be established (72-74).

Body fluid balance

Water is the body's principal constituent and the body fluid homeostasis is essential in human life (75). The fluid within the human body is involved with intracellular communication,

removal of waste products and nutrition supply. Any disturbances in interstitial fluid homeostasis may lead to organ dysfunction which can be fatal. Composition of body fluid and the balance in different compartments are strictly regulated to ensure stability in response to fluctuations in the outside environment. Total body water (TBW) make up approximately 60% of the body weight in an adult man. The fluid is distributed among three major fluid compartments with 2/3 being intracellular volume (ICV). The extracellular volume (ECV), which consists of the intravascular volume (plasma volume 25%) and extravascular volume (interstitial volume 75%), makes up 1/3. The total water content in the body varies according to gender, age and content of body fat. There is an increase in body water volume with increasing obesity together with an enlarged portion of extracellular water to TBW (75). In a newborn baby 75-80% of the body weight is water, a percentage that rapidly declines during the first 12 months. A more stable level is reached before 3 years of age, and at around 9-10 years, a TBW similar to adults is achieved (76, 77).The reduction of TBW throughout childhood is primarily due to a gradual decrease of extracellular water content from approximately 40% of body weight in a neonate to 20% at puberty.

The solute composition in ECV and ICV differs with potassium and magnesium as the major cations and proteins and organic phosphates being the major anions in ICV, whereas in ECV the major cations is sodium and chloride and bicarbonate being the main anions. This asymmetric transmembrane distribution is maintained by cellular ATPase and the net flux of water is driven by ECV osmolality. In ECV the fluid exchange between plasma and the interstitium depends on the permeability properties of the capillary membranes separating the intravascular and extravascular compartments. The interstitial fluid (IF) and plasma will have similar ionic composition, but plasma will have a higher protein concentration.

Transcapillary fluid exchange

The transcapillary exchange and formation of interstitial fluid is determined by properties of the capillary wall, hydrostatic pressure and protein concentrations in the blood and interstitium. The capillaries consist of a single endothelial layer and are semipermeable for water and electrolytes. This ultrafiltration across a semipermeable membrane is considered to be a passive phenomenon where the filter is the endocapillary coating of biopolymers, the glycocalyx. The glycocalyx interpolymer spaces function as a system of small pores that are permeable to water and small solutes but are too narrow to transmit plasma proteins (78). Transport of solutes depends on their chemical and physical properties (79). The primary force opposing filtration is the osmotic pressure of the plasma proteins. Ernest Starling postulated in 1896 that transcapillary fluid movement is determined by the imbalance between colloid osmotic and hydrostatic forces (80). This theorem is now referred to as the “Starling hypothesis” (81). It was later expressed formally in the Starling equation (82)

$Jv = CFC [(Pc-Pi) -\sigma (COPp-COPi)] = CFC \times \Delta P$ = flow of lymph

Where Jv is the net capillary filtration, CFC is the product of capillary hydraulic permeability of the capillaries per unit surface area available for filtration, σ is the capillary reflection coefficient. Pc and Pi are the hydrostatic pressure in the capillary and the interstitial compartments, respectively, and COPp and COPi are the colloid osmotic pressures in the plasma and interstitial fluid.

The CFC is called the hydraulic conductance and measure the efficiency of which water crosses the capillary wall which varies amongst different vascular beds (83) (84, 85). A lowered CFC might reduce the net filtration rate that can be handled by the lymph, but CFC by itself cannot prevent an increase in interstitial volume. CFC is presumed to be constant under different fluid pressures given steady state conditions (86). However, studies show a moderate increase with systemic low-grade inflammation (87), and decreases in situations of elevated transmural pressure.

The colloid osmotic pressure difference is due to the relative impermeability of the vessel wall to macromolecules and proteins. Sigma (σ) is the capillary reflection coefficient for macromolecules and proteins which corrects for the overall effective permeability to these proteins. It ranges between 0 and 1 where $\sigma = 0$ if the barrier freely allows passage of proteins and $\sigma = 1$ in an impermeable barrier. During inflammation, the disruption of the endothelial barrier will change protein permeability and hence reduce the reflection coefficient for proteins, causing edema.

Pc-Pi is the difference in hydrostatic pressure between the capillaries and IF. Pc is the most variable of the four pressures in the equation and is the only one under nervous control (88). Pc is influenced by arterial (Pa) and venous pressure (Pv) together with microvascular resistance. Increase in either Pa or Pv will raise Pc, but an increase in Pv in humans is four times as effective as the same change in Pa in altering Pc (78). This is due to the moderately low venous resistance, in which a change in Pv is readily transmitted back to the capillary. Acute inflammation will cause arteriolar vasodilatation, which lowers the pre- to post-capillary ratio and, consequently, raises Pc, favoring filtration to the interstitium. Conversely, arteriolar vasoconstriction elevates this ratio and lowers Pc. Pi is marginally sub atmospheric in the skin of an adult, given a relative state of interstitial dehydration. This occurs by a small net capillary filtration pressure and removal by lymph (86).

The plasma colloid osmotic pressure (COPp) in human is quite stable at 25 mm Hg in adults and children. It is lower in newborn babies at ~19 mmHg and increases during the first months of life, reaching values comparable to adults at one year of age (20, 89). The oncotic pressure is created by the plasma proteins where albumin is responsible for up to 80%. The IF colloid osmotic pressure (COPi) varies in different tissues and under different conditions (between 30-60% of plasma COP) and has been the subject of debate mainly caused by the difference in methods for harvesting interstitial fluid that have been used to determine its true value (82, 88, 90).

The question of the relevance of COPi for fluid exchange has been debated. Levick and Michel (82) claimed that the true net filtration forces depends not so much of the global COPi but the COP just below the endothelial glycocalyx (COPg). COPg is significantly lower than COPi because of continuous fluid filtration and dilution of proteins and therefore creates a higher COP gradient. In vivo studies in rats and frogs indicate that this substantial gradient in COPg and COPi depends on filtration rate, with a global COPi close to COPg near the endothelial border under normal filtration pressures (91, 92) In an extensive review by Wiig and Swartz they conclude that although less important in high filtration states, COPi is still a major determinant for normal fluid filtration (93).

Compliance is the ability of the tissues to resist distension and is expressed as the change in volume related to pressure changes. Different tissues have different compliance and consequently, the low compliant tissue will oppose expansion, resulting in an increase in Pi, even at small increases in volume. By contrast, in highly compliant tissue the amount of fluid may be substantial before Pi rises. The tissue architecture, the tissue organization and the composition of interstitium define the compliance and are influenced by both acute and chronic modulation, such as injury, inflammation and tissue remodeling (93).

Visible edema appears in human subcutis when interstitial volume increases with 50-100% (84). An overall net increase of fluid with a raised interstitial pressure and/or lowered interstitial COP was first described by Guyton et al. as, ‘edema preventing mechanisms’ (94). Disturbing this delicate balance of exchange between IF and blood may have profound effects on the human body, and both age-related differences in COP and P, as well as homeostasis or sickness, may influence the balance.

ΔP is the net pressure gradient across the capillaries and is estimated to be between 0.5-1.0 mmHg (86) which leads to a net fluid filtration. In a steady state condition this will equal the lymphatic flow which plays an important role in edema formation.

The lymphatic system

Interstitial fluid is created by transcapillary filtration and cleared by lymphatic vessels. For the maintenance of fluid homeostasis in the interstitium, the net flux of filtered plasma must be balanced by lymph formation into the initial lymphatic vessels. The IF volume is kept fairly constant under normal condition at 20% of body weight by several buffering mechanisms including structural changes, adjustments of forces acting across the capillary wall and lymph flow. IF, proteins, immune cells, and other solutes of high molecular weight is continuously filtrated and are absorbed by the lymphatic capillaries and returned via lymph nodes back to the blood circulation (93). The IF flow through the interstitium and into the lymphatic capillaries has an important role in modulating important biological events that contribute to morphogenesis, extra cellular matrix remodeling, cell migration and signaling, and immune response in addition to fluid homeostasis. Lymph vessels are present in almost all tissues. The

lymphatic vasculature is a drainage network that originates in the interstitium as lymphatic capillaries and ends in the great veins of the neck or thorax. The lymphatic vessels can be divided into three different types: initial lymphatics, pre-collectors and collecting lymphatics (95). The initial lymphatics are non-contractile and considerably larger than surrounding blood capillaries. They consist of a single layer of endothelial cells with little or discontinuous basement membrane (93). Fluid entry is by absorption and depends on a pressure gradient favoring fluid movement from interstitium into the lymphatics. A pressure gradient of ~0.09 mmHg/mm has been found to drive filtrate from capillaries to the initial lymphatics. An even lower gradient may be sufficient in edema which can increase the hydraulic conductivity (96).

These initial lymphatics drains to larger collecting lymphatic who consist of smooth muscle cells, continuous endothelium, basement membrane and valves to aid lymph propulsion and prevent retrograde flow. These unidirectional valves divide the collecting vessels into segments called lymphangions. These lymphangions contract spontaneously and act as pumping units and are responsible for propulsion of lymph (93).The three layers of the vessel wall are less well organized than in blood vessels. Their smooth muscle cell content increases proximally. The collecting lymphatics pump the lymph further centrally, which, after passing through the regional lymph nodes, reaches the thoracic duct or the right lymphatic trunk. The human thoracic duct is comprised of smooth muscle cells arranged in bundles with no distinct longitudinal or circumferential orientation.

The lymphatic system has three cardinal functions: (1) maintenance of interstitial fluid balance, (2) immune surveillance and (3) absorption of fat. The lymphatic vessels play an important role in host defense by carrying antigens and immune cells from the tissues. Excessive fluid will be returned to the blood circulation via the lymphatic vasculature. Edema occurs when there is a mismatch between microvascular filtration and lymphatic removal and can thus be caused by an increase in filtration, reduced lymphatic removal or both (78).

Lymph transport is dependent on a combination of intrinsic and extrinsic factors. Extrinsic factors like skeletal muscle contraction, respiration and arterial pulsation cause tissue deformations leading to compression and expansion of the initial lymphatics. The intrinsic factor is the lymphangions. The systolic pressure generated by the spontaneous contractions in humans can reach up to 100 mmHg, and several studies show averages of around 40–60 mmHg in arms and legs in adult humans (97, 98). The contractions are influenced by many different stimuli: humoral, neuronal, pressure and shear stress. Human lymphatic collecting vessels are myogenically active and show reactivity, either inhibitory or stimulatory to a vast panel of vasoactive substances such as nifedipine, noradrenaline, histamine, endothelin and prostaglandins (99). The lymphatic vessels have been shown to be innervated and respond to sympathetic stimulation. A study by Telnius at al found that functional innervation of the human thoracic duct is predominantly adrenergic (100). Lymph propulsion is dependent on phasic contractions of the lymphatic vessels (101). Several channels have been studied in the regulation of this contractility. In vitro studies have shown that they are sensitive to drugs like calcium channel blockers and diuretics, which inhibit contractile activity and diminish

noradrenaline- induced phasic contractions but in vivo administration have not shown reduced lymphatic contractile activity (102, 103).

A study of the mechanical properties of the human thoracic duct demonstrates a role for adrenoceptors, thromboxane, and endothelin receptors in human lymph vessel function and that the human thoracic duct can develop wall tensions that permit contractility to be maintained across a wide range of transmural pressures and that isolated ducts contract in response to important vasoactive agents (99). Both increases in preload and afterload lead to changes in contractility. Similar to arteries, there is a flow-mediated nitric oxide (NO) production and myogenic constrictions as a response to increased stretch (95).

Despite the evolving knowledge in lymphatic biology the last two decades, the lymphatic is still called the forgotten circulation. Recent research includes the discovery of lymphaniogenic factors and identification of lymphatic vascular markers along with how the microenvironement can influence lymphaniogenesis (93). Fluid drainage is one of the main functions of lymphatic capillaries and interstitial flow regulates lymphatic capillary regeneration. Inflammatory lymphaniogenesis plays a key role in clearing edema fluid and antigen. Studies on lymph vessels in mouse models of inflammation and inflamed human tissues have shown that acute inflammatory reactions and chronic inflammatory diseases are accompanied by both the growth of new lymphatic vessels and the expansion of preexisting lymphatic vessels. Also, chronic cardiac allograft lymphangiogenesis is inflammation-driven and the lymph vessels are important for migration of donor and host antigen-presenting cells.

Aims

The main purpose of this project was to evaluate transcapillary fluid balance in children with congenital heart defect and to investigate whether the edema seen during cardiac surgery is related to increased microvascular leakage for plasma proteins.

Specific aims

1. To determine the age-related values of COP in plasma and subcutaneous tissue of healthy children aged 2-10 years. Evaluate the relationship of COP for this specific population at different implantation times and to assess the effect of gravity (Paper 1).
2. To assess the net COP gradient across the capillaries in children with a simple cardiac defect (ASD) during a procedure with and without the use of cardiopulmonary bypass (CPB) to evaluate the contribution of surgery with the use of CBP and hypothermia on the COP changes. To compare values of COP in children with a simple cardiac defect to healthy children (Paper 2).
3. To assess and evaluate the net COP gradient across the capillaries in children at different stages of Fontan procedure. Determine COP prior to, during and after cardiac catheterization and surgery and to examine whether fluid accumulation and pleural effusion could be predicted by changes in COP. To compare COP in children with a complex cardiac defect to healthy children (Paper 3).

Methods

Study population

Paper 1

This study was designed as a non-blinded, sequential descriptive study taking place at Haukeland University Hospital and Akershus University Hospital. Ninety-nine healthy pediatric patients, ranging in age from 2 to 10 years were included if they met the criteria for tonsillectomy and/or adenectomy and/or tympanic paracentesis, judged by a physician at the Departments of Ear, Nose and Throat. Forty-five patients were females. Children were excluded if they had a medical history of acute illness, chronic disease, or were presently on medication that might interfere with protein metabolism.

Paper 2

This study was designed as a prospective, non-blinded, cross-sectional study and took place at the Pediatric and Surgical Departments, Oslo University Hospital. Forty-one patients scheduled for closure of an isolated ASD were enrolled. Twenty-three had an interventional device closure and 18 children had a surgical repair. The treatment of choice was based on anatomical reason where the size of the atrial septal rim was of importance, and the decision was made by the physicians at the Department of Pediatric Cardiology, Oslo University Hospital in collaboration with the surgeons at the Department of Cardiothoracic Surgery at the same Hospital. Patients were excluded if they had other co-existing cardiac malformations or liver or renal diseases.

Paper 3

This study was designed as a prospective, observational study and took place at the Pediatric and the Surgical Departments, Oslo University Hospital, Norway. Thirty-nine patients undergoing Fontan procedure, 18 patients scheduled for Bidirectional cavopulmonary connection (BCPC) procedure and 21 patients scheduled for Total cavopulmonary connection (TCPC) procedure were enrolled. All of the patients had a diagnostic cardiac catheterization prior to surgery. We were able to obtain COP during catheterization in 14 patients in the BCPC group and 16 in the TCPC group. The only exclusion criteria were lack of informed parental consent.

Ethical considerations

All studies were performed in accordance with the Declaration of Helsinki of 1964 including later amendments (104). The European Union legislation also regulate clinical research and

has presented ethical consideration in clinical research in the pediatric population emphasized on ethical standards in a vulnerable group of patients (105). Their limited ability to informed consent, their limited personal gain of the research, and the need to avoid unnecessary procedures and pain were all part of the consideration prior to and during conduction of these studies. All studies were approved by the Regional Committee for Medical and Health Research Ethics, Western Norway (REK 063.09) prior to commencement and were registered in the Norwegian Biobank Registry. The study for paper 1 was approved by the Norwegian Data Inspectorate and the Data Protection Officer at Haukeland University Hospital and Akershus University Hospital. The studies for paper 2 and 3 were approved by Data Protection Official and central research committee at Oslo University Hospital. We had a formal agreement for transferring biological material between Oslo University Hospital and Haukeland University Hospital. Written informed consent was obtained from the parents or legal guardian after careful explained information. All three studies were done in accordance with good clinical practice and were registered at ClinicalTrials.gov.

The wick method

Sampling of interstitial fluid (IF) was performed using the wick method. The wick technique was developed by Aukland and coworkers to allow simple and direct sampling of IF (106, 107). It was later modified and refined, and is now recognized as an acceptable and representative method for sampling of native IF given optimal time for equilibration (108-110). The method is based on the assumption that a time limited, subcutaneous implantation of a wick absorbs fluid that reasonably represents the actual innate IF in situ. The assumption is supported by the idea that a saline-soaked implanted wick does not interfere with subcutaneous homeostasis. The disadvantages will be implantation trauma with the possibilities for bleeding and inflammation (93).

Sampling of IF for determination of COPi were achieved by means of multifilamentous nylon wicks with a diameter of ~ 1mm (Polyamid no. 8, Norsk Fletteri AS, Bergen, Norway) sewn into subcutaneous tissue. All procedures were performed under sterile conditions. All wicks were sterilized by gamma irradiation (Institute for Energy Technology, Kjeller, Norway). Double threaded wicks were soaked in saline for 10 min., placed on straight sterile suture needles (Acufirm, 210/3, Germany) and tied with a knot at the end. The skin was disinfected with a solution of 0.5% chlorhexidine before inserting the wick in lengths of ~3-5 cm. Adhesive plastic film (Tegaderm, 3 M Canada Inc.) was placed over both ends of the wick in order to reduce evaporation of fluid. At the end of the implantation period, the central part of each wick was rapidly transferred to 1.5 ml Eppendorf centrifuge tubes with funnel (Sarstedt, Nümbrecht, Germany) containing mineral oil. Heavily blood-stained wicks (judged visually) were discarded and blood-stained parts of wicks were cut off if possible. Wicks were centrifuged for 10 minutes at 16060 *g* and frozen at -70°C until analysis. Before the analysis, room tempered samples were transferred by pipette to a non-heparinized glass capillary tube. The capillary tube was sealed in one end with plasticine (Hematocrit sealing compound,

No.74950; BRAND, Germany) and centrifuged in a hematocrit centrifuge (SABA, Haematocrit 20, Hettich) for 4 minutes in order to separate mineral oil from wick fluid.

In paper 1, seventy-nine patients had one wick implanted in the upper arm and one wick in the medial part of the leg, with an implantation time of 60 minutes. An additional 20 patients, of any age between 2-10 years, had one wick implanted in each leg, and wicks were withdrawn after 60 and 90 minutes to evaluate implantation time.

Blood samples for COPp analysis were collected from a venous cannula before the administration of general anesthesia. For paper 2 and 3 blood samples were also collected from the venous cannula at the timepoint for wick removal. The blood samples were allowed to clot and were then centrifuged for 10min at 1881*g*. Serum was isolated and frozen at -70 $^{\circ}$ C for later analysis. Serum, a surrogate for circulating plasma, was later analyzed in the above-mentioned colloid osmometer and was denoted as COP_p.

Colloid osmotic pressure analysis

A colloid osmometer measures a pressure difference between a sample and a reference solution separated by a semipermeable membrane. If the sample contains particles with a molecular weight more than 30 k Dalton, a shift of water from the reference chamber to the sample chamber will create negative pressure, which is detected by a transducer in the reference chamber. The disadvantage of extracting IF from wicks is the small amount of sampled liquid obtained and the minimum volume needed for analyzing the COP. A typical commercial colloid osmometer often requires a sample size of > 100 μL, where a wick of 5 cm in length will produce only 5 μL of fluid.

In 1974 Aukland and Johnsen designed an alternative colloid osmometer for small samples, requiring< 5 μL of fluid (111). With additional technical refinement and the use of a low compliant industrial transducer, Wiig et al. made a reliable and accurate osmometer for sample volumes as little as 0.1-0.2 μL (108). This device is made of pellucid polymethylacrylate (Plexiglas®) and acrylnitrilmethylacrylate plastic (Plexidur plus) (Röhm Chemische Fabrik, Darmstadt, Germany). It contains of a transducer (SensoNor A/S, Horten, Norway) connected to an amplifier and a recorder (Easy Graph P930, Gould Inc., Ohio, USA). It is separated from the sample chamber by a semipermeable membrane that is impermeable to molecules > 30 kDalton (PM-30 Amicron, Lexington, MA, USA).

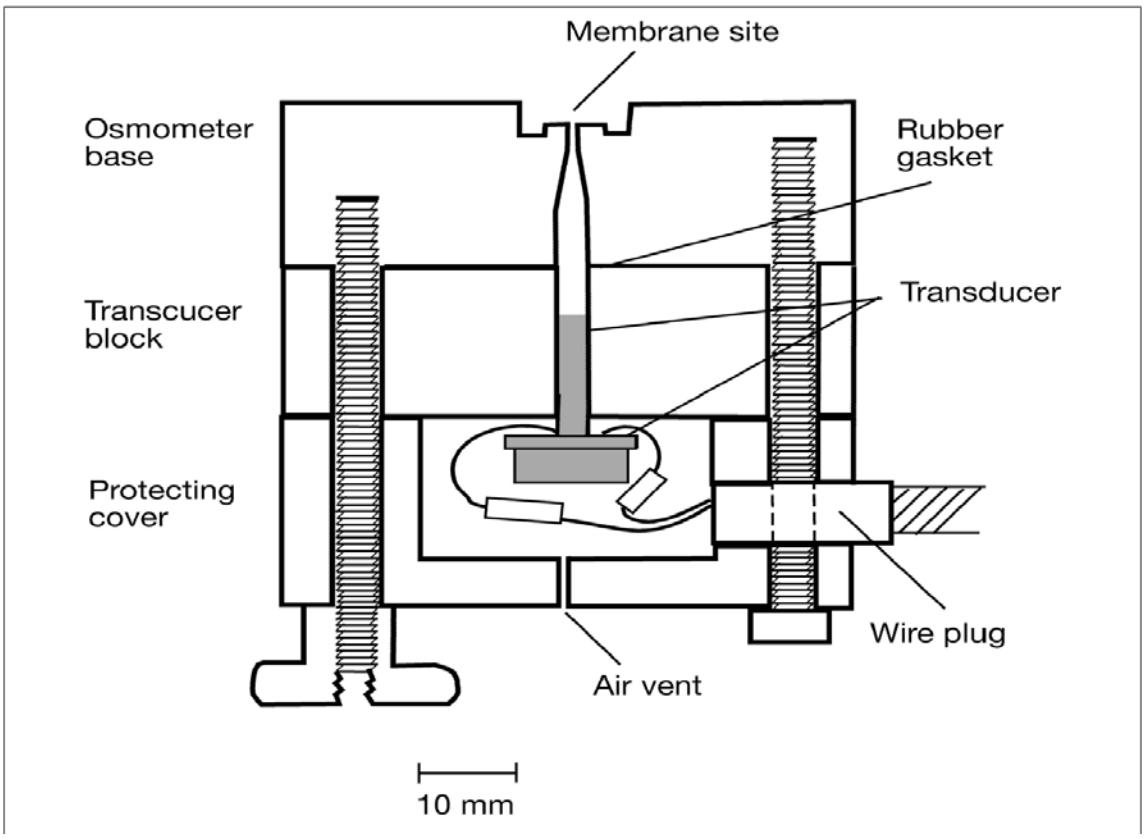


Fig.2 The colloid osmometer **(**From Wiig et.al (108) with permission from Wiley © 1988 Scandinavian Physiological Society)

This type of colloid osmometer was used in all three papers. The colloid osmometer was pre-calibrated hydrostatically with a saline filled column representing a pressure of 20 mmHg. In order to verify membrane response and to test the accuracy of the whole system, a solution of serum with a known colloid osmotic pressure was used as a standard reference. After flushing the sample chamber 4 times, 2 ml saline was left for recording zero pressure. Both saline, serum and wick fluid were sucked up by a soft absorbing tissue paper.

Echocardiography

The 2D echocardiography and Doppler echocardiography were performed in all the study population in paper 2 and 3. Echocardiography was conducted with a Vivid E9 ultrasound scanner with offline data analyses using EchoPAC® (GE Healthcare, Vingmed Ultrasound, Horten, Norway). In paper 2 it was performed the day before the procedure, during the hospital stay and at 1 month and 12 months following the procedure. Standard 2D echocardiography included short-axis and long-axis, 4-chamber and subcostal views, according to recommendations (112, 113). Areas of the right and left atrium and ventricles were measured (114). Standard Doppler color flow was used to assess stenosis or regurgitation. The presence and magnitude of pericardial effusion (PE) were recorded from

the para- sternal long-axis and 4-chamber views. Less than 3 mm of fluid at the ventricular level was considered minor and up to 10 mm was considered moderate. Transesophageal echocardiography was done prior to catheterization and throughout the procedure.

In paper 3 a structural segmental echocardiography had established the diagnosis and the morphology of the systemic ventricle during the first week of life in all of the patients. Standard 2D echocardiography was performed before procedure in unsedated patients according to local guidelines by the attending physicians or echo technicians. The systolic ventricular function was visually graded by four levels from normal to poor. Standard Doppler color flow was used to assess valvar stenosis or regurgitation. Regurgitation was graded in four levels from no regurgitation to severe. Any restriction to flow across the atrial septum was noted. Transesophageal echocardiography was in use during surgery in case of intra cardiac repair to evaluate any regurgitation or stenosis.

Anesthesia

For paper 1 all patients fasted for eight hours before induction of anesthesia. They received weight-related doses of sodiumthiopental/ propofol, fentanyl/ remifentanil, morphine, atropine and mivacurium chloride. Sevoflurane gas induction was in use if venous cannulation failed.

In paper 2 and 3, all the patients received standard general anesthesia. Induction and maintenance were conducted by the anesthesiologist according to local clinical guidelines. All patients received oral premedication with midazolam (0.3–0.5mg/kg). They all had a fasting period of 4-8 hours according to age. General anesthesia was induced using thiopental (4–6 mg/kg) and fentanyl. Muscle relaxation was achieved using cisatracurium (0.15mg/kg) with additional doses given in longer procedures. Analgesia was provided by fentanyl (total 20 μg/kg) and propofol (4–5 mg/kg/h) or midazolam (0.1–0.15mg/kg) infusion according to the patient's age and weight. In the case of gas anesthesia, sevoflurane was in use for induction, and isoflurane was used for maintenance. During surgery, heparin was used to achieve an activated clotting time above 400 s, and after the end of the bypass, protamine was used to reverse the effects of heparin. Dexamethasone was given to three patients during pre-BCPC catheterization and to two patients during surgery for TCPC.

Surgery

For paper 1 the surgeries were minor procedures, that is tonsillectomy, adenectomy and/or tympanic paracentesis. The procedures were performed by the ear-nose-throat surgeon according to local guidelines.

For paper 2 all surgical procedures were performed by a senior pediatric cardiac surgeon or by a younger colleague under the guidance of a senior surgeon. The procedure was performed

through a median sternotomy on CPB and mild hypothermia (31–34°C). The surgical technique consisted of approximating the edges of the defect with direct sutures in small ASDs or closing the defect using autologous glutaraldehyde-treated pericardium or a synthetic patch in larger ASDs.

For paper 3 all surgical procedures were performed by one of three senior pediatric cardiac surgeons. BCPC and TCPC were performed through a median sternotomy on CPB and mild hypothermia (31–34°C).The aorta was cross-clamped in four patients during BCPC surgery and in five patients during TCPC surgery who received a concomitant intracardiac procedure. Additional procedures included mitral and tricuspid valve plasty, collateral and vena azygous closures, pulmonary artery plasty and resection of left ventricular outflow tract obstruction. The standard surgical method for TCPC in our unit consisted of creating an extracardiac conduit using a polytetrafluoroethylene (Gore-Tex) graft (W.L. Gore and Associates, Flagstaff, AZ). Conduit diameter was based on the patient's weight, morphologic relationships and inferior vena cava diameter. High pulmonary artery pressure was considered an indication for creating a fenestration. The fenestration was created using a 4 mm punch in the conduit and anastomosed directly to the atrium.

Cardiopulmonary bypass

CPB was in use during surgeries for paper 2 and 3. The CPB circuits consisted of a Maquet Quadrox-i oxygenator with a hard-shell reservoir, a roller pump and an integrated arterial line filter. The miniaturized circuits were custom packed, all heparin coated. Arterial cannulas (Medtronic DLP) and venous cannulas (Sorin) were not coated. The hemofilter used was a Maquet BC 20 Plus hemofilter. Modified ultrafiltration was not in use. Circuit prime volumes were calculated according to standard protocol for our unit, where children up to 7.5 kg are administered packed red blood cells (PRBCs), 40 ml of albumin, 30-40 ml of sodium bicarbonate and 150 mg/ml mannitol at 3 ml/kg of body weight in their priming solution, whereas in children weighting more than 7.5 kg, the priming solution consists of PRBCs, dextran 70 (16 ml/kg) and the same calculated amounts of mannitol and sodium bicarbonate as in smaller children. A total of 1000 IU of heparin was added to all priming solutions. All patients were in mild hypothermia with temperatures between 31°C and 34°C during bypass. Cardioplegic arrest was used in all patients in paper 2 and for short periods of time in cases of intracardiac repair in paper 3. The cardioplegia in use was a modified St. Thomas solution prepared at the pharmacy at Oslo University Hospital, where 500 ml contains 465 ml of lactated Ringer solution, 25 ml of sodium bicarbonate (0.5 mmol/ml) and 10 ml of cardioplegia (magnesium chloride hexahydrate 163 mg/ml, potassium chloride 60 mg/ml and procaine hydrochloride 14 mg/ml).

Catheterization

Cardiac catheterization was in use in paper 2 and 3. The catheter laboratory in use was Artis zee biplane (Siemens Healthcare, Erlangen, Germany) Cardiac catheterization was performed with the patients under general anesthesia. All procedures were performed by experienced interventional pediatric cardiologists.

In paper 2 accesses was by the femoral vein. After access, 100 IU heparin/kg was administered, and if the procedure lasted more than 90 min, another 50 IU heparin/kg was administered. Pressures were measured in both atrias, the right ventricle and the pulmonary artery by fluid filled catheters calibrated at the start of the procedure. The shunt size was determined by oximetry according to the Fick principle. An Amplatzer ®(ASO, St. Jude Medical, St Paul, MN, USA) ASD septal occluder was in use in all cases except 2, where the Occlutech® (OFSO; Occlutech GmbH, Jena, Germany) was used.

For paper 3, a routine preoperative cardiac catheterization was performed 2-4 months prior to surgery. The hemodynamic and angiographic assessments were dependent on the clinical characteristics of the patient. All procedures were performed while the patients were under general anesthesia by experienced pediatric interventional cardiologists by accessing the femoral vein and artery, and the internal jugular vein in the pre-TCPC catheterization. Hemodynamic measurements included the pressures in the left and right pulmonary artery, the left and right atrium, the inferior and superior caval veins, the ventricle and the aorta. Angiography was performed in the venous, pulmonary and systemic circulations to demonstrate the anatomy and signs of obstruction and collaterals. The McGoon ratio was calculated by measuring the right and left pulmonary artery dimensions before the first branching and dividing the sum by the dimension of aorta at the diaphragmatic level. Case-specific investigations and interventions were performed if indicated. The aorta was balloon-dilated in three patients in the BCPC group for whom the pressure difference across the aortic arch was more than 5-10 mmHg. In one additional patient a stent was placed in the ventricle-to-pulmonary artery shunt (Sano). During pre-TCPC catheterization one patient had a left superior caval vein coiled, and one had an aortopulmonary collateral coiled.

Statistical methods

In paper 1 the SigmaPlot 11 (SyStat Software, Inc., Germany) was used for statistical analyses. The results were presented as numbers with proportions (% of total) and means ± one standard deviation (SD). One-way ANOVA was used for evaluating the COPp and COPi for the different age groups, followed by an all-pairwise Holm-Sidak multiple comparison procedure, if a factor was significant in the one-way ANOVA. When comparing smaller groups with a skewed distribution, a nonparametric test (Mann-Whitney U Test) was used. A 2-tailed P value of < 0.05 was considered statistically significant.

In paper 2 statistical analyses were performed using SPSS software (IBM SPSS statistics 24). All the analyses were double checked, and the figures were made using SigmaPlot 13 (SyStat Software, Inc., Germany).
Continuous variables were reported as mean with standard deviation (SD) or median with range in case of a skewed distribution. The independent-samples t-test was used to assess differences between the two groups when a normal distribution was found, and if a Shapiro–Wilk test for normality failed, a non-parametric test, the Mann–Whitney U-test, was used. Logistic regression was used to predict correlation between pericardial effusions and changes in COP. Multiple linear regression was used to explore the relationship between COP changes and clinical data. A 2-tailed P-value of <0.05 was considered statistically significant.

In paper 3 Statistical analyses were performed using SPSS software (IBM SPSS Statistics 25). Continuous variables were reported as mean ± one SD or as median with range in case a skewed distribution. The independent-samples t-test was used to assess differences between the groups when a normal distribution was observed. The paired t-test was used to assess differences at the various conditions within the groups in case of normal distribution. If a Shapiro–Wilk test for normality failed, non-parametric tests, the Mann–Whitney U-test and Wilcoxon signed rank test, were used. Pearson product-moment correlation coefficient and, in cases of skewed distribution, Spearman correlation were used for correlations of fluid accumulations and changes in COP. Linear regression was used to explore the possible association between COP changes and fluid accumulations. Variables associated with the length of pleural effusion in the univariate analysis ($P<0.01$) were entered into the multiple linear regression. To avoid the multi testing problem only clinical parameters previously known to correlate with pleural effusion were entered into the multiple linear regression. A 2-tailed p-value of <0.05 was considered statistically significant.

Summary of results

Paper 1

“Interstitial fluid colloid osmotic pressure in healthy children.”

Interstitial fluid was harvested at the heart level in 99 presumably healthy children between 2-10 years old. The children were under general anesthesia during a minor surgical procedure.

We found that plasma COP values for children were close to those of adults at 25.6±3.3 mmHg. It was a significant rise from 24.6± 3.2 mmHg at 2-3 years to 28.0±4.2 mmHg at 8-10 years of age P= 0.02. Mean interstitial COP was 13.9±3.5 mmHg with a higher value in the oldest age group at 17.2±3.2 mmHg.

There were no significant differences in interstitial COP between arm and leg, indicating less effect of gravity in children. Eighteen patients had wicks implanted for both 60 and 90 minutes. There was no significant difference with different implantation time, indicating that both 60 and 90 minutes of implantation time can be used in further research.

In this study we were able to provide age-related values of COP in plasma and interstitial fluid in healthy children and could evaluate the relationship of COP at different implantation times and assess the effect of gravity.

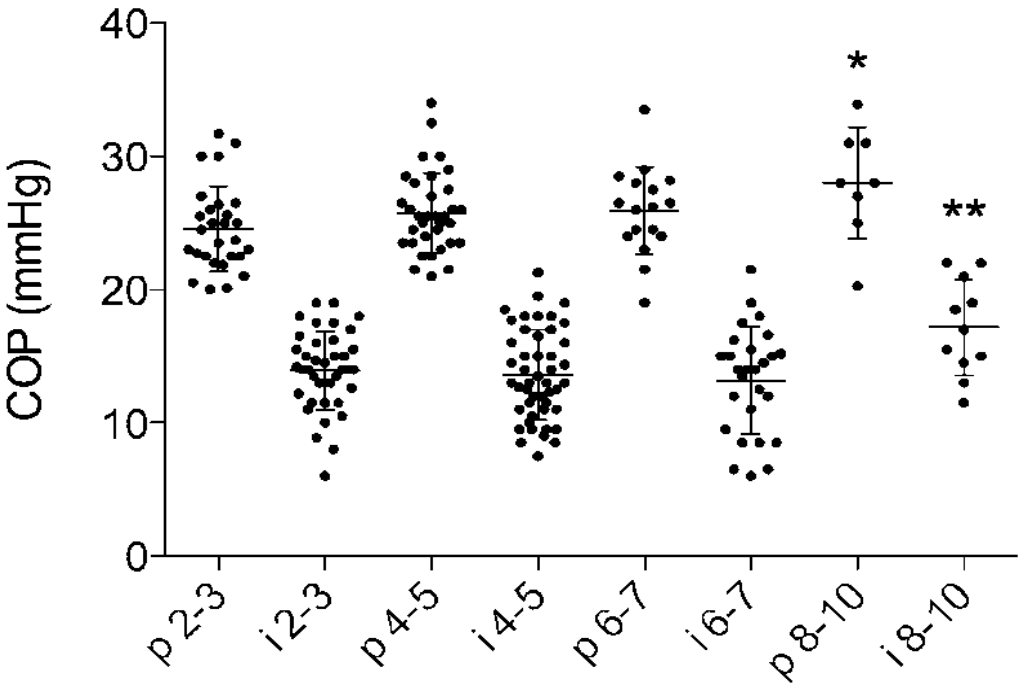


Fig.3 Colloid osmotic pressure in plasma (p) and interstitium (i) related to age. From Guthe et al. (115) © 2015 under the terms of the Creative Commons Attribution License.

Paper 2

"Fluid accumulation after closure of atrial septal defects: the role of colloid osmotic pressure"

ASD can be closed either by closure with device during interventional catheterization or by surgical closure with the use of CPB. Interstitial fluid was harvested using the wick method in 23 patients during interventional closure and in 18 patients during surgical closure of ASD. IF was collected before and after closure with concomitant blood samples.

Baseline COPp and COPi were lower in ASD patients compared with age-matched healthy children from the population in paper 1. COPp was 21.9 ± 2.8 and 21.4 ± 2.2mmHg and COPi was 12.7±3.2 and 12.6±3.7 mmHg in the interventional and surgical groups, respectively.

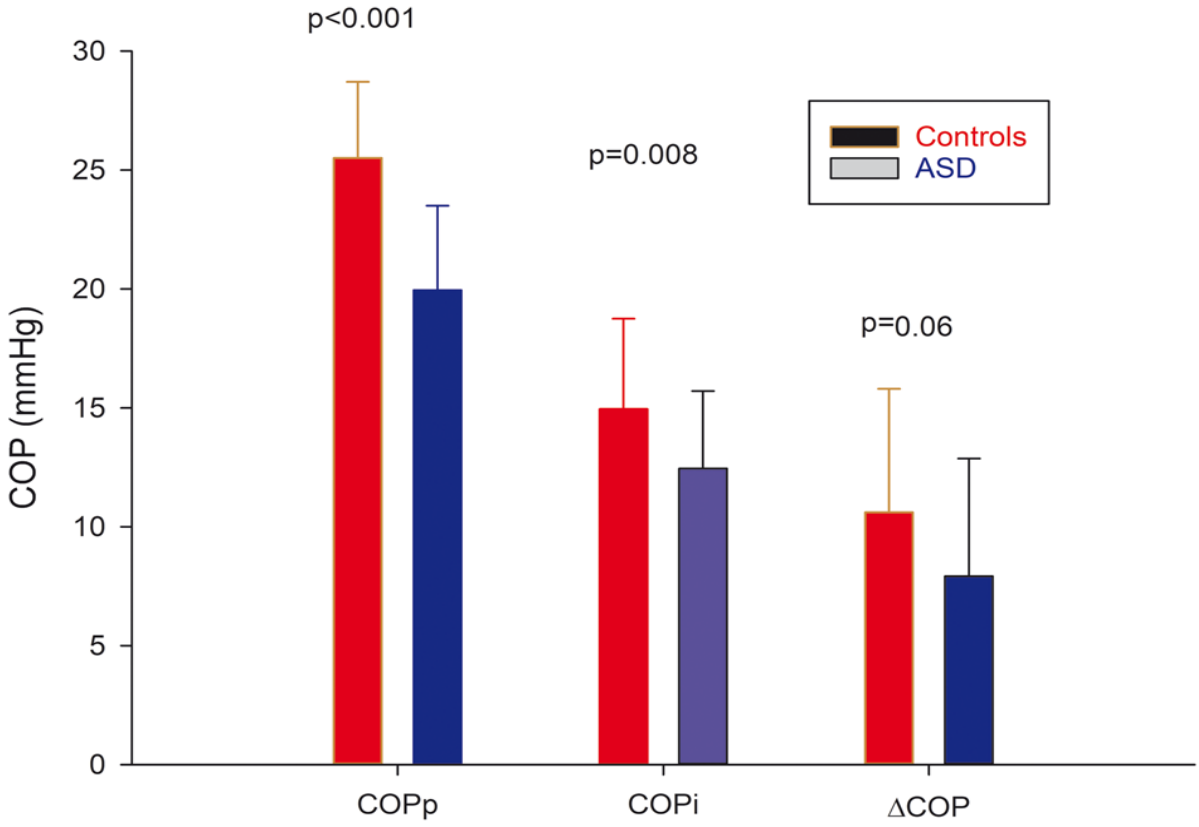


Fig.4 COP_p, COP_i and ΔCOP ($COP_p0 - COP_i1$) in patients with ASD compared with healthy controls. From Indrebo et al (116) © with permission from Oxford University press on behalf of the European Association for Cardio-Thoracic Surgery.

In the surgical group, the use of CPB significantly reduced COPp to16.9 ± 2.9mmHg ($P < 0.001$) and the colloid osmotic gradient [ΔCOP (COPp - COPi)] to 2.9 ± 3.8mmHg ($P < 0.001$) compared with the interventional procedure. The significantly lower COP gradient during CPB may explain the tendency for more fluid accumulation with pericardial effusion in the surgical group.

One hour after the procedure, COPi was 15.6 ± 3.8mmHg and 9.9 ± 2.1mmHg (P < 0.001) and the ΔCOP was 5.4 ± 3.0mmHg and 9.1 ± 3.1mmHg (P < 0.003) in the interventional and surgical groups, respectively. The reduction in COPi after surgery with CPB leads to a higher COP gradient and may represent an edema-preventing mechanism in children undergoing cardiac surgery.

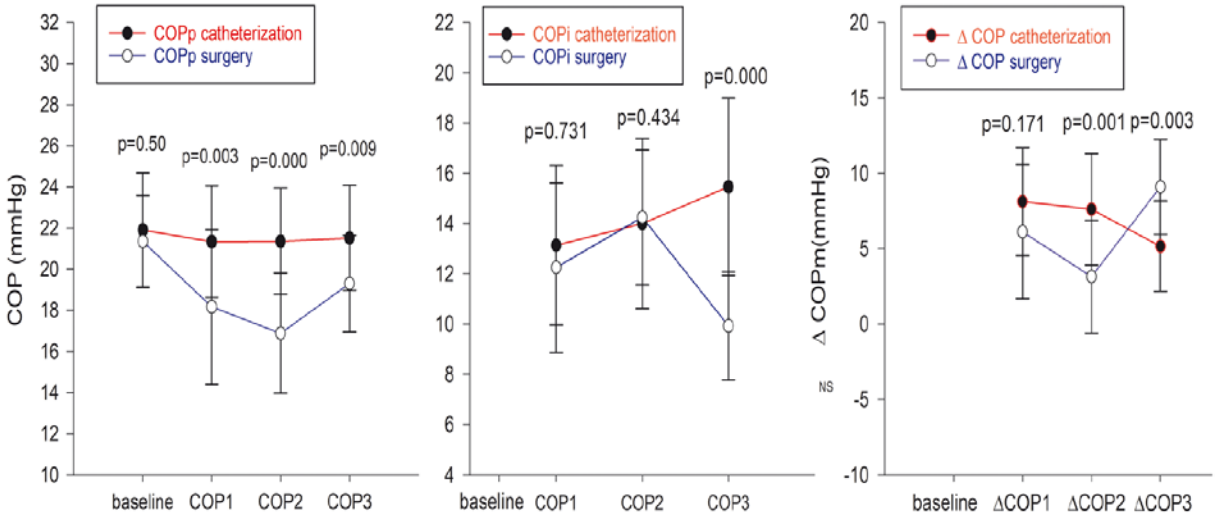


Fig.5 COP_p (left panel), COP_i (middle panel) and colloid osmotic pressure gradient (ΔCOP = COP_p − COP_i (right panel) in patients with atrial septal defect closed by interventional catheterization or surgery. CPB: cardiopulmonary bypass; COP: colloid osmotic pressure; COP_i : colloid osmotic pressure of interstitial fluid; COP_p : colloid osmotic pressure of plasma; COP1: before start of CPB/detachment of the device; COP2: end of procedure/CPB; COP3: 1 h after the procedure/surgery.
From Indrebo et al (116) © with permission from Oxford University press on behalf of the European Association for Cardio-Thoracic Surgery.

This was the first report on COP in both plasma and interstitial fluid following pediatric cardiac surgery. This knowledge is of clinical importance for prevention of the excessive fluid retention that occurs in some patients after cardiac surgery.

Paper 3

"Fluid accumulation in the staged Fontan procedure: the impact of colloid osmotic pressures"

In paper 2 we had evaluated COP changes in a simple cardiac defect and an evaluation of these changes in a more complex cardiac defect were requested. We hypothesized that staged Fontan surgery would affect the colloid osmotic pressures and that these changes were related to interstitial fluid accumulation.

As in paper 2 we observed that COPp and COPi at baseline were lower in patients scheduled for TCPC than in age-matched healthy controls. We assessed the net COP gradient across the capillaries in children at different stages of Fontan surgery. All of the procedures, both in catheterization and surgery, caused a decrease in COPp, returning to baseline values following the procedure. Twenty hours after the surgery we observed a decreased COPi, which resulted in an increased ΔCOP. The reduced COPi was associated with fluid accumulation.

Changes in COPi can be a marker of excessive fluid filtration and was associated with longstanding pleural effusion and hospitalization which contribute significantly to morbidity in children undergoing Fontan surgery. Our findings emphasize the importance of microvascular circulation in edema formation in these patients.

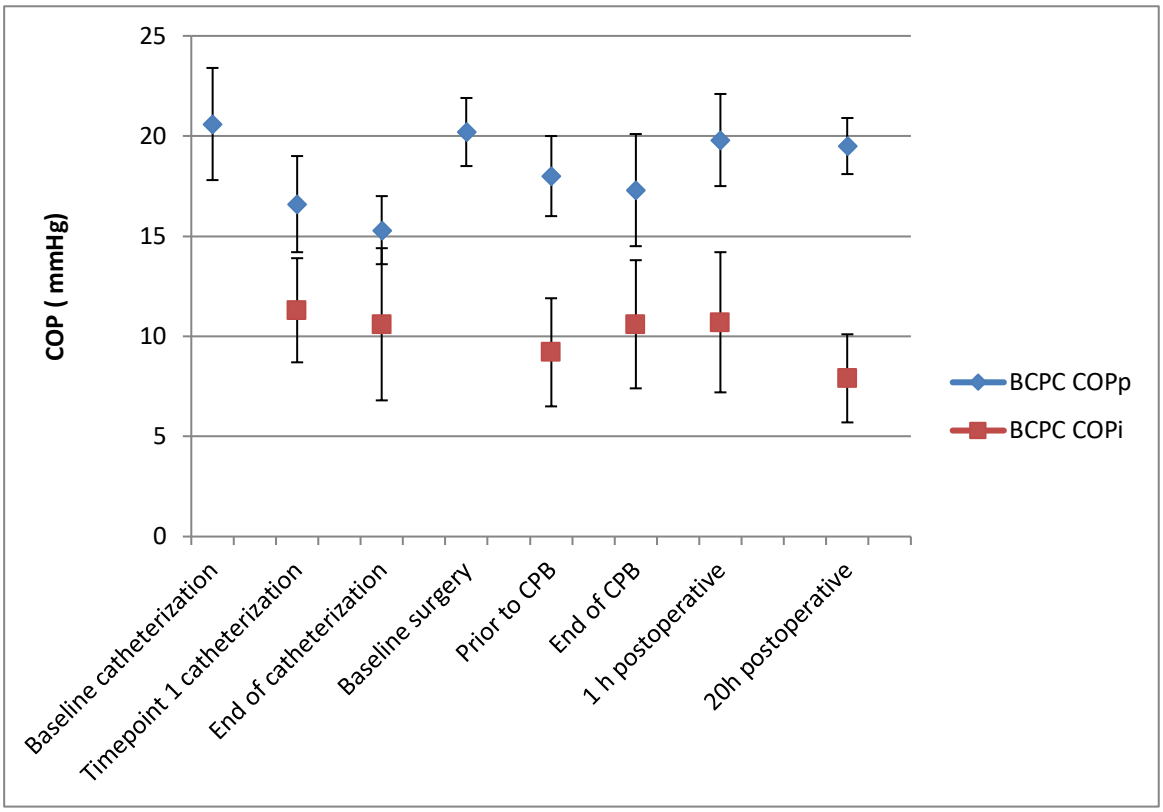


Fig.6 Colloid osmotic pressures in plasma (COPp) and interstitium (COPi) during catheterization and surgery for the BCPC procedure.

BCPC

	COPp (mmHg)	p-value	COPi (mmHg)	p-value
Baseline catheterization: surgery	20.6±2.8:20.2±1.7	0.626	11.3±2.6: 9.2±2.7	0.119
Baseline catheterization: End catheterization	20.6±2.8:15.3±1.7	0.001	11.3±2.6:10.6±3.8	0.462
Baseline surgery: Prior to CPB	20.2±1.7:18.0±2.0	0.000		
Baseline surgery: End of CPB	20.2±1.7:17.3±2.8	0.001	9.2±2.7:10.6±3.2	0.309
Baseline surgery: 1 h postoperative	20.2±1.7:19.8±2.3	0.595	9.2±2.7:10.7±3.5	0.196
Baseline surgery: 20 h postoperative	20.2±1.7:19.5±1.4	0.200	9.2±2.7:7.9±2.2	0.267
End of CPB: 1 h postoperative	17.3±2.8:19.8±2.3	0.002	10.6±3.2:10.7±3.5	0.094
End of CPB: 20 h postoperative	17.3±2.8:19.5±1.4	0.008	10.6±3.2:7.9±2.2	0.086
1 h postoperative: 20 h postoperative	19.8±2.3:19.5±1.4	0.392	10.7±3.5: 7.9±2.2	0.047

Table 2. The corresponding values of COPp and COPi during BCPC procedures with p-values for different timepoints.

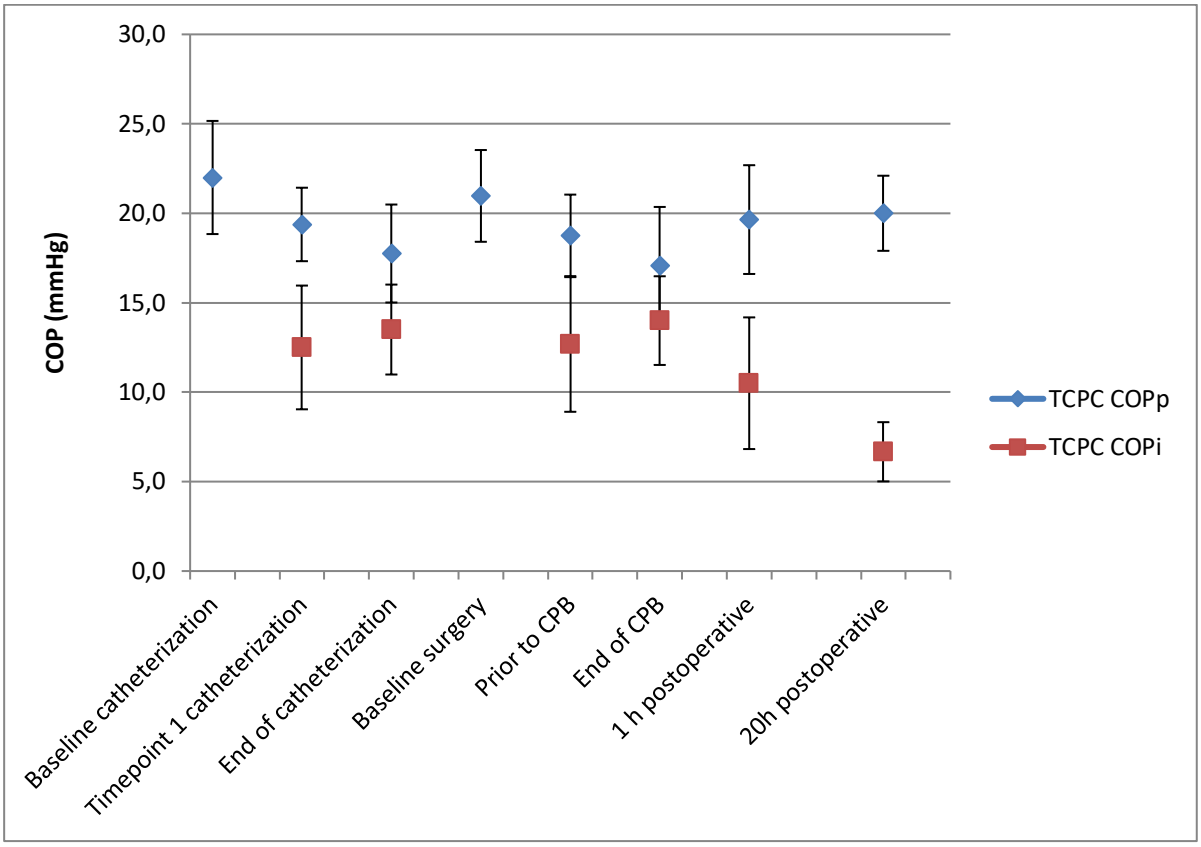


Fig.7 Colloid osmotic pressures in plasma (COPp) and interstitium (COPi) during catheterization and surgery for the TCPC procedure.

TCPC

	COPp (mmHg)	p-value	COPi (mmHg)	p-value
Baseline catheterization: surgery	22.0±3.2:21.0±2.5	0.180	12.5±3.5:12.7±3.6	0.694
Baseline catheterization: End catheterization	22.0±3.2:17.8±2.7	0.000	12.5±3.5:13.5±2.5	0.220
Baseline surgery: Prior to CPB	21.0±2.5:18.8±2.2	0.001		
Baseline surgery: End of CPB	21.0±2.5:17.1±3.2	0.001	12.7±3.6:14.0±2.3	0.546
Baseline surgery: 1 h postoperative	21.0±2.5:19.6±2.9	0.107	12.7±3.6:10.5±3.5	0.079
Baseline surgery: 20 h postoperative	21.0±2.5:20.0±2.0	0.181	12.7±3.6:6.7±1.6	0.005
End of CPB: 1 h postoperative	17.1±3.2:19.6±2.9	0.001	14.0±2.3:10.5±3.5	0.023
End of CPB: 20 h postoperative	17.1±3.2:20.0±2.0	0.002	14.0±2.3: 6.7±1.6	0.001
1 h postoperative: 20 h postoperative	19.6±2.9:20.0±2.0	0.534	10.5±3.5: 6.7±1.6	0.028

Table 3. The corresponding values of COPp and COPi during TCPC procedures with p-values for different timepoints.

Discussion

Methodological consideration

Fluid balance derangement during cardiac surgery is of major importance for the increased mortality and morbidity seen. The development of capillary leak and edema formation is more profound in the pediatric population (5, 117). The knowledge of the mechanism governing fluid flux across the capillaries is based on animal experience and clinical research in adults. The challenge of conducting randomized clinical trials with sufficient number of participants in children with congenital heart defects together with the need for extra care in conducting research in a very vulnerable group of patients has, to some degree, restricted the knowledge.

This thesis studies focused on assessing COP in healthy children and children with congenital heart defects and to evaluate whether changes in transcapillary COP could be associated with the fluid accumulation seen in these patients. In order to evaluate COP in children with congenital heart defects we needed knowledge of the normal COP values in children. The first study was to sample interstitial fluid (IF) and plasma in healthy children.

The wick method

To sample IF the wick method was used. To accurately determine COP of IF the sample must represent native IF in undisturbed tissue and represents the true COP acting outside of the capillary wall. The methodology of isolating IF from subcutaneous tissue has been developed and refined over the last 50 years, and different techniques have been compared to assess the validity of different sampling methods (86). All sampling with a device through the tissue will to some degree disrupt the natural state. All traumatic implantation will cause an inflammatory reaction and can to some degree causes bleeding. In studies including live patients the sampling method must also be applicable with only minor discomfort. The ideal method would be a non-traumatic real-time collection of fluid without the need for equilibration. Experience and studies suggest that the wick technique is currently the most appropriate for studies of human IF (93).

In the wick method, a prerequisite for analyzing the content is an equilibration time. The wick volume must equilibrate with the relatively larger volume of the interstitium and preferably without any disrupting influence on the true COPi. Introducing a needle into the IF will cause some tissue trauma and local inflammatory reaction (107). Inflammation may alter local endothelial permeability to some degree increasing the leakage of both plasma proteins and red blood cells. Noddeland et al. found increased uptake of labelled albumin from the blood into the wick after 30 minutes of implantation (109). They systematically studied implantation times ranging between 30 to 180 minutes and their effects on osmotic equilibrium in human subjects. The time dependent equilibrium for water and protein associated with less severe

inflammation for COPi is close to 60 minutes. However, in an animal study of native IF free from inflammatory and dilution influence of the wick, only 1-3 % of labelled albumin was found (118). Implantation for longer than 120 minutes seems to increase protein concentration in wick fluid from local inflammation with extravasation of plasma proteins (106). Guthe et al found an implantation time between 60-90 minutes to be optimal (119). We found no difference in COPi in healthy children at 60 or 90 minutes. This indicates that short-term implanted wicks have an insignificant influence on interstitial protein.

It is essential for the wick method that the extracted liquid is comparable to the native IF of the tissue. The trauma caused by the needle needed for implantation of the wick may cause some degree of bleeding and associated leakage of products from the bloodstream. An admixture of erythrocytes might lead to an elevated COPi if this represents more than 5 % of the total protein concentration (106). Experiments of the effect of bleeding to the true COPi found that wicks that are clearly smeared with blood contain more than 0.2g/dl hemoglobin and should be rejected whereas pink colored wicks are equal to clear wicks and can be valid (106, 109). In the studies leading to this thesis 31/198, 11/123 and 30/184 wicks were discarded due to blood staining in paper 1, 2 and 3, respectively. Previous human studies using wicks have reported bleeding to be a minor problem with 7-15% of wicks which needs to be discarded due to protein contamination (109, 120).

Discarding of wicks will affect the number of measurements in each study and thereby the sample size. In study 2 and 3, heparin was given prior to the catheterization procedure, and heparin was added to the priming fluid in the CPB-circuit. Bleeding leading to bloodstained wicks was more often seen during the use of CPB.

The wick method in children allows for only small sample volume of IF. In the present studies in children repeated measurements of COPi on the same sample was not possible, due to limited volume that could be extracted, and testing of reproducibility was therefore not performed. However, the samples were analyzed by the same osmometer in all the patients and the values obtained were comparable to other studies (16, 18, 121).

Evaluation of fluid accumulation

In the studies leading to paper 2 and 3 we wanted to obtain values of COP in children with congenital heart defects and to evaluate whether changes in COP was correlated to fluid accumulation. Interstitial fluid for analyzing COPi was harvested from subcutaneous tissue. Fluid extravasation into subcutis was considered a marker for general edema. General edema may compromise cellular function with reduced oxygen supply. That said, it is the fluid accumulation in the lung and the myocardium that are the main contributor to the increased morbidity together with reduced intravascular volume. Fluid accumulation was measured by peri- and postoperative fluid balance, weight gain, and the development of pleural and pericardial effusion. Due to the difference in capillary filtration coefficient (CFC) among

tissues, the fluid extravasation will be tissue dependent (93, 122). In animal studies it is possible to measure the actual total tissue water content in different tissue (123) Studies in adults undergoing coronary artery bypass graft surgery has used extravascular lung water (EVLW) accumulation as a marker for fluid accumulation (124). This marker is difficult to obtain in children with an atrial shunt which was the case in our studies. In a study by Tassani et al of extravasation of albumin during surgery, the radiologic edema index was used (6). This method needs an experienced radiologist to be accurate and would not add sufficiently value to our estimation of fluid accumulation.

Healthy children

Even if the wick method is relatively free of discomfort for the study object the implantation of wicks is considered a painful procedure. Implantation of wicks in adults has usually been done after intradermal injections of a local anesthetic. Minimizing both unpleasant and distressing procedures and trauma from physical and emotional pain is obliged when a pediatric population is involved in clinical research (105). Topical anesthesia has been shown to reduce the pain during implantation of wicks without interfere with the measured COPi but the pain relief was not considered enough to perform the wick method in children without general anesthesia (119). It would be considered unethical to give children general anesthesia only for research purpose. To get access to a healthy population of children without causing them harm, children scheduled for a minor surgical procedure under general anesthesia were chosen. Children scheduled for adenoidectomy or tympanic paracentesis were considered to represent the healthy child population. The reason for these surgical procedures can be recurrent infections and these children may therefore be subject of a generally higher state of inflammation. Recurrent infections are not uncommon in the pediatric population and no patients had sign of infection before surgery.

The effect of anesthesia

The use of anesthetic has effect on the microcirculation which may have affected the true value of COP. Pre- and post-anesthetic measurement in a dog model revealed a reduction in COPp at 5 mmHg (125). Sano et al showed a decrease in COPp of 4 mmHg after induction of anesthesia with propofol and vecuronium bromide (126).

General anesthesia and sedation with propofol and sodium thiopental are known to produce a dose-response decrease in blood pressure caused by a general systemic vasodilatation as well as negative inotropic and chronotropic effects, and propofol is known to lower vascular resistance accompanied by diminished baroreceptor reflex and reflex tachycardia (127) De Blasi et al observed a propofol induced increase in muscle blood flow and decreased resistance in muscle microcirculation compared to sevoflurane, which facilitated a possible fluid shift to the interstitium (128). In a related study, Bruegger et. al measured CFC in the lower limb with a non-invasive, computer-assisted venous plethysmograph and found that sevoflurane decreases CFC with less perioperative fluid substitution in contrast to propofol in

human (129). On the other hand Husby et al found in a study in pigs that anesthesia with midazolam, fentanyl and isoflurane had no effect on COPp (130). In another pig model where propofol were administered during cardiopulmonary bypass, fluid extravasation was decreased by 30% compared to isoflurane and the pigs receiving propofol had significant higher COPi (131).

Our studies measured baseline COPp before onset of general anesthesia whereas the first measurement of COPi were taken after one hour equilibration under general anesthesia. This may have influenced the true COPi. We did not measure additional COPp in the healthy population whereas in the other studies all but patients undergoing catheterbased ASD closure had a decrease in COPp one hour after onset of general anesthesia.

The entire study population had a fasting period before measurement of baseline COPp. Preoperative fasting is traditionally thought to reduce intravascular blood volume and is identified as the main opponent of stable hemodynamics which can have a significant and potentially harmful influence on cardiac preload (132). Nevertheless, there are data indicating that blood volume is normal even after an extended overnight fasting period (133).

General discussion

Colloid osmotic pressure in plasma

To evaluate plasma COP, blood samples were allowed to clot and centrifuged to isolate serum, a surrogate for circulating plasma, and was later measured by the osmometer and denoted as COPp. According to an evaluation by Noddeland COP determined from either plasma or serum is equivalent (109).

The normal mean adult COPp is reported to be 25-27 mmHg (109, 134). In paper 1 we found the COPp in healthy children to be close to adults values at 25.6±3.3 mmHg. The increase in COPp with increasing age from 24.6± 3.2 in the youngest to 28.0±4.2 mmHg in the oldest is in accordance with the continuum reported by Sussmane et al (20) who found increasing COPp from 24.3 to 26.0 mmHg during the first year of life.

The increase in COPp is thought to reflect the reduction in TBW and extracellular fluid which occur during the first year of life together with increased serum albumin. Albumin accounts for 70-80 % of the total COP. The increase in serum protein during childhood may explain the increase in COPp with age found in paper 1.

In paper 2 and 3 children with congenital heart defect had lower baseline COPp than age-matched healthy controls. All had serum albumin levels within normal range. The albumin level was measured the day before procedure and hence the sampling of blood for COPp measurements. We did not measured total protein level in these patients.

Lower baseline COPp values could be explained by excessive fluid administration prior to the anesthesia causing a dilution effect, but there were no indications for such an extensive fluid administration. On the contrary, all patients had a fasting period before procedure like the healthy controls.

Low baseline COPp is, however, in accordance with values reported in infants and children by Golab et al and Riegger et al, who found lower values in children with congenital heart defects (8, 135). In a study by Crook et al, extremely low baseline COPp (13.9 ± 2.5 mmHg) was reported in high-risk infant cardiac surgery and a strong negative correlation with outcomes was demonstrated (21). We found lower baseline values in patients both with a simple cardiac defect, paper 2, and with a complex cardiac defect, paper 3.

Cardiopulmonary bypass effect on COP

From the first successful application of extracorporeal circulation in the 1950s through the evolving of CPB, the basic concepts remain the same. Which is the oxygenation and carbon dioxide elimination, the perfusion of the organs, the need for systemic cooling and rewarming, and the removal of the blood from the heart to provide a blood-free surgical field. Despite clear understanding of the basic concepts and many improvements, CPB management in infants still remains a challenge.

The use of CPB causes profound alterations of physiological fluid homeostasis which can result in interstitial fluid accumulation and edema.

The use of CPB can affect transcapillary fluid exchange by several ways. Both by the continuous low flow altering the intra vascular hydrostatic pressure, the composition of priming fluid altering the osmotic pressure together with the priming volume causing hemodilution, and through the inflammatory reaction thought to be responsible for the degrading of glycocalyx membrane leading to increased flux of protein from the capillaries caused by the use of CPB together with hypothermia and the surgical trauma.

In both paper 2 and 3 we could evaluate the contribution of CPB with the use of mild hypothermia on transcapillary fluid exchange.

Pulsatile vs non-pulsatile flow

In both papers CPB was done using a roller pump creating a continuous non-pulsatile low flow. Non-pulsatile flow will affect both the intravascular hydrostatic pressure together with the response in vascular tone. Controversy exists regarding the effect of pulsatile versus non-pulsatile flow during CPB on various physiological outcomes. Whether the pulsatile or non-pulsatile CPB perfusion is of superiority remains debated (123, 136). The pulsatile perfusion has been shown to deliver a surplus of hemodynamic energy that affects tissue perfusion and inflammation beneficially (137) and animal experiments have confirmed better cerebral,

myocardial, renal, lymph flow and pulmonary microcirculation after pulsatile than non-pulsatile CPB perfusion (138, 139) On the other hand, human studies has not been able to confirm the superiority of pulsatile perfusion on mortality, myocardial infarction, stroke or renal failure (140, 141). The effect of pulsatile vs non-pulsatile perfusion on microvascular fluid exchange has been studied in a model in piglets where no benefits of pulsatility was found judged by weight gain, fluid extravasation and edema formation (123, 142, 143) In a study on transvascular fluid filtration in rabbit lungs pulsatile flow lead to higher filtration rate possibly due to higher mean microvascular hydrostatic pressure (144).

Edema formation during CPB may affect tissue compliance. Pulsatile CPB perfusion has been associated with increased myocardial lymph flow whereas continuous perfusion with cardioplegic arrest enhances myocardial edema related to the mismatch between fluid filtration and myocardial lymph drainage (145-147). In paper 2 the surgical patients received cardioplegia and had continuous CPB perfusion which may have led to increased myocardial edema and pericardial effusion. In paper 3 all surgeries were done using continuous CPB perfusion, but only 8 received cardioplegia. Pericardial effusion was not a problem in these patients and only one patient needed pericardial drainage.

Hemodilution

Priming of the bypass circuit with fluid will evidently cause hemodilution. Despite the minimalization of the extracorporal circuit for infant, the size of the patients in comparison to the size of the circuit is more pronounced in children and infants. Also the relatively small blood volume in children makes them more vulnerable to this hemodilution. In accordance with this we found a decrease in COPp during the use of CPB in all our patients.

To provide correct plasma volume expansion the composition of the priming fluid has been rigorously studied. There is a still ongoing debate regarding whether colloid or crystalloid solutions are the best option as the priming fluid (9, 148, 149) Russel et al conducted a meta-analysis of 21 controlled studies and concluded that albumin was better than crystalloids for priming solutions in children and adults because of its positive effect on platelet counts, COP, and fluid balance (150). Albumin has been shown to create a protein coating of the inner surface of the extracorporeal device, thus prevent platelet destruction and activation, and preserve microvascular integrity in addition to its ability to scavenge free radicals (151). Furthermore, in a study by Patel et al, albumin was also found to be better than Hydroxy-Ethyl Starch (HES) and lactated Ringer solution in pediatric cardiac surgery and was associated with less postoperative fluid accumulation (152). However, in that study, COPp was estimated using an equation from total protein, rather than measured by an osmometer. Despite the extensive number of trials regarding the optimal priming solution were both adding human albumin, fresh frozen plasma (FFP) natural colloids such as gelatins, synthetic colloids such as dextrans and HES has proven to be of benefit to maintain adequate COP, improvement in clinical endpoint such as reduced mortality and morbidity is lacking (9). These studies have aimed at keeping COPp adequate during CPB but none of these studies

have measured COPi. The opposing factor for fluid filtration will be the difference in COPp and COPi, the ΔCOP. In paper 3 we found that it was the difference in COPi before and after surgery that was associated with fluid accumulation.

The use of modified ultrafiltration (MUF) during pediatric cardiac surgery is common in some centers and the benefit will be reduced volume load after CPB and higher hematocrit. The use of MUF has been one of the factors associated with favorable outcome in Fontan patients (3, 66). MUF will affect the plasma COP after CPB and thereby the fluid filtration after surgery but will not directly affect COPi or the fluid filtration during CPB. In our studies the COPp after surgery was back to normal value one hour after surgery. Immediately after the use of CPB we measured low COPp, with the lowest value being 16.9 mmHg which is within the physiological limits reported in other studies. The optimal COPp level during CPB in pediatric cardiac surgery has not been established, but a value of 16 mmHg at the end of CPB has been suggested in order to prevent fluid accumulation (153). In a study by Golab et al. with different priming strategies the patients were allocated to either a high COP group where the aim was to keep COP above 18 mmHg or a low COP group where COP at the end of bypass was > 15 mmHg (8). They found no difference in perioperative fluid status indicating that COPp at the end of bypass is not the main determining factor for fluid filtration.

Hypothermia

All surgeries were done in mild hypothermia (31-34 °C) during bypass and we could not distinguish the effect of hypothermia from the effect of CPB. The ideal temperature for congenital cardiac surgery is still under debate. Hypothermia is thought to decrease the metabolism of the tissues and organs as well as the inflammatory response. It can also reduce the pump flow improving the surgical field visibility. On the other hand, it interferes with oxygen and glucose uptake, cellular membrane stability, ATP production and utilization and may cause trombocytic and coagulation dysfunction. In a meta-analysis by Xiong et al. normothermic CPB was found to be as safe as hypothermic CPB in children, although no markers of fluid balance were included in the clinical endpoint (67).

Animal studies have found an effect on fluid filtration during bypass of the hypothermia per se (154, 155) However, in these studies, the temperature was as low as 28°C. Another study in piglets by Kvalheim et al. found fluid extravasation rate to be lowered by 50% at 35°C compared to 28°C but still 25% above normothermic CPB (156, 157).

The use of CPB with hypothermia is thought to create a capillary leak with extravasation of proteins due to the inflammatory response (5). After bypass we found an increase in COPi, although not significant. This is in contrast to the before mentioned study in piglets, where they found a decrease in COPi during CPB with mild hypothermia (156). Another contrasting

finding is the study by Tassani et al. who found no extravasation of albumin during CPB in newborns (6).

Increasing evidence suggest the perfusion temperature affects the inflammatory response. Ali Aydemir et al found that neonates during an arterial switch operation had lower lactate and shorter time on mechanical ventilation on bypass with mild hypothermia at 32° C than bypass with deep hypothermia at 24 ° C (158).

Endothelial glycocalyx

Both the surgical trauma, the use of bypass and hypothermia can induce the systemic immune response (SIRS) seen in congenital cardiac surgery. The effect of this immune response on edema formation is thought to act through increased vascular permeability due to degrading of the endothelial glycocalyx layer (EG). This layer is considered to be the primary structure that provides ionic and colloid osmotic gradients within the vasculature and plays a major role in maintaining the vascular integrity and fluid homeostasis (82). EG is a gel-like layer that, together with associated plasma proteins, covers the lumen of the whole vasculature. Intact EG maintains a separation between vascular endothelial cells and plasma and act as a reservoir of essential plasma proteins trapped within EG (159) . The EG has a central position between the bloodstream and the endothelium and act as a regulator of vascular function, including shear stress response and regulation of vascular permeability. During CPB both shear stress due to flow alteration and the inflammation response will cause degradation of the EG (160, 161). Shedding of EG has been observed during inflammation, sepsis and during ischemia/ reperfusion during cardiac surgery (162). Unlike the synthetic colloids, the natural colloids like albumin and plasma provides a protection of the EG. Some authors claim the properties of the EG to be a reason not to conduct trials with synthetic colloid as volume replacement (163, 164).

The immune response is well recognized even though the exact details in how the cascade develops are not completely understood. Steroids attenuate the inflammatory responses and might improve outcome in patients after CPB. A meta-analysis by Whitlock et al found a non-significant reduction in mortality with the use of steroids (165). In a later large randomized multicenter study, they found that steroids given prior to CPB did not have a significant effect on mortality or major morbidity. In these studies, fluid accumulation was not included as clinical endpoints (166).The routine use of steroids during cardiac surgery is not recommended and was not in use in routine use in our studies.

The COP below the glycocalyx layer (COPg) will be lower than COPi and some claim the COPg to be the true opposing factor to fluid filtration. Although global COPi is found to reflect COPg under normal filtration pressures (93), COPi might not reflect the major determinant under high filtration conditions like during CPB. Measuring COPg under these conditions might have been more accurate to determine the colloid osmotic opposing effect to

filtration. The COPg will not be accessible in clinical trials in children during surgery. Also, the wick method will not provide us with data on dynamic changes during bypass as it requires time for the wick to equilibrate. These data might have given us more accurate information to find patients at risk for extensive fluid accumulation.

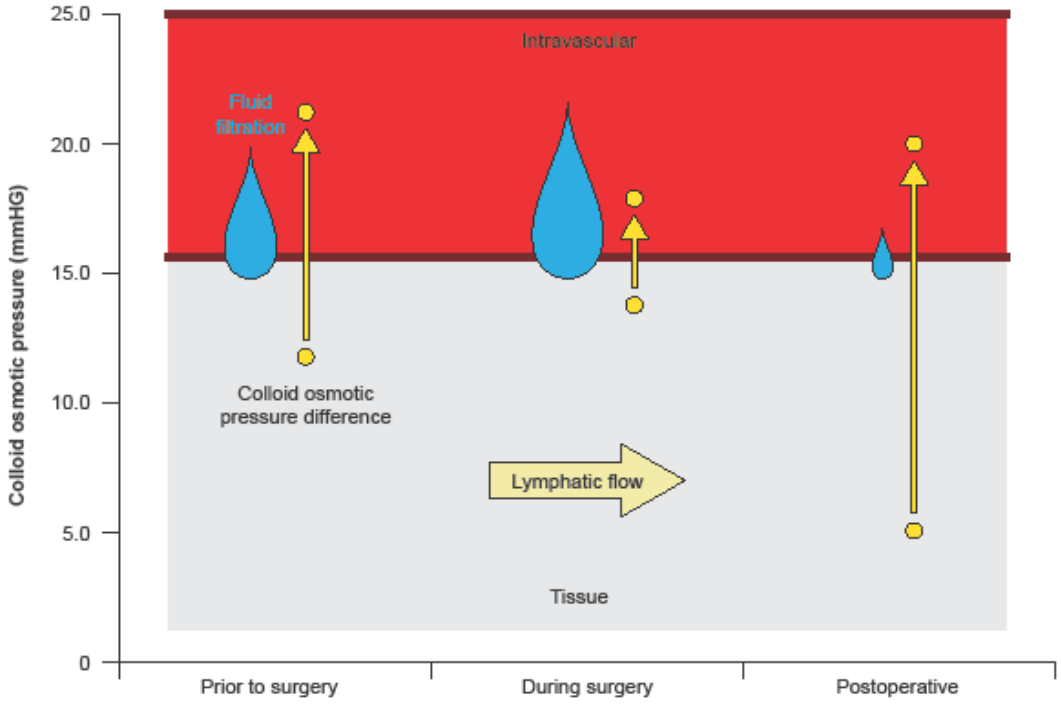


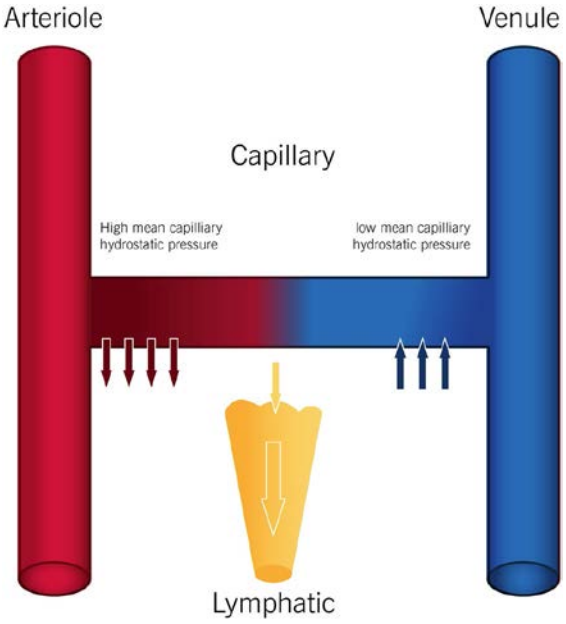
Fig. 8 The colloid osmotic pressure difference as the main opposing factor for fluid filtration. Illustration by Øystein Horgmo in collaboration with the author. From Indrebo et al (167) ©2019 with permission from Oxford University press on behalf of the European Association for Cardio-Thoracic Surgery.

Fontan circulation and fluid accumulation

The knowledge of how the forces in transcapillary fluid filtration is altered in Fontan circulation is of importance in understanding the fluid accumulation and edema formation seen in these patients. The hydrostatic pressure, the osmotic pressure, lymphatic flow and capillary permeability are all affected by the circulatory changes.

To ensure flow through the pulmonary vascular bed there is a mandatory elevated central venous pressure (CVP). The capillary hydrostatic pressure relies on differences in arterial and venous pressure and will be altered in a state of elevated CVP. This can increase fluid filtration into the tissue. We did not measure the hydrostatic pressure in our patients but found reduced osmotic pressure after surgery in our patients in paper 3. We did measure the mean arterial pressure (MAP) and CVP and found only minor increase in CVP during surgery. The factors in the Starling equation are interdependent and there might be an osmotic adaption to elevated hydrostatic pressure.

We found however changes in the osmotic pressure during and after staged Fontan surgery and the decrease in COPi was associated with longstanding pleural effusion.



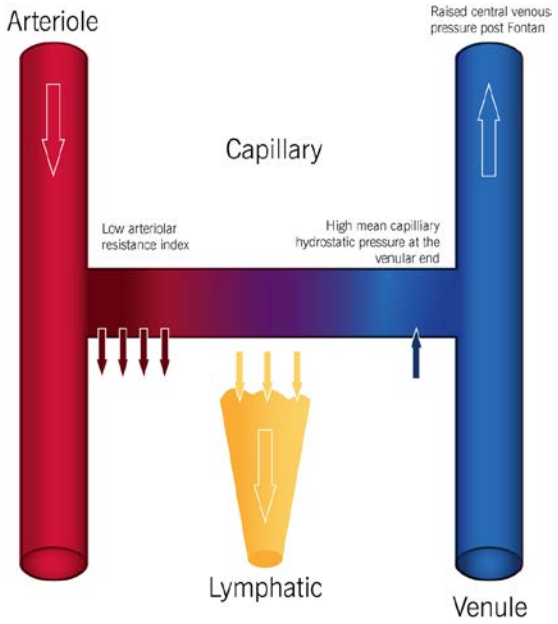


Fig. 9 Normal capillary filtration **Fig. 10**Abnormal capillary filtration post Fontan
From Menon et al (168) Reprinted by permission from Springer Nature, Pediatric Cardiology © 2017

The fluid balance derangement with development of edema and pleural effusion is a well-known contributor to the increased mortality and morbidity during Fontan surgery. Many studies have tried to identify risk factor for increased pleural effusion, most of them retrospective studies. We found more patients with longstanding pleural effusion than in these studies (3, 37, 38, 41, 44, 48, 49).

One of the known risk factors is elevated pulmonary artery pressure (PAP) (169).Historically this was predominantly seen in patients transitioning directly from arterial shunt physiology to total-cavopulmonary connections. The development of the staged procedure, which is now the preferred procedure, has reduced this risk factor and has better results (170). In our study elevated PAP was not a problem before completion of the Fontan pathway.

In case of elevated PAP a fenestration in the Fontan pathway is created. Only two patients had a fenestration created in our population. Some authors advocate the use of a fenestration in all cases and have found evidence for its superiority in reducing the risk of fluid accumulation by reducing the pulmonary pressure (3, 42, 44, 170-172).

In the Fontan circulation cardiac output is highly dependent on pulmonary flow. Adequate pulmonary flow is dependent on the size and architecture of the pulmonary arteries. During

fetal life, growth of the pulmonary arteries in a single ventricle circulation is often not optimal. The first palliation is created to ensure adequate pulmonary flow in order to enhance development of these arteries (170). The size of the pulmonary arteries can be expressed by the McGoon ratio and a ratio less than 1.3 is correlated with more fluid accumulation and poor outcome (42). We found no correlation with pleural effusion in our study, but then, all our patients had a favorable McGoon ratio both before BCPC and TCPC.

In patients developing PLE there will be a loss of albumin into the intestinal lumen which will lower serum albumin and reduce intravascular COP.

We found fluid accumulation to be associated with changes in COPi during Fontan surgery. Our study could not answer if this is related to increased hydrostatic pressure or vulnerability for glycocalyx degradation during the procedure. The question of whether some individuals are more susceptible to glycocalyx degradation and if this can explain the increased fluid accumulation is yet to be addressed.

Limitations

Our studies were all clinical observational studies with a vulnerable group of participants. No study intervention was done, and we could only report observed values without any specific causality. Apart from measuring COP no additional clinical measurements were performed and therefore no highly specific measurement of fluid accumulation could be reported.

Before conducting these studies, no data was available regarding COPi in children. Neither normal variation in children nor the clinical implication were known. A power analysis was therefore not performed.

The number of possible actual candidates for participation in paper 2 and 3 were limited and thus the numbers of participants are small. This gives limited statistical opportunities and cautions were made to avoid the problem of multiple comparisons.

The patients with univentricular heart are a very heterogeneous group and subgroup analyses could add value to the results. The number of participants was low and subgroup analyses could not be performed in paper 3.

Conclusions and implications

Measuring COP in interstitium using the wick method in children is feasible. This method can be used to gain more information about transcapillary fluid balance in different pathological condition in infant and children. Applied under general anesthesia this method has minimal discomfort and complication for the participants.

We have provided data for the normal variation in COP values in children aged 2-10 years. Children in this age have COP values close to adults and are increasing with age. This is necessary information in further studies regarding fluid balance in children.

Under normal condition there is no difference in COPi after implantation time of 60 or 90 minutes (Paper 1). An implantation time of both 60 and 90 minutes will provide reliable values of COPi and was in use in paper 2 and 3.

Unlike in adults we found no effect of gravity on COPi. This offers an explanation for the clinical observation seen in children with heart failure, were peripheral edema is not a problem.

Baseline COPp and COPi are lower both in children with a simple and a complex cardiac defect compared with healthy children. The transcapillary gradient (ΔCOP), the main opposing factor for fluid filtration, is not affected.

Pericardial effusion is more pronounced after surgical closure than interventional catheter-based closure for ASD. The actual procedure time is not different, but time on mechanical ventilation and length of stay in hospital was significantly longer. Thus, the recommended treatment of choice for ASD closure would be the catheter-based interventional procedure when applicable.

During the catheter-based ASD closure, the COPp values were stable and the ΔCOP was reduced after procedure. In contrast the COPp were reduced during surgery with a significant increase in ΔCOP after closure. This reflects the increased transcapillary fluid filtration during surgical procedure and may explain the tendency for more fluid accumulation.

In staged Fontan surgery, there is more fluid accumulation in the TCPC procedure than the proceeding BCPC procedure with more positive fluid balance during surgery, weight gain and pleural effusion.

There was a significant decrease in COPi after surgery resulting in an increase in ΔCOP during both surgical procedures indicating a substantial amount of fluid being filtrated into the tissue during surgery. This was more pronounced the day after surgery and in the TCPC group.

The length of pleural effusion correlated with changes in COPi. This could be viewed as a marker of fluid filtration and indicates that early microcirculatory changes after surgery can be detected. This may identify individuals with an increased risk for later maladaptation.

Future perspectives

The first study in this thesis provided values for COP in plasma and interstitial fluid in healthy children age 2-10 years. Given the substantial changes in fluid proportion and TBW during the first year of life there is still a knowledge gap in normal values and the development in COPi from newborn to 12 months of age.

We found lower values of COPp and COPi in children with congenital heart defect. There were changes both in a simple and a complex cardiac defect. Whether these changes are adaptations to the development of heart failure or present at birth remains an unanswered question. Children born with congenital cardiac defect with altered flow in utero might have early changes in COP and these questions needs to be addressed.

Our study in children with univentricular heart was a cross sectional study. To further evaluate the role of COP in fluid accumulation in these patients a longitudinal study from birth to Fontan completion, and even to the development of the failing Fontan, could more precisely identify individuals at risk.

Given the interdependence in the forces in Starlings equation future research should include hydrostatic pressure together with colloid osmotic pressures. The end fluid filtrations contribution to edema formation is highly dependent on the lymphatic flow. Further studies are needed to clarify the role of lymph flow in the fluid accumulation seen in congenital cardiac defect and during the use of CPB.

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Errata

In paper 1 page 4 line 20 the original text is 5 l of interstitial fluid, the correct is 5µl of interstitial fluid.

In paper 3 page 513 line 28 the original text is 12.5± 35 mmHg, the correct is 12.5± 3.5 mmHg

In paper 3 page 514 line 45 the text is “ either or neither in healthy or” , the correct should be “ neither in healthy nor”

I

PLOS | ONE

RESEARCH ARTICLE

Interstitial Fluid Colloid Osmotic Pressure in Healthy Children

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Abstract

Objective

The colloid osmotic pressure (COP) of plasma and interstitial fluid play important roles in transvascular fluid exchange. COP values for monitoring fluid balance in healthy and sick children have not been established. This study set out to determine reference values of COP in healthy children.

Materials and Methods

COP in plasma and interstitial fluid harvested from nylon wicks was measured in 99 healthy children from 2 to 10 years of age. Nylon wicks were implanted subcutaneously in arm and leg while patients were sedated and intubated during a minor surgical procedure. COP was analyzed in a colloid osmometer designed for small fluid samples.

Results

The mean plasma COP in all children was 25.6 ± 3.3 mmHg. Arbitrary division of children in four different age groups, showed no significant difference in plasma or interstitial fluid COP values for patients less than 8 years, whereas patients of 8-10 years had significant higher COP both in plasma and interstitial fluid. There were no gender difference or correlation between COP in interstitial fluid sampled from arm and leg and no significant effect on interstitial COP of gravity. Prolonged implantation time did not affect interstitial COP.

Conclusion

Plasma and interstitial COP in healthy children are comparable to adults and COP seems to increase with age in children. Knowledge of the interaction between colloid osmotic forces can be helpful in diseases associated with fluid imbalance and may be crucial in deciding different fluid treatment options.

Trial Registration

ClinicalTrials.gov NCT01044641

Introduction

Maintenance of body fluid homeostasis requires a delicate balance between the hydrostatic and colloid osmotic pressure (COP) acting across the intravascular and interstitial compartments. According to the classical Starling hypothesis, the net fluid shift across the capillary membrane is based on the interaction between two opposing forces: the difference in hydrostatic pressures and COP on either side of the membrane separating the capillary and the interstitial fluid (IF) spaces [1]. Studies of these parameters under various conditions with fluid retention (e.g. nephrotic syndrome [2], premenstrual syndrome [3], normal pregnancy [4] and cardiopulmonary disease [5]) in humans have had great significance for understanding the pathophysiology of these disorders, and have in some cases been important in the choice of fluid treatment [5]. The normal values for colloid osmotic and hydrostatic pressures in the resting state of healthy children have not been established, largely because of methodological difficulties in measurements of each of the parameters. In the case of interstitial colloid osmotic pressure (COP_i), this is likely due to lack of generally accepted methods for isolating IF. What is clear, however, is that plasma colloid osmotic pressure (COP_p) increases during the first months after birth, reaching at one year of age, values comparable to those reported in adult subjects [6]. Furthermore, decreased COP_p in disease states, like congenital analbuminemia and during surgery for congenital cardiac malformations, is associated with compromised pulmonary function and tissue edema [7, 8]. A commonly accepted method for IF sampling and thus COP_i determination is implantation of wicks [9, 10] within the proximity of the heart assuming that this position represents the average capillary pressure [11]. Gravity is thought to influence the transcapillary pressures measured below the heart level with decreased interstitial COP from an increased hydrostatic pressure gradient [11]. Harvesting IF has traditionally been managed by extraction of fluid from subcutaneously implanted nylon wicks [9]. Although evaluation studies have shown that an implantation time of 60 minutes is appropriate in adults, this supposition is not necessarily true for the pediatric population. Establishing normal values of COP_i in healthy children can be vital for proper fluid therapy in critically ill children. The aim of this study was primarily to evaluate the relationship between COP_p and COP_i in healthy children of different age. Secondly, we wanted to test if gravity would induce a difference in COP_i obtained from arm and leg and thirdly to evaluate implantation times of wicks of 60 and 90 minutes.

Materials and Methods

The study was designed as a non-blinded, sequential, descriptive study, taking place between 05. January 2007 and 22. January 2012. Patient enrolment began before study trial was registered (ClinicalTrials.gov Identifier: NCT01044641, https://clinicaltrials.gov/ct2/show/NCT01044641?term = guthe&rank=2) due to the authors not being aware of the need for registration when the study was initiated. The authors confirm that all ongoing and related trials for this intervention are registered. The protocol was approved 29. September 2006 by the local ethics committee (Regional Committee for Medical and Health Research Ethics, Western Norway) and conducted at the outpatient clinic, Department of Ear-Nose-Throat, Haukeland University Hospital. Patients were also recruited from the Department of Ear-Nose-Throat,

Akershus University Hospital from May 2010 to June 2010 to increase number of participants. Ethics approval was not necessary from Akershus University Hospital because of the existing approval from the Regional Committee for Medical and Health Research Ethics, Western Norway. For CONSORT checklist, Ethical Confirmation and Trial Protocol in Norwegian and English; see S1 CONSORT Checklist, S1 Ethics approval, S1 Protocol and S2 Protocol. The time period for patient recruitment was prolonged after approval from the local ethics committee, due to delayed enrolment of patients.

Patients were included in the study, after written informed consent was obtained from the parents or guardian. All subjects were recruited from otherwise healthy patients, 99 children (aged between 2 and 10 years), who were scheduled either for tonsillectomy and/or adenotomy and/or tympanic paracentesis. Enrolled subjects were excluded if they had any sign or significant medical history of acute febrile illness, underlying chronic diseases like cardiac anomalies, liver disease, nephropathy or any disease states and present medication that could interfere with protein metabolism. Age, gender and weight were plotted in pediatric national growth charts according to Juliusson [12].

Experiments were performed after a fasting period of at least 8 hours (no food, or chewing gum allowed after midnight the day on admission, only small amounts of water were allowed 2 hours before operation). Induction and maintenance of anesthesia consisted of weight related doses of sodium thiopental/propofol, fentanyl/remifentanil, morphine, atropine and mivacurium chloride (sevoflurane gas induction was used initially if intravenously cannulation failed). Rectal administration of paracetamol/paracetamol-kodein and non-steroidal anti-inflammatory drugs (NSAIDs) was performed after intubation, and controlled mechanical ventilation was adjusted according to end-expiratory carbon dioxide concentration and pulse oximetry. Maintenance fluid of Ringer`s solution was administered according to local guidelines during the procedure and all medication were requested and handled by anesthetists who were not involved in the study.

Procedures: Included patients were divided into age specific subgroups (2–3, 4–5, 6–7, 8–10 years) according to National Institute of Child Health and Human Development (NICHD) pediatric terminology where early childhood is defined from 2 to 5 years and middle childhood from 6 to 11 year [13]. After induction of anesthesia, sterilized multi-filamentous nylon wicks of approximately 5 cm were introduced in subcutis after skin disinfection and covered with adhesive plastic film in accordance with earlier wick studies [14]. Each patient had one wick implanted subcutaneously in one arm (lateral upper arm) at the level of the heart. This site was chosen since it was technically preferable to the thorax used in the original publication by Noddeland [11] and due to less and difficult accessible subcutaneous tissue of a small thorax. A second wick was placed in the medial part of one leg. All wicks were removed after 60 minutes according to optimal implantation/equilibration time in adults [15]. In an additional group, irrespective of age, where general anesthesia was expected to last for over 90 minutes, one wick was implanted in each medial part of the leg. One wick was retracted after 60 minutes and one wick after 90 minutes.

Plasma colloid osmotic pressure: Before surgery, in connection with peripheral intravenously cannula insertion, 0.5 ml venous blood was collected after light hemostasis. Since coagulating factors are known to have sparse effect on COP [16], plasma were allowed to coagulate in unheparinized tubes and serum was separated from the sample by centrifugation, 3000 rpm for 10 minutes, and immediately frozen in plastic tubes (Sarstedt, Reagiergefaße, micro tubes, 1,5ml) at -20°C. COP_p was measured directly with a colloid osmometer designed for small fluid samples [15, 17, 18] using a membrane impermeable for molecules > 30 kDa (PM-30 Amicron, Lexington, Ma, USA). Signals were amplified and recorded (Easy Graph P930, Gould Inc., USA).

Interstitial colloid osmotic pressure: After induction of anesthesia, two double threaded multi-filamentous nylon wicks were sewn into subcutaneous tissue. Methods and procedures were performed under sterile conditions and according to previous studies [15]. Blood stained wicks were discarded, and only clear and pink wicks with presumably none or low hemoglobin contamination were accepted for further evaluation [19]. COP_i was measured directly by a colloid osmometer as described above.

Circulating blood volume (CBV) for estimation of blood loss during surgery was calculated by the following formula estimated from a recent meta-analysis by Riley et al [20].

$$CBF = 75ml/kg\ bodyweight \times kg\ bodyweight \quad (1)$$

Serum albumin and hemoglobin concentration were analyzed in an automatic analyzer (Cobas 8000 c702, Roche Diagnostics, USA and CELL-DYN Sapphire, Abbott Diagnostics, USA) respectively, both by colorimetry.

Bedside hemodynamic monitoring (blood pressure, heart rate and SpO_2) was performed immediately before, under and after the procedure according to local protocol.

Statistical Analysis: Data were evaluated using SigmaStat 11 (Sy Stat Software/Inc.; Germany). Results are presented as numbers with proportions (%) and means with standard deviation (SD). Statistically significance was defined as a P value < 0.05. One-way ANOVA was used for evaluating the COP_p and COP_i between the different age groups, followed by an all-pairwise Holm-Sidak multiple comparison procedure if there was found a statistical significance with the One-way ANOVA. When comparing smaller groups, not normally distributed, we used a non-parametric test (Mann-Whitney).

Results

Patients and outcome: A total of 99 children (45 girls and 54 boys), were included in the study (Fig 1), and 92 (93%) had weights within ± 2 SD according to Norwegian growth charts (S1 Fig). Eleven participants (mean age 4 years 8 months) had blood loss greater than 10% of estimated CBV (Fig 2). Nineteen percent of wicks were discarded due too blood staining of wick or insufficient sample size (Fig 2). In average, 5 l of interstitial fluid was harvested from each wick. Preoperative hemoglobin (Hb) measured in 83 patients (84%) less than eight days before surgery averaged 12.5 g/dl (range 10.5–15.0). Serum Albumin was measured before surgery in 32 patients (32%) with mean albumin of 44 g/l (range 38–52) and no statistical difference was found between the different age groups. There were no immediate (before discharge) or long time (phone interview 7 days after the procedure) complications due to wick implantation or blood sampling.

Plasma COP: Mean COP_p for all age groups was 25.6 ± 3.3 mmHg, and there was a significant rise from 24.6 ± 3.2 mmHg at 2–3 years to 28 ± 4.2 mmHg at 8–10 years of age, P = 0.02 (Fig 3).

Interstitial COP: Mean COP_i (arm and leg together) for all children was 13.9 ± 3.5 mmHg. There were no significant differences between the 2–3, 4–5 and 6–7 year age groups, (14.2 ± 3.2 mmHg, 13.6 ± 3.4 mmHg and 13.2 ± 4.1 mmHg respectively), but the 8–10 age group had a higher value (17.2 ± 3.2 mmHg) than the younger children, $P < 0.05$ (Fig 3). There were no significant differences between arm and leg COP_i within any of the groups or between groups (Fig 4). In contrast to the other groups, the COP_i tended to be lower in the leg than in the arm in the 8–10 year old group, but observations were few. The mean COP_i (arm and leg altogether) from patients with blood loss over 10% of CBV was significantly higher than the COP_i obtained from the other patients (16.0 ± 4.0 mmHg vs.14.0 ± 3.6 mmHg, P = 0.048).

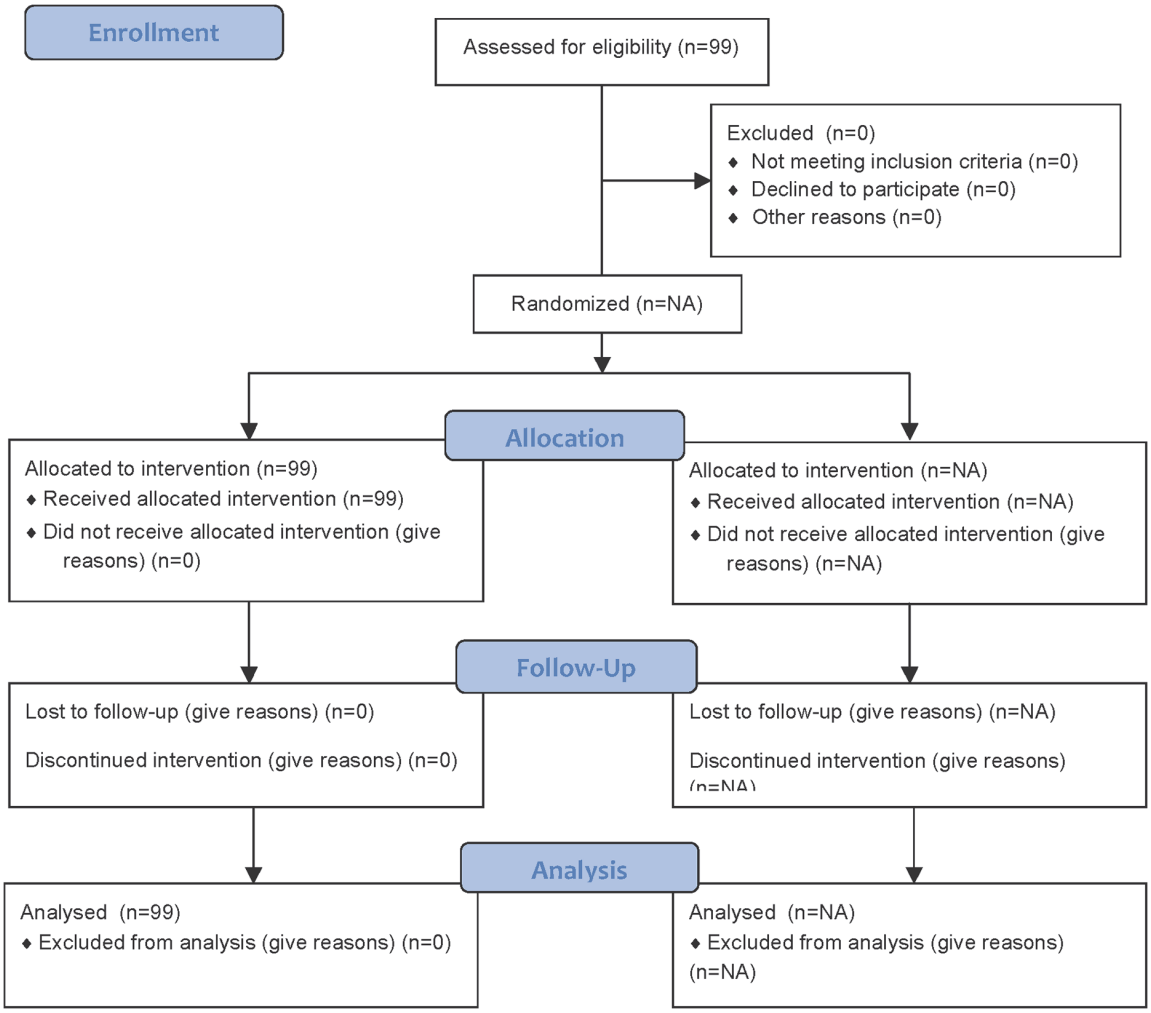

Fig 1. CONSORT flow diagram.

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Twenty-two patients who received NSAIDs in combination with paracetamol had almost identical COP_i to those who did not receive NSAIDs (14.1 ± 3.9 mmHg vs. 14.0 ± 3.5 mmHg P = 0.9).

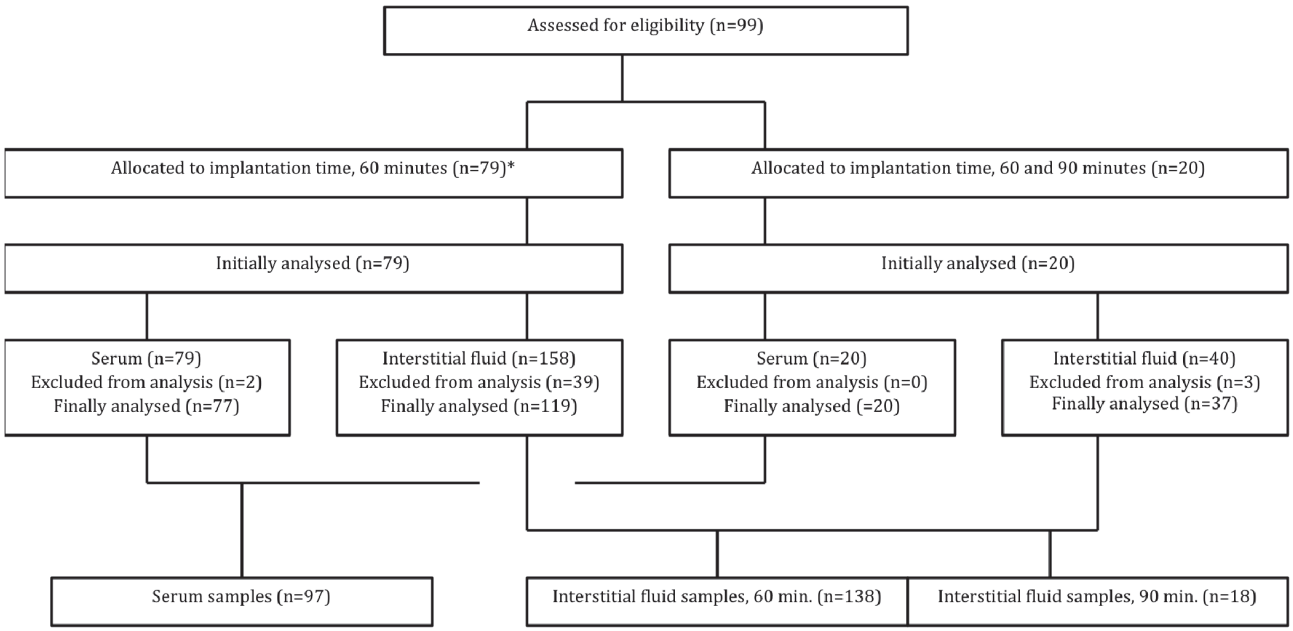

Fig 2. Recruitment of patients and distribution of samples. *Blood loss greater than 10% of estimated CBV (n = 11).

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Eighteen patients had wicks implanted in the leg for 60 and 90 minutes. There was no significant difference in mean COP_i between these endpoints (11.3 ± 2.8 mmHg vs.12.9 ± 3.1 mmHg, P = 0.11).

There was a significantly increasing colloid osmotic pressure gradient between plasma and interstitium (ΔCOP) from 10.1 ± 2.8 mmHg at 2–3 years to 14.5 ± 3.9 mmHg at 6–7 years (Fig 5). The difference tended to be smaller for the 8–10 year old group (12.5 ± 2.6 mmHg), but there were only five observations.

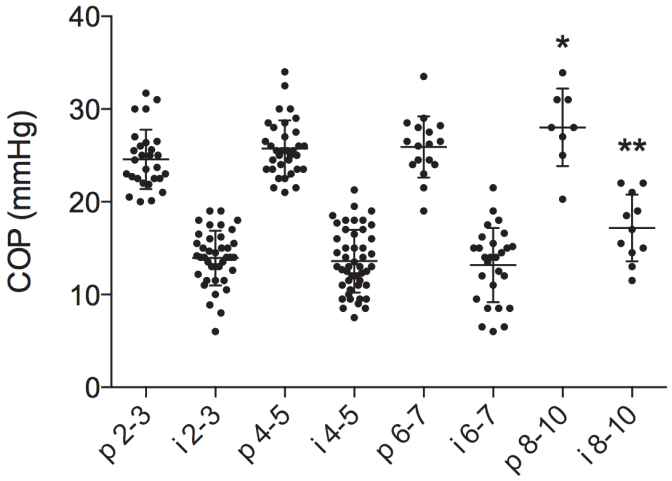

Fig 3. Colloid osmotic pressure in plasma and interstitium. Colloid osmotic pressure in plasma (p) and interstitium (i) (arm and leg merged) related to age. There was significant difference in pressures between 2–3 years and 8–10 years for plasma ($P < 0.05$, *) and between first three age groups and 8–10 years ($P < 0.01$, **) in interstitial fluid.

doi:10.1371/journal.pone.0122779.g003

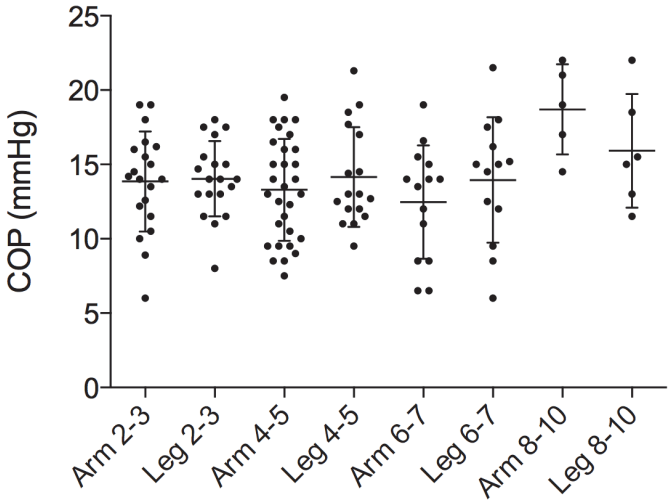

Fig 4. Colloid osmotic pressure in arm and leg. Colloid osmotic pressure from wicks after implantation in arm vs. leg related to age. There was no significant difference in the pressures obtained in arm and leg.

doi:10.1371/journal.pone.0122779.g004

Discussion

In this study, COP_p and COP_i in healthy children were close to what has been reported for healthy adults [21], but both COP_p and COP_i were significantly higher at 8–10 years than in younger children. Whether COP_i was obtained at or below heart level, or after 60 or 90 minutes, did not influence the results. There were no complications to using nylon wicks, suggesting that this method is safe for harvesting IF in anesthetized children. Furthermore, the method also gave sufficient volumes of IF to allow COP measurements. In rats, the optimal time needed for fluid and protein transport into the wick with a minimum of inflammation is between 30 and 120 minutes [19]. As suggested for healthy adults [14, 15], we found that 60 minutes was sufficient implantation time for collection of IF.

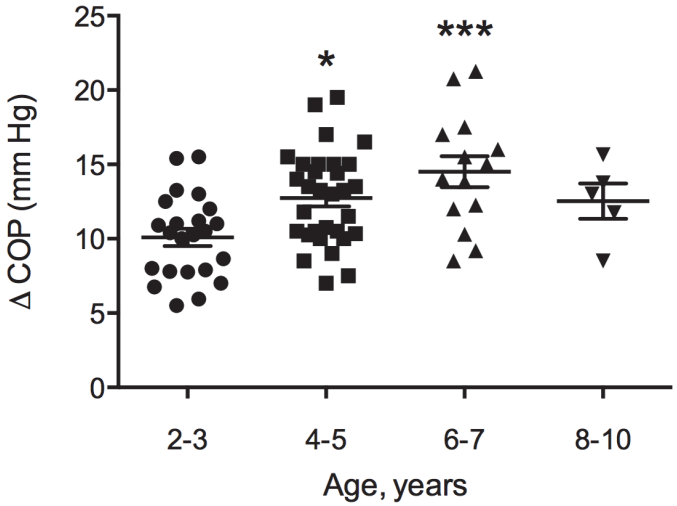

Fig 5. Transcapillary gradient in COP. ($\Delta COP = COP_p—COP_i$ (arm and leg merged)) ΔCOP related to age, with significant difference between 4–5 years and 2–3 years (P = 0.017 *) and between 6–7 years and 2–3 years ($P < 0.001$ ***).

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Earlier studies by Noddeland showed a significant higher COP_i in the thorax wall than in the calf close to the ankle in adults who were examined both in the upright and horizontal position, and that the duration of horizontal positioning did not change COP_i significantly up to 40 hours [11]. We did not observe such difference in our population although the children had been freely ambulatory until shortly before the relatively minor surgical procedures. The duration of horizontal position was not recorded, but lasted at least 1 hour before sampling. Our finding of no significant difference between arm and leg COP_i suggests that children do not experience the same orthostatic effects on COP_i as adults. This conclusion is underscored by the observation that the COP_i in the leg was lower than in the arm for the age group closest to adulthood, but not in the younger children. The difference between adults and children may have several explanations, e.g. a larger orthostatic effect due to a greater height and poorer venous drainage as a consequence of the characteristics of the veins or less physical activity.

In accordance with earlier data of COP_i in ankle [11], this finding indicates that duration of implantation within these limits did not cause sufficient trauma and subsequent inflammation to produce changes in interstitial protein distribution, which may have been expected to increase with increased implantation time [22].

COP_p in healthy full-term (19.4 ± 2.2 mmHg) [23] and pre-term (15.4 ± 1.3 mmHg) [24] babies have been reported to be significantly lower than in healthy infants from 1 to 9 months of age [6], who may have values almost identical to that of adults (25 mmHg) [21]. Due to lack of data on changes in COP in infants up to 2 post-natal months, we anticipate a sharp increase in COP to occur within the first months of life. Our findings of COP_p and COP_i similar to what has been reported for patients from two years of age are in line with studies beyond the neonatal period, suggesting that the major change occurs at around 1–2 months of age, although our study documents that a small, but significant increase also occurs during early childhood. This rise of COP_p is probably due to increasing serum concentration of proteins other than albumin, which is known to occur with increasing age [25] since we experienced a concomitant elevation in both COP_p and COP_i and no age dependent change in preoperative serum albumin.

COP_p in healthy subjects is predominantly dependent on plasma albumin and to a lesser extent on the total plasma protein concentration [26]. Albumin concentrations in the premature infant are lower than in term newborns [27], increase gradually up to 1 years of age and then undergo a modest rise towards adulthood [25]. The apparent parallel increase in COP_p and albumin concentration, therefore, suggests that albumin is part of the cause of the age dependent COP_p.

The use of hyperoncotic albumin in diseased states for fluid replacement and maintenance of COP_p is controversial and has not proven superior to saline with respect to mortality for adults admitted to the intensive care unit needing fluid resuscitation e.g. [28, 29]. In critically ill patients with increased vascular permeability, administration of albumin may actually worsen edema due to leakage of albumin into the interstitium with concomitant elevation of COP_i and intensified accumulation of interstitial fluid. There is a correlation between low COP_p at birth and severity of respiratory distress syndrome [30] and detection of early hypoproteinemia in sick preterm babies is associated with unfavorable outcome [31].

Blunt et al found a reduced contribution of albumin from 80% to 17% in relation to COP_p in sick patients [32] with no proven effect of albumin administration on mortality. Although there is a reasonable link between COP_p and albumin these findings may advocate reduced serum albumin simply as a marker of serious disease rather than an indicator of decreased COP_p. There are few studies addressing albumin for fluid resuscitation in the pediatric population, and albumin is now less used as standard plasma expander in infants although potentially beneficial for children undergoing cardiopulmonary bypass [8].

Accurate measurement of CBV in children is difficult due to lack of a "gold" standard method. However, a recent meta-analysis of values from many small studies showed that a CBV of 75 ml/kg appears to be normal for children from 2 years of age [20]. A blood loss between 10% to 15% of estimated CBV (equivalent to Class 1 hemorrhage in adults, i.e. mild blood loss) in healthy children will only cause no more than mild tachycardia [33]. By excluding patients with blood loss above 10%, which may possible influence hemodynamic, untoward effects of blood loss on COP were probably eliminated. Excessive provision of fluid will decrease COP_p and also decrease COP_i subsequent to increased filtration of fluid into the interstitium. Approximately 80% of the patients received fluid in excess of basic needs during the surgical procedure, but they were not given fluids during several hours before surgery and it is likely that the fluid balance was within normal levels at the time of sampling. Therefore, it is reasonable to assume that our data on COP represented normal physiological values.

We found a significant increase in ΔCOP from 2 to 7 years. Such increase will favor transport of fluid into the capillaries and concurrent reduced absorption of fluid by the lymphatic system in order to preserve homeostasis. Earlier studies have shown that a local rise in COP_p will counteract edema formation in tissue with reduced blood flow [34], as observed in our study, where COP_p and lymph drainage is thought to be important. A reduced ΔCOP for 8–10 years is probably due to higher COP_i compared to a net increase in COP_p with age. Whether this decline in delta COP is caused by a hydrostatic effect of patients being taller or a result of few observations remains uncertain.

COP_i is, together with interstitial hydrostatic pressure, COP_p and lymph flow, important regulators of interstitial fluid balance. These factors may also counteract edema formation in situations with hyperfiltration. Recording of interstitial fluid pressure (P_i) in relation to COP_p and COP_i would have been useful, but this was not feasible under the current clinical conditions.

In a revision of the traditional form of Starling`s principle, Levick and Michel advocate that COP_i in the "global" tissue at a distance from the capillary has less effect on capillary filtration than previously anticipated due to the presence of a semipermeable endothelial glycocalyx layer (EGL) built up of glycoproteins and glycosaminoglycanes [35]. The EGL will result in a COP gradient into the capillary that is higher than that calculated from ΔCOP. A recent review by Woodcock and Woodcock emphasizes that maintenance of the integrity of EGL might be of significance for fluid resuscitation to support the circulation in high filtration states [36]. The conclusions of a reduced importance of the COP_i in fluid filtration originates from experiments with high filtration pressures, whereas studies in mesenteric capillaries of rats suggests that the oncotic transcapillary pressure gradient is highly filtration dependent and that COP_i is close to COP of EGL at normal filtration pressures [37]. As recently discussed by Wiig and Swartz [38], with these reservations, we may still conclude that COP_i is of major importance for normal fluid filtration.

The present study has some limitations. All subjects were under general anesthesia and, therefore, may have had altered homeostasis, which could impinge upon normal COP values, although maintenance of anesthesia with propofol is associated with minimal fluid extravasation [39]. Due to our protocol, additional implantation of wicks was not accepted when traumatic bleeding occurred, which reduced the number of simultaneous determination of COP in plasma and IF. Plasma COP should optimally be sampled at the same time as harvesting the wicks, not only before implantation. This is especially important for patients with traumatic bleeding over 10% of CBV since a fall in hematocrit probably would alter COP_p as well as COP_i. Due to our protocol, and the wish to avoid harmful procedures in non-therapeutic research, it was not possible to sample blood after discontinuation of anesthesia.

In conclusion, children between 2 and 10 years of age have plasma COP values similar to adults, with raised COP_p and COP_i with increasing age. These findings are important to acknowledge, since small alterations in the pressure gradient over the capillary membrane can cause substantial fluid shifts. Increasing knowledge of COP in both health and disease and the influence of crystalloids, colloids and diuretics on COP may be beneficial optimizing clinical care both in adults and the pediatric population.

Supporting Information

S1 Checklist. CONSORT Checklist.
(PDF)

S1 Ethics approval. Ethical Confirmation from the Regional Committee for Medical and Health Research Ethics, Western Norway.
(PDF)

S1 Fig. Weight and standard deviation according to Norwegian growth charts. 2 SD equals 97.7 percentile and -2 SD equals 2.3. percentile.
(PDF)

S1 Protocol. Trial protocol in Norwegian.
(PDF)

S2 Protocol. Trial protocol in English.
(PDF)

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Author Contributions

Conceived and designed the experiments: AB HW HJTG TN GN MI. Performed the experiments: HJTG AB MI GN TN HW. Analyzed the data: HJTG MI TN AB HW GN. Contributed reagents/materials/analysis tools: HW AB HJTG TN MI GN. Wrote the paper: HJGU AB HW TN MI GN.

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