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## Quantification of left-to-right shunt through Patent Ductus Arteriosus by colour Doppler

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Quantification of left-to-right shunt through  
Patent Ductus Arteriosus  
by colour Doppler

Clinical and experimental studies

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Akademisk avhandling

Som med vederbörligt tillstånd av Medicinska Fakulteten vid Skåne Universitet för avläggande av  
doktorsexamen i medicinsk vetenskap, kommer att offentlig försvaras i Segerfalksalen, WNC,

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Faculty of Medicine  
Lund University  
Sweden

# Quantification of the left-to-right shunt through Patent Ductus Arteriosus by colour Doppler

Experimental and clinical studies



**LUND**  
UNIVERSITY

Solweig Harling  
2011



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Clinical Sciences, Lund

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*“In physiological terms the measure of hemodynamic significance is the size of the shunt relative to the baseline cardiac output. Accurate measurement of this requires cardiac catheterisation and is clearly not practical or ethical in preterm infants. So which echicardiographic criteria have the best correlation with these invasive measurements?”*

*Nick Evans 1993*

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## ORIGINAL PUBLICATIONS

This thesis is based on the following papers

I. Harling S, Oskarsson G, Gudmundsson S, Pesonen E. Acute decrease of coronary flow after indomethacin delivery in the newborn lambs.

*Acta Paediatr.* 2007; 96:1460-63.

II. Harling S, Jansson T, Gudmundsson S, Pesonen E. Quantification of left to right shunt in patent ductus arteriosus by color Doppler. An experimental study in newborn lambs.

*Ultrasound Med & Biol.* 2009;35:403-8.

III. Harling S, Hansen-Pupp I, Baigi A, Pesonen E. Echocardiographic prediction of patent ductus arteriosus in need of therapeutic intervention.

*Acta Paediatr.* 2011; 100:231-5.

VI. Harling S, Jansson T, El-Segaier M, Pesonen E. Quantification of left to right shunt through patent ductus arteriosus by color Doppler in children admitted for a device closure.

*Cardiology in the Young*, in print.

V. Harling S, Hansen-Pupp I, Dumitrescu A, David Ley, Pesonen E. Increased levels of Interleukin-8 correlate in preterm infants to reduced systemic blood pressure and increased diameter of ductus arteriosus. *Submitted*

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In addition to the five publications included in this PhD thesis, the following paper and abstract have been published and presented during this PhD period:

Harling S, Jansson T, Pesonen E. Quantification of shunt through ductus arteriosus by colour Doppler. Published in the E-book “*Treatment Strategies/Echocardiography/Interventional Cardiology*” (<http://viewer.zmags.com/publication/80ebc97c#/80ebc97c/1>) June 2011

## SUMMARY

The aim of this thesis was to develop a non-invasive method to quantify the size of a shunt through a patent ductus arteriosus (PDA) by ultrasound and to test its usability in clinical settings. There is no consensus regarding the optimal management strategy for a PDA in premature infants. Non-steroidal anti-inflammatory drugs (NSAID) are the first treatment of choice. The use of NSAIDs, especially indomethacin, should be carefully balanced, as they have their disadvantages. In our experimental study in lambs, indomethacin acutely reduced the coronary flow by up to 50% and the effect lasted for up to one hour. In our lamb model, we developed a non-invasive method to quantify the ductal shunt by ultrasound. The flow was measured with electromagnetic flow meters in the ascending aorta and in the ductus and a colour Doppler image was obtained simultaneously over the main pulmonary artery longitudinal cross-section including ductal inflow. The percentage of colour pixels representing ductal flow was quantified in the main pulmonary artery outlined by anatomic landmarks. There was a correlation between the ratio of pulmonary to systemic flow ( $Q_p/Q_s$ ) and the percentage of total colours covering the cross-section and there was an even better correlation with green pixels alone. When the  $Q_p/Q_s$  was  $\geq 1.5:1$ , the percentage of green pixels in PALS was  $\geq 50$ . In children admitted for the device closure of the open ductus, the method had 92% sensitivity for a measured  $Q_p/Q_s$  of  $\geq 1.5$ . In preterm infants during the first three days of life, the ductal diameter but not the quantified ductal shunt predicted the need for treatment. We showed further that the perinatal cytokine burden during the first three days of life is not associated with an increased need to close the ductus, but it is associated with increased ductal diameter and reduced systolic blood pressure.

We suggest that our method could be used as a non-invasive tool to determine a haemodynamically significant ductal shunt. Using the evaluated  $Q_p/Q_s$  of  $> 1.5:1$  as a guide for treatment decisions might reduce the need for unnecessary interventions and reduce complications.

Key words: Colour Doppler, image analysis, ductal flow, patent ductus arteriosus, indications for closure

## ABBREVIATIONS

AUC	Area under the curve
APV	Average peak velocity
BPD	Bronchopulmonary dysplasia
CD	Colour Doppler
CO	Cardiac output
COX	Cyclo-oxygenase
DA	Ductus arteriosus
ECG	Electrocardiogram
ELBW	Extremely low birth weight
GA	Gestational age
IDGW	Intracoronary Doppler guide wire
IL	Interleukin
IVH	Intraventricular haemorrhage
LA/Ao	Ratio between left atrium and aorta
LAD	Left anterior descending coronary artery
MAP	Mean artery pressure
NEC	Necrotising enterocolitis
NO	Nitrogen oxide
NSAID	Non-steroidal anti-inflammatory drug
PALS	Pulmonary artery longitudinal cross-section
PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
PDA	Patent ductus arteriosus
Qp/Qs	Ratio of pulmonary to systemic flow
RDS	Respiratory distress syndrome
ROI	Region of interest
SGA	Small for gestational age
UCG	Echocardiography
VATS	Video-assisted thoracoscopic surgery
VEGF	Vascular endothelial growth factor
2-DE	Two dimensional echocardiography

## INTRODUCTION

The openness of the ductus arteriosus is a prerequisite for fetal life. The majority of the cardiac output from the right ventricle bypasses the lungs and supplies the body of the fetus. After birth, long-term openness of the ductus arteriosus could lead to neonatal morbidity. Doppler echocardiography has made it possible to detect a patent ductus arteriosus (PDA) and its response to treatment. However, determining the pathological significance of the PDA remains a problem. An echocardiography method to evaluate the size of shunts through a PDA could be of value in optimising the timing of interventions and preventing serious complications of a PDA and its treatment.

### Physical factors underlying closure of the ductus arteriosus

Fetal ductal patency is maintained by low blood oxygen tension, high circulation levels of prostanoids, especially prostaglandin (PGE<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>), and by nitrogen oxide (NO).<sup>1, 2</sup> The medial layer in the ductus arteriosus (DA) is composed of longitudinal and spiral layers of smooth muscle cells within concentric layers of elastic tissue. Intimal cushions are formed in the intimal layer of the DA in the second trimester, accompanied by the separation of endothelial cells from the internal elastic lamina and the migration of smooth muscle cells from the arterial media into the subendothelial space.<sup>3</sup> During the last trimester, the duct becomes more muscular and the smooth muscle cells become less sensitive to the dilating PGE<sub>2</sub> and more sensitive to the vasoconstricting effect of oxygen. The duct normally constricts shortly after birth, due to the postnatal drop in circulating PGE<sub>2</sub> levels, as well as the rise in systemic oxygen tension, which induce an increase in the potent vasoconstrictor endothelin-1 in ductal smooth muscle cells.<sup>4</sup>

Oxygen-sensing mechanisms in the DA smooth muscle cells cause cell-membrane depolarisation, which allows for calcium influx and concentration. Developmentally regulated potassium channels allow voltage-gated calcium channels to open and increase calcium influx.<sup>5</sup> The immaturity of potassium and calcium channels leads to ineffective oxygen-mediated constriction in the preterm rabbit DA.<sup>5, 6</sup> Endothelin 1 acts to increase intracellular calcium through G-protein coupling. Reduced intraluminal blood pressure in the DA, due to vasoconstriction, contributes to ductal closure with the development of a hypoxic zone and induces vascular endothelial growth factor (VEGF) expression or cell death, depending on the severity of hypoxia.<sup>7</sup> VEGF plays an important role in the formation of neointimal mounds and vasa vasorum ingrowths during permanent ductus closure.<sup>8</sup> Platelets appear to be crucial for PDA closure by promoting the thrombotic sealing of the constricted DA and by supporting luminal remodelling.<sup>9</sup> Closure occurs in two stages: *functional closure* as a consequence of smooth muscle contraction and *anatomic closure* contributed to by ischemia due to transductal flow decrease, when the wall becomes progressively more ischemic and eventually fibrotic.<sup>8, 10</sup> The remodelling effects start at the pulmonary end of the ductus and progresses towards the aortic end.<sup>11</sup>

### Patency of the ductus arteriosus

#### *Preterm infants*

The immature ductus has been shown in vitro to have less intrinsic tone and to lack both intimal folds and circumferential medial musculature.<sup>12, 13</sup> It is less responsive to oxygen and more sensitive to PGE<sub>2</sub> and NO and endothelin 1 is increased.<sup>2, 12, 14</sup> It is possible for the immature infant to develop ischemia of the medial muscle, but only if transluminal flow is

completely obliterated. Failure to generate the hypoxic zone by insufficient constriction prevents true anatomic DA closure. This makes it possible for the DA to re-open.<sup>15</sup>

Prostaglandins and nitrogen oxide both play a role in inhibiting ductus closure in vitro. PGE<sub>2</sub> acts through G-protein-coupled receptors that activate adenylyl cyclase and produce cyclic adenosine monophosphate (cAMP) to relax the vascular smooth muscle layers.

Concentrations of cAMP also depend on phosphodiesterase-mediated degradation. Likewise, NO activates guanylyl cyclase to produce cyclic guanosine monophosphate (cGMP). More immature animals have less ability to degrade cAMP or cGMP and thereby more sensitivity to PGE<sub>2</sub> and NO.<sup>16</sup> The co-administration of a nitrogen oxide synthetase (NOS) inhibitor (N-nitro-L-arginine (L-NA) with a cyclo-oxygenase (COX) inhibitor leads to increased contractility and luminal obliteration of the preterm ductus arteriosus in baboons.<sup>17</sup>

A progressive increase in nitrogen oxide production in the ductal wall after birth makes the preterm ductus less sensitive to prostaglandins, which may play a role in the decreasing effectiveness of COX inhibitors with increasing postnatal age.<sup>12, 18</sup> In preterm infants with a lung disease, the lower clearance of circulating dilatory PGE<sub>2</sub> in the lungs may contribute to a higher incidence of PDA. The administration of cortisol to immature fetal lambs in utero has resulted in a ductus that responds to oxygen and cyclo-oxygenase (COX) inhibitors similar to that in a mature fetus, which explains the decreased incidence of PDA in preterm infants who are born to mothers treated with antenatal corticosteroids.<sup>19, 20</sup>

The late re-opening of the ductus arteriosus is common in association with neonatal infections. Possible explanations include the fact that infection-associated inflammatory mediators such as tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) increase prostaglandin levels and reactive oxygen intermediates along with other inflammatory mediators, thus favouring persistent ductal patency or re-opening.<sup>21-23</sup> It is possible that other proinflammatory cytokines affect platelet function and thereby inhibit thrombotic sealing of the constricted DA.<sup>9</sup>

#### *Full-term infants*

A patent ductus in full-term infants is abnormal and is related to significant structural abnormalities. Histologically, the internal elastic lamina of the duct is intact and the internal cushions are absent or less well formed in these ductuses.<sup>24</sup> In the majority of cases, there is no identifiable cause, representing the influence of multifactor inheritance.<sup>25</sup> The existence of an open duct is a prerequisite for life in children with ductus-dependent cardiac anomalies.

### **Incidence of patent ductus arteriosus**

#### *Newborns*

A patent ductus arteriosus (PDA) is a congenital heart abnormality defined as a persistent patency in term infants older than three months.<sup>26</sup> The ductus is functionally closed in > 90% of healthy term babies after three days.<sup>27</sup> Isolated PDA is found in around 1 in 2,000 full-term infants.<sup>28</sup> In preterm infants and especially in extremely low birth weight infants (ELWB), every third preterm infant with a birth weight of 500 to 1,500 g can be expected to have a PDA.<sup>29</sup> About fifty to sixty per cent of infants who weigh < 1,000 g have a symptomatic PDA that leads to medical treatment.<sup>30</sup> In these infants, failure of the PDA to close is due to the incomplete development of ductal tissue, its increased sensitivity to vasodilating prostaglandins and increased concentrations of circulating prostaglandins.<sup>1, 12</sup> The absence of major lung disease predicts spontaneous closure in much the same time frame as in term infants, but it is dependent on gestational maturity.<sup>31</sup> A PDA may cause respiratory and heart failure, intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD), necrotising

enterocolitis (NEC) and death.<sup>32-34</sup> The treatment for a PDA with a symptomatic shunt is medical or surgical therapy.

### *Children and adults*

After the neonatal period, a heart murmur, with or without other clinical signs, leads to the diagnosis of PDA. It accounts for approximately 5-10% of all types of congenital heart disease.<sup>35</sup> Unlike the ductus arteriosus in premature infants, in whom failure to close is due to physiological developmental retardation, a PDA in full-term infants is abnormal, related to significant structural abnormality.<sup>24</sup> A PDA is associated with chromosomal aberrations, specific genetic defects (trisomy 21 and 18, Rubenstein-Taybi and CHARGE syndrome, birth at high altitude and congenital rubella). Studies have revealed a recurrence rate of between 1-5% among siblings with a PDA.<sup>36</sup>

## **Physiological changes after birth**

### *Foramen ovale*

In the fetus, the right ventricle takes care of the main part of the total cardiac output. A small part of the blood from the right side goes through the foramen ovale and less than 5% through the lungs to the left atrium in lambs.<sup>37</sup> After birth, pulmonary vascular resistance falls, resulting in a ten-fold increase in pulmonary blood flow. Thereafter, the left atrial pressure exceeds the pressure in the right atrium and the redundant flap of tissue of the foramen ovale that previously bowed towards the left atrium is pressed against the septum, leading to the closure of the foramen ovale. However, it remains physiologically open in 30% of people.<sup>38</sup>

### *Ductus arteriosus*

During intrauterine life, the output from the right ventricle goes mainly through the main pulmonary artery via the ductus arteriosus to the aorta. The separation of a low-resistance placental circulation after birth leads to an increase in the systemic vascular resistance and the reversal of ductal flow. After birth, the ductus arteriosus is exposed to a sudden increase in arterial oxygen tension and a reduction in circulating prostaglandins, resulting in the constriction of the vessel.

### *Pulmonary circulation*

During intrauterine life, the lungs are compressed. At birth, the lungs expand and improved oxygenation leads to a decrease in pulmonary vascular resistance. After the initial rapid fall in pulmonary vascular resistance and pulmonary arterial blood pressure, there is a slow, progressive fall, with adult levels reached after 2 to 6 weeks.<sup>39</sup> This is due to vascular remodelling. Resistance is further lowered by several vasoactive substances, such as acetylcholine, bradykinin and prostacyclin (PGI<sub>2</sub>).<sup>40, 41</sup> Pulmonary vasoconstriction may be caused by hypoxemia, acidosis, increased production of thromboxane and vasoconstricting leukotrienes.<sup>40, 42, 43</sup> Meconium aspiration leads to pulmonary hypertension.

## **Pathophysiological consequences of patent ductus arteriosus**

### *Preterm infants*

In preterm infants, the consequences may include pulmonary over-circulation and/or systemic hypo-perfusion. The clinical impact is dependent on the magnitude of the shunt determined by the ratio of systemic pressure to pulmonary vascular resistance, the size of the duct and the ability of the infant to initiate compensatory mechanisms. The predominant direction of left-to-right flow leads to pulmonary oedema, whereas right-to-left flow is associated with hypoxemia.

Pulmonary over-circulation and increased lung interstitial fluid secondary to the large system-pulmonary ductal shunt contribute to decreased lung compliance and pulmonary haemorrhage.<sup>45</sup> The increased capillary penetration of serum proteins to lung tissue leads to the inactivation of surfactant and increases the risk of respiratory distress syndrome of the newborn.<sup>46</sup> The cumulative effects of increasing or prolonged ventilator requirements (secondary to hypoxemia, hypercapnia or abnormal pulmonary resistance) and myocardial dysfunction may increase the risk of chronic lung disease (CLD).<sup>47-49</sup>

Systemic hypo-perfusion occurs with large ductal shunts where over 50% of flow can go backwards up the aorta, resulting in the relative under-perfusion of all of the systemic arteries, such as renal, cerebral or mesenteric arteries. The distribution of systemic flow is significantly altered even with small volume shunts. One of the characteristics of a PDA is a diastolic backward flow from the descending aorta to the ductus and further to the lungs. There may be significant hypo-perfusion to the brain, kidneys and gastrointestinal tract even before a haemodynamically significant ductus is clinically suspected. This may lead to significant morbidity.<sup>50-54</sup> Absent or retrograde diastolic cerebral blood flow is said to be present in babies requiring ductal ligation and rare in babies without a ductus.<sup>55</sup>

Left ventricular failure in preterm infants may develop as early as the second or third postnatal day. Extremely low birth weight infants are less capable of compensating for the haemodynamic instability and are prone to develop left ventricular failure. This may lead to low cardiac output syndrome (delayed capillary refill, oliguria, hypotension, lactic acidosis) and/or alveolar oedema.<sup>53</sup> The immature myocardium contains fewer contractile elements per unit weight and the left ventricle is less compliant in preterm than in term infants.<sup>56, 57</sup> Preterm infants with a symptomatic PDA may frequently have ST-segment depression on an electrocardiogram (ECG), suggestive of subendocardial ischemia that normalises after the surgical closure of PDA.<sup>58</sup> This is a result of aortic run-off to low-resistance pulmonary circulation, which reduces the diastolic pressure and flow to the coronary circulation in particular. Coronary blood flow is almost entirely diastolic. The decreasing pressure gradient between the coronary ostia and the endocardium may jeopardise the myocardial oxygen supply.

### *Term infants*

In mature infants and older children, symptoms of PDA are related to the same factors as in premature infants. Symptomatic children are rare in the developed countries, due to the early detection and treatment of PDA. In countries with limited health resources, the pathophysiological consequences of PDA remain a significant health issue.<sup>59</sup>

**Heart failure** frequently develops due to pulmonary over-circulation and left heart volume overload in children with a moderate to large PDA. Increased flow returning to the left heart results in increased left atrial mean pressure and left ventricular end-diastolic pressure. Neuroendocrine adaptation occurs with increased sympathetic nerve activity. Circulating catecholamine concentrations increase and this results in increased contractility and heart rate. The diastolic blood pressure is decreased as a result of diastolic “run-off” through the patent ductus. The diastolic time is shorter due to tachycardia, while intramyocardial tension is increased due to left ventricle dilatation. These factors and increased sympathetic nervous tone increase myocardial oxygen demand, which may result in subendocardial ischemia.<sup>60</sup> In children with a moderate to large PDA, pulmonary vascular resistance remains modestly elevated, which limits the shunting sufficiently to alleviate its physiological impact and permits survival and growth.<sup>59</sup> Those with significant chronic volume overload of the left heart may develop congestive heart failure in adulthood, starting in the third decade.<sup>41</sup>

**Pulmonary hypertension** due to a large shunt through the PDA leads to unfavourable vascular remodelling. Endothelial cell dysfunction, wall stretch and imbalance in vasoactive mediators promote vasoconstriction, inflammation, cell proliferation and fibrosis. Patients with a large, non-restrictive patent ductus may develop irreversible pulmonary vascular disease.<sup>61</sup> Ductal closure in the first 2 years of life prevents the development of irreversible vascular damage.

**Endocarditis** was a fatal illness in the pre-antibiotic era. In the last 30-40 years, the incidence of this illness has declined and it is now almost non-existent. Of nearly three million deaths in Sweden during the period 1963-1993, two cases were due to infective endocarditis as a complication of PDA. In both cases, a large shunt was present.<sup>62</sup> This is very rare considering that the incidence of a silent ductus in children (incidentally discovered by echocardiography performed for another purpose) is 1 in 500.<sup>63</sup> Factors such as the early detection of large PDAs, the antibiotic treatment of common diseases, changes in socio-economic circumstances and dental health might explain the reduced incidence of endocarditis as a complication of PDA.<sup>59</sup>

### **Identification of a haemodynamically significant ductus arteriosus**

#### *Clinical presentation*

Preterm infants with a clinically significant shunt have a murmur which, due to high pulmonary vascular resistance, is mainly systolic but can be continuous.<sup>64</sup> The precordial activity is increased and the peripheral pulses bound. The systemic diastolic pressure is low and pulse pressure is widened. There is tachycardia and, in spontaneously breathing infants, there is tachypnea, intercostal and subcostal retractions and frequent episodes of apnea. The arterial pCO<sub>2</sub> level is often increased and the infant may require a higher concentration of ambient oxygen to maintain adequate oxygenation.<sup>65</sup> Clinical signs of a symptomatic ductus usually develop with declining pulmonary vascular resistance during the second half of the first postnatal week, or occasionally during the second or third weeks of life. Preterm infants appear to experience a more rapid decline in pulmonary vascular resistance due to a less well developed pulmonary vascular smooth muscle cell layer, which leads to the earlier development of a symptomatic shunt through the PDA.<sup>66, 67</sup>

Silent ductus in the first week of life is common. The existence of a haemodynamically significant yet “silent ductus” has been confirmed by cardiac catheterisations and echocardiography studies.<sup>68, 69</sup> By assisting the natural postnatal fall in pulmonary artery pressure, surfactant has been shown to alter the timing of clinical presentation by increasing the volume of the systemo-pulmonary shunt.<sup>70</sup> An effect of a PDA, such as reduced systemic blood flow, might appear in the first 12 h, leading to increased morbidity and intraventricular haemorrhage in particular.<sup>71</sup> A symptomatic ductus arteriosus should be suspected in a setting of delayed hypotension (days 2–3), oxygenation failure, increasing ventilation requirements or metabolic acidosis. An ELBW infant is more likely to present with both systolic and diastolic hypotension due to the inability of the immature myocardium to compensate for high volume shunting throughout the cardiac cycle.<sup>72</sup>

Full-term infants with a small duct are asymptomatic with normal physical findings. In children with a moderate to large duct, the patency of the arterial duct is recognised by a continuous murmur, located at the upper left sternal border, often referred to as “machinery” murmur. Large shunts may lead to failure to thrive, recurrent infection of the upper respiratory tract, pulmonary hypertension, bacterial endocarditis and heart failure, even if patients of this kind are extremely rare in the developed countries.



### *Echocardiography findings*

Echocardiography is the gold standard diagnostic method for a PDA.<sup>73</sup> It is a bedside, non-invasive procedure with minimal risks to the patient.<sup>74</sup> By using Doppler information, it has been possible to determine the time of ductal closure in newborn infants and its response to COX inhibitors.<sup>75, 76</sup> Echocardiography findings may suggest a haemodynamically significant flow 1-2 days before the physical signs develop in preterm infants.<sup>77</sup>

The ductal diameter can be measured on a standard two-dimensional echocardiography (2-DE) view and assessments of ductal blood flow using pulsed-wave Doppler and colour flow mapping. A shunt through the ductus arteriosus of haemodynamic importance leads to secondary changes, such as the enlargement of left heart chambers.

The following echocardiographic and Doppler criteria are used to confirm a symptomatic patent ductus arteriosus: *ductus diameter* and *transductal flow patterns*, which can be obtained by using a pulsed-wave Doppler from a high left parasternal short-axis view, *retrograde flow in the descending aorta* seen from a suprasternal view, volume loading of the heart by measuring the ratio of the diameter of the left atrium and aorta ascendens (*LA/Ao ratio*) in a parasternal long-axis view, *diastolic flow in pulmonary branches* from a high left parasternal short-axis view, *flow pattern in the superior vena cava* obtained from a subcostal view and *flow in arteria cerebri media* measured by ultrasound of the head through an open anterior fontanelle.

### *Biomarkers for PDA*

Elevated plasma B-type natriuretic peptide (BNP) or NT-pro-BNP levels are used as biomarkers of heart failure and congenital heart disease in infants and children.<sup>78, 79</sup> Their elevated concentrations may indicate a “symptomatic” PDA and guide its treatment.<sup>80</sup> Cardiac troponin T (cTnT) levels are higher in preterm infants (<32 weeks gestation) with a PDA who subsequently develop IVH grade III/IV or death, compared with those with a PDA without complications.<sup>78</sup>

## **Quantification of a left-to-right shunt through the ductus arteriosus**

### *Echocardiography*

Right and left ventricular output can be calculated from the 2-DE and Doppler echocardiograms using the following equations:

$$SV = \frac{V \times CSA}{1000} \quad \text{mL/L}$$

$$CO = SV \times HR$$

(SV= stroke volume (mL/beat), V = mean velocity (cm/s), CSA= cross-sectional area of flow (cm<sup>2</sup>) in the pulmonary artery or aorta, CO = cardiac output (mL/minute), HR = heart rate (beat/minute))

The pulmonary artery mean velocity and diameter are used to calculate pulmonary blood flow, while the mean velocity and diameter of the ascending aorta are used to calculate systemic blood flow.

### *Cardiac catheterisation*

The quantification of a left-to-right shunt through the ductus arteriosus is usually performed oximetrically with blood samples taken from the vena cava superior, main pulmonary artery and a systemic artery during cardiac catheterisation according to Fick's principle (Qp/Qs):

$$\text{Pulmonary flow (Qp)} = \frac{\text{VO}_2}{\text{C}_{\text{PV}} - \text{C}_{\text{PA}}}$$

$$\text{Systemic flow (Qs)} = \frac{\text{VO}_2}{\text{C}_{\text{AO}} - \text{C}_{\text{MV}}}$$

(VO<sub>2</sub> = oxygen consumption, C = oxygen content, PV = pulmonary vein, PA pulmonary artery, AO = aorta, MV = mixed systemic venous blood (superior vena cava))

In the event of a PDA, measurements of mixed venous and arterial blood samples from the pulmonary artery are problematic. The sample should not be taken directly from the ductal jet.

### *Magnetic resonance imaging*

A set of coronal spin-echo images is used to localise the ascending aorta and pulmonary trunk. Flow is calculated as a product of the area of the great vessels and the net mean velocity within it. The mean flow rate over the cardiac cycle is calculated over the mean R-R interval, determined from the image software for the calculation of blood volume per heart cycle to assess left and right ventricular stroke volume.

### *Radionuclide scanning*

Technetium-99m is injected via a cannula into a peripheral vein. Data analyses assume exponential indicator clearance from normal cardiac chambers by dilatation. The late prolongation of tracer disappearance compared with the initial clearance rate indicates an abnormally early return of the indicator to the cardiac chamber. Patients with left-to-right shunts demonstrate prolonged clearance of radioactivity from all cardiac chambers distal to the site of the shunt. The magnitude of curve distortion is quantitatively related to the size of the shunt and counts recorded from the right lung are used for shunt quantisation.

## **Management of patent ductus arteriosus**

In preterm infants, the goals of the treatment are to reduce pulmonary over-circulation and subsequent left ventricle failure and to improve systemic and/or end-organ perfusion. Sixty to seventy per cent of preterm infants of < 28 weeks GA receive medical or surgical therapy for a PDA.<sup>14</sup> COX inhibitors blocking the prostaglandin synthesis, such as indomethacin or ibuprofen, remain as the first treatment of choice. The timing of the intervention and the dosage of pharmacological treatment remain an unresolved issue. COX inhibitors are less effective in severely preterm infants; a fact that was suggested as a result of the failure of intimal cushion formation and NO-mediated fibronectin synthesis.<sup>81-83</sup> No statistical difference has been shown in effectiveness between ibuprofen and indomethacin.<sup>84</sup> Early surgical intervention is considered if the PDA remains large despite medical treatment, in infants with renal impairment, gastrointestinal sickness, platelet dysfunction or progressive cardiorespiratory deterioration. Whenever possible, treatment should not be attempted without a prior echocardiography evaluation to exclude duct-dependent cardiac lesions.

### *Cyclo-oxygenase inhibitors*

Indomethacin, first produced in 1976, has long been the drug of choice.<sup>81, 85</sup> Although indomethacin results in ductal closure in the majority of cases, it is ineffective in up to 40-50% of patients.<sup>86</sup> In addition, in up to 35% of the infants who initially respond to the drug,

the ductus will re-open.<sup>87</sup>

Ibuprofen has been introduced as an alternative to indomethacin because it has fewer side-effects.<sup>84</sup> Meta-analysis suggests that ibuprofen may be as effective as indomethacin in closing a PDA.<sup>88</sup> Oral ibuprofen administration appears to have similar efficacy compared with intravenous indomethacin administration. However, a case report of spontaneous gastrointestinal perforation after oral ibuprofen highlights the need for larger studies before this treatment regimen can be recommended.<sup>89</sup>

Adverse effects of COX inhibitors, such as renal failure/oliguria, appear to be more frequent with indomethacin than ibuprofen (19% vs. 7%), although they are reversible.<sup>88</sup> Indomethacin in conjunction with postnatal corticosteroids may increase the risk of intestinal perforation.<sup>90</sup> It has been reported that prophylactic ibuprofen is associated with severe pulmonary hypertension.<sup>91</sup> There is also potentially a higher risk of kernicterus, as ibuprofen interferes with the binding of serum bilirubin to albumin at the usual dose of the drug.<sup>92</sup>

In the case of COX inhibitors, three approaches are used, where the most aggressive is to give indomethacin to all high-risk babies prophylactically to be started in the first 24 h of life. The least aggressive approach is to treat only when the duct becomes clinically apparent. Between these two approaches, there are a variety of strategies for targeting treatment in the pre-symptomatic period, such as three bolus injections (0.2 + 0.1 + 0.1 mg/kg in a 12 h interval) or a 36-h infusion (0.4 mg/kg) with indomethacin, or, as an alternative, bolus injections of ibuprofen (10 mg/kg followed 24 and 48 hours later by a dose of 5 mg/kg).<sup>93-95</sup> The optimal time for treatment is the first weeks of life. In the treatment decision, the spontaneous closure rate of about 60% must be considered.<sup>96</sup> Therapeutic closure is not recommended in a setting of suprasystemic pulmonary hypertension or right heart failure, as a patent ductus helps unload the stressed right ventricle and may support pulmonary perfusion.

### *Surgery*

Surgical ligation is normally indicated after the failure of medical therapy or if therapy with COX inhibitors is contraindicated.<sup>97</sup> The surgical ligation of the ductus in preterm neonates is usually successful, with a minimal complication rate, but there may be significant morbidity, including bleeding, pneumothorax, chylothorax, infection, haemodynamic instability and thoracic scoliosis.<sup>98-100</sup> Infants with a lower gestational age and birth weight are more likely to be treated surgically. The procedure is most commonly performed using a lateral subcostal approach. Video-assisted thoracoscopic surgery (VATS), as a means of managing a patent ductus, has been successfully performed. Endoscopic instruments through a 3 mm incision in the chest wall are used to approach the ductus.<sup>101</sup> The increased number of surviving ELBW infants has led to a larger number of infants requiring surgical intervention. In preterm infants weighing less than 800 grams, ligation may be preferred to COX inhibitors.<sup>98</sup> The prophylactic surgical ligation of the duct is reported to reduce the risk of severe NEC but not other major complications in preterm infants. The lack of significant benefit and growing data suggesting the potential harm of such treatment do not support the early surgical ligation of a PDA in the management of preterm infants.<sup>102</sup>

### *Device closure*

In children outside the neonatal period, the treatment of choice is device occlusion under interventional heart catheterisation, which is usually offered to all children with a PDA of haemodynamic importance.

Transcatheter closure can be performed safely in infants who have a body weight of 6 kg or more, even if it has been performed in young infants with lower body weights.<sup>103, 104</sup> The immediate occlusion rates are in excess of 90-95% and complication rates are low.<sup>105</sup> At most

centres, PDAs are closed with a device if the shunt does not look too small in colour Doppler evaluations and a murmur can be heard. The indications for closing the ductus are unclear, even if they are based on the prevention of heart failure, pulmonary hypertension and bacterial endocarditis. The risk of endocarditis is almost non-existent, although studies have reported an increase in incidence in these patients, which has led to some centres offering elective closure of the PDA to all patients.<sup>106, 107</sup> Surgical ligation remains an option for those unsuitable for transcatheter closure. This is rare, but it might be necessary in treating very large ducts.

### **Interaction of cytokines on the systemic and lung circulation**

Interleukins (IL) are a subset of a larger group of cellular messenger molecules which modulate cellular behaviour. They were first seen to be expressed by white blood cells. The first interleukins were identified in the 1970s. There are currently 35 well-known interleukins, but many more are still to be found and characterised. They promote the development and differentiation of T and B lymphocytes and haematopoietic cells and trigger a cascade of signals within the target cell that ultimately changes the behaviour of the cell. This can cause cellular proliferation, cell activation, inflammation, physiological changes, such as a reduction in blood pressure, fever and pain, such as allergies.<sup>108-110</sup>

#### *Interaction of cytokines on the systemic and pulmonary lung circulation*

There is increasing experimental and clinical evidence to suggest that a number of cytokines play a major role in the response to injury and infection and in the development of organ damage in critically ill patients. There are several studies indicating that cytokines are potent vasodilators. IL-6 and TNF- $\alpha$  plasma levels are increased in adult patients with a hyperdynamic circulation with tachycardia, mild hypotension, increased cardiac index, peripheral vasodilatation and myocardial depression.<sup>111</sup> Increased levels of IL-6 and IL-8 are associated with a decrease in mean arterial blood pressure and are good predictors of treatment for arterial hypotension in preterm infants.<sup>112</sup>

Inflammatory cytokines cause endothelial dysfunction, a hallmark of pulmonary hypertension. In pulmonary hypertension, there is reduced availability of vasodilators and antiproliferative factors and increased production of vasoconstrictors and vascular proliferative factors. The up-regulation of inflammatory cytokines and perivascular inflammatory cell infiltration have been detected in the lungs of patients with idiopathic pulmonary hypertension.<sup>113</sup> Pulmonary oedema, pulmonary hypertension and increased pulmonary lymph flow have been noted after the administration of IL-2 over 72 h in sheep.<sup>114</sup> TNF- $\alpha$  and other cytokines have been identified in the tracheal lavage fluid of infants and may contribute to the neonatal respiratory distress syndrome.<sup>115</sup>

## **AIMS OF THE PRESENT STUDY**

The aim of this study was to

Assess the haemodynamics of patients with a PDA and to determine whether transthoracic Doppler techniques can be used to quantify the size of the shunt via a persistent ductus arteriosus from pixel counts in colour Doppler flow images and to test the clinical applicability of the method.

### **Specific aims**

- ◆ To evaluate the effect of cyclo-oxygenase inhibitors (indomethacin) on the coronary circulation (Study I)
- ◆ To establish a non-invasive method for the evaluation of ductal shunt size by colour Doppler by measuring pixel counts in colour Doppler flow images in an experimental setting (Study II)
- ◆ To determine the predictive value of this method for preterm infants (Study III)
- ◆ To apply the method to patients admitted for the device closure of a PDA (Study IV)
- ◆ To evaluate the effects interleukin-6 and interleukin-8 have on the ductus arteriosus and ductal flow (Study V)

## **MATERIAL AND METHODS**

### **Subjects**

#### ***Experimental studies***

##### ***Effects of indomethacin on coronary flow in lambs (Paper I)***

Nine newborn lambs of mixed breed and gender were studied during their first day of life. Their gestational age varied between 132 and 134 days (term 145 days) and they weighed between 3- 4.7 kg.

##### ***Quantification of shunt through PDA by colour Doppler in lambs (Paper II)***

Four newborn lambs of mixed breed and gender were studied during their first day of life. Their gestational age was between 133-135 days (term 145) and they weighed between 3-5 kg.

#### ***Clinical studies***

##### ***Prediction of symptomatic PDA in preterm infants by echocardiography (Paper III)***

Forty-five infants with a mean gestational age of 27.7 weeks ( $SD \pm 1.9$ ) and a mean birth weight of 1,012 grams ( $SD \pm 302$ ) were studied. Echocardiography examinations were performed at  $24 \pm 6$  and  $72 \pm 6$  hours of age. Twenty-eight of the infants with a mean gestational age of 26 weeks ( $SD \pm 1.4$ ) and a mean birth weight of 895 grams ( $SD \pm 107$ ) had a PDA with a left-to-right shunt. Infants in need of ductal closure ( $n = 12$ ) had a mean gestational age of 25.7 weeks ( $SD \pm 1.4$ ) and a mean birth weight of 871 grams ( $SD \pm 107$ ). Infants treated surgically had a mean gestational age of 25 weeks ( $SD \pm 1$ ) and a mean birth weight of 815 grams ( $SD \pm 98$ ).

##### ***Quantification of shunt through PDA by color Doppler in children (Paper IV)***

Of children scheduled for the device closure of a PDA between 1998 and 2007 in Lund, 20 infants fulfilled the image criteria for colour pixel measurements. The size of their ductal shunt varied from 1:1 to 2.3:1. The median (range) age of the included children was 2 years (0, 6-15) and the median (range) weight was 12 kilos (7-48).

##### ***The effect cytokines (IL-6, IL-8) have on ductal diameter, systolic blood pressure, pulmonary resistance and the treatment of PDA in preterm infants (Paper V)***

The patients were the same as in the Paper III.

## Methods

### *Experimental studies*

#### *Animal models*

The lambs included in the studies were delivered by caesarean section. The pregnant ewes were premedicated with 6-8 mg of xylazine i.m. Intravenous sedation and anaesthesia with 35 mg of ketamine and 650-800 mg of thiopental were used. The trachea was intubated and anaesthesia was maintained with isoflurane in nitrous oxide/oxygen. The lungs were ventilated with a Servo Ventilator, keeping the end-tidal  $p\text{CO}_2$  at 4.5-6 kPa. Fluid balance was maintained by infusing a balanced glucose/salt solution. Arterial pressure was monitored via an arterial cannula. Systolic blood pressure was maintained between 90 and 110 mmHg by adjusting the isoflurane concentration and infusing Ringer's acetate as necessary. The abdominal wall and uterus of the ewes were opened and the head and neck of the lamb were exteriorised. A 3.5 or 4 mm inner diameter tracheal tube was inserted through an incision in the trachea. Air leaks were prevented by securing the tube. Catheters were inserted in the right jugular vein and right carotid artery. The lamb was then exteriorised and 8 mg of ketamine and 0.4 mg of pancuronium were given i.v. immediately after the umbilical cord was cut.

The lambs were weighed, dried with towels, placed in an open incubator and covered with thin plastic sheets to reduce evaporative heat loss. The oesophageal temperature was kept at 38-39°C with radiant heat lamps as needed. The tracheal tube was connected to a Servo Ventilator (model 900C; Siemens-Elcoma, Solna, Sweden) in the pressure control mode. The initial ventilator settings were inspiratory pressure 29 cm  $\text{H}_2\text{O}$  with 4 cm  $\text{H}_2\text{O}$  PEEP and ventilator rate 50/min. The inspiratory time was 50% of the cycle and the fraction of inspired  $\text{O}_2$  ( $\text{Fi O}_2$ ) was 0.5. The ventilator settings were subsequently adjusted to maintain  $\text{PaO}_2$  at 6-8 kPa and  $\text{PaCO}_2$  at 5-6 kPa.

A catheter was placed in the umbilical artery. The tip position in the lower abdominal aorta was confirmed with fluoroscopy. Systemic arterial blood pressure, as well as pulmonary artery pressure, was monitored continuously. Blood from the ewe, 10 mL/kg, was given if the mean arterial pressure was less than 40 mmHg, but, if Hb exceeded 150 g/L, Ringer's acetate was used instead. Blood was likewise given if Hb was less than 130 g/L. One mmol/kg of sodium bicarbonate was given if the pH was less than 7.25 and the base deficit more than 5 mmol/L. Sedation and analgesia after delivery were maintained with 1 mg/mL of ketamine in 5% glucose with an infusion rate of 4 mL/kg/h and 10 mg/kg/h of fentanyl after an initial bolus dose of 20 mg/kg. To maintain paralysis, pancuronium was administered i.v. as needed. The lamb was allowed to stabilise for at least 2 hours after birth before further preparations.

A left lateral thoracotomy was performed in the fourth intercostal space. The left lung was retracted and the pericardium opened. A pre-calibrated ultrasonic blood flow transducer connected to a Transonic T101 flow meter was applied around the ascending aorta and the ductus arteriosus to measure cardiac output (Papers I and II) and ductal flow (Paper II). Subcutaneous electrodes were sutured to the chest wall for continuous ECG monitoring and a pulse oximeter probe was placed on the tail for the continuous monitoring of  $\text{O}_2$  saturation ( $\text{Sp O}_2$ ) during the whole experiment. Haemodynamic stabilisation for 30 to 60 minutes was allowed before starting measurements of coronary flow velocity (Paper I), ductal flow and ultrasound registrations (Paper II).

#### *Coronary flow velocity (Paper I)*

In sedated newborn lambs, a 4F right coronary angiography catheter (Judkins) was advanced through the introducer in the right carotid artery to the aortic root. A selective left coronary angiography was performed by the contrast injection of Omnipaque 240. To measure

coronary flow velocities, an Intracoronary Doppler Guide Wire (IDGW), 0.014 inch (0.36 mm), (Flowire, Cardiometrics, Inc, Mountain View, California, USA) was advanced through the coronary catheter into the proximal left anterior descending coronary artery (LAD) and its position was confirmed by fluoroscopy. The position was kept constant by securing the probe tightly within the coronary catheter and the position was confirmed by repeated fluoroscopy.

A Doppler signal was acquired with a 15-MHz piezoelectric ultrasound transducer at the end of the guide wire. The transducer permitted velocity acquisition with a pulse repetition frequency of up to 90 kHz from a sampling depth of 5 mm. The forward-directed ultrasound beam with a 25-degree divergent angle sampled the coronary flow profile. A high-quality signal was obtained by torque adjustment with reference to the amplitude display. Continuous flow profiles with simultaneous ECG were registered from the LAD and recorded on a video cassette. Doppler flow velocity spectra were analysed on-line for average peak velocity (APV), where APV was the time average value of the instantaneous peak velocities over two cardiac cycles. Diastolic peak flow velocity and systolic peak flow velocities were measured off-line and were averaged over three cardiac cycles.

A single bolus dose of 0.2 mg/kg of indomethacin (0.4 mL/kg) was given i.v. over 1 minute. The coronary flow velocity was registered continuously until the flow returned to its preinjection level. Recovery time was defined as the time between the lowest APV after indomethacin injection until the APV had returned to the pre-injection level. Arterial pressure was measured with pressure transducers, using the midchest level as the zero reference. Heart rate was obtained via continuous ECG monitoring. Blood gas tensions, pH and Hb were measured with a Radiometer OSM 3 blood gas analyser (Radiometer, Copenhagen). SpO<sub>2</sub> was additionally monitored continuously by a pulse oximeter.

#### *Ductal flow velocity (Paper II)*

In sedated newborn lambs, a cotton band was placed around the ductus arteriosus for adjustments of ductal flow and the compartment around the heart was filled with an ultrasound gel with a temperature of 38 degrees. An Acuson Sequoia scanner equipped with a 7 MHz transducer and a colour Doppler program was placed directly on the right ventricle to detect the pulmonary artery. An image was obtained over the pulmonary vessel, including the pulmonary valve and the bifurcation. At the same time, an ECG was recorded. Images were recorded and saved on a magnetic optic-disc. The ductal shunt was changed by different degrees of strangulation of the ductus arteriosus by the cotton band. Colour clips were obtained from ductal shunts of 24 different sizes measured by an electromagnetic-flow probe placed around the ductus (0-390 mL/min).

#### Clinical studies

The shunt through the ductus arteriosus was quantified by counting the percentages of total colour and green pixel density in diastole in pulmonary artery longitudinal cross-sections in an ultrasound colour Doppler image. Images registered from infants born before 32 weeks of gestational age (Papers III and V) and from children scheduled for the device closure of a PDA (Paper IV) were analysed. The correlation of the obtained values with the in-heart catheterisation measured ratio of pulmonary to systemic flow was tested (Paper IV). In Paper III, the predictive value of echocardiography parameters for a clinically significant ductus, including an analysis of the percentages of colour pixels, was evaluated. In Paper V, the effect cytokines had on ductus diameter, systolic blood pressure, pulmonary resistance and the development of symptomatic ductal shunting was evaluated.



## ***Echocardiography equipment***

Echocardiography was performed (Papers II-V) with an Acuson Sequoia™ C256 echocardiography system (Acuson Mountain View, Ca, USA). This system uses a coherent image former, with sampling of both the amplitude and phase data from multiple beam formers, to reconstruct reflected information. For pulsed-wave Doppler, the solo™ mode, with a dedicated beam former optimised for digital Doppler signal processing, and vector array transducers with 5 to 7 MHz frequencies for 2-dimensional imaging and 3.5 to 5 MHz frequencies for pulsed-wave Doppler were used. The colour scale maximum settings had a variation of 50-130 cm/s and the gain was 43-61. Images were saved on a magnetic optic-disc and analysed subsequently by one observer in Studies II, III and V and by two observers in Study IV.

## ***Colour Doppler scanning***

### ***Experimental study in lambs (Paper II)***

A 7 MHz colour Doppler transducer was placed directly on the right ventricle close to the apex to detect the pulmonary artery with a ductal flow jet. The colour scale maximum was 64 cm/sec, CD frequency 5 MHz, CD gain 50. The image was obtained over the main pulmonary artery longitudinal cross-section (PALS), including the pulmonary valve, the origin of the ductal jet close to the plane of the origins of pulmonary artery branches. An ECG was recorded simultaneously.

The ductal flow was changed by different degrees of strangulation of the ductus arteriosus by a cotton band around it. Colour clips including PALS were obtained in 24 different flows, varying between 0 and 390 mL/min.

### ***Clinical study in preterm infants (Papers III and V)***

In a standard M-mode, 2-dimensional, with colour Doppler, transthoracic echocardiography was performed with a 7 MHz transducer with confirmation of normal anatomy and function of the heart. In colour Doppler registration, an image was obtained from a high left parasternal view over the main pulmonary artery longitudinal cross-section, including the pulmonary valve, the origin of the ductal jet close to the plane of the origins of pulmonary artery branches with an ECG recorded simultaneously. From this view, both the internal diameter of the ductus arteriosus, measured from colour Doppler, and the ductal shunt flow pattern were registered. The left atrium/aortic root (LA/Ao) ratio was obtained by M-mode from a parasternal long-axis view. The colour scale maximum was 110 cm/sec, CD frequency 3.5 MHz, CD gain 50.

### ***Clinical study in term infants (Paper IV)***

Saved images obtained with a 5 or 7 MHz transducer, used from a high left parasternal short-axis view to detect the pulmonary artery and ductal flow, were used. The colour scale varied between 55 and 130 cm/sec, the colour Doppler frequency varied between 2.5 and 5 MHz and the gain was 43 to 61.

## ***Quantification of ductal shunt by colour Doppler by computer analysis***

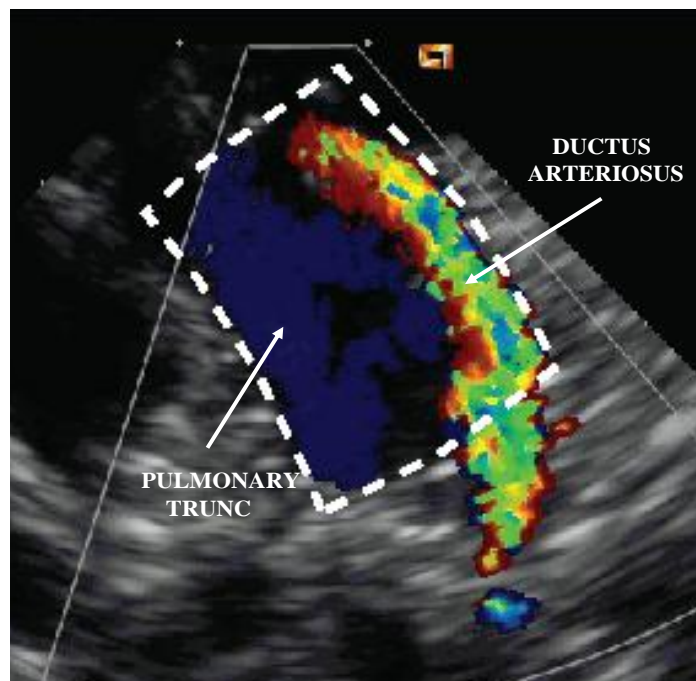
Colour clips from both lambs and infants were saved on magnetic optic-discs in the DICOM format. An image frame in diastole closest to the beginning of the R peak in the ECG was selected using Showcase® computer software (Trillium Technology, Inc., Ann Arbor, Michigan, USA) and converted to the Bitmap (BMP) format. The analysis was made in a custom-designed program written in MATLAB® (The MathWorks, Natick, MA).

A region of interest (ROI) was delineated in PALS, as illustrated in Figure 1. In the BMP format, the hue in a pixel is represented by three numbers corresponding to the amount of the

base colours red (R), blue (B) and green (G) that make up the colour in question. In the chosen display format, colour pixels in the image represented the direction of flow (red towards the transducer, blue away) or turbulence, i.e. a large variance in the velocity estimate (green). The images were thus read into MATLAB® in three separate matrices, each containing one of the three base colours.

To find the velocity that corresponded to a certain colour, the values of red, green and blue along the colour bar were analysed. In this case, a monotonic increase in the sum of R and G channels and the sum of the G and B channels was observed along the colour bar in each direction and these sums were therefore set to represent velocity towards (if considered to be a mainly red pixel) and away from (if considered to be a mainly blue pixel) the probe. Anatomic structures were shown in greyscale.

Within the chosen ROI, a pixel was considered to show one of the base colours and thus represent velocity to or away from the probe, or turbulence, and not greyscale (anatomic information), when the colour channel for a given pixel reached a value that was more than the sum of the other two channels. Green pixels, i.e. showing turbulence, had a less clear-cut border with the other colours and an arbitrary bias value was added (here set at 30) to the sum of the R and B channels. The maximum velocity in colour bars towards the probe was coded in yellow (represented by high values in the R and G channels). Likewise, the maximum velocity away from the probe was coded in a cyan nuance, represented by high values in the G and B channels. The total number of pure green colour pixels were separated from the red and blue pixels in Papers IV and V.



*Figure 1. Region of interest lined out in the pulmonary arterial longitudinal cross-section, from the pulmonary valve to the pulmonary bifurcation, including the ductal flow*

### ***Correlation of colour Doppler information with Qp/Qs***

*Pixel density*: the areas covered by pixels that indicated flow towards or away from the transducer, or turbulence, within the ROI were measured and divided by the area of the ROI. Pixel density of green pixels: the total sum of green was calculated, separated from the other two base colors and expressed as the percentage of total green colour in the ROI.

*The sum of velocity*: the velocity was determined as the sum of R and G and G and B values respectively, normalised to the maximum values these sums could have, as established from the colour bar. A pixel could therefore represent a velocity between 0 and 1, where 1 was the highest indicated velocity on the colour bar. The mean velocity was then found as the average of the pixels indicating flow towards or away from the probe and divided by the area of the ROI.

*Kinetic energy*: because the kinetic energy is proportional to velocity squared, the average of the pixel velocities squared within the ROI was calculated.

*Volume*: the volume of the jets was calculated by multiplying the flow area with the velocity of the pixels, where the area of the ductus lumen was calculated as:  $\pi \times \text{radius}^2$  divided by body surface area.

### ***Calculation of the ratio of pulmonary to systemic flow (Qp/Qs)***

In the *experimental study* (Paper II), the ratio of the pulmonary to the systemic flow (Qp/Qs) was calculated as aortic flow + ductal flow/aortic flow. In the *clinical study* (Paper IV), the Qp/Qs was calculated according to Fick's principle from the blood saturation values in the vena cava superior (VCS), left pulmonary artery (LPA) and aorta (Ao).

### ***Ultrasound markers in detecting symptomatic ductus arteriosus***

The power of three different UCG markers in daily use to predict a clinically significant ductal flow was tested in preterm infants delivered before the gestational age of 32 weeks (Paper III). Infants with congenital heart disease were excluded. Echocardiography measurements were performed by two investigators at the age of  $24 \pm 6$  and  $72 \pm 6$  hours.

The LA/Ao ratio was measured from M-mode registrations obtained from a parasternal long-axis view. A significant shunt was considered to be present when the ratio was more than 1.4:1. The internal diameter of the ductus arteriosus was measured from colour Doppler registrations from a high left parasternal short-axis view. The diameter was corrected with the infant's weight, expressed as mm/kg. A significant shunt was considered to be present when the diameter was larger than 2 mm/kg. The ductal shunt flow pattern was also registered from a high left parasternal short-axis view. A significant shunt was considered to be present when a pattern of pulsatile flow was present at the age of 72 hours.

### ***Quantitative analyses of plasma cytokines***

Sampling was performed from umbilical cord blood and from arterial blood at 6, 24 and 72 h through the indwelling arterial line (Paper V). Blood samples were collected in a Vacutainer tube containing the anticoagulant EDTA (BD Biosciences, San Jose, CA), put immediately on ice and delivered within 20 min to the local chemical laboratory where the plasma was separated into several aliquots and then stored in a freezer ( $-70^{\circ}\text{C}$ ) until it was analysed in one batch 7 months after the termination of the study. Levels of pro-inflammatory IL-6 and IL-8 cytokines in plasma were determined by cytometric bead array (CBA; BD Biosciences) and flow cytometry according to the manufacturer's recommendations. The assay is based on a mixture of microbead populations with distinct fluorescent intensities (FL-3) precoated with capture antibodies specific to each cytokine and uses the sensitivity of fluorescence detection by flow cytometry to measure soluble cytokines in a particle-based immunoassay. Each bead

provides a capture surface for a specific cytokine and is analogous to an individually coated well in an ELISA plate. Briefly, 50 µL of mixed beads coated with cytokine-specific capture antibodies were added to 50 µL of patient plasma and incubated for 1.5 h at room temperature. After washing, 50 µL of phycoerythrin-conjugated (PE) anti-human inflammatory cytokine antibodies were added.

Simultaneously, 50 µL of standards for each cytokine (0–5,000 pg/mL) were treated likewise to generate a standard curve. Two-colour flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD Biosciences). Data were acquired and analysed using BD Biosciences CBA software. Forward- versus side-scatter gating was used to exclude any sample particles other than the 7.5-µm polystyrene beads. Flow cytometric analysis was performed and analysed by a single operator and cytokine concentrations were determined based on the standard curves using CBA software. The lower limit of detection for the various cytokines that were evaluated ranged from 2 to 10 pg/mL. For results above the upper limit of detection, serial dilution of the sample was performed accurately to determine cytokine levels. A level of  $\leq 0.1$  pg/mL was regarded as not detectable.

Plasma levels of the respective cytokines from the umbilical cord and at 6, 24 and 72 h of postnatal age were used to calculate an AUC as an assessment of cytokine burden over time in each subject. The AUC was calculated according to the trapezium rule. The AUC was only calculated in subjects with three or more valid plasma samples. The calculated AUC was adjusted for the total sampling period, which was either 66 or 72 h, thus achieving a weighted average level over time. Cytokine levels at the respective sampling points, as well as the calculated AUC, were logarithmically transformed to obtain a normal distribution of values for statistical analysis.

## **Statistical methods**

Statistical analysis was performed using SPSS 14-17.0 for Microsoft Windows (SPSS Inc., Chicago, IL). A simple regression analysis was performed and correlation coefficients ( $r$ ) were calculated in Papers I, II, IV and V. Curvilinear regression was performed for Paper II and multivariate linear regression analysis for Paper V. The Mann-Whitney U test was used for variables between groups in Papers III and V and Student's  $t$  test for variables within groups in Papers I and IV. In Papers III and IV, the predictive power was calculated as sensitivity, specificity and likelihood ratio, as well as the efficiency of the tests in Paper III. In Paper IV, reliability analysis was used to test the significance of intra- and inter-observer variability. Relationships between continuous outcome variables were assessed using Spearman's rank correlation coefficient and Fisher's exact test for binary variables in Paper V. A  $p$ -value of  $< 0.05$  was considered significant.

## **Ethics**

The study protocols for the experimental studies (Papers I and II) were approved by the Animal Regional Ethical Board at Lund University. The study protocols for clinical studies (Papers III-V) were approved by the Regional Ethical Board at Lund University. The parents gave written and informed consent before enrolment in clinical studies III and V.

## **RESULTS**

### **Effect of indomethacin on coronary flow in lambs (I)**

Nine preterm newborn lambs were included in the study. Indomethacin was given intravenously as a single bolus dose of 0.2 mg/kg (0.4 mL/kg) over 1 minute i.v. The flow velocity in the left coronary artery was registered continuously until the flow had returned to its pre-injection level.

Indomethacin had significant effects on the haemodynamics. A decrease in average peak velocity (APV) was seen in all the lambs ( $p < 0.05$ ), as shown in Figure 2. Heart rate and cardiac output decreased, while rate pressure product and mean arterial pressure increased significantly, as shown in Table 1.

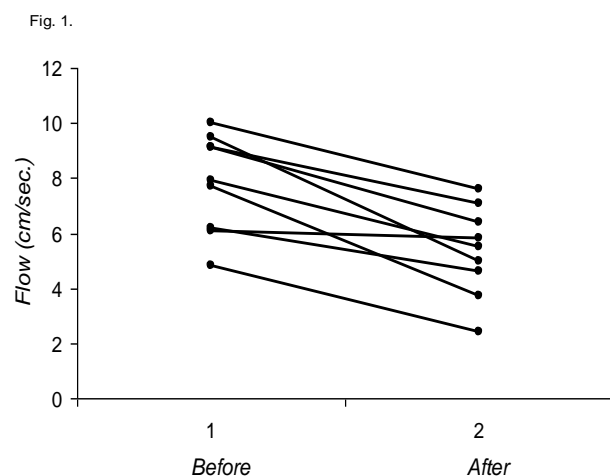


Figure 2. Coronary flows before and after indomethacin injection

Table 1. Heart rate (HR); mean arterial pressure (MAP); rate-pressure product (RPP); and cardiac output (CO) before and after intravenous injection of indomethacin 0.2 mg/kg.  
(bpm = beats per minute)

		Median before	Range before	Median after	Range after	p-value
HR	bpm	146	126-167	141	115-165	< 0.05
MAP	mmHg	49	36-72	68	57-92	< 0.05
RPP	bpm x mmHg/ 100	69	58-110	102	73-135	< 0.05
CO	mL/min	462	228-890	420	293-730	< 0.05

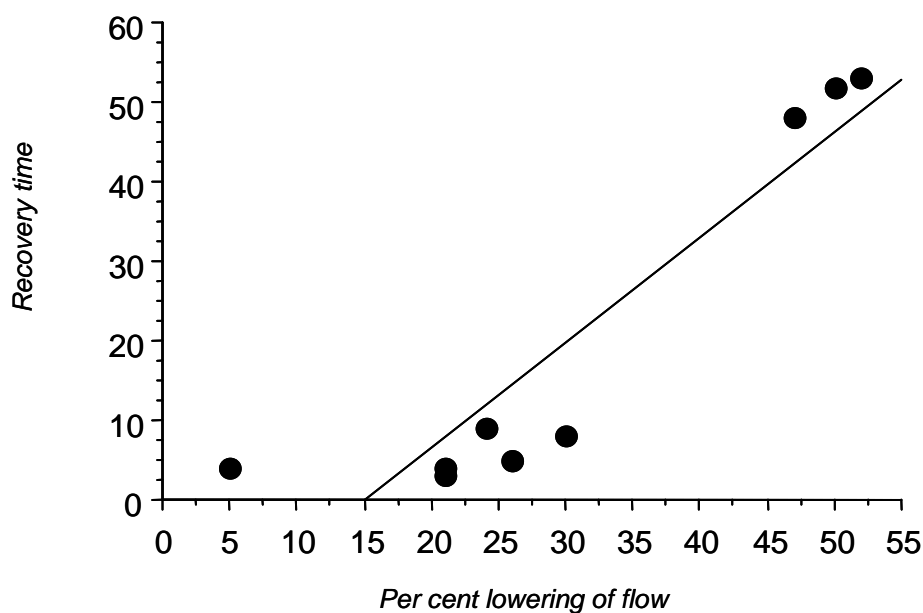
The maximum decrease in APV was 5 to 52 (median 26) per cent and appeared one to seven (median 3) minutes after the administration of indomethacin, as shown in Table 2. There was no correlation between the basal APV and its maximum reduction after indomethacin. There was a large variation in the response of APV to indomethacin. In a group of six lambs, the median of the percentage of APV reduction was 23 (range 5-26). In the remaining three

lambs, the median of the reduction was 50% (range 47 - 52). The reason for this difference in response is not clear.

*Table 2. Effect of indomethacin on Average Peak flow Velocity (APV) in the coronary arteries (n=9). \*  $p < 0.05$  between flow before and after indomethacin injection .(IND = indomethacin)*

		<i>Median</i>	<i>Range</i>
APV before IND	(Cm/sec.)	7	4.8-10 *
APV after IND	(Cm/sec.)	5	2.4-7.6 *
Reduction of APV	(%)	26	5-52
Time to minimum APV	(minutes)	3	1-7
Time to recovery of APV	(minutes)	10	4-53

There was a variation in recovery time from 4 to 53 minutes, with an almost linear correlation to the maximum degree of decrease in APV ( $r = 0.91$ ,  $r^2 = 0.84$ ,  $p < 0.002$ ), as illustrated in Figure 3. When the flow was reduced to more than 40 per cent, the recovery time was almost an hour.



*Figure 3. Maximal lowering of an average peak flow velocity in the left coronary artery as found as a function of recovery time*

## Quantification of shunt through PDA by colour Doppler in lambs (II)

In four lambs, twenty-four different ductal flows, varying between 0 and 390 mL/min. were measured. The shunt (Qp/Qs) was calculated and varied between 1:1 and 1.9:1. The cardiac output varied between 225 and 547 mL/min. The percentage of colour pixels per cm<sup>2</sup> in the region of interest (ROI) in the pulmonary artery longitudinal cross-section (PALS) is presented in Figure 4.

Various measurements were derived from the colour information. They were the percentage of colour pixels in the region of interest, the mean velocity of pixels indicating flow towards and away from the probe, the mean velocity as an average of flow towards or away from the probe and kinetic energy, which is proportional to velocity squared. Their correlation to Qp/Qs is presented in Table 3.

*Table 3. Correlation of Qp/Qs to pixel velocities, velocities squared and pixel area percentages in pulmonary artery longitudinal cross- section.*

\*  $p < 0.001$

<i>Sum</i>	<i>R</i>	<i>R<sup>2</sup></i>
Velocity of red pixels*	0,67	0,45
Velocity of blue pixels	0,46	0,21
Velocity <sup>2</sup> of red pixels	0,67	0,45
Velocity <sup>2</sup> of blue pixels	0,32	0,1
Velocity of red and blue pixels*	0,65	0,42
Velocity squared of red and blue pixels*	0,61	0,37
Red pixel area percentages*	0,64	0,41
Blue pixel area percentages*	0,65	0,43
Green pixel area percentages	0,48	0,24
Total pixel area percentages*	0,87	0,75

Ductal flows as estimated from the colour pixels showed a significant correlation ( $p < 0.05$ ) between Qp/Qs and the percentage of the colour area in the ROI, the mean velocity of the pixels and for colour pixel velocity squared apart from velocities squared for blue pixels. All the separate pixel area percentages showed a significant correlation to Qp/Qs; blue ( $r = 0.65$ ,  $r^2 = 0.43$ ,  $p = < 0.001$ ), red ( $r = 0.64$ ,  $r^2 = 0.41$ ,  $p = < 0.001$ ) and green ( $r = 0.48$ ,  $r^2 = 0.24$ ,  $p = < 0.01$ ). The velocity of red pixels ( $r = 0.67$ ,  $r^2 = 0.45$ ,  $p < 0.001$ ) had a better correlation than the area of red pixels, but the correlation was poorer for the blue pixels ( $r = 0.46$ ,  $r^2 = 0.21$ ,  $p < 0.02$ ). The velocity of red pixels squared showed the same correlation ( $r = 0.67$ ,  $r^2 = 0.45$ ,  $p = < 0.001$ ) as velocity alone. Velocities squared were ( $r = 0.32$ ,  $r^2 = 0.10$ ,  $p = < 0.2$ ) for blue and ( $r = 0.61$ ,  $r^2 = 0.37$ ,  $p = < 0.001$ ) for red + blue, as shown in Table 3.

The best correlation was seen between the size of ductal shunting and the percentage of the total sum of pixels including red, blue and green in the region of interest, outlined in the pulmonary artery longitudinal cross-section, including the pulmonary valve, the origin of the pulmonary artery branches, and the ostium ( $r = 0.87$ ,  $r^2 = 0.75$ ,  $p < 0.001$ ), as shown in Figure 4.

In the curvilinear regression model, the correlation was even better ( $r^2 = 0.84$ ,  $p < 0.001$ ). When the shunt was 1.4:1 or more, 10/10 measurements had 40 per cent or more of the region of interest covered with colour pixels, with a sensitivity of 100 per cent and a specificity of 76 per cent.

When the shunt was larger than 1.5:1, there was no clear linear correlation between flow measurements and pixel areas.

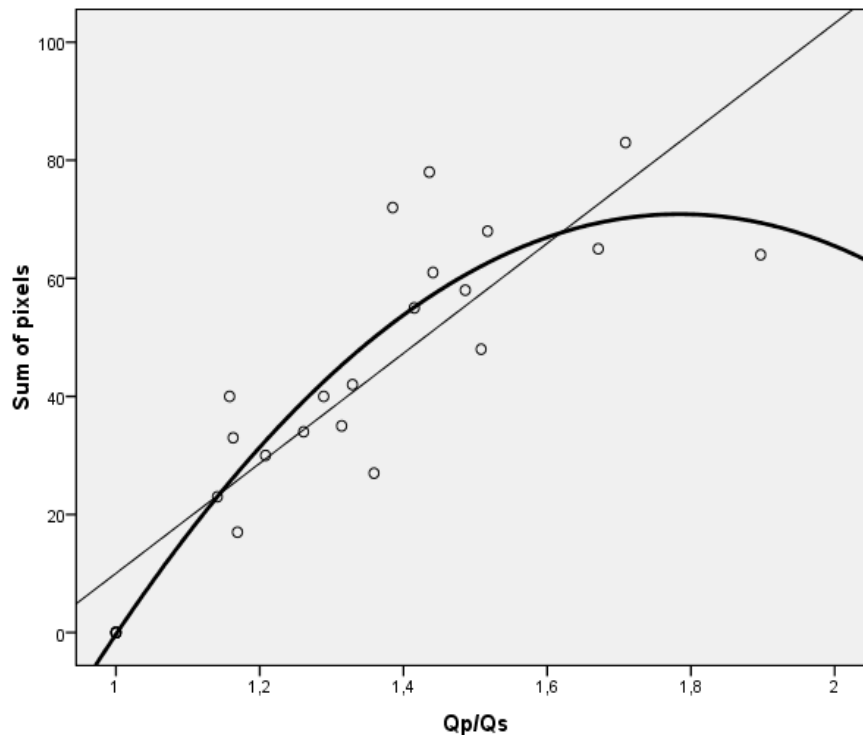


Figure 4. Per cent of pixels in pulmonary artery longitudinal cross-section correlated to  $Qp/Qs$ . ( $r = 0.87$ ,  $r^2 = 0.75$ ,  $p < 0.001$ , curvilinear regression ( $r^2 = 0.84$ ,  $p < 0.001$ ,  $n = 24$ ))

### Prediction of symptomatic PDA by echocardiography (III)

A significant PDA demanding treatment developed in 12 (27%) of forty-five infants included in the study. Indomethacin with a median of 3 (range 1-6) doses was used as the first-line treatment. Five of these infants had their first dose of indomethacin before the age of 72 h (mean 69 h). Seven of the twelve treated infants with a mean gestational age (GA) at birth of 25 weeks ( $SD \pm 1$ ) required surgery because of the lack of response to medical treatment for the closure of a PDA at a median age of 8 days (range 7.5-8.2). With the exception of one infant (GA 25 weeks), the most immature infants were the ones who required surgery. Infants treated for a PDA had a significantly lower GA ( $p < 0.05$ ), but birth weight was not a significant factor. All the infants treated with surgery required mechanical ventilator support before surgery.



*Table 4. Comparison of clinical values between patients with or without a PDA in need of therapeutic intervention by the age of 24 h post partum (n=28)*

	<i>All patients with PDA (n = 28)</i>	<i>Not treated for PDA (n = 16)</i>	<i>Treated for PDA (n = 12)</i>	<i>p-value treated/ untreated</i>	<i>Ligation of PDA (n = 7)</i>
Birth weight, g (mean $\pm$ SD)	895 $\pm$ 107	1109 $\pm$ 356	871 $\pm$ 107	NS	815 $\pm$ 98
GA, weeks (mean $\pm$ SD)	26 $\pm$ 1.4	27.7 $\pm$ 1.9	25.7 $\pm$ 1.4	p $\leq$ 0.05	25 $\pm$ 1
Mechanical ventilation	13 (46%)	6 (37%)	7 (58 %)	NS	7 (100 %)

At the age of 24  $\pm$  6 hours, 28 infants of 45 had a detectable left-to-right shunt through the ductus arteriosus. By the age of 72  $\pm$  6 hours, these infants were reduced to 22, with a median age of 27 weeks.

A ductal diameter of  $> 2$  mm/kg was the most accurate echocardiography marker in predicting a subsequent significant PDA at both 24 and 72 h of age, with a highest sensitivity of 89% and specificity of 70%, a positive likelihood ratio of 2.97 and a lowest negative likelihood ratio of 0.16 at the age of 72 h. When  $> 1.5$  mm/kg was used as a cut-off point for ductal diameter, the sensitivity was 91%, but the specificity was only 18% at 24 h and 100% and 50% respectively at 72 h of age. For a PDA in need of therapeutic intervention, the Mann-Whitney U-test at 24 h was significant for a ductal diameter of  $> 2$  mm/kg (p = 0.01) and a pulsatile flow curve (p = 0.01). At 72 h, the test was only significant for a ductal diameter of  $> 2$  mm/kg (p= 0.01).

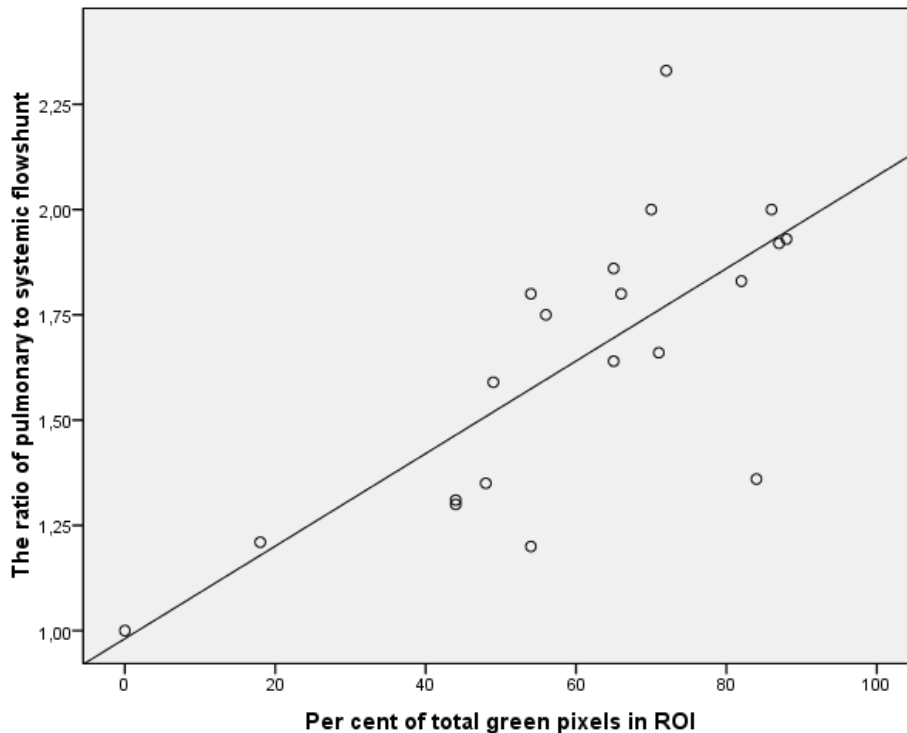
The efficiency of the test by the age of 72 hours was best for a ductal diameter of  $> 2$  mm/kg (84%). For the existence of a pulsatile Doppler curve, it was 72% and, for the percentage of green colour pixels representing ductal flow, it was 63%. The LA/Ao ratio had the weakest power in predicting a symptomatic PDA in this study (53%). The sensitivity, specificity, positive-negative likelihood ratio and efficiency of the test for a ductal diameter of  $> 2$  mm/kg, pulsatile Doppler curve, left atrial/aortic root ratio of  $\geq 1.4$  and green pixels of  $> 50\%$  in the ROI are presented in Table 5. Birth weight, surfactant treatment and the appearance of respiratory distress syndrome were not significant for the development of a significant ductal shunt, according to stepwise logistic regression analysis.

*Table 5. Predictive values for echocardiography parameters for demanding a ductal shunt in need of therapeutic intervention at the age of 24  $\pm$  6 h (n = 28) and 72  $\pm$  6 h (n = 19)*

	<i>Sensitivity %</i>		<i>Specificity %</i>		<i>Efficiency %</i>		<i>Positive LR</i>		<i>Negative LR</i>	
	<i>24 h</i>	<i>72 h</i>	<i>24 h</i>	<i>72 h</i>	<i>24 h</i>	<i>72 h</i>	<i>24 h</i>	<i>72 h</i>	<i>24 h</i>	<i>72 h</i>
<i>Diameter (<math>&gt; 2</math> mm/kg)</i>	91	89	59	70	71	84	2.22	2.97	0.15	0.16
<i>Doppler curve (pulsatile)</i>	91	67	59	78	71	72	2.22	3.04	0.15	0.42
<i>LA/Ao <math>\geq 1.4</math></i>	70	56	29	50	54	53	0.99	1.12	1.03	0.88
<i>Green pixels <math>&gt; 50\%</math></i>	64	70	47	44	44	63	1.21	1.25	0.77	0.68

#### Quantification of shunt through PDA by colour Doppler in children (IV)

A further improvement to our pixel method reported in Study II was used. The methods separate the number of pure green pixels from all colour pixel data. The color pixel percentages during diastole, representing ductal flow, are illustrated in Figure 5. The shunt size ( $Q_p/Q_s$ ) was calculated on the basis of the oxygen saturation data according to Fick's principle and varied from 1:1 to 2.3:1.



*Figure 5 Per cent of total green pixels in pulmonary artery longitudinal cross-section correlated to the ratio of pulmonary to systemic flow between 1:1 and 2.3:1 ( $r = 0.73$ ,  $r^2 = 0.54$ ,  $p < 0.001$ )*

The ductal diameter correlated with the ratio between colour and non-colour pixels ( $r = 0.68$ ,  $p < 0.05$ ). The linear correlation between  $Q_p/Q_s$  and the ductal diameter measured from the angiograms was of borderline significance ( $r = 0.59$ ,  $r^2 = 0.34$ ,  $p < 0.05$ ). No better result was seen when the area of the ductus lumen was calculated and divided by the body surface area ( $r = 0.5$ ,  $r^2 = 0.25$ ,  $p < 0.01$ ). The ratio between colour and non-colour pixels correlated to  $Q_p/Q_s$  ( $r = 0.59$ ,  $r^2 = 0.33$ ,  $p < 0.05$ ).

The total sum of colour pixels to  $Q_p/Q_s$  was high ( $r = 0.69$ ,  $r^2 = 0.48$ ,  $p < 0.002$ ). The estimated jet volume showed a similar correlation ( $r = 0.67$ ,  $r^2 = 0.45$ ,  $p < 0.002$ ).

In the multiple regression analysis,  $Q_s/Q_s$  was explained by the sum of green pixels, ductus diameter, body surface area, the difference in diastolic blood pressure between the pulmonary artery and aorta and weight ( $r = 0.82$ ,  $r^2 = 0.67$ ,  $p < 0.03$ ). In a stepwise regression analysis, only green pixels remained as a significant explanatory variable.

The ratio of pulmonary to systemic flow correlated best to the sum of the percentage of pure green colour pixels ( $r = 0.73$ ,  $r^2 = 0.54$ ,  $p < 0.001$ ), as shown in Figure 5. When the shunt was

1.5:1 or more, 12/13 infants had 50 per cent or more of the region of interest covered with green pixels (sensitivity 92 per cent, specificity 71 per cent). The correlation between ductal diameter and pulmonary to systemic flow ratio was less significant ( $r = 0.59$ ,  $r^2=0.34$ ,  $p < 0.05$ ). The intraclass correlation of repeated measurements of the percentage of total green pixels was 0.99 between the same observer and 0.86 between two observers, as shown in Tables 6 and 7.

*Table 6. Intraclass correlation (ICC) between two analyses made by the same examiner A (n=20) (SD = standard deviation; Md = median)*

<i>Examiner</i>	<i>Variable</i>	<i>Mean</i>	<i>SD</i>	<i>Md</i>	<i>ICC</i>	<i>95%</i>
A (1)	% total green	0.60	0.23	0.65	0.99	(0.97-0.99)
A (2)	% total green	0.62	0.23	0.62	0.99	(0.97-0.99)

*Table 7. Intraclass correlation (ICC) between examiner A and B (n = 20) (SD = standard deviation; Md = median)*

<i>Examiner</i>	<i>Variable</i>	<i>Mean</i>	<i>SD</i>	<i>Md</i>	<i>ICC</i>	<i>95%</i>
A	% total green	0.60	0.23	0.65	0.86	(0.69-0.94)
B	% total green	0.64	0.23	0.71	0.86	(0.69-0.94)

### **The effect IL-6 and IL-8 have on ductal diameter, systolic blood pressure, pulmonary resistance and treatment of PDA (Paper V)**

The patient population is the same as in Study III. Of the forty-five mothers, 13 (29%) had a suspected infection, of whom 3 (7%) had clinical chorioamnionitis, and 30 (67%) received antenatal antibiotic treatment based on the clinical evaluation. The infants of infected mothers had a significantly lower GA ( $p = < 0.001$ ) and birth weight ( $p = < 0.05$ ). Of the infants included, 31 (69%) were delivered by caesarean section. Thirteen (29%) of the infants were small for gestational age (SGA), i.e. had a birth weight  $<$  two standard deviations below the mean. Fourteen (31%) of the infants received dopamine treatment for systemic hypotension and thirty-nine (87%) underwent volume expansions during the first 72 h of life. Maternal, delivery and neonatal characteristics are listed in Table 8.

A left-to-right shunt though the DA was present in 41 infants (91%) at  $6 \pm 4$ , 28 (62%) and 22 (49%) at  $24 \pm 6$  and  $72 \pm 6$  h of age respectively. The percentage of pixels, representing the shunt size, measured diameter of ductus, ductal flow velocity or estimated pressure in the pulmonary artery are shown in Table 9.

Twelve (27%) of the infants were treated for a PDA with cyclo-oxygenase inhibitors (indomethacin) and 7 (16%) were further treated surgically. These twelve infants had a lower GA ( $p = 0.002$ ), an increased incidence of RDS ( $p = 0.003$ ) and were less frequently delivered by caesarean section ( $p = 0.004$ ). Six (46%) infants of mothers with infection developed an sPDA, all of which needed to be surgically closed.

No significant differences in IL-6 and IL-8 levels were seen between infants with or without a left-to-right shunt through the DA. The correlation between the levels of IL-6 and IL-8 and systolic blood pressure, DA diameter and flow velocity respectively at the studied time points is given in Table 10. IL-8 correlated negatively to systolic blood pressure at 24 and 72 h of age and IL-6 correlated to a level of borderline significance at all time points, IL-8 positively to DA diameter at 72 h and IL-6 and IL-8 negatively to DA flow velocity at all time points. Multivariate analysis, including systolic blood pressure, DA diameter and flow velocity as independent variables and IL-6 or IL-8 as the dependent variable, showed a significant correlation to reduced systolic blood pressure. The levels of IL-6 and IL-8 in infants who did or did not develop an sPDA were similar and can be seen in Table 11, even though infants with an sPDA were significantly more immature (25.7 vs. 27.7 gestational weeks), ( $p = 0.002$ ). Thirty-one infants (69%) were delivered by caesarean section, but, in those that did or did not develop an sPDA, no significant difference could be seen with regard to cytokine levels. No correlation was observed between the levels of IL-6 and IL-8 and estimated lung resistance.

For all infants included, the median levels at 6, 24 and 72 h for IL-6 (40, 38, 29 pg/mL) and IL-8 (70, 67, 55 pg/mL) decreased with increasing age. However, increased levels of IL-8 at the age of 72 h were higher in infants subsequently treated with the surgical closure of the DA ( $p < 0.05$ ). In a multivariate comparison, the lower GA was not a significant determinant factor for the need for surgical closure of a PDA. The median (range) level by the age of 72 h in those treated surgically was 101 (440-636) pg/mL ( $n = 7$ ) and in those treated with indomethacin 46 (23-86) pg/mL ( $n = 5$ ). In non-treated infants, the median (range) level was 46 (23-260) pg/mL ( $n = 33$ ).

*Table 8. Maternal, delivery and neonatal characteristics of infants treated ( $n = 12$ ) or not treated ( $n = 33$ ) for patent ductus arteriosus*

	<i>Treated</i>		<i>Not treated</i>		<i>p-value</i>
	<i>Number or value</i>		<i>Number or value</i>		
		(%)		(%)	
Maternal infection	6	(50)	8	(24)	0.07
Maternal antibiotic	10	(83)	20	(61)	0.14
Caesarean section	4	(33)	28	(85)	0.004*
Gestation age (weeks)	25.7	± 1.4	27.7	± 2	0.002*
Birth weight (g)	871	± 106	1063	± 334	0.05
Gender (male)	8	(67)	16	(48)	0.23
SGA	1	(8)	12	(36)	0.07
Dopamine treatment	5	(42)	9	(27)	0.28
Volume expansion	9	(75)	30	(91)	0.11
RDS	12	(100)	19	(58)	0.003*
IVH	5	(42)	5	(15)	0.07

(PDA, patent ductus arteriosus; SGA, small for gestational age; RDS, respiratory distress syndrome; IVH, intraventricular haemorrhage. Treatment with dopamine and/or volume expansion during the first 3 days post partum. \*  $p < 0.05$ )

Table 9. Echocardiography measurements and systolic blood pressure in preterm infants (n 41) (diameter and flow velocity of DA; pressure (s) = mean systolic blood pressure; pressure (p) = calculated pressure in pulmonary artery)

Age	Diameter (mm/kg)	Velocity (m/s)	Pressure(s) (mmHg)	Pressure(p) (mmHg)	Shunt size (% pixels)
6 ± 4 h	2,6	1,7	47	35	50
24 ± 6 h	2,4	1,9	49	32	52
72 ± 6 h	2,4	2,5	58	31	49

Table 10. Correlation between IL-6, IL-8 and ductus diameter, flow and systemic blood pressure

Age	Diameter	Velocity	Pressure (s)
<i>IL-6 (pg/mL)</i>			
6 ± 4 h	p=0.46	p=0.03	p=0.052
24 ± 6 h	p=0.43	p=0.017	p=0.057
72 ± 6 h	p=0.98	p=0.005	p=0.085
<i>IL-8 (pg/mL)</i>			
6 ± 4 h	p=0.5	p=0.011	p=0.144
24 ± 6 h	p=0.13	p=0.001	p=0.001
72 ± 6 h	p=0.032	p=0.002	p=0.001

Table 11. Logarithmic levels of IL-6 and IL-8 (mean, SD) in infants treated or not treated for patent ductus arteriosus (n 45) (AUC = area under the curve)

	Treated (n = 12)	Not treated (n = 33)	p-value treated/not treated	PDA ligation (n = 7)
<i>IL-6 pg/ml</i>				
Age				
6 ± 4 h	1.78 ± 0.6	2.05 ± 1.17	Ns	2.0 ± 0.3
24 ± 6 h	1.68 ± 0.57	1.74 ± 0.68	Ns	1.8 ± 0.6
72 ± 6 h	1.91 ± 0.12	1.51 ± 0.39	Ns	2.3 ± 1.4
0-72 h (AUC)	2.15 ± 0.92	1.99 ± 0.85	Ns	2.4 ± 1.1
<i>IL-8 pg/ml</i>				
Age				
6 ± 4 h	1.93 ± 0.28	1.99 ± 0.58	Ns	2.0 ± 0.3
24 ± 6 h	1.98 ± 0.34	1.87 ± 0.36	Ns	2.1 ± 0.4
72 ± 6 h	2.0 ± 0.57	1.71 ± 0.24	Ns	2.3 ± 0.6
0-72 h (AUC)	2.07 ± 0.39	1.93 ± 0.44	Ns	2.2 ± 0.4

## DISCUSSION

Effective tools for evaluating the clinical and prognostic significance of ductal shunting are needed. Indications for medical treatment and the device closure of a PDA should be defined. Doppler echocardiography has made it possible to detect a PDA and its response to treatment. However, determining the pathological significance of the PDA is difficult and when and how to treat a PDA in children is the subject of an ongoing debate.

We tested the effect indomethacin, a drug used for closing PDAs in newborn infants, has on the coronary circulation in a lamb model (Paper I). Further aims of the thesis were to develop an echocardiography method for evaluating the size of a shunt through the PDA which could be used as a guideline in treatment decisions relating to PDAs. The method was developed in a lamb model (Paper II) and the applicability of the method was tested in clinical studies (Papers IV and V). A good quality image with the right projection is essential for reasonable colour measurements, which can easily be performed even in very preterm infants without complications. We further tested the power of the method to predict PDAs in need of treatment in preterm infants (Paper III). Neither the size of the child nor echocardiography settings appeared to be critical for the method (Paper IV).

### Identification of a haemodynamically significant ductus arteriosus

This is usually based on clinical signs together with echocardiography findings. Clinical signs of a symptomatic ductus often develop during the second half of the first postnatal week in preterm infants, but a silent ductus in the first week of life is common. The reduction of systemic blood flow, caused by ductal steal phenomena, in preterm infants might be most harmful in a lamb model in the first 12 h and might lead to increased morbidity.

It is possible to calculate pulmonary and systemic flows by echocardiography. This can be done by calculating the right and left ventricular output with 2-DE and Doppler echocardiograms. The stroke volume is calculated as a mean velocity (cm/s) x the cross-sectional area of flow (cm<sup>2</sup>)/1,000. The product of stroke volume and heart rate gives the cardiac output (CO), where the left ventricle CO minus the right ventricular CO should be equal to the ductal shunt. In small infants, this calculation may not be accurate, because there is always a degree of incompetence of the foramen ovale and thereby a left-to-right atrial shunt, which explains why this method should not be used in newborn infants. The method is not used in daily clinical work due to the complexity and unreliability of the measurements. A size of DA diameter of  $\geq 1.4$  mm/kg, a ratio of  $\geq 1.4:1$  between left atrium/aorta diameter (LA/Ao ratio), left ventricular enlargement and a holodiastolic flow reversal in the descending aorta are echocardiographic and Doppler criteria commonly used for the identification of a shunt of haemodynamic importance.<sup>116</sup> Unfortunately, they all have their weak points and their specificity is low.

Determining the left-to-right shunt under cardiac catheterisation using Fick's principle is also unreliable and a source of inaccuracy in our studies. In the case of PDA, measurements of mixed venous and arterial blood samples from the pulmonary artery are problematic. The sample should not be taken directly from the ductal jet.

In our experimental study in lambs (Paper II), we evaluated the size of the shunt through the ductus arteriosus by colour Doppler. We aimed to develop a method where, in association with shunt lesions, a Qp/Qs ratio of 1.5 could be used. Using the flow measured with electromagnetic flow transducers in the aorta and ductus, Qp/Qs correlated very significantly with the percentage of colour pixels in a region of interest (ROI) in the pulmonary artery cross-section. When the Qp/Qs was 1.4:1 or greater, the sum of the colour pixels covered  $> 40\%$  of the ROI. The sensitivity was 100 per cent and the specificity 76 per cent. In the curvilinear regression model, the explanatory rate was ( $r^2$ ) 0.84. The correlation between the

percentage of colour pixels and Qp/Qs disappears in high shunt values, apparently due to the saturation of colours in the pulmonary artery in Qp/Qs values of  $> 1.5$ , illustrated in the curvilinear correlation curve in Figure 5.

The correlations obtained in this experimental study are high when taking account of the fact that ductal shunting continuously varies in these newborn animals, which must lead to slight discrepancies in the timing of the flow and colour measurements. Further, there is uncertainty about whether the mixed venous sample taken from the left pulmonary artery can be used in calculating Qp/Qs. The estimated size of the shunt through the ductus using colour pixels is based on ductal flow into the pulmonary artery, which would make the method more specific when it came to judging the shunt size.

A further improvement in our pixel method by better defining the area of green pixels is reported in Paper IV. Data from children admitted for the device closure of a PDA were evaluated. A significant correlation was seen between the Qp/Qs measured according to Fick's principle and the percentage of green pixels in pulmonary artery longitudinal sections. When the shunt was 1.5:1 or more, 12/13 infants had 50 per cent or more of the region of interest covered with green pixels (sensitivity 92 per cent, specificity 71 per cent). The model appears to be adequate for diagnosing a significant shunt, which we consider to be about 1.5:1. This corresponds to a colour pixel area of more than 50% in pulmonary artery longitudinal sections. Even if two different transducers with different settings were used regarding colour scale maximum, colour Doppler frequency and gain, as well as the frame rate, and the fact that the 20 children included were a heterogeneous group (0.6-15 years and weight 7-48 kilo grams), a good correlation was seen, which indicates that the method is practical. The intraclass correlation of repeated measurements of the percentage of total green pixels between the same observer was 0.99, while it was 0.86 between two observers.

### **Prediction of a symptomatic patent ductus arteriosus**

The ductus diameter appeared to have a strong power to predict a symptomatic PDA, as has also been shown in several other studies. Kluckow et al. report that a ductal diameter of  $\geq 1.5$  mm predicts the development of a symptomatic PDA with a sensitivity of 83% and a specificity of 90%.<sup>117</sup> Doppler flow patterns predict the development of a subsequently clinically significant PDA, with a sensitivity of  $> 90\%$  and a specificity of up to 100%, according to Su BH et al. in 1997.<sup>118</sup> Left ventricular output of more than 60 mL/min/kg consistently preceded a symptomatic PDA by at least 24 h in preterm infants, as reported by Walther et al.<sup>119</sup> An LA/Ao ratio of  $> 1.5:1$  gives a sensitivity of 88% and a specificity of 95%, according to Iyer et al.<sup>120</sup> Using multiple parameters in the statistical model usually increases the sensitivity and specificity, indicating that the parameters reflect partially different kinds of physiological phenomena, such as an LA/Ao ratio of  $> 1.3$ , a left ventricular pre-ejection period/left ventricular ejection time ratio of  $< 0.25$  and a left ventricular isovolumic contraction time of  $< 0.015$  sec. which is reported to have a sensitivity of 100% and a specificity of 85% at 3 days of age when it comes to predicting a symptomatic shunt.<sup>121</sup> The highest sensitivity and specificity are reported by Kupferschmid et al. for a disturbed cerebral blood flow with absent or retrograde diastolic perfusion with a sensitivity and specificity each of 100%.<sup>55</sup>

In clinical practice, a ductal diameter of  $\geq 1.4$  mm/kg together with an LA/Ao ratio of  $\geq 1.4:1$  and the presence of diastolic flow in the aorta descendens, are commonly used as guidelines in treatment decisions relating to PDAs in preterm infants. Difficulty in measuring its diameter by colour Doppler exists. It is easy to interpret the diameter of the ductus ampulla as the ductal diameter, which leads to an overestimation of the diameter. False positives, regarding the LA/Ao ratio, can result from left-to-right shunting through a ventricle septum defect, of left ventricular dysfunction and mitral valve abnormalities. The M-mode beam can also have an angle error.

A ductus diameter of  $> 2$  mm/kg was the most accurate marker of a PDA in need of treatment, with a sensitivity of 89% and a specificity of 70% in our clinical study (Paper III). The diameter was obtained from colour Doppler registrations which possibly led to an overestimation of the diameter of the shunt that can explain a low specificity. The efficacy of the markers at 72 h of age was calculated with a highest efficacy for ductus diameter (84%) followed by a pulsatile Doppler curve (72%), percentage of green pixels (63%) and LA/Ao ratio (53%).

There are other methods for quantifying ductal shunting, such as magnet resonance imaging, but the method is time consuming, expensive and demands more resources compared with ultrasound, while invasive methods like cardiac catheterisation and radionuclide scanning are clearly not practical or ethical to perform in preterm infants.

A growing body of evidence suggests that elevated plasma BNP or NT-pro-BNP levels may indicate a symptomatic PDA and guide its treatment. In neonates of  $< 28$  weeks' GA, BNP levels of  $> 550$  pg/mL by the age of 48 h were suggested to predict PDA intervention (sensitivity 83%, specificity 86%).<sup>122</sup> For neonates born at 25-34 weeks' GA, the best cut-off BNP concentration by the age of 72 h to diagnose a hPDA was 1110 pg/mL (sensitivity 100%, specificity 93%).<sup>123</sup> Similarly, plasma NT-pro-BNP levels from infants born at  $< 33$  weeks' GA were a good indicator of a PDA in need of treatment, with a suggested cut-off concentration of 10,280 pg/mL at 48 h of age (sensitivity 100%, specificity 91%).<sup>124</sup>

## Management of patent ductus arteriosus

Management is based on both clinical and echocardiographic findings in preterm infants.<sup>64</sup> The timing of the intervention and the dosage of pharmacological treatment remain an unsolved issue. Using the evaluated Qp/Qs as a guideline could help in treatment decisions. COX inhibitors blocking the prostaglandin synthesis remain the first treatment of choice. The coronary flow velocity decreased acutely in lambs almost immediately (median 3 min.) after the administration of the COX inhibitor indomethacin. In some of the animals, the decrease was substantial, with a flow velocity reduction of 50%, and the effect remained for up to one hour (Paper I). This corresponds to other studies in which the effect of indomethacin on renal, cerebral and intestinal flow produces a similar reduction in blood flow.<sup>125-127</sup> The lambs that had the largest decrease in coronary average peak velocity also had the longest recovery time.

### *Prophylactic treatment with indomethacin, ibuprofen or surgery*

Prophylactic indomethacin has produced a reduction in the incidence of symptomatic PDA and especially the incidence of severe intraventricular haemorrhage as a complication, but prophylactic treatment exposes a large proportion of infants unnecessarily to a drug that has side-effects and potential short- and long-term complications.<sup>128</sup> Without conferring any important long-term benefits, the use of prophylactic therapy is not indicated in the management of preterm infants. Our quantification method should reduce the need for prophylactic treatment.

### *Surgical ligation or medical treatment with indomethacin*

The data regarding the net benefit/harm are unclear and insufficient to draw any conclusion about what should be preferred as the initial treatment for a symptomatic PDA in preterm infants. Surgery may be preferred as a first treatment of choice in infants with a birth weight of less than 800 g, since cyclo-oxygenase inhibitors are reported to be less effective in these infants. It should be noted that recent observational studies indicated an increased risk of one or more of the following outcomes associated with PDA ligation; chronic lung disease, retinopathy of prematurity and neurosensory impairment.<sup>102</sup> Infants whose DA is ligated may run a greater risk of poor developmental outcome compared with infants treated medically.<sup>129</sup> It is possible that the delay due to the "waiting time" and transport to another facility with the



surgical capacity for PDA ligation could adversely affect outcomes, as could the peri-operative care. To date, no consensus supports surgical treatment as a first treatment regimen. Again, an objective method like our pixel method should help when it comes to making decisions between these difficult choices.

#### *Conservative treatment*

In a selected subgroup of ELBW preterm infants, conservative treatment consisting of fluid restriction (maximum: 130 mL/kg/day beyond day 3) and the adjustment of ventilation (lower inspiratory time and higher positive end-expiratory pressure) may be an alternative treatment. No higher rates of major complications were reported by Vanhaesebrouck et al., although the efficacy of this treatment needs to be evaluated in larger trials.<sup>130</sup>

#### *Device closure*

The development of devices for closing the ductus under interventional cardiac catheterisation has greatly increased the number of patients treated, but this practice has produced evident problems. At some centres, closure of an echocardiographically diagnosed PDA is performed, even if the shunt appears to be insignificant. The closure might be offered without taking account of the fact that the use of devices exposes the patients to the risks of cardiac catheterisation and possible embolisation by the device. Moreover, the cost is relatively high. A fairly widely accepted indication for closing an open ductus is the existence of a continuous heart murmur associated with ductal flow documented by echocardiography. However, the continuous murmur might also be due to the amount of flow, as well as to the direction of the ductal jet. It has been suggested that a continuous murmur is not due solely to the amount of shunt flow but also to the direction of the jet.<sup>131</sup> When the jet is directed against the wall of the pulmonary artery, a continuous murmur is heard. This might lead to a large amount of unnecessary treatment.

Clear indications for device closure are needed. A pulmonary to systemic flow ratio ( $Q_p/Q_s$ ) over 1.5:1 is used as an indication for closing a ventricular septal defect by surgery and, before the era of echocardiography, for the closure of atrial septal defects, and it would be logical to use the same indication for ductal closure.

### **Cytokine interaction in the systemic and lung circulation**

Cytokines play a major role in the response to injuries and infection with endothelial dysfunction. Cytokines appear to be potent systemic vasodilators, as well as pulmonary vasoconstrictors.<sup>112, 113</sup> A large number of preterm births are associated with infection, in turn associated with increased fetal and postnatal levels of pro-inflammatory cytokines, and extremely low gestational age newborns are reported to be less able to metabolise inflammatory cytokines, owing to their immaturity.<sup>132, 133</sup> Cytokine IL-8 showed a significant negative correlation with systolic blood pressure and a decreased ductal flow velocity was documented, probably secondary to reduced systemic pressure at 24 and 72 h. A correlation between cytokine levels and ductal diameter was seen at 72 h of age (Paper V). There was no significant correlation between cytokine levels and calculated shunt size, ductal diameter, ductal flow velocity and estimated pulmonary resistance in a stepwise regression analysis, which may be explained by the complex interaction of cytokines with parameters involved in the development of a shunt through a PDA. Levels of IL-6 and IL-8 were significantly decreased by 72 h compared with levels during the first 24 h of life, as reported earlier.<sup>112</sup> This finding, together with the documented lack of difference in the levels of IL-6 and IL-8 between the infants who were or were not treated for a PDA, speaks against perinatal infection as an important factor in the development of symptomatic PDA in preterm infants.

## **Practical applications and future perspectives**

Neither the size of the patient nor the echocardiography settings appear to be critical for our pixel method. A good quality image with the right projection is essential for reasonable colour measurements. This should not be too difficult, because the anatomic landmarks for projections are clear. Following the landmarks makes the ductal flow come almost directly toward the transducer, which is important when comparing different registrations. Angle dependence in this sense is a minor problem because the view is standardised.

The standard of care at many centres is to perform routine echocardiography in infants with an extremely low gestational age to detect and evaluate a ductus arteriosus within the first 72 hours of life. Determining the shunt size is important when it comes to giving the infant the best treatment at the right moment to prevent complications of the disease or the given treatment. Evaluation of the ductal shunt size can be made by colour Doppler and is easy to perform at neonatal care units. The method gives a quantification of the ductal shunt and could be used as guidance in the treatment decision relating to preterm infants with a patent ductus arteriosus. Closure of a preterm PDA is currently only justified by the reduction in severe IVH with the prophylactic administration of indomethacin and, potentially, the reduction of NEC with prophylactic surgery ligation; however, given the risk of therapy and the high rate of spontaneous closure, these prophylactic strategies cannot be recommended for all preterm newborns.<sup>134</sup>

The early detection of a large shunt through a PDA could select infants with an increased risk of severe intraventricular haemorrhage and exclude a large number of infants exposed to unnecessary treatment in a prophylactic treatment regimen. The treatment for closing the duct might have serious side-effects and the indications for closure of a PDA should therefore be clear.

The treatment of choice in term children after the neonatal period is intervention by device occlusion under heart catheterisation, providing that the shunt is significant. A quantitative analysis of ductal flow by colour Doppler helps in evaluating the pathological significance of the PDA and could be used as a useful non-invasive tool to select children for device closure. The method could be of great value in preventing unnecessary complications of treatment, but the significance of the method must be further validated in clinical studies.

## CONCLUSIONS

- ◆ A bolus dose of indomethacin is able to reduce the coronary flow velocity in lambs to less than half in less than 10 minutes and this effect may remain for up to almost one hour.
- ◆ Indomethacin can increase myocardial oxygen demand, while simultaneously reducing myocardial flow and cardiac output.
- ◆ Quantification of shunt size through a PDA can be performed by colour Doppler by measuring the percentage of colour pixels in the pulmonary artery.
- ◆ Quantification of the percentage of green pixels (turbulence) correlates more specifically to the ductal shunt size than the total number of colour pixels.
- ◆ Clinically significant shunts with a Qp/Qs of  $> 1.5:1$  can be diagnosed by colour Doppler, where neither the size of the patient nor the echocardiography settings appear to be critical.
- ◆ Ductus diameter appears to be the most important echocardiography variable in determining the need for intervention for a PDA in preterm infants.
- ◆ Increased levels of IL-8 can reduce systemic blood pressure during the three first days of life in preterm infants.
- ◆ Increased levels of IL-8 are associated with increased ductal diameter in preterm infants.
- ◆ Increased levels of IL-6 and IL-8 during the age of 0-72 h in preterm infants are not associated with increased or decreased shunt size through the DA.

## SWEDISH SUMMARY

### (Populärvetenskaplig svensk sammanfattning)

Ductus arteriosus är ett kärl som finns hos foster för att styra blodet förbi lungorna. Kärlet stänger sig normalt inom 3 dagar efter födelsen men kan kvarstå öppet framför allt hos mycket för tidigt födda barn. Vid födelsen leder tryckförändringar i cirkulationen till att flödet i ductus vänder och blodet styrs istället till lungorna. Flödet genom ductus hos för tidigt födda barn kan vara mycket skadligt varför detta behandlas med medicin som stänger kärlet.

Alternativt används kirurgi vid utebliven effekt av medicinen.

Huvudsyftet med inkluderade studier har varit att utveckla en metod att med hjälp av ultraljud storleksbedöma hur mycket blod som styrs genom ductus arteriosus till lungorna.

Det finns ingen enhetlig behandlingsstrategi för prematurfödda barn med skadligt flöde genom ductus. Då läkemedel som används för slutning av ductus arteriosus, speciellt indomethacin, har allvarliga biverkningar måste man noga överväga behandlingen.

I vår djurstudie av lamm visade vi att indomethacin reducerar flödet i hjärtats kranskärl i upp till 50 %, en effekt som kunde sitta i upp till en timme. I en djurmodell har vi utvecklat en icke invasiv metod för storleksbedömning av blodflödet genom ductus arteriosus. Flödet i aorta och genom ductus mättes med flödesmätare placerade runt kärnen samtidigt som ultraljudsregistreringar gjordes över lungpulsåderns huvudstam med inkommande ductusflöde. Ductusflödet, som i en ultraljudsbild presenteras som färgpixlar, kvantifierades som procent färgpixlar/ytenhet, där ytan avgränsades med anatomiska landmärken. Där fanns en signifikant korrelation mellan uppmätt flöde till lungorna (shuntstorlek) och procent färgpixlar/ytenhet som blev mer signifikant när enbart gröna pixlar jämfördes med flödet. När shuntstorleken var  $\geq 1.5:1$  så täcktes mer än  $\geq 50$  % av den uppmätta ytan av färgpixlar. I vår kliniska studie av barn som skulle genomgå ductus stängning med device hade metoden en sensitivitet på 92 % hos barn med en shunt  $\geq 1.5$ .

Vi visar också att inflammation under barnets 3 första levnadsdygn inte ökar risken för utvecklandet av öppen ductus med skadligt flöde som behöver behandlas men att dessa barn får lägre blodtryck och vidgning av ductuskärlet.

Vi föreslår att vår metod på ett enkelt och säkert sätt kan användas för att bedöma storleken av flödet genom ductus. Att använda uppmätt shuntstorlek  $> 1.5:1$  som vägledning för behandling kan minska antalet onödiga behandlingar samt komplikationer till följd av dessa.

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## REFERENCES

- 1.Coceani F, Olley PM, Bishai I, Bodach E, Heaton J, Nashat M, et al. Prostaglandins and the control of muscle tone in the ductus arteriosus. *Adv Exp Med Biol.* 1977;78:135-42.
- 2.Clyman RI, Waleh N, Black SM, Riemer RK, Mauray F, Chen YQ. Regulation of ductus arteriosus patency by nitric oxide in fetal lambs: the role of gestation, oxygen tension, and vasa vasorum. *Pediatr Res.* 1998;43(5):633-44.
- 3.Slomp J, van Munsteren JC, Poelmann RE, de Reeder EG, Bogers AJ, Gittenberger-de Groot AC. Formation of intimal cushions in the ductus arteriosus as a model for vascular intimal thickening. An immunohistochemical study of changes in extracellular matrix components. *Atherosclerosis.* 1992;93(1-2):25-39.
- 4.Coceani F, Kelsey L, Seidlitz E. Evidence for an effector role of endothelin in closure of the ductus arteriosus at birth. *Can J Physiol Pharmacol.* 1992;70(7):1061-4.
- 5.Thebaud B, Michelakis ED, Wu XC, Moudgil R, Kuzyk M, Dyck JR, et al. Oxygen-sensitive Kv channel gene transfer confers oxygen responsiveness to preterm rabbit and remodeled human ductus arteriosus: implications for infants with patent ductus arteriosus. *Circulation.* 2004;110(11):1372-9.
- 6.Thebaud B, Wu XC, Kajimoto H, Bonnet S, Hashimoto K, Michelakis ED, et al. Developmental absence of the O<sub>2</sub> sensitivity of L-type calcium channels in preterm ductus arteriosus smooth muscle cells impairs O<sub>2</sub> constriction contributing to patent ductus arteriosus. *Pediatr Res.* 2008;63(2):176-81.
- 7.Clyman RI, Chan CY, Mauray F, Chen YQ, Cox W, Seidner SR, et al. Permanent anatomic closure of the ductus arteriosus in newborn baboons: the roles of postnatal constriction, hypoxia, and gestation. *Pediatr Res.* 1999;45(1):19-29.
- 8.Clyman RI, Seidner SR, Kajino H, Roman C, Koch CJ, Ferrara N, et al. VEGF regulates remodeling during permanent anatomic closure of the ductus arteriosus. *Am J Physiol Regul Integr Comp Physiol.* 2002;282(1):R199-206.
- 9.Echtler K, Stark K, Lorenz M, Kerstan S, Walch A, Jennen L, et al. Platelets contribute to postnatal occlusion of the ductus arteriosus. *Nat Med.* 16(1):75-82.
- 10.Gittenberger-de Groot AC, van Ertbruggen I, Moulaert AJ, Harinck E. The ductus arteriosus in the preterm infant: histologic and clinical observations. *J Pediatr.* 1980;96(1):88-93.
- 11.de Reeder EG, Poelmann RE, van Munsteren JC, Patterson DF, Gittenberger-de Groot AC. Ultrastructural and immunohistochemical changes of the extracellular matrix during intimal cushion formation in the ductus arteriosus of the dog. *Atherosclerosis.* 1989;79(1):29-40.
- 12.Kajino H, Chen YQ, Seidner SR, Waleh N, Mauray F, Roman C, et al. Factors that increase the contractile tone of the ductus arteriosus also regulate its anatomic remodeling. *Am J Physiol Regul Integr Comp Physiol.* 2001;281(1):R291-301.

13. Clyman RI, Campbell D, Heymann MA, Mauray F. Persistent responsiveness of the neonatal ductus arteriosus in immature lambs: a possible cause for reopening of patent ductus arteriosus after indomethacin-induced closure. *Circulation*. 1985;71(1):141-5.
14. Clyman RI, Mauray F, Wong L, Heymann MA, Rudolph AM. The developmental response of the ductus arteriosus to oxygen. *Biol Neonate*. 1978;34:177-91.
15. Weiss H, Cooper B, Brook M, Schlueter M, Clyman R. Factors determining reopening of the ductus arteriosus after successful clinical closure with indomethacin. *J Pediatr*. 1995;127(3):466-71.
16. Liu H, Manganiello V, Waleh N, Clyman RI. Expression, activity, and function of phosphodiesterases in the mature and immature ductus arteriosus. *Pediatr Res*. 2008;64(5):477-81.
17. Seidner SR, Chen YQ, Oprysko PR, Mauray F, Tse MM, Lin E, et al. Combined prostaglandin and nitric oxide inhibition produces anatomic remodeling and closure of the ductus arteriosus in the premature newborn baboon. *Pediatr Res*. 2001;50(3):365-73.
18. Hammerman C, Kaplan M. Comparative tolerability of pharmacological treatments for patent ductus arteriosus. *Drug Saf*. 2001;24(7):537-51.
19. Clyman RI, Mauray F, Roman C, Heymann MA, Ballard PL, Rudolph AM, et al. Effects of antenatal glucocorticoid administration on ductus arteriosus of preterm lambs. *Am J Physiol*. 1981;241(3):H415-20.
20. Clyman RI, Ballard PL, Sniderman S, Ballard RA, Roth R, Heymann MA, et al. Prenatal administration of betamethasone for prevention of patent ductus arteriosus. *J Pediatr*. 1981;98(1):123-6.
21. Gonzalez A, Sosenko IR, Chandar J, Hummler H, Claure N, Bancalari E. Influence of infection on patent ductus arteriosus and chronic lung disease in premature infants weighing 1000 grams or less. *J Pediatr*. 1996;128(4):470-8.
22. Lamont RF, Rose M, Elder MG. Effect of bacterial products on prostaglandin E production by amnion cells. *Lancet*. 1985;2(8468):1331-3.
23. Fletcher JR. The role of prostaglandins in sepsis. *Scand J Infect Dis Suppl*. 1982;31:55-60.
24. Ho SY, Anderson RH. Anatomical closure of the ductus arteriosus: a study in 35 specimens. *J Anat*. 1979;128(Pt 4):829-36.
25. Nora JJ. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases. The genetic-environmental interaction. *Circulation*. 1968;38(3):604-17.
26. Cassels DE, Bharati S, Lev M. The natural history of the ductus arteriosus in association with other congenital heart defects. *Perspect Biol Med*. 1975;18(4):541-72.



27. Reller MD, Ziegler ML, Rice MJ, Solin RC, McDonald RW. Duration of ductal shunting in healthy preterm infants: an echocardiographic color flow Doppler study. *J Pediatr.* 1988;112:441-6.
28. Forsey JT, Elmasry OA, Martin RP. Patent arterial duct. *Orphanet J Rare Dis.* 2009;4:17.
29. The Vermont-Oxford Trials Network: very low birth weight outcomes for 1990. Investigators of the Vermont-Oxford Trials Network Database Project. *Pediatrics.* 1993;91(3):540-5.
30. Koch J, Hensley G, Roy L, Brown S, Ramaciotti C, Rosenfeld CR. Prevalence of spontaneous closure of the ductus arteriosus in neonates at a birth weight of 1000 grams or less. *Pediatrics.* 2006;117(4):1113-21.
31. Evans NJ, Archer LN. Postnatal circulatory adaptation in healthy term and preterm neonates. *Arch Dis Child.* 1990;65(1 Spec No):24-6.
32. Dudell GG, Gersony WM. Patent ductus arteriosus in neonates with severe respiratory disease. *J Pediatr.* 1984;104(6):915-20.
33. Osborn DA, Evans N, Kluckow M. Effect of early targeted indomethacin on the ductus arteriosus and blood flow to the upper body and brain in the preterm infant. *Arch Dis Child Fetal Neonatal Ed.* 2003;88(6):F477-82.
34. Noori S, McCoy M, Friedlich P, Bright B, Gottipati V, Seri I, et al. Failure of ductus arteriosus closure is associated with increased mortality in preterm infants. *Pediatrics.* 2009;123(1):e138-44.
35. Campbell M. Causes of malformations of the heart. *Br Med J.* 1965;2(5467):895-904.
36. Wilkins JL. Risks of offspring of patients with patent ductus arteriosus. *J Med Genet.* 1969;6(1):1-4.
37. Rudolph AM, Heymann MA. The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circ Res.* 1967;21(2):163-84.
38. Hagen PT, Scholz DG, Edwards WD. Incidence and size of patent foramen ovale during the first 10 decades of life: an autopsy study of 965 normal hearts. *Mayo Clin Proc.* 1984;59(1):17-20.
39. Krovetz LJ, Goldbloom S. Normal standards for cardiovascular data. II. Pressure and vascular resistances. *Johns Hopkins Med J.* 1972;130(3):187-95.
40. Lewis AB, Heymann MA, Rudolph AM. Gestational changes in pulmonary vascular responses in fetal lambs in utero. *Circ Res.* 1976;39(4):536-41.
41. Campbell M. Natural history of persistent ductus arteriosus. *Br Heart J.* 1968;30(1):4-13.
42. Wojciak-Stothard B, Haworth SG. Perinatal changes in pulmonary vascular endothelial function. *Pharmacol Ther.* 2006;109(1-2):78-91.

- 43.Morganroth ML, Reeves JT, Murphy RC, Voelkel NF. Leukotriene synthesis and receptor blockers block hypoxic pulmonary vasoconstriction. *J Appl Physiol*. 1984;56(5):1340-6.
- 44.Lapointe A, Barrington KJ. Pulmonary hypertension and the asphyxiated newborn. *J Pediatr*.158(2 Suppl):e19-24.
45. Kluckow M, Evans N. Ductal shunting, high pulmonary blood flow, and pulmonary hemorrhage. *J Pediatr*. 2000 Jul;137(1):68-72.
- 46.Robertson B. Surfactant inactivation and surfactant therapy in acute respiratory distress syndrome (ARDS). *Monaldi Arch Chest Dis*. 1998;53(1):64-9.
- 47.Rojas MA, Gonzalez A, Bancalari E, Claure N, Poole C, Silva-Neto G. Changing trends in the epidemiology and pathogenesis of neonatal chronic lung disease. *J Pediatr*. 1995;126(4):605-10.
- 48.Marshall DD, Kotelchuck M, Young TE, Bose CL, Kruyer L, O'Shea TM. Risk factors for chronic lung disease in the surfactant era: a North Carolina population-based study of very low birth weight infants. *North Carolina Neonatologists Association. Pediatrics*. 1999;104(6):1345-50.
- 49.Oh W, Poindexter BB, Perritt R, Lemons JA, Bauer CR, Ehrenkranz RA, et al. Association between fluid intake and weight loss during the first ten days of life and risk of bronchopulmonary dysplasia in extremely low birth weight infants. *J Pediatr*. 2005;147(6):786-90.
50. Jim WT, Chiu NC, Chen MR, Hung HY, Kao HA, Hsu CH. Cerebral hemodynamic change and intraventricular hemorrhage in very low birth weight infants with patent ductus arteriosus. *Ultrasound Med Biol*. 2005;31:197-202.
51. Baptista MJ, Correia-Pinto J, Areias JC, Guimaraes H. Patent ductus arteriosus in neonatal intensive care. *Rev Port Cardiol*. 1999;18:1095-100.
- 52.Bertino E, Giuliani F, Prandi G, Coscia A, Martano C, Fabris C. Necrotizing enterocolitis: risk factor analysis and role of gastric residuals in very low birth weight infants. *J Pediatr Gastroenterol Nutr*. 2009;48(4):437-42.
- 53.Baylen BG, Ogata H, Oguchi K, Ikegami M, Jacobs H, Jobe A, et al. The contractility and performance of the preterm left ventricle before and after early patent ductus arteriosus occlusion in surfactant-treated lambs. *Pediatr Res*. 1985;19(10):1053-8.
- 54.Shimada S, Kasai T, Hoshi A, Murata A, Chida S. Cardiocirculatory effects of patent ductus arteriosus in extremely low-birth-weight infants with respiratory distress syndrome. *Pediatr Int*. 2003;45(3):255-62.
- 55.Kupferschmid C, Lang D, Pohlandt F. Sensitivity, specificity and predictive value of clinical findings, m-mode echocardiography and continuous-wave Doppler sonography in the

- diagnosis of symptomatic patent ductus arteriosus in preterm infants. *Eur J Pediatr*. 1988;147(3):279-82.
- 56.Friedman WF. The intrinsic physiologic properties of the developing heart. *Prog Cardiovasc Dis*. 1972;15(1):87-111.
- 57.Appleton RS, Graham TP, Jr., Cotton RB, Moreau GA, Boucek RJ, Jr. Altered early left ventricular diastolic cardiac function in the premature infant. *Am J Cardiol*. 1987;59(15):1391-4.
- 58.Way GL, Pierce JR, Wolfe RR, McGrath R, Wiggins J, Merenstein GB. ST depression suggesting subendocardial ischemia in neonates with respiratory distress syndrome and patent ductus arteriosus. *J Pediatr*. 1979;95(4):609-11.
- 59.Schneider DJ, Moore JW. Patent ductus arteriosus. *Circulation*. 2006;114(17):1873-82.
- 60.Hoffman JJ, Buckberg GD. Regional myocardial ischemia--causes, prediction and prevention. *Vasc Surg*. 1974;8(2):115-30.
- 61.Espino-Vela J, Cardenas N, Cruz R. Patent ductus arteriosus. With special reference to patients with pulmonary hypertension. *Circulation*. 1968;38(1 Suppl):45-60.
- 62.Thilen U, Astrom-Olsson K. Does the risk of infective endarteritis justify routine patent ductus arteriosus closure? *Eur Heart J*. 1997;18(3):503-6.
- 63.Lloyd TR, Beekman RH, 3rd. Clinically silent patent ductus arteriosus. *Am Heart J*. 1994;127(6):1664-5.
64. Roberson DA, Silverman NH. Color Doppler flow mapping of the patent ductus arteriosus in very low birthweight neonates: echocardiographic and clinical findings. *Pediatr Cardiol*. 1994;15(5):219-24.
- 65.Merritt TA, DiSessa TG, Feldman BH, Kirkpatrick SE, Gluck L, Friedman WF. Closure of the patent ductus arteriosus with ligation and indomethacin: a consecutive experience. *J Pediatr*. 1978;93(4):639-46.
- 66.Rudolph AM, Heyman MA. Fetal and neonatal circulation and respiration. *Annu Rev Physiol*. 1974;36:187-207.
- 67.Skinner JR, Boys RJ, Hunter S, Hey EN. Pulmonary and systemic arterial pressure in hyaline membrane disease. *Arch Dis Child*. 1992;67(4 Spec No):366-73.
- 68.Thibeault DW, Emmanouilides GC, Nelson RJ, Lachman RS, Rosengart RM, Oh W. Patent ductus arteriosus complicating the respiratory distress syndrome in preterm infants. *J Pediatr*. 1975;86(1):120-6.
- 69.Zanardo V, Trevisanuto D, Dani C, Milanesi O, Secchieri S, Guglielmi A, et al. "Silent" patent ductus arteriosus and bronchopulmonary dysplasia in low birth weight infants. *J Perinat Med*. 1995;23(6):493-9.

70. Reller MD, Buffkin DC, Colasurdo MA, Rice MJ, McDonald RW. Ductal patency in neonates with respiratory distress syndrome. A randomized surfactant trial. *Am J Dis Child*. 1991;145(9):1017-20.
71. Kluckow M, Evans N. Low superior vena cava flow and intraventricular haemorrhage in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2000 May;82(3):F188-94.
72. Ratner I, Perelmutter B, Toews W, Whitfield J. Association of low systolic and diastolic blood pressure with significant patent ductus arteriosus in the very low birth weight infant. *Crit Care Med*. 1985;13(6):497-500.
73. Mellander M, Sabel KG, Mellgren G. Single Doppler detection of left to right shunt through the ductus arteriosus. *Acta Paediatr Scand Suppl*. 1986;329:98-102.
74. Groves AM, Kuschel CA, Knight DB, Skinner JR. Cardiorespiratory stability during echocardiography in preterm infants. *Arch Dis Child*. 2005;90(1):86-7.
75. Gentile R, Stevenson G, Dooley T, Franklin D, Kawabori I, Pearlman A. Pulsed Doppler echocardiographic determination of time of ductal closure in normal newborn infants. *J Pediatr*. 1981;98(3):443-8.
76. Smallhorn JF, Gow R, Olley PM, Freedom RM, Swyer PR, Perlman M, et al. Combined noninvasive assessment of the patent ductus arteriosus in the preterm infant before and after indomethacin treatment. *Am J Cardiol*. 1984;54(10):1300-4.
77. Skelton R, Evans N, Smythe J. A blinded comparison of clinical and echocardiographic evaluation of the preterm infant for patent ductus arteriosus. *J Paediatr Child Health*. 1994;30(5):406-11.
78. Ohuchi H, Takasugi H, Ohashi H, Okada Y, Yamada O, Ono Y, et al. Stratification of pediatric heart failure on the basis of neurohormonal and cardiac autonomic nervous activities in patients with congenital heart disease. *Circulation*. 2003;108(19):2368-76.
79. Law YM, Hoyer AW, Reller MD, Silberbach M. Accuracy of plasma B-type natriuretic peptide to diagnose significant cardiovascular disease in children: the Better Not Pout Children! Study. *J Am Coll Cardiol*. 2009;54(15):1467-75.
80. Holmstrom H, Hall C, Thaulow E. Plasma levels of natriuretic peptides and hemodynamic assessment of patent ductus arteriosus in preterm infants. *Acta Paediatr*. 2001;90(2):184-91.
81. Heymann MA, Rudolph AM, Silverman NH. Closure of the ductus arteriosus in premature infants by inhibition of prostaglandin synthesis. *N Engl J Med*. 1976;295(10):530-3.
82. Mason CA, Bigras JL, O'Blenes SB, Zhou B, McIntyre B, Nakamura N, et al. Gene transfer in utero biologically engineers a patent ductus arteriosus in lambs by arresting fibronectin-dependent neointimal formation. *Nat Med*. 1999;5(2):176-82.
83. Mason CA, Chang P, Fallery C, Rabinovitch M. Nitric oxide mediates LC-3-dependent regulation of fibronectin in ductus arteriosus intimal cushion formation. *FASEB J*. 1999;13(11):1423-34.

84. Thomas RL, Parker GC, Van Overmeire B, Aranda JV. A meta-analysis of ibuprofen versus indomethacin for closure of patent ductus arteriosus. *Eur J Pediatr*. 2005;164(3):135-40.
85. Friedman WF, Hirschklau MJ, Printz MP, Pitlick PT, Kirkpatrick SE. Pharmacologic closure of patent ductus arteriosus in the premature infant. *N Engl J Med*. 1976;295(10):526-9.
86. Siassi B, Blanco C, Cabal LA, Coran AG. Incidence and clinical features of patent ductus arteriosus in low-birthweight infants: a prospective analysis of 150 consecutively born infants. *Pediatrics*. 1976;57(3):347-51.
87. Gersony WM, Peckham GJ, Ellison RC, Miettinen OS, Nadas AS. Effects of indomethacin in premature infants with patent ductus arteriosus: results of a national collaborative study. *J Pediatr*. 1983;102(6):895-906.
88. Van Overmeire B, Smets K, Lecoutere D, Van de Broek H, Weyler J, Degroote K, et al. A comparison of ibuprofen and indomethacin for closure of patent ductus arteriosus. *N Engl J Med*. 2000;343(10):674-81.
89. Tatli MM, Kumral A, Duman N, Demir K, Gurcu O, Ozkan H. Spontaneous intestinal perforation after oral ibuprofen treatment of patent ductus arteriosus in two very-low-birthweight infants. *Acta Paediatr*. 2004;93(7):999-1001.
90. Attridge JT, Clark R, Walker MW, Gordon PV. New insights into spontaneous intestinal perforation using a national data set: (1) SIP is associated with early indomethacin exposure. *J Perinatol*. 2006;26(2):93-9.
91. Gournay V, Savagner C, Thiriez G, Kuster A, Roze JC. Pulmonary hypertension after ibuprofen prophylaxis in very preterm infants. *Lancet*. 2002;359(9316):1486-8.
92. Ahlfors CE. Effect of ibuprofen on bilirubin-albumin binding. *J Pediatr*. 2004;144(3):386-8.
93. Christmann V, Liem KD, Semmekrot BA, van de Bor M. Changes in cerebral, renal and mesenteric blood flow velocity during continuous and bolus infusion of indomethacin. *Acta Paediatr*. 2002;91:440-6.
94. Hammerman C. Patent ductus arteriosus. Clinical relevance of prostaglandins and prostaglandin inhibitors in PDA pathophysiology and treatment. *Clin Perinatol*. 1995;22(2):457-79.
95. Van Overmeire B, Allegaert K, Casaer A, Debauche C, Decaluwe W, Jespers A, et al. Prophylactic ibuprofen in premature infants: a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 2004; 364:1945-9.
96. Sahn DJ, Vaucher Y, Williams DE, Allen HD, Goldberg SJ, Friedman WF. Echocardiographic detection of large left to right shunts and cardiomyopathies in infants and children. *Am J Cardiol*. 1976;38(1):73-9.

- 97.Clyman RI. Recommendations for the postnatal use of indomethacin: an analysis of four separate treatment strategies. *J Pediatr*. 1996;128(5 Pt 1):601-7.
- 98.Palder SB, Schwartz MZ, Tyson KR, Marr CC. Management of patent ductus arteriosus: a comparison of operative v pharmacologic treatment. *J Pediatr Surg*. 1987;22(12):1171-4.
- 99.Malviya M, Ohlsson A, Shah S. Surgical versus medical treatment with cyclooxygenase inhibitors for symptomatic patent ductus arteriosus in preterm infants. *Cochrane Database Syst Rev*. 2008(1):CD003951.
- 100.Seghaye MC, Grabitz R, Alzen G, Trommer F, Hornchen H, Messmer BJ, et al. Thoracic sequelae after surgical closure of the patent ductus arteriosus in premature infants. *Acta Paediatr*. 1997;86(2):213-6.
- 101.Burke RP, Jacobs JP, Cheng W, Trento A, Fontana GP. Video-assisted thoracoscopic surgery for patent ductus arteriosus in low birth weight neonates and infants. *Pediatrics*. 1999;104(2 Pt 1):227-30.
- 102.Mosalli R, Alfaleh K, Paes B. Role of prophylactic surgical ligation of patent ductus arteriosus in extremely low birth weight infants: Systematic review and implications for clinical practice. *Ann Pediatr Cardiol*. 2009;2(2):120-6.
- 103.Moore JW, Schneider DJ, Dimeglio D. The duct-occlud device: design, clinical results, and future directions. *J Interv Cardiol*. 2001;14(2):231-7.
- 104.Roberts P, Adwani S, Archer N, Wilson N. Catheter closure of the arterial duct in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2007;92(4):F248-50.
- 105.Rao PS, Gupta ML, Balaji S. Recent advances in pediatric cardiology--electrophysiology, transcatheter and surgical advances. *Indian J Pediatr*. 2003;70(7):557-64.
- 106.Onji K, Matsuura W. Pulmonary endarteritis and subsequent pulmonary embolism associated with clinically silent patent ductus arteriosus. *Intern Med*. 2007;46(19):1663-7.
- 107.Giroud JM, Jacobs JP. Evolution of strategies for management of the patent arterial duct. *Cardiol Young*. 2007;17 Suppl 2:68-74.
- 108.de Oliveira CM, Sakata RK, Issy AM, Gerola LR, Salomao R. Cytokines and pain. *Rev Bras Anesthesiol*. 61(2):255-65.
- 109.Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J Endotoxin Res*. 2004;10(4):201-22.
- 110.Sehra S, Yao Y, Howell MD, Nguyen ET, Kansas GS, Leung DY, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol*. 184(6):3186-90.
- 111.Hartemink KJ, Groeneveld AB. The hemodynamics of human septic shock relate to circulating innate immunity factors. *Immunol Invest*. 39(8):849-62.

- 112.Hansen-Pupp I, Harling S, Berg AC, Cilio C, Hellstrom-Westas L, Ley D. Circulating interferon-gamma and white matter brain damage in preterm infants. *Pediatr Res*. 2005;58(5):946-52.
- 113.Mathew R. Inflammation and pulmonary hypertension. *Cardiol Rev*.18(2):67-72.
- 114.Glauser FL, DeBlois GG, Bechard DE, Merchant RE, Grant AJ, Fowler AA, et al. Cardiopulmonary effects of recombinant interleukin-2 infusion in sheep. *J Appl Physiol*. 1988;64(3):1030-7.
- 115.Hitti J, Krohn MA, Patton DL, Tarczy-Hornoch P, Hillier SL, Cassen EM, et al. Amniotic fluid tumor necrosis factor-alpha and the risk of respiratory distress syndrome among preterm infants. *Am J Obstet Gynecol*. 1997;177(1):50-6.
- 116.Tacy TA. Abnormalities of the ductus arteriosus and pulmonary arteries. In: Lai WL, Mertens LL, Cohen MS, Geva T, eds. *Echocardiography in Padiatrics and Congenital Heart Disease*. West Sussex, UK: Wiley-Blackwell; 2009:283-296.
- 117.Kluckow M, Evans N. Early echocardiographic prediction of symptomatic patent ductus arteriosus in preterm infants undergoing mechanical ventilation. *J Pediatr*. 1995;127(5):774-9.
- 118.Su BH,Watanabe T, Shimizu M,Yanagisawa M. Echocardiographic assessment of patent ductus arteriosus shunt flow pattern in premature infants. *Arch Dis Child Fetal Neonatal Ed*. 1997 Jul; 77(1):F36-40.
- 119.Walther FJ, Kim DH, Ebrahimi M, Siassi B. Pulsed Doppler measurement of left ventricular output as early predictor of symptomatic patent ductus arteriosus in very preterm infants. *Biol Neonate*. 1989;56(3):121-8.
- 120.Iyer P, Evans N. Re-evaluation of the left atrial to aortic root ratio as a marker of patent ductus arteriosus. *Arch Dis Child Fetal Neonatal Ed*. 1994;70(2):F112-7.
- 121.Mellander M, Larsson LE, Ekstrom-Jodal B, Sabel KG. Prediction of symptomatic patent ductus arteriosus in preterm infants using Doppler and M-mode echocardiography. *Acta Paediatr Scand*. 1987;76(4):553-9.
- 122.Czernik C, Lemmer J, Metze B, Koehne PS, Mueller C, Obladen M. B-type natriuretic peptide to predict ductus intervention in infants <28 weeks. *Pediatr Res*. 2008;64(3):286-90.
- 123.Choi BM, Lee KH, Eun BL, Yoo KH, Hong YS, Son CS, et al. Utility of rapid B-type natriuretic peptide assay for diagnosis of symptomatic patent ductus arteriosus in preterm infants. *Pediatrics*. 2005;115(3):e255-61.
- 124.Nuntnarumit P, Khositseth A, Thanomsingh P. N-terminal probrain natriuretic peptide and patent ductus arteriosus in preterm infants. *J Perinatol*. 2009;29(2):137-42.
- 125.Van Bel F, Guit GL, Schipper J, van de Bor M, Baan J. Indomethacin-induced changes in renal blood flow velocity waveform in premature infants investigated with color Doppler imaging. *J Pediatr*. 1991;118(4 Pt 1):621-6.

126. Van Bel F, Van de Bor M, Stijnen T, Baan J, Ruys JH. Cerebral blood flow velocity changes in preterm infants after a single dose of indomethacin: duration of its effect. *Pediatrics*. 1989;84(5):802-7.
127. Van Bel F, Van Zoeren D, Schipper J, Guit GL, Baan J. Effect of indomethacin on superior mesenteric artery blood flow velocity in preterm infants. *J Pediatr*. 1990;116(6):965-70.
128. Ment LR, Oh W, Ehrenkranz RA, Philip AG, Vohr B, Allan W, et al. Low-dose indomethacin and prevention of intraventricular hemorrhage: a multicenter randomized trial. *Pediatrics*. 1994;93(4):543-50.
129. Kabra NS, Schmidt B, Roberts RS, Doyle LW, Papile L, Fanaroff A. Neurosensory impairment after surgical closure of patent ductus arteriosus in extremely low birth weight infants: results from the Trial of Indomethacin Prophylaxis in Preterms. *J Pediatr*. 2007;150(3):229-34, 34 e1.
130. Vanhaesebrouck S, Zonnenberg I, Vandervoort P, Bruneel E, Van Hoestenbergh MR, Theyskens C. Conservative treatment for patent ductus arteriosus in the preterm. *Arch Dis Child Fetal Neonatal Ed*. 2007;92(4):F244-7.
131. Bennhagen RG, Benson LN. Silent and audible persistent ductus arteriosus: an angiographic study. *Pediatr Cardiol*. 2003;24(1):27-30.
132. Vedovato S, Zanardo V. [Chorioamnionitis and inflammatory disease in the premature newborn infant]. *Minerva Pediatr*. 62(3 Suppl 1):155-6.
133. Dammann O, Phillips TM, Allred EN, O'Shea TM, Paneth N, Van Marter LJ, et al. Mediators of fetal inflammation in extremely low gestational age newborns. *Cytokine*. 2001;13(4):234-9.
134. Hamrick SE, Hansmann G. Patent ductus arteriosus of the preterm infant. *Pediatrics*. 125(5):1020-30.