

Transcranial Doppler; Relative blood flow measurements and monitoring during  
interventional paediatric cardiac catheterisation

Thesis for the degree of philosophiae doctor (Ph.D.)

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# 1. PREFACE

## 1.1 Acknowledgements

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And finally to Karolina Skagen, my remarkable wife. I tried to sabotage her research career, but despite having three children, she managed to finish her own thesis a few days before me.

## 1.2 List of Papers

1. Wallace S, Logallo N, Faiz K, Lund C, Brucher R, Russell D. Relative blood flow changes measured using calibrated Doppler spectral power at varying hematocrit levels. *Ultrasound Med Biol*. 2014 Apr;40(4):828-36
2. Wallace S, Døhlen G, Holmstøm H, Lund C, Russell D. Cerebral microemboli detection during transcatheter closure of atrial septal defect in a paediatric population. *Cardiol Young*. Feb 2015;25(2):237-44
3. Wallace S, Døhlen G, Holmstøm H, Lund C, Russell D. Cerebral microemboli detection during transcatheter closure of patent ductus arteriosus  
Accepted for publication, *Pediatric Cardiology*, May 2016
4. Wallace S, Døhlen G, Holmstøm H, Tennøe B, Lund C, Russell D. Acquired preoperative brain injury is not linked to cerebral microembolic signals during balloon atrial septostomy.

### 1.3 Abbreviations

AE	Adverse Events
BAS	balloon atrial septostomy
BF	Blood flow
BFV	Blood flow velocity
CBF	Cerebral blood flow
CHD	Congenital heart disease
CPB	Cardiopulmonary bypass
CPP	Cerebral perfusion pressure
CVR	Cerebrovascular resistance
dB	Decibels
DHCA	Deep hypothermic circulatory arrest
EBR	Embolus blood ratio
FV	Flow velocities
HLHS	Hypoplastic left heart syndrome
LF	Low flow
MCA	Middle cerebral artery
MES	Microembolic signals
PI	Pulsatility index
RBCs	Red blood cells
RI	Resistive index
SCA	Sickle cell anaemia
TCD	Transcranial Doppler
TGA	Transposition of the Great Arteries
Vmin	Minimum (cerebral blood flow) velocity
Vmean	Mean (cerebral blood flow) velocity
Vmax	Maximum (cerebral blood flow) velocity
VTP	Vasomotoric Tonus Pressure

### 1.4 Definitions

Embryo	The earliest stage of human development from the first to the eighth week following fertilization. The embryo begins as a multicellular eukaryote.
Foetus	Foetal development occurs between the embryonic phase and birth. There is no clear cut-off between the foetal and the embryonic stage, however, the term foetus tends to imply that major organ development has begun and the embryo is recognizably human



## 2. GENERAL INTRODUCTION and REVIEW OF THE LITERATURE

### 2.1 Transcranial Doppler Ultrasound

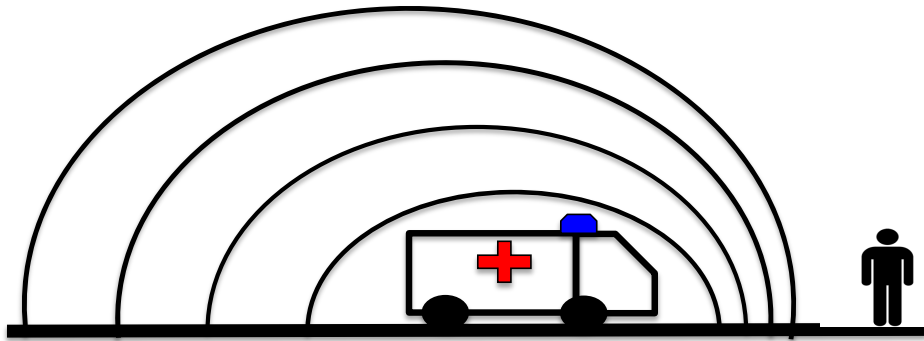
#### 2.1.1 Basic principles

The Doppler effect describes the apparent change in frequency of a wave when either the transmitting or the receiving object moves with respect to the other. When the source of the waves moves towards an observer, each successive wave will be emitted from a point closer to the position of the observer. The time taken for each successive wave crest to reach the observer will therefore be shorter, causing an increase in frequency, where frequency is defined as the number of cycles per unit time (fig 1).

$$T = 1/f$$

*The time period (T) is the duration of one cycle and is reciprocal to the frequency (f)*

This frequency shift, called the Doppler effect, is named after the Austrian physicist Christian Andreas Doppler (1803-1853) who published his work on the colour shifts of stars in 1843. We experience the Doppler effect on sound waves when a vehicle with a siren drives past us; the frequency “received” by the observer is higher as the vehicle approaches and lower as the vehicle drives away. The Doppler effect is commonly used to measure velocities by reflection of a transmitted wave, from a moving object: blood cells in the body (sound waves) or the radar for the speed of cars (electromagnetic wave or laser light waves). It is also used to measure the velocities of distant, moving galaxies (red shift of light).



**Figure 1. Illustration of the Doppler effect:**

Sound waves emitted from a moving vehicle. Sound waves are emitted in a circular pattern from the source. As the vehicle moves forward, each consecutive wave originates from a point closer to the observer and takes less time (higher frequency) to reach an observer standing ahead of the vehicle, compared to an observer standing behind. This theory was first tested in 1845 by the physicist Buys-Ballot with a train and horn player (instead of a siren).

Transcranial Doppler (TCD) is a non-invasive ultrasound technique with a wide variety of uses in both adult and paediatric populations(1). It is used to assess blood flow within the intracranial vessels, most commonly the Circle of Willis or the vertebrobasilar system and their branch vessels. Blood flow velocity (BFV) is measured by emitting sound waves from an ultrasound probe, which are reflected by moving blood cells. The change in ultrasound frequency correlates directly to the speed of the blood cells. The ultrasound frequency shift lies within the audible range and can be expressed as:

$$F = 2 \times F_o \times V \times \cos \alpha / c$$

- F: Doppler shift (Hz)  
 Fo: emitted frequency  
 V: blood flow velocity  
 Cos: is the angle between the emitted sound and blood flow direction  
 C: velocity of sound in tissue

The technique of TCD was introduced by Aaslid in 1982, initially for the diagnosis of cerebral vessel vasospasm following subarachnoid haemorrhage (2, 3). When ultrasound waves meet the bones of the skull, most of the waves are reflected or absorbed, blocking transmission. At areas where the bone is thin, and at low frequencies, more acoustic energy can penetrate into the intracranial space. Lower frequencies, commonly 2 or 2.5 MHz, are therefore used at areas where the bones are thinnest, (such as the temporal bone), referred to as “acoustic windows”. Russell and Brucher introduced the technique of using two different frequencies simultaneously, 2 MHz and 2.5 MHz, for the differentiation of solid and gaseous emboli in 2002 (4, 5).

*This method for automatic embolus detection and differentiation was used in Papers II, III and IV.*

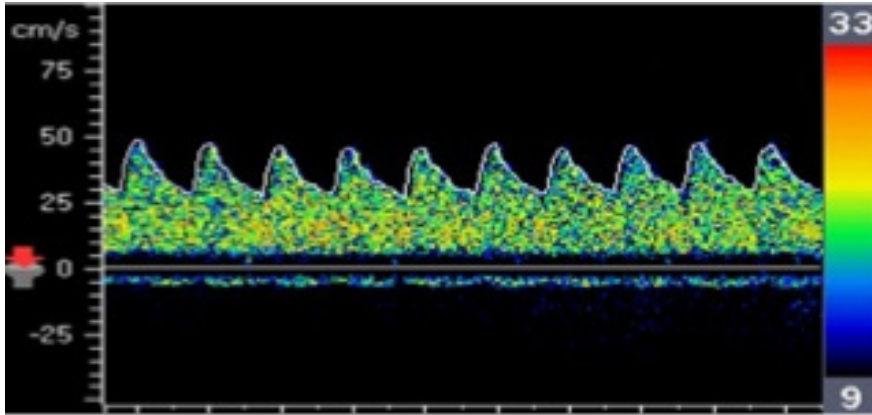
The ultrasound signals reflected from the moving blood cells are made up of a range or “spectrum” of velocities. These are analysed by a spectrum analyser before being displayed as colour coded spectrogram. A “spectral envelope” corresponding to the maximal velocity throughout the cardiac cycle is created (fig 2).

Three key parameters can be obtained from the Doppler spectrum:

- direction of flow (forward/backward in the vessel)
- maximum and mean velocities
- indices for arterial resistance(6).

In addition, TCD can be used for the detection of cerebral microembolic signals (MES).

The most commonly cited velocity measurements within the instantaneous velocity profile in the spectrogram are: the peak systolic velocity ( $V_{\max}$ ), the minimum diastolic velocity ( $V_{\min}$ ) and the mean velocity ( $V_{\text{mean}}$ ).



**Fig 2. Transcranial Doppler spectrum with envelope (outer white line) from the Middle Cerebral Artery:** Velocities in cm/s on the vertical axis and time on the horizontal axis. The crest of each waveform represents the maximal systolic velocity or  $V_{\max}$  while the troughs represent the minimal blood flow velocity (diastolic phase) or  $V_{\min}$ .

Indices, which reflect the downstream vascular resistance, may also be calculated:

The pulsatility index (PI) defined as  $PI = (V_{\max} - V_{\min}) / V_{\text{mean}}$

The resistive index (RI) defined as  $RI = (V_{\max} - V_{\min}) / V_{\max}$

All of these TCD parameters will be affected by both physiological, vasoactive and pathological processes, such as age, blood haematocrit and viscosity, CO<sub>2</sub>, haemodynamic shunts producing large diastolic “run-off” (such as patent ductus arteriosus), raised intracranial pressure, asphyxia and stenosis. Pulsatility index has traditionally been interpreted as an index of resistance in the distal cerebral vessels. Raised intracranial pressure will lead to an increase in both of the indices above, whereas vasodilation will lead to a decrease in both, due to variations in peripheral vascular resistance.

*Changes in PI are described in Paper III, following closure of patent ductus arteriosus; the ductus creates a large shunt, which in some, affects diastolic cerebral blood flow.*

### 2.1.2 Blood flow velocity versus blood volume flow

According to the Hagen-Poiseuille law, the mean velocity ( $V_{\text{mean}}$ ) within the flow profile of a liquid in a rigid tube will be equal to the volume of liquid per unit time ( $Q$ ) divided by the cross sectional area ( $A$ ) of the pipe, given by the equation:

$$V_{\text{mean}} = Q / A$$

Blood vessels are not, however, rigid and the flow velocities (FV) in an artery depends therefore on both the cross sectional area of the blood vessel and the volume of blood passing through it. Both of these factors are subject to changes. Increased BFV may reflect either an increase in the volume flow, (provided the vessel calibre remains constant) or vasoconstriction (in the presence of constant flow volume). In a narrowed vessel, (vasoconstriction, stenosis, atheromatous plaque) with constant blood flow volume, the BFV will increase both at the site of the narrowing and immediately downstream (as a jet stream). Changes in BFV do not therefore necessarily reflect changes in volume flow. Several studies have, however, validated a relationship between BFV and volume flow for specific clinical situations (7-10).

However, in most clinical situations, this assumption is unproven or incorrect and the inference that changes in velocity reflect changes in volume flow is therefore a potential source of error (11). The inability to directly measure blood flow is a major limitation of TCD as it is blood flow and not velocity that determines oxygen delivery to the tissues.

*This discrepancy was the basis for our studies in Paper I*

### 2.1.3 Cerebral blood flow Autoregulation

The brain is dependent on a constant blood flow supply and is particularly sensitive to the effects of over- or under-perfusion due to the high metabolic rate of the brain cells and the lack of storage opportunities for essential nutrients. Cerebral autoregulation refers to the physiological processes by which a relatively stable level of cerebral blood flow is maintained despite changes in arterial blood pressure and is mediated via changes in the diameter (vasodilation or vasoconstriction) of the cerebral arterioles. Autoregulation is effective between mean arterial pressures between around 50 – 150 mmHg; Cerebral blood flow (CBF) will fall or rise in a linear fashion outside of this range causing either an increase in intracranial pressure or ischaemia, respectively (12).

CBF is primarily dependent upon two factors: the cerebral perfusion pressure (CPP) and the cerebrovascular resistance (CVR):

$$CBF = CPP / CVR$$

The CPP may be defined as the pressure gradient causing cerebral blood flow and is determined by both the intracranial pressure (ICP) and the mean arterial pressure (MAP).

$$CPP = MAP - ICP$$

Relatively little is known about the mechanisms of cerebral autoregulation in children(12). It is assumed, however, that as in adults, changes in cerebral arteriolar calibre leads to changes in CVR. Proximal vessels have limited vasodilation capabilities compared to more distal cerebral arterioles, and therefore reduced CBFV in the proximal vessels is more likely to represent reduced blood volume flow (6). Furthermore, a reduction in diastolic BFV, secondary to diastolic steal reducing volume flow, in proximal vessels is described in neonates and infants with patent ductus arteriosus (13).

Factors other than blood pressure will also affect CBF. Three major physiological mechanisms are thought to be of importance:

- metabolic regulation; refers to the balance between metabolic rate (metabolic by-products) and oxygen delivery
- myogenic regulation; refers to the effects of blood pressure changes on the smooth muscle vascular walls and subsequent changes in vessel diameter
- neurogenic regulation; the role of the sympathetic nervous system in controlling arteriolar resistance remains controversial (14)

Hypocapnia, for example, has been shown to lead to distal vasoconstriction and a reduction in BFV. There is an inverse relationship between haematocrit and cerebral BFV (15). Anaemia, on the other hand, will lead to an increase in velocity (16). Several regulatory systems exist in parallel and have quite different temporal responses (14, 17). Static autoregulation is a term used to refer the steady-state of CBF over minutes to hours. Dynamic autoregulation refers to changes in the cerebral pressure-volume flow relationship with transient changes in MAP in the cerebral vessels (for example with changes in posture).

#### 2.1.4 Embolus detection using TCD

Transcranial Doppler is also used for the detection of circulating microemboli within the cerebrovascular system (18, 19). The amount of ultrasound reflected from circulating blood cells is measured as a relative intensity increase in decibels (dB), and is dependent upon the acoustic impedance, (resistance to ultrasound) of the blood cells compared to the fluid media. Red blood cells (RBCs) are by far the most abundant type of cell and are homogenous in their size, shape and density. An embolus, such as a gas bubble or microembolus with a different shape and density, consisting of a different type of material will have different acoustic impedance to the RBCs/fluid media. The detection of microemboli is therefore based on the measurement of the additional increase of intensity in the Doppler signal caused by the microembolus and its duration (Doppler energy increase). The ratio between this additional intensity increase compared to the background intensity caused by the red blood cells is called the Embolus Blood Ratio (EBR). This parameter is used to detect and characterise the embolic events [16].

There will, however, also be other random changes in the received Doppler power due to, for example, clustering of RBCs (speckling) or movement of the probe or patient (4).

Other parameters important in the detection of microemboli include:

- i) The detection threshold (in dBs)
- ii) The size of the sample volume
- iii) The Fast Fourier Transformation;
- iv) The transmitted ultrasound frequency.
- v) The duration of the intensity increase



#### Multifrequency TCD:

The amount of ultrasound being reflected depends not only on the size but also on the acoustic impedance of the embolus, which is characterized by its density and propagation of ultrasound. The greater the difference is between the acoustic impedance of the embolus and that of the surrounding whole blood, the greater the reflection is [18]. Air has an acoustic impedance that is  $<1/4000$  that of whole blood and therefore causes an extremely large reflection. Solid microemboli, on the other hand, have acoustic impedances that are more similar to whole blood and therefore give a much smaller reflection. It is therefore impossible to determine the composition of an embolus by measuring the increase in reflected ultrasound power alone because a small gaseous embolus may cause an increase in reflected ultrasound power similar in size to that caused by a much larger solid embolus.

In 2002 Russell and Brucher published papers introducing new techniques for the automatic identification of emboli and rejection of artefacts, and the discrimination between solid and gaseous microemboli [4, 5]. Differentiation between solid and gaseous microemboli is possible by insonating an embolus with two different ultrasound frequencies simultaneously: 2 MHz and 2.5 MHz. The reflected ultrasound power differs for solid and gaseous elements [4, 5, 18]. Solid microemboli reflect more ultrasound power at a higher (2.5 MHz) compared with a lower frequency (2 MHz), whereas the opposite is the case for gaseous microemboli.

The TCD instrumentation used in the *in-vivo* studies, (papers II, III and IV) uses various parameters to automatically determine if a detected high-energy signal by the ultrasound probe represents an embolus or artefact. These are outlined in the publication by Russell and Brucher [5, 20] but are summarised briefly below:

- i) The quarter Doppler shift: Emboli show quarter Doppler shift characteristics when insonated simultaneously with 2.0- and 2.5-MHz frequencies according to the Doppler formula. The Doppler shift frequency caused by an embolus moving through the vessel is therefore one quarter greater (1.25 kHz) with 2.5-MHz insonation compared with that (1.0 kHz) with 2.0-MHz insonation frequency.
- ii) Maximum time duration: The maximum duration limit is the maximum time it would take an embolus to travel through the sample volume under study. The duration of a detected high-energy signal can therefore not exceed this duration limit if it is due to an embolus.
- iii) Reference gate; This information is obtained by use of a second sample volume not necessarily in the vessel but at a distance at least 10 mm from the

sample volume placed in the vessel under study. An artefact will be detected in both gates at the same time or with a time delay of  $<4$  ms. An embolus will not be detected in the reference gate at all or, if so, with a delay of  $\geq 4$  ms

The TCD monitoring in this thesis was always performed on a middle cerebral artery (MCA), which carries approximately 80% of the blood flow to the ipsilateral cerebral hemisphere.

## 2.2 Transcranial Doppler monitoring in Children

Two types of TCD devices are available for clinical practice. With non-imaging devices, arteries are identified based on the Doppler wave pattern, depth and direction of blood flow. Colour Doppler duplex combines colour Doppler mapping with pulsed Doppler, allowing better visual identification of arteries. In children under one year of age, either the temporal acoustic window or the anterior fontanel is commonly used for TCD monitoring. Age dependant reference values of velocities and resistance indices are available, as these values change with age (6, 21, 22). Velocities increase rapidly in the neonatal and early infant period and then more slowly until 6-8 years of age. This is followed by a slow decrease, which stabilises by the 18 years of age.

Indications for TCD ultrasound examination in children include (6, 23-25):

- i) Evaluation of cerebral BFV in patients with sickle cell anaemia
- ii) Diagnosis and follow-up monitoring of vasculopathies
- iii) Diagnosis and monitoring of acute cerebrovascular disorders, especially following traumatic brain injury and arterial dissection
- iv) Monitoring during and after cardiovascular surgery
- v) Confirmation of brain death following clinical evaluation
- vi) Diagnosis of patent ductus arteriosus
- vii) Monitoring of increased intracranial pressure

### 2.2.1 Sickle Cell Anaemia

TCD plays an important and major role in the screening, management and follow-up of patients with sickle cell anaemia (SCA). Two studies have demonstrated that the risk of stroke in children with SCA increases with cerebral BFV. Annual scans to detect increased cerebral BFVs in neurologically asymptomatic children with SCA are now recommended (26-29). In the STOP I trial, (Stroke Prevention Trial in Sickle Cell Anaemia), children with TCD velocities > 200 m/s were selected for regular transfusion. The trial was discontinued when it became apparent that regular blood transfusions reduced the risk of stroke by 90%. A follow-up trial, STOP II, indicated that children reverted back to their original stroke risk if periodic transfusions were discontinued(30).

### 2.2.2 Asphyxia:

Prolonged, severe asphyxia is associated with impaired cerebral autoregulation due to increased nitric oxide production, increased diastolic blood flow and decreased cerebrovascular resistance (31, 32). TCD can be useful in the assessment of which neonates are at risk of developing severe hypoxic-ischaemic injury.

### 2.2.3 Brain Injury:

Brain trauma may also be associated with various disturbances of cerebral haemodynamics including ischaemia and vasospasm or hyperaemia. As TCD is non-invasive it can be used to monitor changes in cerebral blood flow such as decreased flow following ischaemia or signs of increased vascular resistance with cerebral oedema.

### 2.2.4 Intraoperative monitoring:

TCD has been used to monitor cerebral haemodynamics and the occurrence and timing of cerebral microembolic signals (MES) during various types of operations and cardiac catheterization procedures in both adults and children (33-42).

During the 1970's the use of deep hypothermic cardiopulmonary arrest (DHCA) and deep hypothermic cardiac bypass (DHCPB) during paediatric surgery were introduced (43, 44). The aim of hypothermia is to reduce the metabolic rate of the brain, during either cardiac arrest or periods of reduced cerebral perfusion, ("low flow"). Cerebral autoregulation is, however, affected by body temperature and a number of studies have utilized TCD to monitor the effects of hypothermia on cerebral haemodynamics (22). The autoregulatory mechanisms are altered at temperatures under 25°C and cease to operate during deep hypothermia (18° to 22°C)(45, 46). Changes in cerebral diastolic flow and CVR have also been reported in the post-operative period, following deep hypothermia (47-49). "Showers" of microembolic signals have been detected in children and adults during cardio-pulmonary bypass (42)

## **2.3 Neurodevelopmental outcomes with Congenital Heart Disease**

The brain and the heart develop simultaneously in-utero. These complex processes are orchestrated by a combination of both genetic and environmental determinants. A number of genes and signal pathways are common to the development processes of both of these organs, for example FGF (fibroblast growth factor), Wnt pathway, Sonic hedgehog. The human heart starts to beat around the 22<sup>nd</sup> day of the embryo's development. The development of the heart and circulatory system are necessary for embryogenesis and foetal development. The human brain is the most complex structure known to man and as a highly metabolically active organ is dependent upon the heart for delivery of oxygen and nutrients. It is therefore, not surprising that suboptimal development of one of these organs, can detrimentally affect the development of the other.

### **2.3.1 Congenital heart defects**

Congenital heart defects (CHD) are the most common congenital malformations, affecting approximately 9 per 1000 live births (50, 51). These defects can be classified in a number of ways but are usually initially classified as cyanotic or acyanotic. They can also, or additionally be classified according to the degree of associated symptom severity as either severe, moderate or mild (52).

The majority of patients with severe CHD will present at birth or within the first 3 days of life and will require expert medical and often surgical expertise. Anatomically, these defects may also be termed simple or complex, but a "simple" lesion, for example a large ventricular septal defect, can give rise to clinically severe symptoms, such as heart failure. Examples of defects in this group include transposition of the great arteries (TGA) or hypoplastic left heart syndrome (HLHS).

Patients with moderate CHD do not require the same degree of follow-up and may present at a later developmental stage.

With mild CHD, the patients will initially have few or no symptoms. Some of these patients will have defects that undergo spontaneous repair or never cause symptoms and may therefore not require specialized cardiology follow-up. Small septal atrial or ventricular defects, and patent ductus arteriosus are examples of defects in this group.

### **2.3.2 Treatment and outcome of CHD**

Surgical treatment of CHD can be traced back to 1939 with surgical closure of a patent ductus arteriosus (PDA) (53) with published cases of open-heart surgery appearing in the late 1950's (54). Initial mortality rates were high. It was only in the 1980's and 1990's that results from surgical repair and palliation reached the today's standards (55, 56).

Advances in the medical, transcatheter and surgical management of congenital heart diseases throughout recent decades have led to a significant increase in survival rates. Operative mortality is rare, even with the most complex lesions due to decreasing mortality rates, which have resulted from improved

diagnostic methods, anaesthetic and intensive care treatment in addition to surgical strategies (57). Furthermore, interventional transcatheter based treatments have become an alternative to surgery for a number of defects and conditions (58, 59). There are now more adults than children with congenital heart disease (60).

#### 2.3.3. Neurodevelopmental morbidity with CHD

As mortality rates have decreased, a subsequent increase in interest in the long-term morbidity and quality of life among survivors has developed amongst researchers, clinicians, patients and families. In 2012, the American Heart Association published a scientific statement entitled: Neurodevelopmental Outcomes in Children with Congenital Heart Disease: Evaluation and Management. This paper outlined the risk factors associated with neurodevelopmental abnormalities in children with CHD and recommended early, structured and periodic developmental surveillance for specific groups of patients (59).

Earlier studies that assessed developmental outcomes focused mainly on the domains of intelligence or motor skills. A number of studies have concluded that Full-IQ scores, although within the normal range for the majority of patients, are lower than in control groups: Among patients with complex CHD, a characteristic pattern of persistent neurological and behavioural complications is now recognized [65-68]. This includes inattention, impulsive behaviour, mild cognitive impairment, impaired executive functions and social skills. The prevalence and severity of both neurobehavioral problems and other neurological complications increase with the complexity of CHD.

Several studies have, however, documented decreased cognition and adaptive behavioural outcomes in children after intervention for acyanotic CHD (61-64). Problems with attention, visuospatial information processing and a higher prevalence of low-to-average intelligence scores have all been reported with acyanotic lesion defects, including ASD (65). A recent study, documented a range of neuropsychological problems in children who had undergone either surgical or catheter based ASD closure, with almost no difference between the two treatment groups (65).

#### 2.3.4 Mechanisms

Neurodevelopmental morbidity in children with CHD was previously attributed to operative factors, including the prolonged use of deep hypothermic circulatory arrest (DHCA), cardiopulmonary bypass and problems with perioperative cerebral haemodynamics (22). There is now clear clinical and radiological evidence of neurological abnormalities in the neonatal period, before surgical repair and that these neurodevelopmental abnormalities are due to a combination of innate, genetic, environmental and intervention based factors (59, 66-68).

There is a general consensus that there is a greater risk and incidence of neurological and neurodevelopmental sequelae with more complex and cyanotic CHD (59, 69). It must be noted, however, that acyanotic CHD is also associated with neurological and neurobehavioral difficulties (62-64, 70-72).

Few studies have investigated specific associations between interventional procedures and neurocognitive function.

#### Preoperative factors and findings:

Neurobehavioral and neurological abnormalities have been reported in both neonatal patients and patients less than two years of age, with a range of complex CHD, prior to surgery. Studies have reported clinical and radiological findings in over 50% of newborns with certain forms of CHD, prior to surgery, with microcephaly being reported in over 35% (70, 73, 74). Abnormal findings, such as microcephaly, agenesis of the corpus callosum and cortical abnormalities in infants with hypoplastic left heart syndrome have been reported in 38% of patients prior to operation (75).

Structural abnormalities and various types of white matter lesions have also been demonstrated with Magnetic Resonance imaging prior to surgical management. MRI detected brain injury has been detected in over a third of newborns with CHD, with an additional third showing new lesions following surgery (68, 76-78). White matter injury is most commonly described, but infarction, haemorrhage, volumetric changes and elevated lactate levels on MR Spectroscopy have also been reported. Lower Apgar scores, lower arterial oxygen saturations, balloon atrial septostomy and raised brain lactate on MR have been proposed as risk factors for preoperative brain injury (79-82)

White matter injury is seen in newborns and infants with CHD who are born both prematurely and at term. The pattern of injury is similar to that seen in other preterm patients (82, 83). This similarity in injury pattern is suggestive of abnormalities in brain development and cerebral blood flow in utero. This hypothesis is supported by studies of fetuses with CHD, which have demonstrated that lower pulsatility, and resistance indices are associated with increased mortality and growth retardation (84-86). It has been hypothesized that reductions in either blood volume flow or levels of blood oxygenation reaching the developing brain are associated with white matter changes (67, 87).

#### Patient specific factors

A number of genetic syndromes are associated with both developmental delay and congenital heart disease, for example Downs syndrome, Williams syndrome, Noonan's syndrome and CHARGE association. Other genetic polymorphisms, such as Apolipoprotein E (APOE) genotype, may increase the susceptibility to or risk of brain injury (88). In other studies, low birth weight, younger gestational age birth (prematurity) as well as male gender, Apgar score and ethnicity have been associated with poorer neurodevelopmental outcome (89).

#### Operative causes:

A wide range of causes and factors associated with brain injury during infant and paediatric surgery, have been defined. Cardiopulmonary bypass is associated with changes in cerebral perfusion including the risk of hypoperfusion and the formation of both gaseous and solid microemboli (90, 91). Hypothermia has been used for many decades with the intention of reducing cerebral metabolism during cardiac arrest. Since the 1980's, however, concerns have been raised about the use of prolonged DHCA and its effect on

neurodevelopment (56).

The Boston Circulatory Arrest Study, often described as a seminal work within the field of paediatric cardiac surgery and paediatric cardiology monitored neurodevelopmental outcomes in a group of children over 16 years. The findings of this study helped to define range of neurological, behavioural and educational difficulties experienced by children undergoing paediatric cardiac surgery while at the same time highlighting that it is not only peri-operative factors that affect the neurodevelopmental outcomes of this patient group.

This prospective, randomized controlled study compared DHCA with hypothermic low (*reduced*) cerebral blood flow during surgical correction of Transposition of the Great Arteries (TGA). The study also investigated the effect of duration of DHCA, pH management and optimal haematocrit values during surgery and has provided recommendations regarding these variables for surgeons and perfusionists. One hundred and seventy one children with TGA were initially included in the study. They were divided into two groups: DHCA and low cerebral blood flow (LF); i.e. no blood flow to the brain or reduced cerebral blood flow. Patients were invited back to a medical and neuropsychological follow-up at 1 year, 4 years, 8 years and 16 years of age. With regards to CPB strategy, it was demonstrated that both groups were impaired (lower mean total IQ of 90 in both groups) compared to healthy subjects, but neither was more impaired than the other. Each group had its own “behavioural signature”: The LF group showed significantly more impairment in measurements of attention and impulsive behaviour; the DHCA showed more impairment in areas such as speech and visual-motor tracking.

The results from this, and other studies suggest that when used, the length of DHCA should not exceed 40 minutes (56). There is also evidence that cardiopulmonary bypass (CPB) itself, whether used with DHCA or LF is a risk factor for adverse neurological outcome (62, 90, 92, 93). A systemic inflammatory response to CPB with activation of the complement and coagulation systems can lead to the disruption of the blood brain barrier (91, 94, 95). Timing of cardiac surgery may also be important, with some evidence that brain immaturity may increase the risk of acquired brain injury (76, 83).

#### Post-operative factors

The influence of socioeconomic status, parental IQ and parental stress levels has recently received attention (71, 72, 96). There is evidence that these non-physical factors affect neurodevelopmental outcome (59, 97) with socioeconomic status in particular considered as a strong predictor of neurodevelopmental outcome (69). Interestingly, there is evidence that early-intervention programs to improve mother-child interaction in babies born prematurely and thereby reduce levels of parental stress, significantly improves cognitive outcome at 5 years of age (98).



## **2.4 Paediatric Cardiac Catheterization**

William Rashkind is referred to as the father of interventional paediatric cardiology. In 1966 he introduced the technique of balloon atrial septostomy for infants with TGA (99). In this condition, the aorta arises from the right ventricle and the pulmonary artery arises from the left ventricle. Two, parallel circulatory systems, therefore exist: Venous blood, returning to heart from the body is therefore pumped from the right ventricle into the aorta and returned directly to the body, bypassing the lungs; oxygenated blood returning to the heart from the lungs is immediately returned to the lungs via the transposed pulmonary artery. This situation is incompatible with life. Balloon atrial septostomy (BAS - the creation of an atrial septal defect) allows mixing of blood returning from the body with blood returning from the lungs, at the atrial level. This allows more oxygen-rich blood to circulate through the body (and brain) palliating the patient, for a limited period of time. Surgical repair of the underlying condition can then be carried out when the patient's clinical condition is more stable.

Transcatheter repair of an atrial septal defect in a human, (a 17 year old female) was first described in 1975 (100). However, it was not until 1983 that a new report of transcatheter ASD was published and the procedure was not approved by the US Food and Drug Administration until 2001 (101, 102). In 1982, Jean Khan introduced balloon valvuloplasty for the treatment of pulmonary stenosis (103). Following this, and especially during the 1990's, more and more interventional procedures have been introduced, such that lesions previously requiring open-heart surgery and cardiac bypass are now amenable to transcatheter repair, in some cases as a hospital day-patient.

### **2.4.1 Adverse events:**

Interventional cardiac catheterization in children is considered safe but as with almost medical and surgical procedures is not without risk (104, 105). In 2006 the Congenital Cardiac Catheterization Outcomes Project (C3PO), a multi-institutional study group was established with the aim of prospectively collecting data and developing outcomes measures. This group published results from 3855 cases in 2010, comprising diagnostic, interventional and biopsy cases (106, 107).

Adverse events (AEs) were defined as "any anticipated or unanticipated event, for which avoidable injury could have occurred, or did occur, potentially or definitely as a consequence of performing the catheterization". Adverse events were classified as Low, Minor, Moderate, Major and Catastrophic. Adverse events were reported in 20% of the interventional cases, (compared to 10% and 4% in the diagnostic and biopsy cases respectively). Eleven (0.29%) deaths occurred. Seven of these patients were newborns, less than 24 hours old. The most commonly reported AEs were: Pulse loss, hypotension, atrial arrhythmias and heart block. Only two cases of CNS events (a clinically apparent stroke and a seizure related to sedation) were reported, (CT or MR brain scans were not routinely performed).

A further study from 2014 reported a low incidence, (2.1 %), of life-threatening events based on 8905 cases. The most common events were categorized as: Arrhythmias (25%); vascular or cardiac trauma (24%) and hemodynamic (20%). Neurological events (classified as "other events" – no further details

given), were described in 4 patients (105). Neurological events were classified only as stroke or seizure (108).

A number of catheterization procedures, including occlusion of atrial septal defects and patent ductus arteriosus as well as balloon atrial septostomy involve the placement of catheters (and other foreign materials such as guide-wires, balloons or occluder devices) in the left atrium or arterial circulation. There is therefore, a risk of microembolus formation, with direct communication to the cerebral arterial circulation. Furthermore, paediatric cardiac catheterization has previously been shown to impose transient fluctuations in cerebral blood flow (13, 109, 110).

Several studies in adult patients have documented either the presence of acute brain injury following cardiac catheterization (41, 111-116), the presence of cerebral microembolic signals (MES) during catheterization procedures (33, 39, 40, 117, 118) or both (41, 111). In adult populations microemboli and cerebral injury are thought to be directly related to the catheterization procedure and manipulations of the catheters. MES have been shown to be related to angiography and contrast media injection (33, 41), flushing of catheters (119) as well as dislodgement of atherosclerotic and calcific material and thrombi (120-122).

During the past two decades there has been a marked increase in interest of the effects of cardiac surgery on the brains of both adults and children. Despite its extensive use, the cerebral aspects of paediatric cardiac catheterization have not received similar attention. This is due to the perceived lack of risk factors for cerebral microemboli as well the low incidence of *clinical* neurological complications. Only a very few studies have monitored the possible presence of cerebral MES and changes in CBFV in paediatric (13, 109, 110, 123) or mixed paediatric and adult populations (38).

#### 2.4.2 Transcatheter closure of Atrial Septal Defects

Catheter based closure of ASD can be traced to the 1950's (124) and King and Mills developed an double-umbrella device in the 1970's which was used in clinical practice in 1976 (100). The most commonly reported complications include, device embolization, pericardial effusion or perforation and thromboembolic events (125). Transcatheter closure involves the placement of catheters, a balloon and a septal occluder device within the left atrium. There is therefore a risk of microemboli formation, with direct communication to the systemic and subsequently cerebral arterial circulation.

Itoh et al. used transcranial Doppler to investigate the frequency and factors associated with cerebral microemboli during transcatheter closure of secundum atrial septal defects in 16 patients from a mixed adult and paediatric population (age range 7.8 – 42.3 years). The median number of embolic signals was 31.5, (range 3 – 113). The majority of signals were detected during balloon sizing or long sheath placement and device placement, with median numbers of 9.6 and 6.5 respectively (38). This group did not use multifrequency TCD technology for the differentiation of gaseous and solid MES.

A recent study by Sarrechia et al. compared neurodevelopmental and behaviour outcomes in children undergoing either surgical or transcatheter repair of ASD-II. Compared with healthy controls, patients were shown to have significantly lower scores on testing for attention, executive function, language, working memory, social cognition and visuospatial processing but not overall intelligence (65). In this study, larger defect size and longer hospital stay were associated with poorer neuropsychological outcomes. Visconti et al. have previously shown that, after adjusting for parental IQ, surgical closure of ASD-II was associated with a 9.5 point deficit in Full-Scale IQ, although both had IQ scores which were within the normal range (62). The group that underwent transcatheter repair had more attentional problems. To date, only one study has assessed changes on brain MRI following transcatheter ASD closure (126). This study, (published after our own study on ASD closure began) demonstrated changes on diffusion weighted cerebral MRI, 72 hours after the procedure, in only one of the 30 adult patients.

#### 2.4.3 Treatment of Patent Ductus Arteriosus

The arterial duct connecting the pulmonary artery and aorta is essential in foetal life and is considered abnormal if it remains open after the neonatal period. In preterm infants, a persistently patent ductus may lead to symptomatic heart failure. In preterms, the PDA may be treated medically with indomethacin or ibuprofen or with surgical ligation (127). In recent years, a number of studies have demonstrated the safety and efficacy of transcatheter PDA closure in infants weighing less than 8 kg (128, 129). Transcatheter occlusion is considered the treatment of choice in the majority of patients with persistent PDA (130). While this treatment option is considered safe, there have been no studies investigating the absence or presence of cerebral emboli associated with this procedure using either TCD or cerebral MRI. Several studies have, however, assessed changes in CBFV during transcatheter closure of PDAs (13, 131). PDA is sometimes associated with a “Doppler sign” of low CBFV on transcranial ultrasound examination. Both of these studies documented an increase in the minimum (and therefore medium) but not maximum CBFV following closure of the PDA, in younger patients. This is due to a redistribution of blood volume in the diastolic phase, following closure of the ductus, responsible for the so-called “diastolic steal” where blood is redistributed from the aorta (and subsequently cerebral vessels) during diastole (131).

#### 2.4.4 Transcatheter palliative treatment of transposition of the great arteries

Transposition of the great arteries (TGA) is a cyanotic congenital heart disease in which the aorta and the pulmonary artery are transposed. The aorta arises from the right ventricle, returning deoxygenated blood directly to the body and the pulmonary artery arises from the left ventricle, returning oxygenated blood directly to the lungs. These two, parallel circulatory systems are incompatible with life unless blood can be mixed at either the level of a patent ductus or atrial septal defect. A number of studies have documented changes on cerebral MRI in patients with TGA after birth but before surgical treatment (76, 79, 80, 132). Balloon atrial septostomy (BAS) is a technique that creates a non-restrictive atrial communication allowing the mixing of oxygenated and de-oxygenated blood, prior to corrective, arterial switch surgery. During BAS a deflated balloon is guided into the right atrium and through to the left atrium, during cardiac

catheterization. The balloon is inflated to enlarge the foramen ovale allowing mixing of blood at the atrial level. BAS can be performed either using sedative medications in the neonatal unit or under general anaesthesia in the catheter laboratory. Venous access can be gained via either the umbilical cord or the femoral vein(s).

BAS has, however, been linked to brain injury: McQuillen et al. reported changes on brain MRI following BAS but before operative repair, consistent with embolic injury in 41% of infants with TGA, (63% of the neonates who underwent BAS). Atrial septostomy was found to be an independent risk factor for brain injury and the injuries were reported as being consistent with emboli (79). A number of other retrospective studies have, however, disputed these findings (80, 132, 133).

### 3. AIMS OF THE PRESENT THESIS

The *in-vitro* experiments investigated the accuracy of software designed to calculate changes in a flow index from the sum of frequency-weighted calculations of the Doppler power.

The overall aim of the in-vivo studies was to determine the nature of any cerebral hemodynamic changes and to detect cerebral microemboli, in real-time, during paediatric cardiac catheterization. All three in-vivo studies used multifrequency TCD technology.

The specific research questions were

1. Can a flow index, calculated from frequency-weighted power measurements detect relative changes in blood volume flow?

This hypothesis is discussed in Paper I

2. Can TCD technology be used to provide new and clinically relevant information in real-time use during transcatheter procedures in paediatric populations and how does the cerebral embolic load in paediatric populations compare with previous studies in adults using TCD during cardiac catheterization?.
3. What are the factors associated with cerebral microemboli detected during interventional cardiac catheterization in paediatric populations?
4. Is Raskind-balloon atrial septostomy associated with cerebral microemboli which may cause changes on cerebral MRI?
5. Will findings change current practices in the cardiac catheter laboratory?

## 4. SUBJECTS AND METHODS

The present thesis is based on one in-vitro and three in-vivo studies.

### 4.1 In vitro closed-loop phantom

In theory, each red blood cell (RBC) contributes equally to the reflected Doppler power signal, provided the vessel is insonated with uniform intensity. The power of the reflected Doppler signal would therefore be related to the number of RBCs, the major ultrasound scatters in blood and hence the volume of blood within the sample volume of the ultrasound beam.

The *in vitro* experiments investigated the feasibility of software designed to calculate changes in a flow index from the sum of frequency-weighted calculations of the Doppler power. These studies were performed using a closed-loop system of silicon tubes containing saline and human whole blood.

Blood, which had exceeded its clinical usage date the previous day, was obtained from the local transfusion bank..

Forward flow was generated using a digital roller pump, (Ismatec, MCP Process Pump, Glattburg, Switzerland). The blood was heparinized, kept in constant flow and continuously filtered using a micro-filter to prevent contamination by either gas bubbles or solid microparticles. A Windkessel function was built into the system. A constant temperature of 32°C was maintained within the closed-loop system by passing the tubes through a heated water bath and the temperature was monitored using a digital thermometer (BBC, Goerz Metrawatt, M4051, Austria).

Four different silicone tubes with inner diameters of 1.5, 2, 3 and 4 mm and a wall thickness of 0.5 mm were insonated with a 2 MHz TCD probe (DWL Compumedics, Singen, Germany). Each of the four tubes was insonated through a piece of 5 mm thick Plexiglas and a water bath, and examined with constant power and gain settings. The probe was secured at a 45° angle of insonation with a specially designed Plexiglas holder. The sample volume length and the insonation depth were varied until the maximum Doppler power signal was obtained. By maximizing the received signal power, it can be assumed that the maximum beam intensity lies within the central region of the parabolic flow in the tube being insonated, i.e. the area of highest flow velocities. Maximizing the signal power is also necessary to ensure that the insonating beam intensity distribution is approximately equal for each tube studied.

### Indices

According to the theory of Arts and Roelvros for calculating blood flow, spectral power  $P_i(f_i)$  is proportional to the volume of blood flowing at each velocity ( $v_i$ ) and corresponding frequencies ( $f_i$ ). The spectrum  $P(f_i)$  of Doppler frequencies( $f_i$ ) from an ultrasound beam insonating a vessel with human blood was analysed. A Flow index (FI) can be calculated from the weighted sum of each Doppler frequency  $f_i$  and the corresponding power signal,  $P_i$ , according to the equation:

$$\text{Flow}_{ix} = \sum P_i \times f_i$$

A further variable, an area index (AI), which accounts for changes in cross-sectional area can then be calculated by dividing the Flow index (FI) by the mean or maximum velocity of the Doppler spectrum:

$$\text{Area}_{ix} = F_{ix} / V_{\max}$$

Frequency weighted first moment calculations of the Doppler power were made using specifically designed software. Measurements are therefore weighted towards the higher Doppler shift frequencies, that is to say, those furthest away from the tube wall where velocities are lowest or cells can be stationary. This reduces any theoretical effect of high-pass filtering. The calculated values were then offset to pass through the zero intercept with a Flow value of zero such that with a velocity value of zero the corresponding flow index value would be zero (off-set corrected). In order to enable calculation of relative changes in both Flow and Area Indices the Flow power value in the largest tube, (a 4mm diameter tube) was designated to be 100%. The measurements were recorded and averaged over a ten second period. Flow index measurements were made at flow rates of 150, 240 and 320 ml/min.

Heparinized whole blood with an initial haematocrit (Hct) value of approximately 60% was used for all recordings and injected step by step into the closed-loop system. The closed-loop system was initially filled with 0.9% saline and all gas bubbles removed. Extreme care was taken not to introduce gas bubbles as the blood was injected. Blood was allowed to flow through the system for at least 3 minutes before recordings. Increasing Hct values were measured directly using a centrifuge (Hemokrit 4, Lic Instruments, Stockholm, Sweden) and Hct graph (Heræus sepatech, Osterode/Harz, Germany). Doppler measurements were then carried out at the 3 different flow rates above and at Hct values of 10, 20, 29 and 42%. The Hct value was controlled at the end of each set of recordings for each flow value, to ensure it had not fallen.

## 4.2 In-vivo studies

The in-vivo studies comprise three separate paediatric populations. Each study was a prospective, observational cohort study. Multifrequency TCD ultrasound was used to monitor the presence of MES in consecutive patients during closure of ASD, PDA and the during balloon atrial septosomy.

### 4.2.1 In-vivo Study populations

Oslo University Hospital-Rikshospitalet (OUS-Rikshospitalet, *The National Hospital*) is the sole center for both paediatric cardiac surgery and interventional paediatric cardiac catheterization in Norway. All catheterizations are therefore performed by one of three experienced interventionists.

The atrial septal defect population consisted of 32 consecutive patients admitted for elective transcatheter ASD closure. Eight were excluded due to technical difficulties (poor TCD signal quality or due to technical problems with real time data storage, which made it impossible to control the findings off-line after the procedure). A total of 24 patients were included for the final analysis (eight male and 16 female). The indication for transcatheter closure of the defect was a hemodynamically significant left-to-right shunt.

Twenty six consecutive patients were initially recruited to the PDA study group, however, three patients were excluded due to sub-optimal Doppler signal quality. The study population consisted of 12 male and 11 female patients with a median age of 18 months (minimum 6 months to maximum 4 years). The indication for transcatheter closure of the PDA was a haemodynamically significant left-to-right shunt with a clinically audible murmur.

The TGA group comprised 17 consecutive neonates. One patient was excluded due to poor Doppler signal quality during the BAS procedure. Five patients were diagnosed antenatally. Of the remaining 11 patients, eight were born outside the Oslo area and were transferred to OUS-Rikshospitalet following birth.

### 4.2.2 In-vivo study methods

Each of the in-vivo studies was a prospective, observational, cohort study of a paediatric patients undergoing interventional cardiac catheterization. All ASD and PDA closures were elective procedures. Although five of the patients with TGA were diagnosed antenatally, the actual Rashkind-Balloon atrial septostomy procedures, were all performed as emergency or semi-elective procedures.

ASD closure time intervals;

The entire procedure was divided into 5 periods: right cardiac catheterization; left cardiac catheterization; pulmonary angiography; balloon sizing; long sheath placement and device placement. The number and type of cerebral MES in each of these periods was calculated and compared.

PDA closure time intervals;



The procedure was divided into 5 periods: Arterial catheterization; venous catheterization; ductal catheterization; angiography; device placement and release. A comparison was made regarding the number and type of cerebral microemboli during each of these periods.

PDA closure - pigtail catheter positioning;

The position of the pigtail catheter, relative to the ductus, and subsequent cerebral microembolic load during angiographic studies was noted. Pigtail placement was classified as either: P1, proximal to the ductus; P2, level with the ductus; P3, distal to the ductus: The P3 position being furthest away from the arteries supplying the brain.

#### *Transcranial Doppler monitoring*

All TCD recordings were performed by the lead investigator in patients under general anaesthesia in a horizontal position. Continuous, unilateral monitoring and recording of the middle cerebral artery (MCA) through the temporal bone acoustic window, for both cerebral microemboli and CBFV was commenced 3-5 minutes before vascular access was obtained and continued until all catheters and femoral or umbilical lines were removed.

The bifurcation of the MCA (M1 segment, antegrade flow) and anterior cerebral artery (A1 segment, retrograde flow) was initially identified, ensuring a reproducible window. Range-gated, multifrequency TCD instrumentation was used (EmboDop, DWL, Singen, Germany). Cerebral microemboli were automatically identified and differentiated.

The multifrequency, transcranial Doppler instrumentation insonates simultaneously with 2.0 and 2.5 MHz frequencies. Embolus differentiation is based on the principle that solid microemboli reflect more ultrasound at 2.5M than 2MHz. The opposite is true for gaseous microemboli. The criteria for the automatic detection and differentiation of cerebral MES using multifrequency transcranial Doppler were based on those described previously(4, 5) but were refined for the paediatric population as follows; the detection level for MES was a 9.5 -dB power increase above background level (dEBR; embolus blood ratio), which lasted 4 ms simultaneously in both 2.0- and 2.5-MHz frequency channels and the lower dEBR detection limit for solid emboli was  $y = -0.1 \times 0.12 \text{ dB}$  where  $y = \text{dEBR}$  and  $x = 2.0 \text{ MHz EBR}$ (134).

- All real-time recordings were saved and all automatically detected emboli and decibel levels were manually controlled off-line.
- The timing and occurrence of MES relative to catheter and device or balloon manipulations was simultaneously registered.

#### 4.2.3 Ethical issues

Transcranial Doppler ultrasound is safe and non-invasive. The regional ethics committee approved each of the three in-vivo study protocols. Written, informed consent to participate in each of the studies was obtained from all participating families (parents).

## 5. SUMMARY OF PAPERS

### 5.1 Paper I

Wallace S, Logallo N, Faiz K, Lund C, Brucher R, Russell D.

Relative blood flow changes measured using calibrated Doppler spectral power at varying haematocrit levels. *Ultrasound Med Biol.* 2014 Apr; 40(4):828-36

**Aims:** A major limitation of transcranial Doppler instrumentation is the inability to measure either blood volume flow or vessel size. Currently, changes in cerebral blood flow are inferred from changes in blood flow velocity. This limitation is important as it is blood volume flow and not velocity that determines oxygen delivery to the tissues.

Direct measurement of the vessel cross sectional area is not possible but a method that potentially avoids the need for such measurement uses changes in the Doppler signal power. In theory, each red blood cell (RBCs) contributes equally to the reflected Doppler power signal, provided the vessel is insonated with uniform intensity. The power of the reflected Doppler signal would therefore be related to the number of RBCs, the major ultrasound scatters in blood and hence the volume of blood within the sample volume of the ultrasound beam.

The aim of this *in vitro* study was to derive indices of changes in blood flow (Flow index – FI) and vessel size (Area index – AI) from the both the reflected Doppler power signal and the full spectrum of Doppler frequencies. We examined the ability of these indices to measure relative changes in flow and vessel size at varying haematocrit values.

**Results:** The calculated FI for each of the 4 tube sizes at each of the defined Hct values showed a strong correlation with the actual flow values, as determined by the digital roller pump: r-value ranging from 1 to 0.95.

The correlation between the calculated area index (AI) compared to the absolute cross-sectional area (CSA) was strong for each absolute volume flow at each Hct value.

Plotting either FI or AI against Hct demonstrates an initial rise and then fall in FI values. Such a relationship has previously only been predicted based on earlier experiments detailing the effects variation in Hct has on the received Doppler power signal (15).

**Conclusion:** The results of this study support the hypothesis that indices derived from the full Doppler spectrum, specifically a Flow Index and percentage Area Index, can be used to measure changes in flow and vessel diameter over a wide range of red blood cell concentrations but highlight that the Hct value should remain constant throughout recordings. Both FI and relative AI changes correlated well with actual flow and cross-sectional areas within each tube diameter at all constant Hct levels investigated.

One possible clinical application of this method may be represented by monitoring of the flow index before and after manoeuvres or agents inducing changes in the peripheral resistances. This may be an accurate and sensitive method to test the cerebrovascular reserve capacity (vasoreactivity) in patients

with acute ischemic stroke and also in other neurological conditions.

The feasibility and accuracy of this method depends on the quality of the Doppler signal. The advent in the near future of probes which can continuously and automatically search for the optimal Doppler signal may further improve our ability to measure relative changes in blood volume flow.

Figure 2. Correlation between the calculated flow index and the absolute flow (150 ml/min, 240 ml/min and 340 ml/min). Each line represents one of the four different tube diameters. Pearson correlation values were > 0.98 for each line. • 1.5 mm tube; ▲ 2 mm tube ■ 3mm tube; ◆ 4 mm tube.

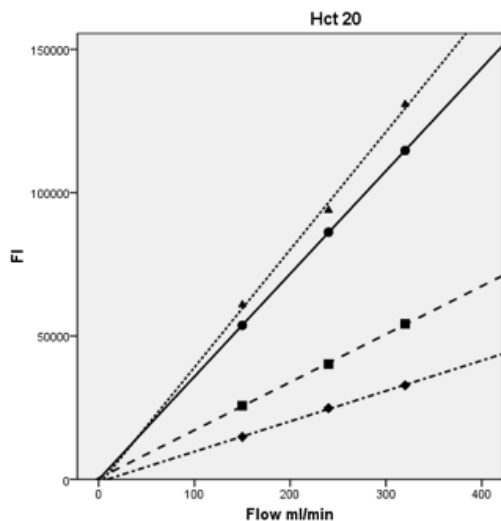


Figure 3. Correlation between the calculated area (AI) and absolute cross-sectional area  
The absolute cross-sectional area was calculated from each of the four different sized tubes. The 4 mm tube ( $CSA = 12.56\text{mm}^2$ ) was defined as 100% for AI-calculations. . Pearson linear correlation values were  $> 0.98$ : Flow rates  $\circ$  150 ml/min  $\diamond$  240 ml/min  $\square$  340 ml/min.

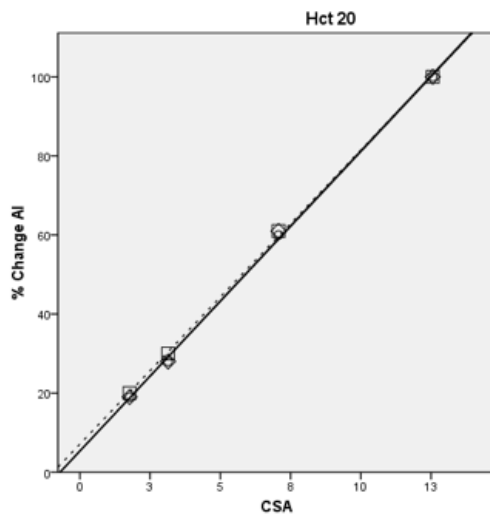
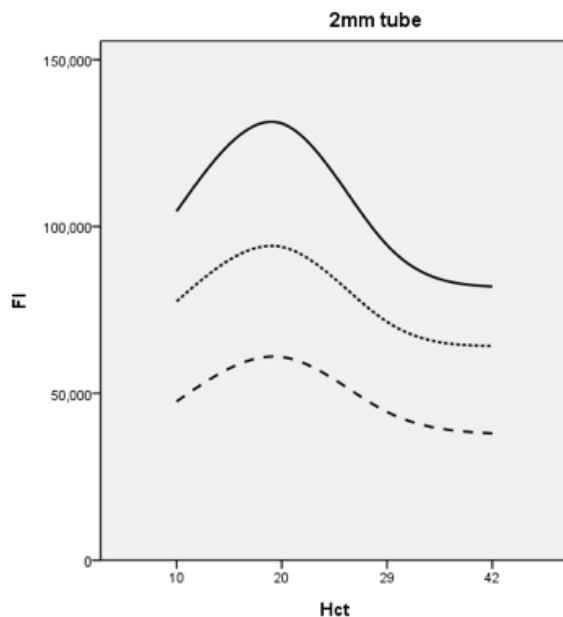


Figure 4. Relationship between the flow index and haematocrit Plot of Flow Index (FI) against rising haematocrit (Hct) values in presence of constant volume flow and tube size. Each curve represents a different absolute flow volume flows of 150 ml/min , 240 ml/min and 340 ml/min.



## 5.2 Paper II

Wallace S, Døhlen G, Holmstøm H, Lund C, Russell D.

Cerebral microemboli detection during transcatheter closure of atrial septal defect in a paediatric population. *Cardiol Young*. Feb 2015; 25(2):237-44

**Aims:** During the past 2 decades there has been a marked increase in interest regarding the effects of cardiac surgery on the brains of both adults and children. Despite its extensive use, the cerebral aspects of paediatric cardiac catheterization have not received similar attention. Interventional cardiac catheterization in both the adult and paediatric populations is, however, known to be associated with the formation of cerebral microemboli. Transcatheter closure involves the placement of catheters, a balloon and a septal occluder device within the left atrium. There is therefore a risk of microemboli formation, with direct communication to the systemic and subsequently cerebral arterial circulation. Studies in adults and mixed adult and paediatric populations have demonstrated the presence of cerebral emboli during closure of ASDs.

The aim of this prospective study was to use multifrequency transcranial Doppler to determine the quantity, timing, composition and factors associated with the formation of cerebral microemboli during transcatheter closure of secundum atrial septal defects using the Amplatzer septal occluder.

**Results:** Cerebral microemboli were detected in all patients. A total of 1688 signals were detected during the 24 procedures: 95.3% were gaseous and 4.7% were solid. The median number of all microembolic signals was 67, (range 9 to 242). Solid emboli were detected in 16 of the 24 patients. All microembolic signals were detected after vascular access was obtained and over 99% of the signals were temporally associated with specific catheter or device manipulations.

Detection of microembolic signals in two periods, balloon sizing and device placement and release, was significantly higher than during the other periods, (right cardiac catheterization, left cardiac catheterization and pulmonary angiography). Of all the microembolic signals, 84.4 % were detected during these two time periods.

There was no correlation with either the number of either total or gaseous cerebral microemboli and total procedure time, fluoroscopic time, device placement time (both including and not including long sheath placement) or combined balloon sizing and device placement time.

In eight of the patients the occlusion device was deployed several times in order to ensure an optimal and secure placement. In all but one of these patients, (patient 1), the number of microembolic signals detected decreased with each successive deployment. In patient 1, the device was deployed 3 times before it became attached to fixed structures within the atrium as an attempt to close it again was made. With each of these 3 consecutive deployments, the number of embolic signals decreased. Following further

manipulation, however, the number of embolic signals increased with a fourth deployment. This device was then completely withdrawn and a new device introduced and successfully placed.

All catheterization procedures were successful with regards to device placement. No episodes of systemic hypotension or decreases cerebral blood flow velocity were observed in any of the patients included in this study. All patients were discharged the following day.

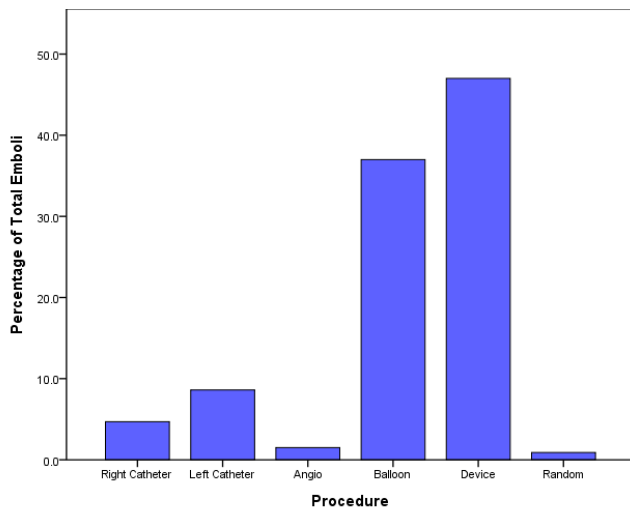
Conclusion: Cerebral microemboli were detected in all patients and are frequent during paediatric cardiac catheterization. Microemboli were associated with specific catheter manipulations. No correlation between the number of embolic signals and fluoroscopic time or duration of procedure was demonstrated. Although the majority of these emboli are gaseous, it cannot be excluded that both solid and gaseous emboli may be harmful to the brain. (REF FOR GASEOUS EMBOLI ?)

Importantly, in patients in whom the device was opened more than once, the number of MES detected fell with each successive opening.

Transcranial Doppler is a beat-to-beat on-line monitoring system which can inform the interventionist that microemboli are beginning to enter the brain's circulation. This may allow immediate procedure adjustments which may prevent further microembolization.



**Figure 5:** Graph showing the percentage of total number of microembolic signals detected during each of the stages during transcatheter closure of atrial spetal defect. A significantly higher number of embolic signals were detected during two periods, balloon sizing and device placement & release. Of all microembolic signals, 84.4 % were detected during these two time periods.



**Table 1.** Table showing the number of microembolic signals detected during successive opening of the ASD closure device in those patients in whom the device was opened more than once. The number of signals fell with each opening, except in Patient 1 when the device became attached to fixed structures in the atrium.

Patient	Total Number of device deployments	Number of microembolic signals						
		Deployment	1	2	3	4	5	6
1	4		12	2	1	10		
1	1 (new device)		5					
2	2		10	3				
5	2		15	0				
6	9		12	4	1	1	0	0
11	2		14	0				
13	4		34	16	0	0		
15	5		22	10	9	0	0	
23	3		26	9	2			

### 5.3 Paper III

Wallace S, Døhlen G, Holmstøm H, Lund C, Russell D. Cerebral microemboli detection during transcatheter closure of patent ductus arteriosus

**Aims:** Transcatheter occlusion is considered the treatment of choice in the majority of older patients with persistently patent ductus arteriosus. The procedure involves arterial hemodynamic studies, contrast injection into the aorta and the placement of catheters in the heart, ductus arteriosus and aorta. There is therefore a risk of microembolus formation within the systemic circulation with direct communication to the cerebral arteries. Furthermore, transcatheter PDA occlusion specifically, has been shown to lead to an increase in diastolic cerebral blood flow velocity in younger children and neonates, with abolition of the transcranial "Doppler sign" of low diastolic blood flow velocity in the middle cerebral artery in this population.

The aims of this prospective, observational study were to use multifrequency TCD to determine the quantity, timing, composition and factors associated with the formation cerebral MES signals and to monitor changes in cerebral blood flow velocities during transcatheter closure of a PDA in a paediatric population.

**Results:** Cerebral MES were detected in all patients. A total of 213 MES were detected throughout the 23 procedures: 95.3% were gaseous and 4.7% were solid. The median number of total MES detected per patient was seven, (minimum 1, maximum 28). Solid emboli were detected in four of the 23 patients, with a median 0 and a maximum of 5 solid MES. Although MES were detected during all of the five time periods, only 1.4% were not directly, temporally associated with specific catheter or device manipulations.

MES were detected during device placement in eight patients and accounted for 11% of all MES detected. Detection of MES was significantly higher during periods of angiographic investigation compared to any of the other periods (Freidman's test,  $P < 0.001$ ). Of the total embolic signals, 64.3 %, (139) were detected during angiograms. A further 16.5 % were detected during arterial catheterization, (hemodynamic studies). Of the total number of MES detected during angiograms, 47.5% (67), 37.5% (53) and 15% (21) were detected with the first, second and third angiogram respectively.

Linear regression analysis revealed a moderate relationship between the total number of MES detected and the volume of contrast used, ( $R\ 0.622$ ,  $P < 0.01$ ), figure 3. The amount of MES detected increased by 0.24 units per unit patient weight ( $P < 0.05$ ). There was no correlation between either the number of total MES or the number of gaseous MES detected and total procedure time, fluoroscopy time or radiation dose, or operator.

Nine angiographs were performed with the pigtail catheter in a position proximal to the PDA (and therefore closer to the origin of the vessels supplying the cerebral circulation (P1) with a mean number of

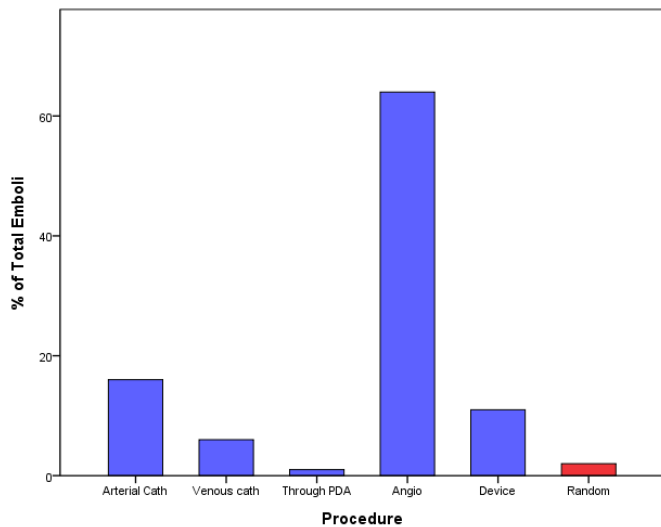
MES of 2.67. A total of 27 angiographs were performed at the level of the PDA (P2), mean number of MES 1.93 and a further 33 angiographs distal to the PDA (P3), with a mean number of MES of 1.97. There was therefore a trend towards a higher number of MES detected when contrast was injected proximally but this was not statistically significant. Multiple regression analysis for pigtail positioning, patient weight and the amount of contrast used revealed the same trend, but was not significant. Despite variations in the pigtail catheter positioning, contrast was observed in the carotid vessels during all angiographic studies.

In five patients a statistically significant increases in the  $V_{\min}$  and  $V_{\text{mean}}$  was detected following occlusion of the patent ductus. These patients were under 16 months of age. No change in the maximum, systolic CBFV ( $V_{\max}$ ) was observed. In all other patients, including four who were less than 16 months of age, no such changes were observed.

All procedures were successful with regards to device placement. There were no significant changes in oxygen saturation, MAP or temperature during the procedures. No adverse events (moderate or severe) were noted during any of the procedures. No patients developed signs or symptoms consistent with neurological dysfunction prior to discharge on the same or the following day.

Conclusion: Cerebral microemboli were detected in all patients but the vast majority, (over 95%) were gaseous. A minority, 11%, of the MES, were detected during device placement. The majority of MES were detected during angiographic studies and an association between the number of embolic signals detected and contrast used was demonstrated. Minimizing the amount of contrast used could therefore, reduce the embolic load. Additional changes to the procedure are not indicated based on the results of this study. The clinical significance of a low diastolic cerebral blood flow velocity in the minority of younger patients remains unclear.

Figure 5 Graph showing the percentage of total number of microembolic signals detected during each of the stages during transcatheter PDA closure. The number of signals detected during angiographic studies, 64 % of all MES was significantly higher than during any other period ( $P<0.001$ ). A further 16 % were detected during arterial catheterization and 11% with occluder device placement.



## 5.4 Paper IV

Wallace S, Døhlen G, Holmstøm H, Lund C, Russell D.

Acquired preoperative brain injury is not linked to cerebral microembolic signals during balloon atrial septostomy.

**Aims:** Preoperative brain injury in neonates with transposition of the great arteries (TGA) is common, and changes on brain MRI consistent with stroke have been linked to balloon atrial septostomy (BAS). Other retrospective studies, also looking at acquired brain injury, following the BAS procedure but prior to surgery have concluded that BAS is not associated with stroke. This prospective study investigated the presence of microembolic signals (MES) during septostomy using transcranial Doppler technology. In addition, MRI studies of the brain were performed after BAS but prior to surgical repair of TGA to look for changes consistent with acquired, preoperative, focal or multifocal ischemic brain injury.

**Results:** MES were detected in 10 patients (63%) of the 16 patients. The median number of MES was 2 (range 0-19). The majority of signals (94.6%) were classified as gaseous.

In three patients, a single lesion consistent with acute ischemia was found on brain MRI. Preoperative brain MRI was performed in 14 of the 16 included patients. The imaging protocol included T2 and Diffusion weighted imaging only and was designed to allow detection of recent, acquired, preoperative injury as well as structural abnormalities, whilst minimising patient time outside of the neonatal unit. In two of these patients, no MES were detected during BAS. In these patients TCD monitoring was performed on the same cerebral hemisphere as the changes on the cerebral MR. In the third patient only a single, gaseous MES was detected with TCD monitoring of the MCA on the contralateral side of the single change on cerebral MR.

For all patients, the mean gestational age was 40 weeks. Mean birth weight was 3.6 kg and mean head circumference was 35 cm. No patients were microcephalic. Nine patients had a ventricular septal defect (VSD); including one of the three in whom changes on MRI were detected. The group with findings on preoperative brain MRI were similar to the group without findings on DWI with regard to gestation, birth weight, head circumference, PO<sub>2</sub> levels and saturation levels. No risk factors for preoperative brain injury were, therefore, identified.

**Conclusion:** This is the first study we are aware of to have investigated for the presence of cerebral microemboli in real-time during the BAS procedure. The median number of MES detected (2) was low when compared to previous studies in both adults and children. The majority of MES, 94.4%, were classified as gaseous. Acquired brain injury was present on MRI scan in three (18%) of subjects, but MES were not detected during BAS in two of these patients. An association between physiological or demographic variables and brain injury was not found.

This study does not support changes in current clinical practice.

## 6. GENERAL DISCUSSION

### 6.1 Main findings in relation to previous studies

Our in-vitro experiments used both transcranial Doppler instrumentation and human blood to investigate the feasibility and reliability of software designed to calculate relative flow changes using frequency-weighted Doppler power measurements. It was then also possible to calculate relative tube diameter changes. A number of in-vivo studies have previously attempted to utilize information from the full Doppler power spectrum in an effort to measure flow variations (17, 135). These studies did not, however, correct for the effects of inhomogeneous vessel insonation on the reflected Doppler power signal and the FI/velocity relationship.

We have confirmed that a Flow index which correlates closely with actual blood flow values, can be calculated using a frequency-weighted Doppler power signal and velocity measurements.

The in-vivo studies presented here represent the first studies documenting the number, timing and type of cerebral microemboli in paediatric populations during cardiac catheterization. It has been demonstrated that TCD, a non-invasive, beat-to-beat on-line monitoring system can be used in the catheter laboratory and does not interfere with or inhibit the procedures or the interventionist.

Despite the widespread and common use of interventional paediatric cardiac catheterization, the neurological and specifically cerebral-hemodynamic effects of these invasive procedures have received little attention. This is likely due to a combination of low prevalence of clinical neurological complications and the lack of obvious risk factors for solid microembolus formation, specifically the presence of atherosclerosis, in children compared to adults. It is, however, common practice to use heparin to reduce the risk of micro-thrombus formation during both ASD and PDA closure. It is also common practice to prepare the closure devices in such a way that as much air as possible is removed before they are introduced into the patient's vascular system. Precautions and strategies are therefore already in place to reduce the risk of both gaseous and solid embolus formation.

The number of MES detected in the ASD study (median 63) and the PDA study (median 7) were lower than in previous studies investigating the number and/or timing of cerebral microemboli in adults. Lund et al. for example reported a median number of 754 MES during left heart catheterization in adults (41). The low number of MES in the PDA study, specifically, reflects that during PDA closure, both angiographic studies and device manipulation occur distal to the origin of the head and neck vessels in the aorta.

In both the PDA and ASD studies, the vast majority of MES were associated with specific manipulations of the catheters, for example during angiography with PDA closure and with balloon sizing and initial device placement with transcatheter ASD closure. The MES tended to come in "clusters" or "showers". Both these findings are in keeping with previous studies in adult populations.

During balloon atrial septostomy in patients with TGA, however, solitary embolic signals were the norm. This is reflected in the very low number of MES detected, with a median number of two. Potential sources for embolus formation with this procedure have previously been postulated to include: thromboembolism related to vascular access, including dislodgement of thrombus from within the umbilical vein; tracking of gas along the catheter; administration of medicines associated with the procedure; the procedure itself, and thrombus formation around the defect in the days following the procedure but prior to surgery. The majority of emboli in our study, (38%), were detected during advancement of the catheter towards the heart. These MES were all classified as gaseous. A further 30% of all MES were detected during balloon inflation, most likely due to microcavitation or air-trapping around the uninflated balloon.

This study is the first to investigate for the presence of cerebral emboli in real-time during BAS. Previous studies have assessed MRI findings within the first week following the procedure. It must be remembered, however, that due to the nature of the arterial transposition where the venous system is in direct communication with the aorta and therefore the cerebral arterial circulation, patients are at risk of cerebral embolic injury from various sources, including all venous injections and infusions, not only BAS.

## 6.2 Clinical implications

The overriding concern is that cerebral microemboli can cause brain injury, detectable on MRI scanning (brain structure) or with neuropsychological testing (brain function). There remains some debate regarding the pathological significance of such microemboli. Diffusion-weighted MRI has identified silent, acute brain injury identified following percutaneous cardiac catheterization in adult populations (41, 136). Some studies have demonstrated a link between embolic load and onset of new neurological symptoms or brain MRI changes (36, 37, 41) while other studies have not found this association (137, 138).

Gaseous microemboli often occur in showers within a very short time frame after injections of saline or contrast agent. Clearance of these showers of gaseous microemboli may be impaired if there is a decreased perfusion pressure distal to the stenosis (139, 140). It should also be noted that the passage of gaseous or solid microemboli through the cerebral microvasculature may cause damage to the endothelium, with subsequent activation of leukocytes and inflammatory processes (141, 142)

Ferrari et al monitored 35 adult patients (age range 24-59 years) with TCD who underwent neuropsychological testing before and after transcatheter closure of ASD. The highest rates of MES were observed when the left atrial disc was deployed and with balloon sizing. There was no evidence of a general neuropsychological impairment following the procedure and no association between the number of cerebral microemboli and changes in neuropsychological scores (40). A recent study published in 2015, has, however, documented a range of neuropsychological problems in children who had undergone either surgical or catheter based closure of ASD, with almost no difference between the treatment groups (65).



To date, only one study has looked at changes on brain MRI following transcatheter ASD closure (126). This study, (published after our own study on ASD closure began) demonstrated changes on cerebral MRI, 72 hours after the procedure, in only one of the 30 adult patients. Cerebral MRI, although sensitive will not, however, detect all changes due to microemboli. This is supported by the findings of a study by Brown et al, who performed neuropathological examinations in patients who died after cardiopulmonary bypass. The authors describe extensive cerebral pathological findings with small capillary and arteriolar dilatations caused by microembolization.

As stated above, the number of MES detected during ASD closure and PDA closure were much lower than other interventional cardiac catheterization and cardiac bypass studies in adults. No major adverse events were reported in any of the patients in the three in-vivo studies (ASD closure, PDA closure or balloon atrial septostomy). There were no reported symptoms or findings consistent with acquired central nervous system injury in patients following ASD or PDA closure prior to discharge from the hospital. The majority of emboli detected were classified as gaseous.

### 6.3 Specific findings

In both the ASD and PDA studies, no correlation between the number of embolic signals and fluoroscopic time or duration of procedure was demonstrated.

In the PDA study, a moderate correlation between the number of MES and volume of contrast used ( $R=0.622$ ,  $P<0.01$ ) was found. This is keeping with the fact that the majority of MES (68%) were detected during angiographic studies, while only 11% were detected during device placement. The majority of MES detected throughout the entire procedure therefore appears to reflect the effects of arterial hemodynamic and angiographic studies and not the manipulation and release of the PDA closure device.

Minimizing the amount of contrast used during transcatheter PDA closure could therefore, reduce the embolic load. Additional changes to the procedure are not indicated based on the results of the studies presented.

Similarly, changes to transcatheter ASD closure procedures are not indicated by the findings in the ASD study. In eight patients in this study, the device was opened more than once and the number of embolic signals decreased with each successive device deployment. This likely represents “air-trapping” around the device prior to its introduction. With the initial opening, the majority of small amount of trapped air is released which is reduced with each successive opening until no further gaseous emboli were formed.

In the BAS study no direct link between the procedure and injury causing cerebral emboli was demonstrated. Acquired brain injury was seen in three patients: in two of these patients, no MES were detected during the procedure; in the other patient, only one, gaseous MES was detected. Furthermore, MES were not detected in all patients. In those patients in whom signals detected, they were detected in very low numbers (median 2). This study does not support changes in current, bedside BAS procedures

and provides further evidence that the BAS procedure is not associated with emboli causing cerebral infarction.

#### 6.4 Future perspectives

The findings in all three in-vivo studies will be reassuring to interventional paediatric cardiologists: The number of MES detected was low compared to adult studies and over 90 % of all signals were classified as gaseous: This will be in-part due the lack of atherosclerosis in children and also due the nature of the procedures, whereby the majority of emboli were gaseous and were associated with contrast injection or introduction of air into the circulatory system (eg opening the ASD occlude device). There was no correlation between the duration of procedures or fluoroscopic time and the number of MES detected. We have proposed that reducing the amount of contrast used as well as positioning the pigtail catheter used for administering contrast distal to the ductus may lead to a reduction the cerebral embolic load. It should be noted, however, that contrast was seen in the brachiocephalic trunk, left common carotid and left subclavian arteries in all of the patients in our study in whom the pigtail was positioned distally. A larger, prospective study could further investigate the effects of pigtail catheter positioning and lower volumes of contrast administration on the cerebral embolic load.

The majority of procedures in our BAS study were carried out in the neonatal unit. The largest number of MES were detected during the only procedure carried out with the patient under general anesthetic in the catheter laboratory. It would be interesting, therefore to perform a similar study comparing the embolic load on patients undergoing BAS at the bedside versus the catheter laboratory.

Finally, analysis of the full Doppler spectrum has been shown to provide information of the cerebral hemodynamics beyond mere velocities. Analysis of the Doppler signal power has provided reliable measurements of relative changes in blood flow volume, which represents a first step towards a more accurate evaluation of the cerebral hemodynamics and autoregulation using Doppler technology. This method detects relative changes in flow volume, but does not provide absolute flow values.

It has also been shown, however, that such measurements are dependent on a stable probe position and constant haematocrit. The feasibility of such a method is therefore inherently dependent upon the quality of the Doppler signal. The development of new transducer probes which automatically and continuously search for the optimal, most powerful Doppler signal will therefore be important.

Potential clinical applications for this method include monitoring flow changes during surgical or catheter based manoeuvres or during the administration of pharmacological agents which cause peripheral resistance changes. This may be applied in both clinical (neurosurgery, cardiovascular surgery, patients with acute ischemic stroke) and research settings as a measure of the cerebrovascular reserve capacity.

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