Assessment of Systemic Blood Flow in the Newborn Infant

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Medicine, The University of Auckland, 2006.

Abstract

Preterm infants are vulnerable to brain injury which is thought to be caused partly by abnormalities in cerebral perfusion. However the accurate assessment of cerebral and general systemic perfusion remains a challenge in the newborn infant. Commonly used clinical parameters such as blood pressure and blood lactate concentrations are imperfect predictors of blood flow. Cardiac output measurements used in older children do not reflect true systemic perfusion in the neonate due to shunting of blood through persisting fetal pathways. Echocardiographic measurements of descending aortic (DAo) and superior vena caval (SVC) blood flow may provide more reliable assessment of neonatal systemic perfusion. This thesis evaluates these techniques in the first days of postnatal life.

Measures of flow volume in the SVC and DAo were found to be feasible in the vast majority of infants, and were performed without significantly affecting cardiorespiratory status. Assessment of SVC flow volume showed similar repeatability to other measures of blood flow in neonates when assessed by a single observer, as did assessment of velocity of flow in the DAo.

We then used these techniques to further assess the transitional circulation, and found no evidence of a positive association between arterial blood pressure and volume of systemic perfusion. Contrary to previous assumptions that ductal shunting compromises systemic perfusion, we found that left ventricular output tended to increase with increasing shunt through the ductus arteriosus, thereby maintaining upper, though not necessarily lower, body perfusion.

There was an association between very low levels of flow in individuals and some adverse outcomes that had a strong circulatory component to their pathophysiology (periventricular haemorrhage and necrotising enterocolitis). However low blood flow in the SVC or DAo did not predict poor outcome within the entire cohort.

Assessments of SVC and DAo flow in the neonate are feasible, relatively repeatable and have already enhanced our understanding of the pathophysiology of the transitional circulation. These and other techniques to monitor systemic blood flow in the neonate may aid identification of circulatory failure, act as short-term endpoints in clinical trials of interventions supporting the circulation, and eventually improve neurodevelopmental outcome in preterm infants.

Acknowledgements

I am extremely grateful to my supervisors who provided invaluable support and guidance while writing this thesis. Carl Kuschel gave me the opportunity to come to Auckland, showed consistent enthusiasm for the project, and even allowed me free time by performing some of the echocardiograms. Professor Jane Harding showed boundless patience both in reading multiple drafts of this thesis and in steering me through my early statistical encounters; her wise words were always well chosen. Jon Skinner has an incredible understanding of haemodynamics, took interpretation of the data to a whole additional level, and was never shy in telling me when I was coming up short!

The staff at National Women's in Auckland were incredibly supportive throughout the duration of the study; recruitment would have been impossible without the input of the consultants, registrars and nurse practitioners, and my sanity would have been compromised without the friendship and support of Claire West. The unit nursing staff acted as strong advocates for the infants studied, but also showed great patience and good humour.

I am also most grateful to the infants who took part in the study, and to their parents for granting consent at what is always a very stressful time.

Financial support was provided by The Southern Trust, via the Starship Foundation, to whom I am deeply indebted. I hope they are pleased with the outcome of the project.

My sincere thanks go to all the staff at Sunderland Royal Hospital where I undertook my initial registrar training. My time on the neonatal unit there cemented my passion for neonatology. I would especially like to thank Majd Abu Harb whose outstanding

echocardiographic skills I have tried to emulate, and to Sam Richmond whose example as an inspiring, intelligent and complete neonatologist I will always try to follow.

This thesis is dedicated to my family and in particular to my parents who have always been there for me. Likewise to my brother, sister and my friends both in New Zealand and the UK, who helped me keep my ‘work life balance’.

Preface

The incidence of premature birth in industrialised nations shows no signs of decreasing(1). While survival rates have improved dramatically in recent decades there is little or no evidence for similar improvements in neurodevelopmental outcome(2). Many of the causes of adverse neurodevelopmental outcome are poorly understood. However there is increasing evidence that circulatory factors may play a critical role in the pathophysiology of brain injuries in preterm infants(3).

The circulation of the newborn infant undergoes a transition from the fetal to the adult pattern in early extra-uterine life. In the fetus the ductus arteriosus carries deoxygenated blood from the pulmonary artery away from the high resistance pulmonary circulation towards the placenta for oxygenation. Oxygenated blood returning to the right atrium from the placenta is diverted by the foramen ovale towards the brain and upper body via the left atrium, left ventricle and ascending aorta. The central features of the transitional circulation are the removal of the low resistance placental circulation by clamping of the umbilical cord, and an abrupt increase in pulmonary blood flow due to falling pulmonary artery pressure as the lungs take on the role of gaseous exchange. In term infants the fetal shunt pathways of the ductus arteriosus and foramen ovale are generally functionally closed within 24 hours of birth(4).

In term infants the changes occurring in the transitional circulation are rapid and lead to significant increases in cardiac output to match metabolic demand(4). In the extremely preterm infant the heart, like many other organs, may not be adequately prepared for the rigours of extra-uterine life. The immature myocardium may be less able to contract against the increase in vascular resistance produced by removal of the low resistance

placental bed(5). The fall in pulmonary vascular resistance is often delayed(6), as is functional closure of the fetal shunt pathways(4). Severe respiratory disease and the requirement for mechanical ventilation may further impair cardiac function in preterm infants(5).

Attempts to monitor cardiac function during this transitional period in preterm infants are hindered by the persistence of the fetal shunt pathways. Measurements of left and right ventricular output are useful in assessing systemic perfusion in older children and adults. However in the presence of shunting through the ductus arteriosus and foramen ovale, neither left nor right ventricular output assesses the volume of blood actually reaching the tissues.

In the absence of reliable guides to systemic perfusion, clinicians have limited ability to detect circulatory failure in preterm infants. Furthermore, even if circulatory failure was to be detected, the optimal treatment required to support the circulation is unclear, since adequacy of perfusion cannot easily be assessed as an outcome measure in clinical trials.

The work described in this thesis was prompted by a series of journal articles published in 2000-2001. These highlighted the vascular component of the pathophysiology of preterm brain injury(7), the importance of provision of appropriate cardiovascular monitoring and support to preterm infants(4, 8) and the potential for superior vena cava (SVC) flow to be measured as a marker of systemic perfusion that was unaffected by fetal shunt pathways(9) and that predicted subsequent brain injury(10).

We elected to use echocardiography to study a cohort of preterm infants born at the National Women's Hospital, Auckland, New Zealand. By studying a large cohort of

preterm infants we aimed to evaluate the feasibility of using echocardiography to make repeated haemodynamic measures in the early postnatal period in preterm infants.

A principle objective of the study was to further examine the utility of the technique of measurement of SVC flow volume. It was important to establish whether this measure was feasible and reproducible in the hands of a group of researchers distinct from those who first described the technique. As volume of descending aorta (DAo) flow is increasingly being monitored in paediatric(11) and adult(12) intensive care units, we also undertook the first systematic evaluation of this technique in preterm infants to assess its suitability as a further marker of systemic perfusion unaffected by fetal shunt pathways.

We aimed to assess the safety of these echocardiographic techniques, to establish reference ranges for SVC and DAo flow, and to carefully assess the repeatability of the measurements.

To allow interpretation of the patterns of SVC and DAo flow volume in the unique context of the transitional circulation we also quantified left and right ventricular outputs, assessed ductal and atrial shunt patterns and quantified arterial blood pressure. We were particularly interested in whether findings from our cohort of infants studied during the transition from the fetal to the adult circulations would provide further insights into the relationship between arterial blood pressure and systemic blood flow, and the impact of shunting through the fetal channels on adequacy of systemic perfusion during this critical time.

Finally, and perhaps most importantly, we hoped to further assess the association between markers of blood flow and outcome following preterm birth, particularly the association between early low SVC flow and subsequent brain injury. Specifically we

aimed to examine whether use of echocardiographic markers of perfusion in clinical practice could add to the information obtained through the currently routine practice of monitoring of arterial blood pressure.

The aim of the research described in this thesis is above all to increase awareness and understanding of the pathophysiology of the transitional circulation. In the future we hope to continue working to improve cardiovascular monitoring and support in preterm infants with the goal of improving long term neurodevelopmental outcome.

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1 Literature Review

1.1.1 Incidence of preterm birth

Preterm birth remains the most important cause of perinatal mortality in industrialised nations(13). Despite increasing focus on the prevention of preterm birth, its incidence continues to rise. Incidence of delivery prior to 32 weeks gestation increased by 8% between 1981 and 1999 in the USA, and by 20% in Canada between 1978 and 1996(14). In the UK incidence of delivery at the extremes of prematurity (22-27 weeks gestation) increased by 32% between 1983 and 1994(15). Incidence of delivery at very low birth weight (VLBW, <1500g) is also increasing. The Vermont Oxford Network Database recorded a 24% increase in NICU admissions of infants weighing 501-1500g between 1991 and 1999(1).

In New Zealand the incidence of prematurity mirrors that seen elsewhere, with 1.1% of all live births occurring prior to 32 weeks gestation, and 0.96% having birth weights of <1500g in 1998-1999(16). In absolute numbers this equates to approximately 620 infants/year of <32 weeks, or 540 infants/year of <1500g throughout New Zealand.

1.1.2 Mortality rates in preterm birth

There is no doubt that survival rates following preterm birth have improved dramatically in the last 25 years, though improvements in neurodevelopmental outcome are less impressive, as will be discussed in section 1.1.3. Surprisingly only a few studies have looked at outcome in comparable cohorts of infants over time. Data from Victoria, Australia in VLBW infants showed survival increasing from 25% to 73%

between 1979 and 1997(17). Data from the UK Northern Neonatal Network show that survival to one year post-delivery in infants of 24-27 weeks gestation increased from 37.8% to 49.2% between 1983 and 1994(15). Similar degrees of improvement were seen in the North West of England(18). Data from the United States also show improvements in survival for VLBW infants, survival increasing from 68.4% to 81.6% of live births between 1989 and 1995(19).

Recent data from a cohort of infants born in New Zealand in 1998-1999 show that survival to discharge home (as a proportion of live births) is 72% at 24-25 weeks, 87% at 26-27 weeks and 95% at 28-29 weeks(16).

1.1.3 Morbidity rates in preterm birth

The long-term adverse sequelae of extreme preterm birth are often severe. The major morbidities are chronic lung disease and cerebral insults including periventricular haemorrhage (PVH) and periventricular leukomalacia (PVL). The pathophysiology of PVL and PVH will be discussed in sections 1.1.4 and 1.1.5, but their combined effects are principally responsible for a range of neurodevelopmental disabilities, including impaired mental development, cerebral palsy, blindness and deafness.

The incidence of chronic lung disease has shown signs of improvement over the last 2 decades, both in the United States(20) and in New Zealand(21), though rates remain high with 15-25% of surviving VLBW infants being affected. Although the functions of the cardiac and respiratory systems are inextricably linked and abnormal postnatal pulmonary perfusion may inhibit alveolisation(22), this thesis will focus on assessment of systemic perfusion rather than its relationship to chronic lung disease.

Extensive data on neurodevelopmental outcome following preterm birth have been produced by the National Institute of Child Health and Human Development (NICHD)

Neonatal Research Network. They evaluated a cohort of 1,151 babies born at extremely low birth weight (≤1,000g) for neurodevelopmental and functional outcomes at 18 to 22 months corrected age(23). In these babies born in 1993-94 they found an overall incidence of abnormal neurodevelopmental or sensory findings of 49%. Rates for specific abnormalities were abnormal neurological examination 25%, cerebral palsy 17%, seizures 5%, vision impairment 9% and hearing impairment 11%. Thirty seven per cent of children had a Bayley II mental development index (MDI) of <70. Twenty nine per cent had a psychomotor development index (PDI) of <70.

There is little evidence that rates of neurodevelopmental deficit are decreasing significantly in survivors of preterm birth. Rates of cerebral palsy, developmental delay and disability have been shown to be static in Australia between 1979 and 1997(17). These figures are presented as a proportion of survivors and since survival rates are increasing the absolute numbers of infants surviving preterm birth with disability must be increasing. Likewise the absolute number of infants surviving preterm birth without disability must also be increasing.

In a relatively recent review of neurodevelopmental outcome following extremely preterm birth (≤26 weeks) approximately 25% of surviving extremely preterm infants had at least one major disability (impaired mental development, cerebral palsy, blindness or deafness), with around half of these having more than one major disability(2). A further 50% of survivors with birth weights below 1000g 'will have one or more subtle neurodevelopmental disabilities in the school and teenage years'(2). While more recent improvements in outcome are possible, this disability rate of up to 75% in survivors, combined with the conclusion that there "is little evidence to suggest

that long-term neurodevelopmental outcome has changed from the late 1970s to the early 1990s or with increasing survival", make grim reading.

The improving survival rate following premature delivery, combined with the relatively static morbidity rate emphasises the importance of improving our understanding of the pathophysiology of brain injury in preterm infants in the hope that this can lead to improvements in neurodevelopmental outcome.

1.1.4 Pathophysiology of periventricular haemorrhage

In the NICHD Neonatal Research Network study, severe periventricular haemorrhage (grade 3 or 4) and periventricular leukomalacia were consistently shown to be the strongest and most statistically significant risk factors for low mental and developmental index scores, as well as lack of independent walking and feeding at 18-22 months of age(23).

Periventricular haemorrhage occurs in approximately 25% of VLBW infants, with severe PVH occurring in around 8%(1). A variety of anatomical and physiological factors specific to preterm infants appear to be central to the development of PVH. The subependymal germinal matrix is highly active in the second and early third trimesters of pregnancy producing neuronal precursor cells. For this period of rapid cell production the germinal matrix demands an extensive blood supply. However the structure's blood vessels have relatively poor connective tissue support(24) and the vessel themselves may be structurally immature(25). This temporary arrangement is sufficient in the constancy of the intrauterine environment, but following preterm birth the circulation may be much less stable. Arterial flow to the germinal matrix may be low due to acute episodes of systemic hypotension, high due to hypercarbia, or fluctuating due to respiratory instability, and all of these are associated with increased

risk of PVH(26-28). The critical role of cerebral ischaemia in development of PVH and PVL will be discussed following a background discussion of the techniques available to assess systemic and cerebral perfusion in section 1.3. Further discussion of circulatory instability and PVH/PVL follows in section 1.1.6.

Initial bleeding may be purely subependymal (grade 1), may extend into the lateral ventricle (grade 2), and may then cause ventricular dilatation (grade 3). Parenchymal bleeding extending dorsal and lateral to the lateral ventricle (grade 4) was previously thought to be due to extension through the ventricular lining but is now appreciated to be haemorrhagic venous infarction following obstruction of venous drainage by germinal matrix haemorrhage(3).

While grade 1 and 2 PVH have little predictive value for outcome, combined mortality and morbidity rates are approximately 35% in grade 3 PVH, and approach 100% in grade 4 PVH(24).

1.1.5 Pathophysiology of periventricular leukomalacia

Periventricular leukomalacia includes both focal necrotic and more diffuse patterns of brain injury(7). The overall incidence of PVL is hard to quantify, as only the focal component is visible on ultrasound, with an incidence of between 5 and 15% in VLBW infants(3). Increased use of magnetic resonance imaging may detect more subtle areas of PVL not detected by ultrasonography(29).

As in PVH, a variety of anatomical and physiological factors specific to preterm infants are central to the development of PVL. These have been extensively reviewed by Volpe and colleagues(3, 7), but the major circulatory components are anatomical aspects of the developing cerebrovascular system and immature cerebrovascular

regulation. The impact of any circulatory insults is compounded by the inherent vulnerability of immature neuronal precursors to ischaemic damage(7).

At 24 - 32 weeks gestation both the cortex and white matter are poorly vascularised(25), leaving watershed areas particularly at risk of hypoperfusion(3).

Cerebrovascular autoregulation appears to be impaired in some preterm infants(30), which may lead to critical ischaemia during periods of acute hypotension. There is evidence for and against maintenance of autoregulation in preterm infants, and this will be reviewed in section 1.4.3.

The influence of PVL on neurodevelopmental outcome is not yet fully understood, although focal cystic changes are strongly associated with spastic diplegia(7). There is growing evidence that diffuse patterns of injury are associated with decreased cerebral volume on MRI at term(31) and that the specific patterns of injury are associated with specific long-term impairments(32). This supports the hypothesis that, despite the presence of a normal cranial ultrasound scan, diffuse patterns of PVL may be responsible for cognitive and behavioural deficits which are frequent complications of extreme preterm birth(7).

1.1.6 Relation of circulatory instability to outcome

A proposed mechanism of perinatal cerebral vascular insult from a cycle of hypoperfusion and reperfusion has been suggested for at least a quarter of a century(33). Short term beat-to-beat variability in blood pressure and cerebral blood flow may contribute to cerebral injury as may fluctuating blood flow values over more prolonged periods.

A recent study using near infrared spectroscopy to assess fractional oxygen extraction in preterm infants has suggested that infants with fluctuating patterns of cerebral perfusion in the first 3 postnatal days are more likely to develop PVH than infants with more stable flow patterns(34).

The majority of studies examining the relation of instability to PVH have looked at more short term variability. Perlman and colleagues found that nearly half of VLBW infants had a clearly fluctuating pattern of cerebral blood flow in the anterior cerebral artery, as well as fluctuating arterial blood pressure. They defined a coefficient of variation (CV) of velocity in the anterior cerebral artery of more than 10% as abnormal. 91% of infants with this fluctuating pattern developed PVH, compared to 26% infants with stable (CV <10%) flows. Fluctuating patterns of flow were closely linked to severity of respiratory distress(28).

In a prospective trial of neuromuscular paralysis to prevent PVH in those infants with fluctuating cerebral blood flow velocities, only 1 of 14 treated infants developed PVH while paralysed with pancuronium, compared to all 10 of the untreated controls(35). A subsequent meta-analysis of 3 studies suggested that neuromuscular paralysis reduces the risk of PVH by 46%, though no data were available concerning long-term neurodevelopmental outcome, and paralysis showed no apparent long-term respiratory benefit(36).

In contrast, Bada showed that 28 VLBW infants who sustained PVH had the same mean CV for mean arterial pressure as 72 unaffected infants(37), though the PVH cohort tended to spend a higher proportion of time with periods of high CVs. Miall-Allen and colleagues reported that infants with PVH tended to have lower CVs than healthy controls(38). However no infant in this study had a CV over the 10% which

Perlman considered to be excessive. The authors postulated that the lower CVs may be seen in the sickest infants who were less responsive to stimulation and fought less against mechanical ventilation. It is also important to remember that a degree of variability of haemodynamic status, such as the variability in heart rate seen in CTG monitoring of the fetus in labour, is often a healthy physiological sign.

While it seems sensible to conclude that excessive fluctuation in cerebral blood flow is associated with an increased risk of PVH(39), it is perhaps more important to question why different centres have such disparate rates of circulatory instability. Presumably the many factors associated with instability including ventilatory asynchrony(40), sedation use(41), intubation method(42) and PDA rates(43) must differ between the units, and few would dispute that improving stability of haemodynamics in sick preterm infants is likely to be beneficial(39).

1.1.7 Summary

Rates of premature birth are increasing and survival rates are improving. There are therefore an increasing number of infants vulnerable to long-term neurodevelopmental morbidity following preterm birth, and morbidity rates do not appear to be improving. Periventricular haemorrhage and periventricular leukomalacia are strongly linked to adverse outcome, and both are felt to have a circulatory component to their pathophysiology. Improved understanding of the pathophysiology of these brain lesions, along with improvements in circulatory monitoring and support may lead to improvements in neurodevelopmental outcome following premature birth.

1.2 Role of the ductus arteriosus

The role that shunting of blood through a patent ductus arteriosus may play in compounding any systemic hypoperfusion in the preterm transitional circulation will be a key focus of this thesis.

The presence of a patent ductus arteriosus (PDA) in preterm infants is associated with a wide range of adverse outcomes, including PVH, PVL, chronic lung disease, renal impairment and necrotising enterocolitis. However distinguishing *causal* from *casual* relationships (where it is simply the most unwell infants who develop PDA and other complications of preterm birth) is more difficult, as will de discussed below

1.2.1 Incidence of PDA

Gestation, birth weight, pulmonary disease(44) and possibly antenatal steroid use(45) and postnatal fluid management(44) appear to have an impact on the incidence of PDA. There is no single cut-off of postnatal age at which prolonged patency is considered abnormal in preterm infants, and crucially it is not clear whether ductal shunting in the first postnatal day when patency is still clearly physiological has significant haemodynamic consequences(46). Knight studied 110 infants at National Women's Hospital, Auckland (mean gestation 27.9 weeks and mean birth weight 1120g) and found that 39% had closed their ducts by 72 hours, 26% had small (<1mm) ducts and 34% had larger ducts(44). Phillipos et al similarly found that 34% of ducts had closed by 72 hours in 30 slightly more mature infants(47).

Evans et al examined 54 VLBW infants requiring ventilation for greater than 24 hours and found that 50% of ducts had closed by 72 hours, with a further 15% closing spontaneously thereafter(48). The remaining 35% of ducts became clinically apparent, and were closed by conservative, medical or surgical management(48).

Skinner et al, in a study focusing on pulmonary arterial pressure, found that 75% of ventilator dependent infants (median gestation 30.5 weeks, median birth weight 1651g) had a patent duct at day 4, compared to 6% of healthy controls (no ventilator requirement, median gestation 32 weeks, birth weight 1608g)(6). This study, and work by Reller et al(49) suggest that infants >28 weeks gestation are likely to have prolonged ductal patency only in the presence of significant lung disease. Prior to 28 weeks gestation it is increasingly difficult to find infants without lung disease to act as 'healthy' controls.

Overall, around half of infants born at or before 30 weeks are likely to have prolonged patency of the ductus arteriosus, and these are most likely to become symptomatic in infants with significant respiratory distress. Shunt through the duct on the first day of life (before patency would be considered abnormal in any group of infants) could also have haemodynamic significance in infants with little cardiovascular reserve(46) (See section 1.5.4)

1.2.2 Impact of PDA on blood pressure

Ductal patency is associated with systemic hypotension in preterm infants, even relatively early in the postnatal period. Knight followed 110 infants (mean gestation 27.9 weeks, birth weight 1120g) in the first 3 days of life with echocardiography and intra-arterial blood pressure measurement. He found that infants with a large duct (>2mm minimum diameter on day 3) had lower systolic and diastolic blood pressures from 12 hours of age when compared to those with closed or small ducts(44). The disparity in the blood pressures of the two groups seemed to widen after day 3 of life. These differences in blood pressure could not be accounted for by differences in gestation or birth weight, though infants with patent ducts did have more severe

respiratory disease. Knight concluded that significant left-right shunting was the most likely cause for the lower blood pressures, even in the early postnatal period.

Evans followed 41 VLBW infants with daily echocardiography and hourly intra-arterial blood pressure measurement in the first 5-7 days of life. No differences were found in the blood pressures of infants with birth weights >1,000g with and without ducts. However extremely low birth weight (ELBW) infants with 'haemodynamically significant' ducts (defined as having strong Doppler signals from ductal flow, and left atrial to aortic root ratios of >1.5) had lower mean blood pressures(50). Differences in systolic pressure were small in the first 3 days, but as in Knight's study, again became more marked after 72 hours. Differences in diastolic pressure were significant throughout, becoming larger after 72 hours. Some of these differences could be accounted for by the group with patent ducts being slightly smaller and less mature, as well as having a trend towards more severe respiratory disease as assessed by mean airway pressure(50). The authors hypothesised that infants with normal cardiac function will respond to significant ductal shunting (and therefore increased pre-load) by raising stroke volume and therefore maintain systolic blood pressure. Diastolic blood pressure would fall despite this increased left ventricular function as it depends more on pulmonary vascular resistance than cardiac output. It may be that as the pulmonary arterial pressure continues to fall after the third day of life(6), myocardial reserve can no longer compensate for the increased ductal shunt.

1.2.3 Impact of PDA on pulmonary function

As noted above, proving an association between presence of PDA and adverse neonatal outcomes is straightforward, but proving a causative association is more difficult.

Presence of a PDA is clearly correlated with severity of acute respiratory disease(48), though not necessarily with long-term respiratory outcome. There is biological plausibility behind the concept that PDAs can lead to adverse respiratory outcomes. Significant left to right ductal shunt may increase left atrial pressure and therefore pulmonary capillary hydrostatic pressure. This in turn may increase pulmonary interstitial and alveolar lung fluid, decreasing lung compliance(51) and increasing the risk of haemorrhagic pulmonary oedema(52).

Important evidence for the role of the PDA in the pathogenesis of respiratory distress syndrome could come from therapeutic trials. The majority of studies of treatment with indomethacin or surgical ligation have shown short-term benefits in terms of improved lung compliance following ductal closure(51, 53, 54). However these improvements have not consistently been shown(55) and could be related to a degree of publication bias.

Perhaps more significantly there is no evidence for long-term respiratory benefit from therapeutic intervention for PDA (either prophylactically or in the presence of prolonged patency(56)) with surgery(56), indomethacin(57), or ibuprofen(58).

1.2.4 Impact of PDA on cerebral perfusion

The cerebral vasculature of the healthy newborn is a low resistance circuit, such that antegrade flow in the major vessels occurs throughout the cardiac cycle, being maximal in cardiac systole(59). The relative contribution of the diastolic forward flow has not been quantified in the cerebral circulation. As the velocity of diastolic flow is generally approximately a third of systolic(59), and diastolic flow occurs for up to two thirds of the cardiac cycle(60), it seems reasonable to predict that, even allowing for a narrower

vessel diameter during diastole, around 30% of total perfusion in the healthy brain could occur during diastole.

The presence of systemic-pulmonary shunting across a PDA can dramatically alter the pattern and possibly the volume of cerebral blood flow in preterm infants.

However the evidence for this comes almost exclusively from Doppler ultrasound studies of cerebral artery flow. Perlman et al were among the first to study patterns of anterior cerebral artery (ACA) flow in preterm infants with clinically apparent PDA(59). In a cohort of 10 infants with PDA diagnosed between 3 and 10 days of age, pulsatility in the ACA was significantly increased at the time of the PDA, and returned to normal levels following ductal closure(59). The increased pulsatility was almost exclusively due to decreased velocity of diastolic flow, with minimal change in systolic velocity. Four infants had a slight increase in systolic flow velocity, and 6 infants had no change. By monitoring blood pressure invasively in 4 infants the authors showed that changes in diastolic ACA flow velocity followed diastolic blood pressure changes before and after ductal closure. Changes in pulsatility within individuals could not be explained by changes in pCO_2(59).

Perlman's initial finding that ductal shunt can have a dramatic effect on CBF velocity profile has since been supported by others. Lipman et al studied eight preterm infants (mean gestation 29 weeks, mean birth weight 1063g) with clinically evident PDA in the first week of life, and found that all had reduced forward diastolic ACA flow, two with retrograde flow(61). Patterns of flow returned to normal following ductal ligation.

Martin et al took the analysis of cerebral flow further by showing that degree of diastolic flow depended on size of PDA (as assessed by clinical signs and dilatation of the left atrium on echo), with infants with small PDAs having normal patterns of

diastolic flow(62). The investigators also examined patterns of flow in the post-ductal descending aorta (DAo), and found that abnormal cerebral flow was seen in all infants with reversed diastolic flow in the DAo, with 3 of 7 infants with reversed diastolic DAo flow also showing reversed diastolic cerebral artery flow(62).

The time at which ductal patency impacts on CBF velocity profile is critical in the consideration of posnatal circulatory adaptation. The three studies mentioned above looked at infants at around 7-10 days. Mellander et al looked at patterns of cerebral flow in 38 infants (mean gestation 29.3 weeks, mean birth weight 1281g) at 72 hours of age(63). Twelve infants had 'haemodynamically significant' PDAs determined by echo criteria of left atrial dilatation and left ventricular function, though only two of these were clinically apparent. Overall, infants with a haemodynamically significant PDA were found to have lower diastolic CBF velocity at 72 hours of age. Infants without respiratory disease had normal systolic CBF velocity in the presence of a haemodynamically significant PDA and produced higher left ventricular output and maintained their mean arterial blood pressure. However they did not appear to increase their systolic CBF velocity to compensate for diastolic steal(63).

Interestingly, those infants with respiratory distress and a haemodynamically significant PDA neither increased their left ventricular output nor maintained their mean arterial blood pressure and were shown to have lower diastolic and systolic CBF velocity(63). It is worth emphasising that the majority of these infants had ducts only detectable by ultrasound, with no clinical signs.

An indication of the potential volume of steal through a PDA comes from Sabila et al's study of cerebral haemodynamics during surgical duct ligation in seven preterm infants at mean age 12 days(64). They showed that systemic diastolic blood pressure and

diastolic CBF velocity increase significantly at the moment of duct occlusion, whereas both systolic blood pressure and systolic CBFV remain unchanged(64). By using area under the CBF velocity curve as a marker of flow volume, the authors estimated that ductal occlusion increased cerebral blood flow to a mean of 228% of pre-ligation values(64), lending a degree of support to the earlier estimation that a significant proportion of total cerebral perfusion occurred during diastole.

A significant limiting factor in all the studies cited in this section is that they relied solely on ultrasound estimation of flow *velocity* patterns. Any change in calibre of blood vessels may have an impact on flow which will not be detected by examining velocity profiles. The anterior and middle cerebral arteries have a distinct muscular layer prior to 24 weeks of gestation(65), and their diameter may therefore change either as an elastic response to changes in distending pressure (i.e. arterial blood pressure) or due to intrinsic vasomotor activity. It is possible then that, in the presence of ductal steal, the cerebral vasculature dilates such that while velocity of flow decreases, volume of flow remains constant. The correlation between ACA flow velocity patterns and cerebral blood flow estimation by other methods will be discussed in section 1.3.2.7.

The ability of left ventricular output to increase as compensation for ductal steal is a central issue in this thesis. Sick preterm infants may be at risk of impaired cerebral perfusion due to ductal steal even in the first 24 hours of life. Retrograde diastolic descending aortic flow, which is associated with retrograde cerebral artery flow(62), has been demonstrated as early as 7 hours of age(48). Evidence that cardiac function may be impaired in the first 24 hours of life will be discussed in section 1.5.2, but a combination of impaired cardiac function and early ductal systemic-pulmonary

shunting at this time could potentially leave the immature brain vulnerable to ischaemic damage.

1.2.5 Impact of PDA on neurological outcome

Many studies have shown an association between PDA and adverse neurological outcome, but proving a causative link has thus far been impossible.

Shortland et al studied 120 infants (median gestation 29 weeks, birth weight 1220g), of whom 33 developed PVH and 22 developed PVL(66). They showed an association between PVL and both ductal patency and retrograde diastolic flow in the ACA but no association between PVH and ductal patency. However this study is limited in that it did not compare cases to matched controls, and the PVH group tended to be smaller than unaffected infants.

Pladys et al showed an association between PVL and PDA in 46 infants (median gestation 29.3 weeks, birth weight 1295g) with specific risk factors for PVL(67). Infants with significant PDA on day 1 of life (as defined by absence of forward diastolic flow in the descending aorta) had significantly increased risk of PVL, even after correction for gestation, birth weight, infection risks and most markers of respiratory disease. The association between PVL and PDA was no longer significant when correction for need for rescue surfactant was included in the analysis. The authors suggested that this association was more likely casual than causal, since they did not find significant differences in markers of cerebral perfusion between the groups, and since a common underlying causative factor (such as respiratory distress syndrome or pro-inflammatory cytokines) could have independently caused both disorders(67).

Evans and Kluckow studied 117 infants (mean gestation 27.3 weeks, birth weight 993g) of whom 31 developed PVH(68). They found ductal diameter at a mean of 19 hours

was associated with PVH, even when correcting for gestation, antenatal steroid use, blood pressure and surfactant treatment. Severe PVH was associated with increased duct diameter and low right ventricular output (suggesting low systemic perfusion). In both cases however, lack of exposure to antenatal steroids was also significantly associated with PVH, and it is equally possible that antenatal steroids are either independently protective for PVH and PDA, or that they protect against PVH through their cardiovascular actions(68).

1.2.6 Impact of therapeutic PDA closure on neurological outcome

Evidence for a role of PDA in the development of PVH/PVL could come from studies of ductal closure. The TIPP trial (Trial of Indomethacin Prophylaxis in Preterms) has shown that early indomethacin treatment both closes the ductus arteriosus and prevents severe PVH(69). The exact mechanism/s by which it achieves these effects is/are less clear. It is possible that indomethacin is protective against PVH purely through inducing ductal closure, and thereby either reducing pulsatility in the cerebral vasculature or reducing systemic-pulmonary shunting and ductal steal. However since both PDA and PVH have been associated with high levels of prostaglandins(70, 71), and as indomethacin has been shown to increase maturity of the germinal matrix in animal models(72) the beneficial effects of indomethacin on PVH are not necessarily related to changes in cerebral perfusion.

In spite of the reduction in PVH in the group receiving prophylactic indomethacin there were no differences in the rates of long-term neurodevelopmental morbidity(69). Given that indomethacin clearly prevents PVH, and that PVH is strongly associated with adverse neurological outcome this is initially surprising. Since indomethacin only

reduced the incidence of severe PVH from 13 to 9% it is possible that an impact on morbidity was absorbed by the overall morbidity rates of around 27% in each group. However the TIPP trial contained such large numbers (1202 infants) that no future trial is likely to detect a true benefit from prophylactic indomethacin. A very real concern is the well known side-effect of transiently reduced cerebral blood flow in the 1-2 hours following dosing(73-75). This indomethacin-induced hypoperfusion could expose many infants to cerebral ischaemia while preventing PVH in a few, thus balancing protective and damaging effects of the drug.

Evidence from any studies of ductal closure by other means (which do not induce transient hypoperfusion) could clarify whether ductal closure itself is neuroprotective. Ductal closure with ibuprofen does not appear to cause transient cerebral hypoperfusion(76), and is effective at closing the ductus, but it does not seem to reduce the incidence of PVH(58, 77). Trials examining long-term neurodevelopmental morbidity are awaited. Studies comparing surgical ligation to indomethacin do not show significant differences in neurodevelopmental outcome(78), but would not have sufficient power to detect small differences.

At present there is no evidence that interventions to promote ductal closure improve neurodevelopmental outcome. Further study of the impact of ductal shunting on systemic and cerebral perfusion in the transitional circulation is important to guide possible further studies of intervention.

1.2.7 Summary

That ischaemia plays at least some role in the pathophysiology of cerebral injury seems clear. However the relative contribution of ductal shunt in ‘stealing’ blood from the cerebral circulation is less clear. Any study assessing the importance of ductal shunting

should attempt to reliably assess systemic and particularly cerebral perfusion, and carefully consider the possible external confounding influences on ductal size, shunt and systemic perfusion.

1.3 Assessment of systemic and cerebral perfusion

Ischaemia plays a role in the pathophysiology of the two principle patterns of brain injury seen in preterm infants. Assessment of the adequacy of the circulation is an integral component of care for the sick preterm neonate. Although research tools are available to specifically assess cerebral blood flow (section 1.3.2), in most clinical scenarios adequacy of the systemic circulation in general is assessed using various surrogates for flow volume(79).

1.3.1 Clinical assessment of systemic perfusion

1.3.1.1 Blood pressure

Blood pressure remains the most frequently monitored indicator of neonatal circulatory status(80). However, since blood pressure is the product of flow and resistance, a high blood pressure may be due to high flow, high resistance, or both. Studies in neonates have suggested a weakly positive(81), weakly negative(82) or no(83, 84) association between blood pressure and left ventricular output. Studies of cerebral perfusion have also failed to show a clear positive association between blood pressure and volume of flow.

Studies of superior vena cava (SVC) flow volume in preterm neonates have demonstrated a positive, though weak, association between blood pressure and volume of flow(10). The same research group have subsequently shown that a mean blood pressure below an infant's gestation in weeks (a common 'rule of thumb' for a low blood pressure(80)) is associated with decreased volume of SVC flow. However this threshold detected only 30% of episodes of low flow. By using a higher threshold of 30 mmHg as an acceptable blood pressure the sensitivity improved, but the positive predictive value of a low blood pressure indicating low SVC flow remained poor. The

authors concluded that blood pressure was an 'imperfect predictor' of systemic perfusion(79).

Chapter 7 of this thesis will address the association of blood pressure and blood flow in more detail.

1.3.1.2 Heart rate

While animal evidence has previously suggested that neonatal stroke volume is relatively constant, such that heart rate should be a strong determinant of cardiac output, it is now appreciated that stroke volume can change greatly in the presence of alterations in preload and afterload(85). While an acute rise in heart rate may be suggestive of hypovolaemia(85), cross-sectional studies have shown no association between heart rate and tissue perfusion(10).

1.3.1.3 Capillary refill time

Capillary refill time is a frequently-assessed marker of systemic perfusion, with a refill time of >2-3 seconds generally considered prolonged(79, 86).

Prolonged capillary refill time has been linked with poor cardiac output in infants(86) and neonates(87). However a strong association of prolonged capillary refill time with decreased left ventricular output in newborns was only seen when infants were subdivided by volume of ductal shunt.

Central and capillary refill times have been systematically assessed as indicators of SVC flow volume in newborn preterm infants(79). Central refill time of ≥3 seconds was shown to have a sensitivity of 55% and specificity of 80% for detecting low SVC flow(79). Combining assessment of blood pressure and capillary refill time appeared to improve the diagnostic performance of the tests(79).

However assessment of capillary refill time has some limitations. The normal refill time may be affected by site at which refill is assessed(88), use of phototherapy(88), ambient temperature(89) and inotrope usage(86).

Despite these limitations, there may be value in assessing capillary refill time as a guide to systemic perfusion.

1.3.1.4 Central-peripheral temperature difference

Central-peripheral temperature difference is a commonly recorded parameter in intensive care units(86). However temperature difference has been shown to have no correlation with measures of flow in infants(86) or newborns(79).

1.3.1.5 Lactate level

When tissue oxygen demand exceeds oxygen supply, tissues increase their rates of anaerobic metabolism. This results in increased production and accumulation of lactate(90). Lactate levels are increasingly monitored in intensive care units(91).

While lactate levels have been directly correlated with cardiac output in adults(92) there are no equivalent published studies in the neonatal period. However high lactate levels have been shown to be of prognostic value after intrapartum asphyxia(93), cardiac surgery(94) and preterm birth(90).

Interpretation of lactate as a marker of tissue perfusion is limited by a number of confounding factors. When perfusion is constant, changes in lactate level may still occur with changes in oxygen saturation, tissue oxygen utilisation or lactate clearance(91). Perhaps most significantly lactate is a marker of prior, rather than current perfusion.

Despite these limitations measurement of lactate is of some value, with trends in lactate concentration probably being of particular value(91).

1.3.1.6 Urine output

The volume of urine production and output is related to renal and therefore systemic perfusion. Decreased urine output in the first 24 hours of life has been associated with decreased volume of SVC flow in preterm infants(95). However, as with lactate levels, urine output is a marker of prior, rather than current, perfusion. Total urine output over at least a 12-hour period is likely to need to be monitored before renal perfusion can be adequately assessed. Urine output cannot therefore be sensitive to acute changes in perfusion.

Preterm infants are often oliguric in the first postnatal day prior to entering a polyuric phase(95). While this may in part be due to changes in renal perfusion, it is also related to a number of hormonal factors(95). These hormone-induced changes in urine output could mask changes due to perfusion alone.

1.3.1.7 Combined clinical assessments

To consider the adequacy of each of the above markers of perfusion on an individual basis is somewhat artificial, since clinicians use multiple tools in combination to assess cardiovascular compromise(96). As discussed above combining information from blood pressure and capillary refill time improves the diagnostic performance of either test(79).

However even when an experienced clinician incorporates information from these multiple factors together into a considered assessment, volume of flow is very difficult to assess(97). In a study of infants and young children on a paediatric intensive care unit, experienced clinicians, having conducted a full clinical examination and in

possession of comprehensive laboratory data, displayed very limited accuracy when estimating cardiac output(97).

1.3.1.8 Echocardiographic assessment of systemic perfusion

The role of echocardiography in the assessment of neonatal haemodynamics will be discussed in section 1.6.

1.3.1.9 Summary

Heart rate and central-peripheral temperature difference are not significantly associated with volume of blood flow. Lactate level and urine output are associated with perfusion, but reflect previous perfusion, and do not adequately assess acute changes in flow. Blood pressure and capillary refill time probably do have some association with flow, but they are both 'imperfect predictors'(79).

Novel reliable methods of assessing perfusion at the cotside would have the potential to improve understanding of the pathophysiology of neonatal brain injury, and could lead to improvements in circulatory support and neurodevelopmental outcome following preterm birth.

1.3.2 Assessment of cerebral blood flow

The technologies currently available to measure cerebral blood flow in neonates have been relatively recently reviewed by Greisen(98), and Pryds and Edwards(99). All available methods are limited by the absence of a 'gold standard' method with which to compare results(100).

Most quantitative methods of measurement assess either the rate of accumulation in, or clearance from, the brain of a detectable tracer. Such methods rely on the Fick principle which states that "the rate of accumulation of a tracer molecule in an

organ…is equal to the difference between the rate of delivery and rate of removal of that tracer"(99). By knowing the concentrations of the tracer in the blood perfusing and draining the brain, blood flow volume can be calculated. Flow calculations however rely on various assumptions about the tracer:- that it is neither produced nor metabolised by the tissue, that rate of flow is constant, and that the tracer must be either fully diffusible or fully non-diffusible within the tissue(99).

Even if volume of blood flow was to be accurately and easily quantifiable the complexities of the brain's metabolism means that many other factors have to be considered in determining whether or not perfusion is 'adequate'.

• Newborn infants may have a lower cerebral blood flow requirement than adults(101).

• CBF requirement differs in different areas of the brain(102)

• CBF requirement may increase over the first days of life(103).

• CBF requirement increases if there is hypercarbia, hypoxia or hypoglycaemia(99)

• Metabolic requirements of the brain may vary over short time periods, for example with seizure activity(104)

• Measures of *flow* at the level of large vessels may not represent *perfusion* at the capillary level if arterio-venous shunting occurs(100)

Despite these caveats, there is growing evidence that low flow states are linked with poor outcomes following preterm birth (section 1.4.2). Methods of accurately (and preferably continuously) monitoring cerebral blood flow at the cot side could aid neuroprotective care.

1.3.2.1 ^{133}Xe clearance technique

Of the currently available methods in the neonatal setting the xenon clearance technique is the closest to an accepted gold standard and is the method with which most new techniques of cerebral blood flow assessment are compared for validation. The technique depends on the rate of clearance from the brain of the inert radioactive tracer xenon.

It is a relatively non-invasive technique, which can be used on the neonatal intensive care unit to quantify both global and regional cerebral perfusion(99). In contrast to other methods using the Fick principle it does not require blood sampling to assess tracer concentration(98). Repeated measurement of cerebral blood flow in individual stable preterm infants have shown coefficients of variation of around 10%(105).

The utility of the technique is limited by the 10-15 minutes required for each clearance measure such that rapid changes in cerebral blood flow can not be detected, and continuous recordings are not possible(99). External scintillators required to detect the emitted gamma radiation are not widely available and each measurement using the technique involves exposure to ionising radiation equivalent to that received from 1-2 chest x-rays(99). This is a significant exposure, particularly were there to be repeated measurements taken.

1.3.2.2 Positron emission tomography (PET)

This technique is the gold standard for cerebral blood flow measurement in adults(100). Injected $H_2{}^{15}O$ emits positrons which in turn collide with electrons in the tissues leading to the production of γ-ray photons which are detectable outside the skull(99).

The technique allows quantification of both global and local cerebral blood flow, as well as markers of cerebral metabolic requirement and oxygen extraction(98).

The utility of the technique is limited by the short half-life of $H_2{}^{15}O$ (which dictates that the cyclotron facilities required to produce the molecule must be nearby(106)) and by the large volumes of blood required to quantify results. Each measurement using the technique involves exposure to ionising radiation equivalent to that received from 4-5 chest x-rays(99). These limitations mean that the technique is rarely used for measurement of cerebral blood flow in neonates.

1.3.2.3 Kety-Schmidt method (nitrous oxide technique)

This was one of the first techniques used for the quantification of cerebral blood flow in humans(107), using inert nitrous oxide as a tracer.

The technique requires continuous monitoring of arterial and venous nitrous oxide concentrations, with venous concentrations being taken from an in-dwelling venous catheter situated in the superior jugular venous bulb. Despite these access requirements the technique has been used successfully for the repeated measurement of cerebral blood flow in infants as young as 2 weeks of age, and as small as 2.4kg(108). Volumes of blood required for the technique (approximately 2.4ml per sequence of measurements) would be significant in the preterm neonate if repeated measures were required.

1.3.2.4 Near infrared spectroscopy (NIRS)

The role of this technique in neonatal care and research has recently been comprehensively reviewed(109, 110). The technique showed early promise as a potential monitor of both cerebral blood flow and oxygenation, but at present NIRS,

along with the other methods reviewed here, remains “very much a developmental technique”(109).

NIRS relies on the relative transparency of tissue to near infrared light (wavelength 600-900nm) and on the presence of chromophores (most notably haemoglobin) whose absorption of near infrared light alters with their oxygen status. Near infrared light is generated by diodes, and an emitter is placed on one side of the preterm skull, with a collector on the other.

To calculate blood flow, a sharp increase in oxygenated haemoglobin concentration is achieved by increasing the inspired oxygen concentration. The gradient of change of HbO_2 level measured by NIRS allows blood flow to be calculated using the Fick principle. The technique relies on assumptions that cerebral blood flow, cerebral blood volume and oxygen consumption remain constant during the change in oxygen saturation - assumptions which do not always hold true(109), particularly since changes in oxygen saturation may themselves change cerebral blood flow(98). The technique is further limited by interference from ambient light(100) and difficulty in rapidly increasing HbO_2 levels in infants with severe lung disease(99).

Reproducibility of the technique is relatively poor, with the standard deviation of repeated measurements within an individual subject of 24%(111).

Despite these limitations repeated measures using the technique improve reliability, and it has been validated against cerebral blood flow measurements by xenon clearance in the newborn(112, 113), and both microsphere(114) and computed tomography(115) techniques in animals.

The ability to use this technique to make rapid repeated measurements of cerebral blood flow at the cot side, non-invasively, and with minimal interruption to normal care means that its early promise as a method of continuously monitoring cerebral perfusion may yet be realised.

1.3.2.5 Magnetic resonance imaging (MRI)

MRI may be used to quantify cerebral blood flow by a number of mechanisms, but its use in the neonatal setting remains at an early stage of development(99). Provisional reports of the use of dynamic susceptability contrast enhanced MRI in infants showed some promise, but movement artefacts affected more than half of the scans, and acquisition of perfusion data was most difficult in the least mature brains(116). Transport and monitoring difficulties, and the limited numbers of MRI scanners available within neonatal units restrict the technique's utility(117).

1.3.2.6 Single photon emission computed tomography (SPECT)

In this technique a radio-labelled tracer (most often 99m-Technetium) is injected intravenously. These compounds act as chemical microspheres, becoming trapped in the brain such that they can be detected during imaging some time later during their decay(99).

This relatively cheap and simple technique has the advantage that tracers may be injected during an acute event, and blood flow examined retrospectively.

However the technique does not allow quantification of global perfusion, only a distribution map of regional flow. Only single assessments of flow may be made, and lateral discrimination is poorer than with PET.

1.3.2.7 Intra-cranial Doppler ultrasonography

Doppler ultrasound permits measurement of blood flow velocity on the basis of the Doppler principle which states that there is a change in frequency of sound waves experienced by the receiver when there is relative movement between the receiver and the transmitter(118). The techniques and limitations of Doppler ultrasonography will be discussed further in section 1.6.1.

Normal range for CBF velocity is well documented in the perinatal period(119, 120). Patterns of flow provide useful information on vascular resistance and the potential impact of the ductus arteriosus on cerebral perfusion (Section 1.2.4).

In Greisen's comprehensive review of cerebral blood flow assessment the author states succinctly "The relation between flow velocity and tissue perfusion is not a simple one"(98). Monitoring changes in cerebral blood flow velocity appear to give information on trends in flow(99). Taylor et al's study comparing changes in cerebral blood flow velocity with changes in cerebral blood flow volume assessed by microsphere techniques in newborn lambs showed reasonable correlations (r=0.79)(121). However a much weaker correlation was found between cerebral blood flow velocity and absolute cerebral blood flow volume (systolic velocity r=0.46, mean velocity r=0.26).

Interpreting changes in flow *velocity* as changes in flow *volume* involves the assumption that the blood vessels studied have maintained a constant diameter. As the major intracranial vessels may change diameter both due to changes in systemic blood pressure and the vessel's own intrinsic vasoactivity(65, 122), it is important to continue to distinguish between flow velocity and flow volume. In the preterm infant the

diameters of the major intracranial vessels are too small to be reliably assessed by current ultrasound techniques.

Some investigators have shown a degree of correlation between CBF velocity and volume of flow. Greisen et al examined the relationship between Doppler ultrasound and xenon clearance measures of cerebral blood flow in 16 newborn infants, of whom 13 were preterm. In virtually all measures they found correlations using pulsed wave (section 1.6.1) Doppler to be superior to those using continuous wave Doppler, and therefore only pulsed wave results will be discussed here. The investigators found reasonable correlations between xenon clearance measures of global cerebral blood flow and Doppler ultrasound measures of both mean blood flow velocity ($r=0.75$) and end diastolic blood flow velocity ($r=0.82$). Their published data only included measures taken in the internal carotid artery. Correlations for flow in the anterior cerebral artery were not quoted, but were said not to be statistically different from those in the internal carotid.

Despite the apparently higher correlation for end diastolic flow velocity (EDFV) than for mean flow velocity (MFV), this study excluded infants with haemodynamically significant PDA. As these infants can have a reversal of end diastolic flow in their cerebral vasculature with high volume left to right shunt, EDFV measurement as an indicator of cerebral blood flow is obviously of limited value.

That there may be some value in measuring cerebral blood flow velocity is also suggested by Hansen et al's study of Doppler flow velocity patterns in the anterior cerebral arteries of newborn piglets, where correlations were performed with cerebral blood flow assessed by microsphere techniques(123). Peak systolic and end diastolic velocities both correlated with microsphere measures ($r=0.76$ and 0.72, respectively),

though correlations were less reliable at higher flow velocities. The area under the flow velocity curve showed the best correlation (r=0.86), and was reasonably consistent across the range of flows. While these correlation coefficients are relatively impressive, it should be remembered that the studies were carried out in paralysed, anaesthetised piglets, all more than one week old. One would expect such subjects to have less spontaneous variation in flow parameters, and the correlations found may not be representative of those in the unstable preterm human infant.

There is certainly some basis for the continued use of anterior or middle cerebral artery blood flow velocity to monitor trends in flow volume, though inconsistencies in the literature suggest that interpretation of absolute volume of flow should be carried out with caution(124).

1.3.2.8 Extra-cranial Doppler ultrasonography

Some attempts have been made to quantify volume of cerebral blood flow (including assessments of vessel diameter) by examining flow in the extracranial arteries. Schoning and Scheel have shown that total cerebral blood flow in adults can be quantified by measuring both diameter and flow velocity in the vertebral and internal carotid arteries(125). The reproducibility of this technique was similar to that of both the xenon clearance and positron emission tomography techniques.

When considering the applicability of extra-cranial Doppler ultrasonography to extremely preterm neonates there are numerous concerns. Kehrer et al found the technique to have a repeatability index of 26% in neonates(126), and it is important to note that the subjects in this study had a corrected gestation of at least 33 weeks. The variability in extremely preterm infants, where vessel diameter is lower, and errors in diameter measures are proportionally larger is likely to be higher still. In addition, no

infant in this study was requiring respiratory support. The accessibility of the neck vessels to ultrasound in an ELBW infant on a ventilator or CPAP circuit must be questionable. The process of scanning all four vessels also took the investigators around 20 minutes per infant, which may constitute a significant period of handling for a single measure of flow.

At present the quantification of volume of cerebral blood flow by ultrasound of the extracranial arteries does not appear promising in individual extreme preterm infants.

1.3.2.9 Superior vena caval (SVC) flow by Doppler ultrasound

Up to 80% of blood returning to the heart in the SVC is said to come from the brain(9, 122). This vessel is a good candidate for Doppler estimation of blood flow velocity as it can be imaged with minimal angle of insonation from the subcostal view(9). Kluckow and Evans estimated SVC flow in a large cohort of term and preterm infants, and found the technique to have reasonably good reproducibility(9). The further assessment of this technique's feasibility, repeatability and clinical utility will form a major component of this thesis.

1.3.2.10 Summary

No single technique can currently offer reliable, repeated and non-invasive measures of cerebral blood flow at the cotside in the preterm infant. Even if available, such a technique may be inadequate to detect cerebral ischaemia since cerebral metabolism is dependent on many additional factors. However cotside cerebral perfusion monitoring would certainly add further insight into cerebrovascular status not provided by blood pressure monitoring (Section 1.4.3). The techniques described above, while currently only research tools, have provided some insight into the pattern of normal cerebral blood flow in preterm infants.

1.4 Cerebral blood flow and neurodevelopmental outcome

1.4.1 Normal patterns of CBF in the preterm infant

As prematurity is essentially a pathological state it is impossible to define 'normality' in any physiological variable. However it is possible, using cohorts of healthy preterm infants without long-term neurodevelopmental morbidity, to define ranges of parameters which are at least compatible with optimal outcome.

The best currently available method for assessing global cerebral perfusion in neonates is the ^{133}Xe technique. Using intra-vascular xenon, Lipp-Zwahlen estimated global CBF in 47 preterm infants (mean gestation 29 weeks, 1081g) in the first week of life(127). The 11 infants from this cohort who subsequently had persistently normal cranial ultrasound scans had a mean global CBF on day 1 of life of 12.5 ml/100g brain tissue/min. These data agree closely with those obtained by Greisen and Trojaborg in a cohort of preterm infants who did not develop severe PVH, and had normal visual evoked potentials (VEPs) at the time of assessment. Mean CBF in this group was 12.3 ml/100g/min, with values as low as 4.3 ml/100g/min(105). While these levels of CBF can be associated with normal cerebral imaging, neither of these studies reported neurodevelopmental outcome.

Using near infrared spectroscopy (NIRS) Meek et al studied 11 preterm infants (median gestation 26 weeks, 825g) and found very similar ranges of global CBF on the first day of life - (6.3-15.2 ml/100g/min)(103). Reassuringly, not only were cranial ultrasound scans normal, but neurodevelopmental outcome at 12-18 months was reported in the majority of these infants, and was found to be normal.

Using positron emission tomography (PET) Altman et al reported that CBF in the first 2 weeks of life in 16 preterm infants ranged from 4.9 to 23 ml/100g/min(101). Infants

were followed up at 6-36 months postnatal age. The lowest CBF in an infant with 'normal' outcome was 4.9 ml/100g/min, but it should be noted that this infant was born at 36 weeks gestation and had CBF measurement carried out while receiving extracorporeal membrane oxygenation, and may therefore not be representative of preterm infants as a whole. The other 4 infants with CBF below 9 ml/100g/min developed PVH, and 3 of these also had parenchymal abnormalities on cranial ultrasound. At follow up one infant had died, 2 had abnormal neurological examinations and follow-up information was not available for the other infant.

Each of these studies show significantly lower levels of global CBF than those found by Pellicer et al, using NIRS in 35 infants (mean gestation 29 weeks, 1112g) where mean CBF on day 1 of life was 21.6 ml/100g/min(128). The reasons for this disparity are unclear, but could include subtle differences in NIRS methodology or genuine differences in flow due to obstetric and neonatal management which are not described in the articles.

Mean SVC flow volume on the first day of life as assessed by Doppler ultrasound in stable preterm infants has been shown to be 73 ml/kg/min(9). To relate these systemic flow figures (per kg total body weight) to previously cited CBF measures (per 100g brain weight) would require accurate measures of brain volume and proportion of SVC perfusing the brain. Studies of cranial volume in preterm infants have found a mean cerebral volume of 246ml in infants with a mean birth weight of 1596g, suggesting an approximation for brain weight as 15% of birth weight(129). The only available estimates of contribution of cerebral to total upper body flow are those quoted (but unpublished) by Skidmore(122). He found that 77% of SVC flow perfused the brain in adults(122) and suggested that the figure would be around 80% in newborn. Using

these estimates, an SVC flow of 73 ml/kg/min reflect a flow from brain tissue of 58 ml/kg/min, resulting in cerebral perfusion rates of 39 ml/100g brain tissue/min.

This figure is obviously very different from most estimates of CBF produced by xenon, PET and NIRS. It is unclear why these measures of cerebral blood flow are so different, but possible mechanisms could include an underestimation of brain volume, an overestimation of SVC flow, an overestimation of the proportion of SVC flow perfusing the brain, or shunting of blood flow through the brain without perfusing the tissues themselves. Of these, the proportion of SVC flow perfusing the brain is the assumption based on the least published evidence. Kehrer et al have recently studied CBF by measurement of flow in the extracranial vessels in ex-preterm infants(130). Although reproducibility of the measures was relatively poor, their population data suggested total CBF volumes of only 33ml/min in infants of 32-34 weeks corrected gestation. Although weights were not quoted, it seems safe to assume that infants at 32 corrected weeks would be >1kg, and therefore CBF would be <33ml/kg/min. Interestingly these authors calculated flow per 100g brain mass, and found a mean of 14 ml/100g/min at 32 weeks, much closer to estimates gained by other methods. If CBF measured in the internal carotid and vertebral arteries is around 30 ml/kg/min, the logical conclusion may be that much less than the quoted 80% of SVC flow has actually perfused the brain, with a high proportion of SVC flow being made up of venous return from the upper limbs, chest, scalp, head and neck musculature and also some lower body flow via the azygous vein. It may be more appropriate then to think of SVC flow as a marker of systemic rather than cerebral perfusion.

1.4.1.1 Additional influences on 'normal' cerebral blood flow

It is clear that trying to delineate a normal CBF for all circumstances in preterm infants is grossly over-simplistic. A great many factors have been shown to alter the 'normal' CBF, and these must be considered when assessing individual cases.

1.4.1.1.1 Time

There is a trend for normal CBF to increase over the first days of life in preterm infants. Xenon clearance studies show a 24% increase in CBF between day 1 and day 2 of life(131), while studies using NIRS have reported a 35% increase over the same time period(103, 128).

Few studies have examined changes in CBF within the first 24 hours of life. Kluckow and Evans used Doppler ultrasound estimates of SVC flow to estimate perfusion at 5, 12 and 24 hours of postnatal life in a cohort of 25 healthy preterm infants(9) and found a stepwise increase in flow from 5 to 24 hours.

Winberg et al used Doppler ultrasound measures of internal carotid artery flow velocity to demonstrate a 29% reduction in CBF velocity between 1 and 5 hours of postnatal age in healthy preterm infants, with a subsequent return to basal velocities by 24 hours(132). Interestingly this reduction was not seen in infants with respiratory distress, who maintained their CBF velocity at a higher level, making the significance of the reduction in CBF velocity at 5 hours in the healthy infants unclear. CBF velocity was not measured between 5 and 24 hours of age.

1.4.1.1.2 Carbon dioxide

The impact of CO_2 levels on CBF is clear. Studies have shown that a fall in CO_2 of 1kPa decreases CBF by 20-50%(105, 131, 133). However there is a great degree of inter-individual variation in the degree of CBF-CO_2 responsiveness seen. A possible role for hypocarbia induced hypoperfusion in the pathogenesis of PVL will be discussed in section 1.4.2.2.

1.4.1.1.3 Oxygen

Stable term and preterm infants have been shown to decrease CBF by 15-30% when FiO_2 increases from 21 to 100%(133, 134).

1.4.1.1.4 Glucose

CBF may increase by up to 50% in the preterm infant at times of hypoglycaemia, with CBF falling after correction of the hypoglycaemia(135). As glucose is the principal energy substrate of the brain, an increase in CBF at times of hypoglycaemia could maintain substrate delivery and protect cerebral function.

1.4.1.1.5 Haematocrit

Pryds' study of intra-individual variation of CBF in relation to haematocrit in preterm infants showed that CBF increased by a mean of 7.4% for each g/dl decrease in Hb(131). A similar strength of association was found by Lipp-Zwahlen et al(127). Interestingly such a degree of change would almost exactly keep the cerebral oxygen delivery constant(131).

1.4.1.1.6 Mode of ventilation

Infants requiring mechanical ventilation have significantly lower CBF (11.8ml/100g/min) than those supported by CPAP (21.3 ml/100g/min) or not requiring

ventilatory support (19.8 ml/100g/min)(136). These lower flow rates appeared to be independent of postnatal age and gestation, and could therefore suggest a lower requirement for CBF in infants receiving mechanical ventilation. However, since some infants in the study had abnormal neurodevelopmental outcomes, and since mechanical ventilation itself may be the cause of reduced CBF, it cannot be assumed that CBF *requirement* is lower in infants receiving mechanical ventilation.

1.4.1.1.7 Regional perfusion requirements

Preterm infants of 29 weeks gestation with normal cranial ultrasound scans have been shown to have higher blood flow to the frontal and parietal regions than to the occipital region(102). More specifically, SPECT studies in preterm infants, again with normal cranial ultrasound scans, have shown that the basal ganglia receive around twice the global rate of CBF, whereas the white matter receives less than half the rate of global CBF(137). The normal white matter perfusion in the healthy preterm infant could therefore be as low as 2.5 ml/100g/min.

1.4.1.1.8 Neonatal and adult CBF

Despite these additional factors to be considered it seems that 'normal' CBF, or at least CBF which is sufficient to maintain cerebral integrity, is significantly lower in newborn infants than in adults. PET studies in adults show that CBF below 10 ml/100g/min is consistently associated with tissue infarction and subsequent necrosis. While some impairment of neonatal cerebral function or development after periods of low CBF cannot be ruled out, the degree of damage at equivalent CBF levels is clearly much less than that which would be seen in the adult brain. Thus data on CBF and outcome in adults cannot be applied to the neonatal population.

Possible explanations for this apparent low requirement for perfusion (and substrate delivery(138)) in the preterm brain include: long periods being spent in electrical quiescence, low numbers of synapses in the brain tissue, high use of non-oxidative metabolism, and slower degradation of high energy phosphate compounds during ischaemia(138).

1.4.1.1.9 Summary

What constitutes normal CBF is dependent on a complex interplay of factors including postnatal age, metabolic demand, oxygen extraction and regional differences in perfusion.

Despite these limitations, the study of normal patterns of cerebral blood flow provides some foundation for the study of abnormal cerebral blood flow and therefore aids examination of the pathophysiology of preterm brain injury.

1.4.2 Abnormal CBF and brain injury in preterm infants

1.4.2.1 CBF and periventricular haemorrhage

The relation of cerebral arterial perfusion and PVH can perhaps best be thought of in terms of the roles of fluctuation of flow, and low flow and high flow states(39). The role of short term fluctuation in flow was discussed in section 1.1.6.

That the region of the germinal matrix is particularly vulnerable to PVH in the presence of any haemodynamic abnormality is appreciated as being related to the area's unique vascular pattern. The structural immaturity of blood vessels in the germinal matrix makes them particularly vulnerable to haemorrhage in the presence of variable CBF patterns(25, 39). The vessels have unusually broad lumens prior to 32 weeks gestation,

meaning that they are exposed to high stretching force(139). In addition they have poor connective tissue support giving them reduced ability to withstand these pressures(24).

There is clear evidence that hypotension in the immediate postnatal period is associated with increased risk of subsequent PVH(26, 37, 140). However hypotension will only lead to hypoperfusion if mechanisms of cerebral autoregulation are impaired. That autoregulation is impaired in at least a proportion of sick preterm infants will be discussed in section 1.4.3.

However the proposed link between low cerebral blood flow and subsequent PVH is not based purely on evidence from studies of blood pressure. As early as 1979 studies using the xenon clearance technique had suggested that low CBF in the first hours of postnatal life was linked to PVH(141), findings that have since been replicated(142). Recent work using NIRS to estimate CBF in the first 24 hours of postnatal life have shown a correlation between low CBF and subsequent PVH(143). Interestingly the infants in this cohort who subsequently developed PVH had a *higher* mean arterial blood pressure on the first postnatal day than the normal outcome group. Differences in CBF in this study were not attributable to differences in CO_2 levels, and the groups did not differ by gestation or degree of respiratory distress(143). This study suggests that low CBF may be a specific predictor of subsequent PVH, irrespective of gestation, respiratory disease and systemic blood pressure.

Of particular relevance to this thesis is the study of Kluckow and Evans linking low SVC flow to subsequent PVH(10). Fifteen of 18 preterm infants in this large cohort who developed PVH in the first days of postnatal life did so after an episode of SVC flow below the range previously identified as normal in the healthy preterm population(9). In addition, the most severe PVH appeared to be associated with the

lowest SVC flow values. By using serial cranial ultrasound scans the authors suggested that haemorrhage was virtually always associated with reperfusion following this period of low SVC flow(10).

Each of the four studies mentioned above looked specifically at levels of CBF prior to the development of PVH. This distinction is important because measures of CBF may be artificially elevated by pre-existing PVH(144, 145). Therefore studies examining early CBF without excluding pre-existing PVH may find different associations(128, 136, 144, 145).

That a period of high CBF, particularly following prior low CBF, may cause PVH is suggested not only by animal models of PVH(146), but by studies in preterm infants temporally linking occurrence of increases in blood pressure with occurrence of PVH(147). While it is important to remember that higher blood pressure may not necessarily be associated with higher cerebral blood flow, it should also be appreciated that sudden or dramatic changes in blood pressure in individual infants may be too rapid or too great to be buffered even by an intact autoregulatory system(148). The clinical instability of the sick preterm neonate means that rapid changes in CBF predisposing to PVH may occur with pneumothorax(149), volume expansion(150), hypercarbia(27, 147) and hypoglycaemia(151).

While all of the evidence linking hypo- and hyper-perfusion to PVH does not prove a causative relationship, the specific association of low CBF, the temporal associations of high CBF, the importance of fluctuant patterns of CBF, and similarities with animal models of CBF all suggest that a hypoperfusion-reperfusion cycle is a plausible pathophysiological model for PVH.

1.4.2.2 CBF and periventricular leukomalacia

The pathophysiology of PVL is multifactorial(152). While there is increasing evidence of an inflammatory component to this complex lesion(153) it is still widely accepted that circulatory factors play a significant role in the pathogenesis of PVL(39). As discussed in section 1.1.5 there is a relative paucity of vascular supply in the developing white matter. This paucity of vasculature is evidenced by the low rates of CBF to the periventricular white matter even in healthy preterm infants(137), leaving a "minimal margin of safety" against ischaemia(7).

To link postnatal ischaemia to PVL definitively would require the prospective study of a large cohort of infants (since the lesion remains rare in ultrasonic terms)(3). The cohort would need to be free from perinatal asphyxia, have PVL excluded at the time of delivery, and have an isolated sub-normal CBF in the absence of confounders. Not surprisingly no such study exists.

However some studies in small numbers of preterm infants looking at CBF with xenon and NIRS techniques do suggest a link between low CBF and PVL(127, 141, 154). Other studies which did not find an association generally measured CBF after cerebral lesions were evident(128, 155).

Prolonged hypotension in preterm infants in the newborn period has been associated with the development of PVL(26). One large prospective study suggested that this link may be due to confounding variables rather than direct causation(156). However this study was only powered to detect a 50% increased risk of PVL in the presence of hypotension, only a single measure of lowest recorded BP was analysed and the authors acknowledge that they may have over-corrected for confounders(156). Assuming impaired cerebrovascular regulation in at least a proportion of infants, there is certainly

scope for episodes of hypotension to cause ischaemic brain injury. A strong association between impaired autoregulation of CBF and PVL has been suggested(157).

Hypocarbia (and presumably therefore a degree of decreased CBF) has been strongly linked with PVL(152), and this association does not appear to be due to prior perinatal events giving increased risk of hypocarbia(158). Other confounding variables such as adverse perinatal events and acidosis(158, 159), sub-optimal cardiac function(160) and ductal steal from the systemic circulation(66) could also compromise cerebral perfusion.

Further evidence for an ischaemic basis for PVL comes from histological comparisons with known asphyxial insults in term infants(39) and from animal models where pure ischaemic insults (in the absence of acidosis, hypocarbia or hypoxia) produce histological PVL(161). Animal studies have also suggested that there may be a selective reduction in white matter perfusion during experimental hypotension, with grey matter flow being relatively preserved(162). Not only would such a mechanism fit with the regional pattern of damage seen in human PVL, but it could potentially explain the lack of strong correlation between hypotension and global CBF. If grey matter flow was relatively preserved during hypotension it could theoretically mask a globally minor, but regionally highly significant drop in white matter perfusion.

1.4.2.3 CBF and neurodevelopmental outcome

That PVH and PVL are linked to low CBF seems highly likely.

That PVH and PVL are strongly linked to adverse neurodevelopmental outcome is also clear(23). However to link low CBF directly to adverse outcome is less straight forward. Studies using cerebral blood flow velocity have consistently failed to find a correlation between intra-cranial blood flow velocity and outcome(163, 164), although

the limitations of this method have been discussed previously. Studies using xenon clearance have in some cases shown a correlation between adverse outcome and both low CBF(154, 165) and low cerebral oxygen delivery(154). However the influence of confounding variables in these studies cannot be ruled out, and equally large studies have failed to find any association(166).

Neurodevelopmental outcome data at 3 years of age have recently been published for preterm infants monitored by SVC flow quantification in the first postnatal day(167). When adjusted for confounding variables (gestation, postnatal steroids and level of maternal education), low SVC flow was found to be significantly associated with adverse outcome, and was found to be a stronger predictor of poor outcome than hypotension(167). While the authors acknowledge that this finding again represents association rather than causation they are currently conducting a prospective study to examine whether prevention and treatment of low SVC flow in the perinatal period reduces the incidence of PVH and adverse neurodevelopmental outcome(168).

1.4.2.3.1 Evidence from therapeutic trials

Prospective interventional studies are the only way to conclusively demonstrate a causative association between abnormal CBF patterns and adverse outcome.

Trials of therapeutic intervention to prevent rapid fluctuations in CBF with muscle relaxants have shown reduced incidence of PVH in the treatment group, as discussed in section 1.1.6. A beneficial effect of a “minimal handling” approach to improve stability has not been proven(39), with the only randomised control study on the issue showing no significant difference between the groups(169).

Prophylactic indomethacin is associated with a reduced incidence of PVH, but as discussed in section 1.2.6 this benefit is not necessarily mediated via effects on CBF, and does not lead to a reduction in neurodevelopmental morbidity(69). Attempts to target early indomethacin treatment to infants with broadly patent arterial ducts have not yet proved beneficial(170).

There are no other published studies which report beneficial effects of postnatal haemodynamic interventions on the incidence of PVH/PVL or adverse neurodevelopmental outcome.

1.4.3 CBF, systemic blood pressure and autoregulation

As discussed in section 1.4.1 the preterm neonate may alter its cerebral blood flow in response to stimuli such as altered CO_2, O_2 and glucose levels. These are all forms of autoregulation which alter CBF in response to changing metabolic requirements. The cerebral circulation is also capable of adapting to changes in blood pressure to maintain constant CBF.

To maintain constant perfusion small arteries and arterioles must vasoconstrict during increases in blood pressure to attenuate flow, and vasodilate during decreases in blood pressure, so producing an 'autoregulatory plateau'(171). This process occurs principally in small resistance vessels, but may also occur in vessels as proximal as the circle of Willis(122). The process can occur within seconds of changes in blood pressure(172, 173). The mechanisms by which the process takes place may be intrinsic in the arteriolar wall, or may be related to changes which occur in other parameters (such as CO_2 and O_2) which in turn lead to alterations in vascular tone to maintain cerebral blood flow(171).

There is evidence that in some preterm infants the process of regulation of CBF in response to changes in blood pressure is intact. Studies using continuous monitoring of CBF by NIRS have shown that some preterm infants keep CBF constant despite fluctuations in mean blood pressure of 10-15mmHg(157).

Animal studies have shown conclusively that the process of autoregulation can be abolished by a preceding hypoxic insult(174). There is strong evidence that hypoxia around the time of delivery can have the same effect on the term human infant(175). The role of intrapartum hypoxia in disrupting autoregulation in the preterm infant remains unclear(30).

While appreciating the limitations of the technique of intracranial Doppler ultrasound, studies examining the relationship between continuously monitored Doppler CBF velocity and invasively monitored blood pressure in infants on the first day of postnatal life have suggested that many preterm infants have impaired autoregulation(173). Two thirds of infants <33 weeks gestation had a change of mean CBF velocity of >0.5% for every 1 mmHg change in mean arterial blood pressure. This apparently impaired autoregulation was seen with both minor and major changes in arterial blood pressure, and was more common in hypotensive and extremely preterm infants(173). It is also interesting to note that some infants showed both intact and deficient autoregulation at different times. A relationship between impaired autoregulation and both degree of prematurity(176) and hypotension(177) has been suggested by others.

Studies using NIRS have also shown a wide range of autoregulatory ability in the first 3 days of life in preterm infants(157). Over 50% of preterm infants may have a significant association between blood pressure change and changes in CBF. Interestingly in this study infants with poorer autoregulation had a significantly higher

risk of abnormalities detected on cranial ultrasound. However ultrasound scans were not performed until the third day of life, and abnormalities may have preceded the disturbed autoregulation rather than been a consequence of it(178). However other studies have linked deficient autoregulation at the time of a normal cranial ultrasound to the subsequent development of lesions(30).

To consider the process of autoregulation as an 'all-or-nothing' phenomenon is overly simplistic - even in healthy adults the autoregulatory plateau has limits of blood pressure beyond which CBF cannot be held constant. It is likely that many preterm infants will have a narrow autoregulatory plateau, such that CBF will be held constant only within a narrow range of blood pressure. Broader swings in blood pressure will lead to changes in cerebral perfusion(7). It may be that 'normal' blood pressures in the preterm infant are already towards the lower end of this plateau, such that infants are particularly sensitive to minor reductions in blood pressure(39, 179). In addition the speed of autoregulatory control may be poor in the preterm brain, leaving CBF unstable in the presence of rapid fluctuations in blood pressure(179).

It is not possible to predict which infants will have relatively intact and which deficient cerebrovascular autoregulation. Irrespective of the presence or absence of autoregulation in any individual infant it is clear that a single measure of blood pressure does not predict volume of CBF(143, 180). While monitoring and maintenance of blood pressure are important, blood pressure cannot be used as a predictor of CBF.

1.4.4 Summary

There is convincing evidence that circulatory factors play a role in the pathophysiology of both periventricular haemorrhage and periventricular leukomalacia, the two major antecedents of adverse neurodevelopmental outcome in preterm infants.

Alterations in blood pressure may lead to alterations in cerebral blood flow either when autoregulation is absent or when the alterations in blood pressure are too broad or too rapid to be adequately compensated for.

The newborn preterm brain may be particularly vulnerable to circulatory compromise when undergoing the transition from the fetal to the adult circulation. The unique physiology of the transitional circulation, including its persistent fetal shunt pathways and its response to changes in vascular resistance, is discussed in the next section.

1.5 The transitional circulation in the preterm infant

In the adult circulation the two ventricles of the heart work in series to supply the body and lungs with adequate perfusion. In physiological terms it is helpful to look at three central factors impacting on left ventricular function and therefore tissue perfusion:

Preload - the initial stretching of the cardiac muscle fibres prior to contraction. Up to a critical point stretching of the fibres enables more sites of contact between the contractile units of the muscle, enhancing contractile force. Further stretching beyond this point will not increase, and may actually decrease contractile force(181). Preload is determined largely by circulating blood volume and venous return to the heart, and to a lesser extent by heart rate, atrial contractility and ventricular compliance. The ability of the heart to increase stroke volume in response to increased preload is known as the Frank-Starling mechanism(181).

Inotropy - The ability to change its inherent contractile force (independent of preload) is unique to cardiac muscle. Increased inotropy (or contractility) manifests itself as increased velocity of muscle fibre shortening and therefore increased ejection velocity of blood. As the time available for ejection of blood is relatively constant, increased ejection velocity over a constant time period will result in increased ejection volume(181). Contractility is determined largely by the interaction of the sympathetic and parasympathetic autonomic nervous systems(181). Circulating catecholamines also have an impact, as do poorly understood interactions with heart rate (the Treppe effect)(181) and afterload (the Anrep effect)(182). Most changes in cardiac contractility appear to be mediated by changes in intracellular calcium levels(181).

Afterload - the load against which the ventricle must eject blood. As afterload increases it increases the wall stress in the ventricle, decreasing the rate at which muscle

fibres contract and (with ejection time being relatively constant) decreasing the ejection volume. Afterload is closely related to blood pressure, but is perhaps best thought of in terms of vascular resistance. Changes in vessel diameter (particularly small arteries and arterioles) regulate blood flow within individual organs, and the total of the resistances created by each of these vascular beds is systemic vascular resistance (SVR)(181). Vasoregulation is determined by intrinsic myogenic mechanisms, local vasoactive mediators and extrinsic factors including the autonomic innervation of blood vessels and circulating vasoactive hormones(181). Blood pressure is the product of vascular resistance and blood flow, and this thesis will discuss the interaction of these three variables. In general it is thought that systemic vascular resistance has a dominant influence over blood flow in the creation of blood pressure(82, 83).

The separation of the effects of these three factors on the control of blood flow is artificial. In fact the three mechanisms are inextricably linked(91). For example an increase in afterload will cause a reflex increase in myocardial inotropy, though this will only partly preserve stroke volume. Residual blood not ejected from the left ventricle will act to increase preload, so enhancing contractile force(181).

In the transitional circulation further factors in the control of systemic blood flow must be considered; the fetal shunt pathways.

In the fetal circulation high volume right-to-left shunts exist within the atria (the foramen ovale) and the great arteries (the ductus arteriosus) to predominantly bypass the fetal lungs, and supply blood to the gas-exchanging placenta. In the immediate newborn period, with clamping of the umbilical cord and inflation of the lungs with the first breaths the process of the transitional circulation begins. Pulmonary vascular resistance begins to fall, pulmonary blood flow increases and right to left ductal shunt

decreases(183). Increased pulmonary blood flow results in increased venous return to the left side of the heart, and shortly after birth left ventricular preload increases(184), and both left ventricular stroke volume and output double(184).

Despite these dramatic changes, significant ductal and atrial shunting persists in the healthy term infant for around 24 hours. In the preterm infant these shunts have a prolonged and accentuated effect.

1.5.1 Preload and the transitional circulation

Preload, as measured by left ventricular end-diastolic dimension (LVEDD) increases abruptly within an hour of delivery in the healthy term infant(184). This increase is due to increased pulmonary venous return to the left atrium, which is itself due to increased pulmonary blood flow in the immediate postnatal period. Preterm infants have similar levels of left ventricular output (LVO) to term infants in the first postnatal hours(84, 185) and it is likely that a similar preload increase has occurred.

Animal studies have suggested that this dramatic increase in preload in the immediate newborn period may stretch myocardial fibres near to their point of maximal contractility, such that further increases in preload do not increase stroke volume(186). This maximal preload may be referred to as the peak of the Frank-Starling curve(181). There is some evidence in human preterm infants for a similarly blunted response to increasing preload in the first hours of postnatal life(187). However the majority of studies assessing change in cardiac output in response to preload increase suggest a beneficial effect in most preterm infants(188, 189). Measures of SVC flow also increase in most preterm infants after fluid volume administration irrespective of the presence of clinical hypovolaemia or hypotension(190), again suggesting that the peak of the Frank-Starling curve has not been reached.

It should be noted that a response to fluid volume (i.e. increasing preload) need not imply that inadequate preload was the cause of low cardiac output; rather that maximal stretching of the myocardial fibres has not yet occurred. Larger volumes of fluid administration may not result in further increases in cardiac output(188) and the duration of beneficial effect of volume administration on cardiac output in the preterm infant is unknown(190).

1.5.2 Cardiac inotropy and the transitional circulation

Studies of the contractility of the newborn left ventricle have been limited by the non-circular shape of the ventricle which prevents accurate interpretation of contractility from measures of fractional shortening(184, 191). In addition measures of fractional shortening may be biased by changes in preload, afterload or heart rate. Despite these limitations, a study of 75 VLBW infants suggested that 47% of the infants who developed shock in the first 24 hours of postnatal life had impaired myocardial contractility(84). These assumptions were based on the group having low fractional shortening despite no differences in LVEDD (reflecting preload) and prolonged left ventricular pre-ejection period(84).

Study of a large cohort of VLBW infants has demonstrated a significant decrease in LVO from 3 to 12 hours of postnatal life, with some recovery by 24 hours(192). This decrease in LVO was most marked in the most premature infants. Changes in left ventricular ejection fraction were closely linked with LVO changes. Decreases in preload or increases in afterload did not appear to account for the fall in LVO, suggesting that contractility had decreased.

Comparing the rate-corrected mean velocity of fibre shortening (mVcFc) to the ventricular end-systolic wall stress (ESS) gives a pre- and afterload independent measure of ventricular inotropy(193). Using this measure it has been shown that VLBW infants in the first 6 hours of life may have significantly impaired contractility compared to term infants(194). Contractility appeared to reach term levels by five days postnatal age in the VLBW infants(194).

By specifically looking at the relation between mVcFc and ESS it can be seen that preterm infants may be particularly vulnerable to impaired myocardial contractility in the presence of high afterload(194, 195), with increases in afterload potentially prompting marked deteriorations in cardiac output(196).

Given this relationship between afterload and contractility it may be that the newborn heart may be particularly capable of raising ejection fraction in the presence of low afterload. A large left to right ductal shunt presents the preterm heart with a high preload and a low afterload. Under such circumstances there is evidence from animals(197) and humans(198) that the left ventricular output can be raised to more than double the normal resting levels.

1.5.3 Afterload and the transitional circulation

Peripheral blood vessels have the ability to vasoconstrict and vasodilate in response to changes in tissue metabolic demand and blood pressure. These mechanisms have been shown to be active even in preterm infants(188). The overall peripheral vascular tone appears to be more important in determining arterial pressure than cardiac output(82, 83). Studies in preterm infants have shown that systemic vascular resistance (SVR) is also a strong determinant of cardiac output itself(82).

Given that high afterload raises blood pressure but lowers cardiac output it is not surprising that blood pressure correlates only weakly with cardiac output(9, 83). It is clear that preterm infants with low blood pressure can have high LVO in the presence of low SVR and that infants with a normal BP can have low LVO in the presence of high SVR(82). As mentioned previously the premature myocardium may be particularly liable to developing low cardiac output in the presence of high afterload(194, 195).

The importance of considering SVR becomes clear when assessing response to interventions to treat hypotension in the newborn infant. Dopamine remains the most commonly used inotrope in the neonatal setting(8) and it is predominantly used to treat hypotension without knowledge of infants' cardiac output. Premature infants are particularly sensitive to the vasopressive effects of dopamine, though whether this is due to impaired clearance of the drug or an inherent tendency to vasoconstriction is unclear(199). Dopamine has been shown to raise blood pressure in preterm infants predominantly by peripheral vasoconstriction(200). The increase in blood pressure produced is often associated with a decrease in cardiac output(201) as well as intestinal(200) and cerebral(190) perfusion. In clinical terms there is a balance to be achieved between maintaining perfusion pressure and reducing afterload in individual patients - a balance that can best be achieved by monitoring cardiac output in addition to blood pressure(91).

1.5.4 Fetal shunt pathways and the transitional circulation

In addition to the concepts of preload, inotropy and afterload, consideration of the transitional circulation must also include awareness of shunt through the fetal shunt pathways; the ductus arteriosus and foramen ovale. In both cases the volume of shunt

through the pathway depends primarily on the area of the vessel and the pressure gradient across it(202).

1.5.4.1 Ductal shunt

The incidence and significance of prolonged patency of the ductus arteriosus was discussed in section 1.2.

Ductal patency in the first 24 hours of life is physiological. Around 50% of healthy term infants will have some ductal shunt at 24 hours postnatal age(203). However ductal patency is not an 'all-or-nothing' phenomenon By 4 hours of age term infants have reduced their mean ductal diameter to half that seen at birth, and this reduces to a quarter by 8 hours postnatal age(203). As vessel area is proportional to the square of the radius a vessel constricting to ½ its previous diameter will have ¼ of its previous area. As area rather than diameter determines shunt volume it can be assumed that volume of ductal shunt is dramatically reduced over the first 8 hours of postnatal life in the healthy term infant(185).

The time course of ductal constriction in the preterm infant is less clear. In the absence of respiratory distress the time to ductal closure is only marginally prolonged, if at all, compared to term infants(6, 49, 204). In preterm infants with respiratory distress patency of the duct is prolonged(6) (as it is in term infants(203)). Only a third of VLBW infants requiring mechanical ventilation will have closed ducts by 48 hours of age and the rate of constriction through this time period is variable(48). Infants who go on to develop clinically apparent systemic to pulmonary shunts through a PDA often have large ducts (1.8 - 3.2 mm) at 24 hours, though some infants have a variable period of constriction prior to the duct re-opening(48). Perhaps most significantly, it is very

difficult to delineate risk factors for delayed constriction of the duct, and large ducts are virtually never clinically apparent in the first 24 hours of life(48).

The second critical factor determining volume of shunt through the ductus arteriosus is the pressure gradient across it; the systemic-pulmonary pressure gradient(6).

Pulmonary arterial pressure drops to approximately half the prenatal level by 48 hours of postnatal age in healthy term and preterm infants, with the majority of this fall occurring in the first 24 hours(205). Over the same time period systemic blood pressure generally increases(206).

The most repeatable echocardiographic measure of pulmonary arterial pressure is peak velocity of tricuspid regurgitation(207). Using this technique preterm infants with minimal respiratory disease have been shown to decrease pulmonary pressure from 87% of systemic levels at 6 hours postnatal life to 53% of systemic levels at 27 hours(6). In contrast, infants with respiratory distress requiring mechanical ventilation have mean pulmonary pressures >70% of systemic levels until after 48 hours of age(6). While a delay in fall of pulmonary pressures was universal in infants with significant respiratory distress, the degree of delay was very variable, and not predictable from clinical signs(6).

Further evidence for a delay in the decrease in pulmonary arterial pressures comes from direct analysis of direction of ductal blood flow. Bidirectional flow (right to left in systole, left to right in diastole) reflects a relative balance of pulmonary and systemic pressures(6). This pattern was seen in between 40 and 80% of preterm infants with respiratory distress in the first 24 hours of life(6, 47).

It is important to consider that most data on postnatal pulmonary pressure in preterm infants was acquired prior to the introduction of surfactant therapy. It is likely that surfactant administration accelerates the decrease in pulmonary arterial pressure(208, 209). Such an effect would be supported by the finding that only 26% of surfactant-treated infants in a recent cohort had bidirectional ductal shunts at 12 hours of age(192). If surfactant administration significantly lowers pulmonary pressure without lowering systemic pressure, it may heighten systemic-pulmonary shunting and potentially further compromise systemic perfusion(208).

While relative pulmonary and systemic pressures are relatively amenable to study, measurement of the volume of ductal shunt remains technically challenging. In the absence of atrial shunting, ratios of LVO and right ventricular output (RVO) will give an indication of ductal shunt volume. LVO and RVO estimated by Doppler echocardiography have been shown to be equal in the absence of ductal shunt(47). Preterm infants with a large duct have been shown to have a mean differences of around 100ml/kg/min between LVO and RVO, presumably reflecting the volume of ductal shunt(47). This estimate of shunt volume is only a mean value. It is not unusual to estimate LVO at double the value of RVO. An associated increase in left to right atrial shunting (secondary to increased pulmonary venous return) may also increase RVO such that true volume of ductal shunt is even higher than the LVO to RVO ratio alone would suggest(202).

A bidirectional pattern of ductal flow suggests a degree of balance between the pulmonary and systemic pressures. However, even in the presence of bidirectional ductal shunting there is generally a net systemic-pulmonary flow. Bidirectional ductal shunting simply shows that pulmonary pressure is higher than systemic during systole,

with systemic pressure being higher during diastole; it has long been appreciated that pulmonary diastolic pressure tends to fall more quickly than systolic(210). As cardiac systole is shorter than diastole the duration of right to left ductal shunting in preterm infants is generally less than a third of the duration of left to right ductal shunting(202). Studies of LVO and RVO in preterm infants suggest that the volume of net left to right flow during bidirectional shunt may be up to half that seen with pure left-right flow(47).

Since infants with significant respiratory distress tend to have broadly patent ducts in early postnatal life, and since direction of shunt is predominantly systemic to pulmonary (even when bidirectional) the volume of blood shunted away from the systemic circulation may be high. Whether left ventricular output is able to increase significantly to compensate for this shunting of blood is an issue central to this thesis. As discussed in section 1.5.2, that a large left to right ductal shunt will automatically increase preload and will only occur in the presence of a low afterload (where pulmonary vasculature resistance is low) may help maintain systemic perfusion despite a high volume of shunt.

1.5.4.2 Atrial shunt

Left to right atrial shunt has been subject to less intensive study than ductal shunt in preterm infants. However its significance is often underestimated. Particularly in infants with an associated ductal shunt and a volume loaded left atrium, more blood may be shunted between the atria than through the duct, such that RVO may exceed LVO(202). An awareness of the volume of atrial shunting (as assessed by colour flow diameter) is essential if using the LVO:RVO ratio as an estimate of ductal shunt.

1.5.5 Systemic blood flow during the transitional circulation

Interactions between these haemodynamic variables which determine systemic perfusion are complex. The interactions are not easily predictable by gestation, clinical condition or current monitoring techniques.

There is a sub-group of preterm infants who have a significant volume of systemic to pulmonary shunt through a widely patent duct in the first 24 hours of postnatal life, even if bidirectional ductal flow is present. Should this shunt be compounded by hypovolaemia, impaired myocardial contractility, high afterload or intra-atrial shunt, significantly impaired tissue perfusion could result.

To optimise systemic and particularly cerebral perfusion during early postnatal life in preterm infants requires an improved understanding of the pathophysiology during this time, as well as improved ability to monitor the circulation in individual infants. Advances in ultrasound techniques may help clinicians to achieve both of these goals.

1.6 Echocardiography in the assessment of neonatal haemodynamics

Echocardiography is becoming an integral component of adequate assessment and management in the critically ill newborn(211). The technique has potential for detecting many treatable causes of circulatory failure(211). Controversies persist as to the role of neonatologists in detecting structural congenital heart disease(212) and to the most appropriate means of training neonatologists in the technique(211). Despite these concerns it seems that there is a demand for the technique(213) and evidence that echocardiography can be 'a reliable tool in the hands of neonatologists with close support from cardiologists'(214).

1.6.1 Basic principles of ultrasonography

The physics, principles and safety of ultrasound scanning in the neonatal environment are discussed in detail elsewhere(118). For the purposes of this thesis a brief summary of the available modes of ultrasound is appropriate.

Cross-sectional (2D, B-mode) scan - a cross-sectional image of the body built up by sweeping a beam of ultrasound sideways through a chosen scan plane(118).

M-mode scan - a beam of ultrasound is transmitted through a single line of tissue, with echoes displayed on a 'sweep' across the screen such that each echo traces out a line representing a single point of reflection. M-mode scanning is particularly valuable as a means of measuring dynamic structures(118).

The Doppler effect - the Doppler effect is the change in frequency of ultrasound (or any wave) experienced when there is relative movement between the receiver and the transmitter. In echocardiography the ultrasound probe acts as both the transmitter and receiver, but as ultrasound waves are reflected off moving red blood cells (or any other

moving structure) these also act as transmitters. Movement of blood cells towards or away from the ultrasound probe produces a Doppler shift in the frequency of the ultrasound waves from which velocity of flow may be calculated.

Continuous wave (CW) Doppler - a CW probe contains two transducers - one that continually transmits and one that continuously receives ultrasound signals. Doppler shifts may be detected from any region where the transmitting and receiving beams overlap. Interfaces within the heart (such as the chamber walls) will generate high intensity Doppler shifts of low frequency which could 'drown out' the low intensity, high frequency shifts produced by moving blood cells. Therefore most Doppler systems include a wall thump filter to reject low frequency Doppler shifts(118).

Pulsed Doppler - It is possible to interrogate velocity of flow within a particular part of the heart or circulation by pulsing ultrasound transmissions and only presenting Doppler signals from pulses that arrive over a time interval corresponding to the time taken for waves to travel to and from the desired area. Should the direction of blood flow at this point not be directly in line with the ultrasound beam an angle correction may be applied to estimate flow velocity(118).

Colour Doppler - a colour map of Doppler frequencies can be overlaid on a real-time cross-sectional image to demonstrate velocities of flow within a structure. The time required to establish cross-sectional information and accurate Doppler assessment of velocity means that Doppler information is generally displayed in relatively large pixels, limiting the lateral resolution of the technique(118).

1.6.2 Repeatability of echocardiographic measures

Any echocardiographic measurement technique must be evaluated in terms of its inherent error(207). Interpretation of quoted estimates of error is complicated by the range of statistical techniques used to report repeatability in the medical literature.

Historically, many techniques have been assessed in terms of the correlation between measured values. For many reasons this is inappropriate, with a more robust statistical approach being to compare differences between pairs of measures with the mean of the two measures(215, 216).

Using this "Bland and Altman" technique one can assess the differences between pairs of measures obtained either by the same operator for intra-observer error, or by different operators for inter-observer errors. Any trend for differences to be affected by mean value can be assessed. If no such trend exists one can calculate the bias between two measures (which should be zero if the same technique is used twice by the same operator) and the standard deviation of the differences.

A widely accepted estimate of error in a measurement is the repeatability coefficient which is twice the standard deviation of the differences between measurements(215). In clinical terms this is the difference one would have to measure in repeat testing of a variable to have a 95% confidence that the change in the measurement is due to real change rather than chance alone(217).

Since different measurement techniques use different units, direct comparison of repeatability coefficients is difficult. This problem can be overcome by expressing the repeatability coefficient as a percentage of the average value, creating a repeatability index(207). Similarly a coefficient of variation (CV) of a set of values is the standard deviation divided by the mean of the set, and is therefore equal to half the repeatability

index. A high repeatability coefficient, repeatability index or CV suggests poor repeatability.

The acceptable error for a measurement technique depends not only its repeatability, but also on the degree of clinical variation expected in a parameter(207). For this reason Skinner et al designed an approach employing 'confidence steps' - the maximum range of values in a parameter in the study population divided by the repeatability coefficient(207). The more confidence steps a technique has the more likely it will be able to reliably detect clinically important changes.

Another commonly used statistical approach to measurement error is to assess variability as the difference between two measures expressed as a percentage of the mean of the two measures(9). The median and range of the variability can then be described.

Since echocardiography is highly user-dependent it is important to consider intra- and inter-observer variability separately; inter-observer variability is often significantly poorer(9, 217). To diminish the impact of inter-observer variability serial echocardiographic measurements in the same infant should be made by the same observer on each occasion whenever possible(217).

1.6.3 Echo assessment of left ventricular output

In the absence of extra-cardiac shunting, LVO is equivalent to systemic cardiac output. Doppler echocardiographic estimation of LVO has been shown to correlate well with invasive estimates of flow in neonates and children(218, 219).

Normal ranges of aortic diameter(220, 221), aortic blood flow velocity(222) and LVO(223) have been described in preterm and term neonates. Mean LVO in the

newborn period has consistently been shown to be 220-260 ml/kg/min, with values tending to be higher in preterm than in term neonates(223)

When considering the repeatability of Doppler estimates of LVO, errors in vessel diameter and flow velocity can be considered separately.

Vessel diameter is the source of greater potential error since any error will be squared in the calculation of vessel area(223, 224). In infants, measures of the aortic root have been shown to be more reproducible than those of the aortic orifice(219). M mode trailing edge-leading edge measures have been shown to be more reproducible than leading edge-leading edge measures(217, 219). Estimates of LVO from diameter measures of end systolic trailing edge-leading edge aortic root diameter have been shown to have the closest correlation with thermodilution estimates of flow(219).

The repeatability of measurements of flow velocity at the aortic valve in infants depends both on the Doppler mode (pulsed or continuous wave) and transducer site (suprasternal, apical or subcostal) used. Continuous wave (CW) Doppler has been shown to be slightly more repeatable than pulsed wave (PW) in newborns(217), but does not allow for estimation of flow at a precise point in the circulation. Since estimates of LVO by PW tend to be higher than those obtained by CW(217), a consistent use of either CW or PW seems to be important. LVO assessed from the suprasternal view appears to have marginally better repeatability than the apical view(217). However this view is limited by poor access to the suprasternal region in small preterm infants requiring respiratory support.

Since changes in aortic diameter in the first week of life appear to be minimal (<5%) in both preterm(222) and term(225) infants; and change in diameter is considerably less than the error inherent in assessment of diameter(226), it seems reasonable to use a

single estimate of aortic diameter for all measures of LVO in the first week of life. Using a single measure of aortic diameter the intra-observer repeatability index for LVO in healthy term infants has been consistently shown to be around 20%(207, 217, 227): a difference of greater than 20% in LVO on repeated measurement by the same observer should be seen before the difference can be interpreted as representing a genuine change.

The almost universal presence of a patent ductus arteriosus in preterm infants in the first 24 hours of postnatal life(46) means that LVO can not be assumed to represent systemic perfusion(228). Ductal steal can account for more than 50% of LVO in the preterm infant(46).

1.6.4 Echo assessment of right ventricular output

In the absence of intra-cardiac shunting, RVO is equivalent to systemic cardiac output. Normal values of pulmonary artery diameter(221), flow velocity(222) and RVO(224) have been described in preterm and term neonates. Mean RVO in the newborn period is around 250 ml/kg/min(224).

Early studies suggested significant difficulty in reliably discriminating the walls of the pulmonary artery(226). Since the pulmonary arterial walls lie parallel to the ultrasound beam when visualised from the praecordium M mode echocardiography can not be used to measure pulmonary diameter, and lateral resolution of ultrasound is relatively poor(224). Despite these limitations the repeatability of RVO measurement in the newborn period appears to be similar to that of LVO(224).

Right ventricular outflow tract diameter is most reliably measured at the hinge points of the leaflets of the pulmonary valve(224). At this point there is no significant difference in measures obtained in either the short or long axis views(224).

Right ventricular outflow velocity can only be reliably assessed by pulsed wave Doppler, as continuous wave Doppler will overestimate flow due to acceleration of flow at the level of the bifurcation of the pulmonary trunk. Pulmonary flow velocity is most reliably measured in the short axis view(224).

Serial studies have shown that the diameter of the pulmonary valve can decrease by up to 10% over the first week of life in term infants(225). This variation is of a similar order of magnitude to the measurement error of the technique, so it would seem reasonable to either use a single estimate of pulmonary valve diameter for all measures of RVO in the first week of life, or to re-measure at each scan.

The intra-observer repeatability index for pulmonary stroke distance in preterm infants has been shown to be around 26%: a difference of greater than 26% in pulmonary stroke distance on repeated measurement by the same observer should be seen before the difference can be interpreted as representing a genuine change.

The potential for shunting through a patent foramen ovale in preterm infants in the first 24 hours of postnatal life means that RVO can not be assumed to represent systemic perfusion(228), as atrial shunt can account for more than 50% of RVO in the preterm infant(229).

1.6.5 Echo assessment of ductal shunt

The limitations of clinical signs in assessing patency of the ductus arteriosus in early postnatal life have been extensively reviewed(46, 85). Use of colour Doppler echocardiography makes detection of a patent ductus relatively simple(85). Assessment of a duct's 'haemodynamic significance' is much less straight-forward. Measures of ductal internal diameter can be taken from the high parasternal (ductal) view, with adjustment of colour gain settings to optimise the colour flow image while eliminating

peripheral colour interference(48). Using this technique a diameter of >1.5mm at the point of maximal ductal constriction has been shown to be the strongest predictor of significant ductal shunting(46, 48). Despite these findings, this technique may be limited by inaccuracies generated by variation in colour Doppler maps and gain settings(85). While repeatability of interpretation of Doppler images from video tape has been shown to be reasonable (CV 12%)(48), there are no published data on intra or inter-observer repeatability of colour Doppler measures of ductal diameter. In addition, shunt volume obviously depends not only on ductal size but on pressure gradient between the systemic and pulmonary circulations(85).

The direction and pattern of blood flow through the duct can be assessed from the high parasternal view with continuous wave Doppler. Bidirectional shunting is often seen on the first day of life in preterm infants, and is due to pulmonary diastolic pressure dropping more quickly than systolic(210), leaving pulmonary pressure higher during cardiac systole and systemic pressure higher during diastole. As diastole lasts longer than systole the net flow of blood during bidirectional shunting is generally still systemic-to-pulmonary(47).

In the presence of pure left-to-right ductal flow, the ratio of systolic to end-diastolic ductal flow velocities also gives a guide to ductal shunt volume. A pattern of high systolic velocity and low diastolic velocity suggests that diastolic systemic and pulmonary pressures are almost equal (presumably due to pulmonary congestion from ductal shunt)(85). This pattern is not seen in healthy infants and suggests a high volume shunt. Somewhat counter-intuitively, a pattern of high velocity ductal shunt throughout the cardiac cycle suggests lower volume of shunt, as there is not sufficient shunt volume to raise the pulmonary diastolic pressure.

Other echocardiographic methods for predicting ductal shunt volume include ratio of LVO to RVO (if there is no inter-atrial shunt), reversal of diastolic descending aortic flow pattern (section 1.6.7), degree of left atrial dilatation(230-232) and the presence of continuous forward diastolic flow in the branch pulmonary arteries(233).

Despite the range of techniques available to assess ductal shunting it remains unclear when treatment for a patent ductus arteriosus is indicated in preterm infants(56).

1.6.6 Echo assessment of atrial shunt

Inter-atrial shunting is seen in the majority of preterm infants(229), particularly when there is also significant ductal shunting(47, 202).

Atrial shunting is best assessed from the subcostal four-chamber view, with the diameter of colour Doppler flow at the level of the atrial septum measured after optimisation of the Doppler settings(229).

Using this technique in preterm infants with a closed duct, diameter of atrial shunt has been shown to correlate strongly with shunt volume as assessed by RVO:LVO ratio(229). This ratio may be >2:1 in the presence of atrial shunt diameter >3mm, suggesting that up to 50% of RVO may be derived from atrial shunt(229). In the presence of ductal shunting RVO is often still higher than LVO, suggesting that the volume of inter-atrial shunt exceeds the volume of ductal shunt(202).

1.6.7 Echo assessment of superior vena cava flow

Superior vena caval flow volume has been suggested as a novel marker of systemic blood flow in newborn infants(9). This study suggested a normal range of SVC flow in preterm infants requiring minimal ventilatory support, demonstrated good repeatability of the technique and showed a correlation between SVC flow and LVO in the absence

of ductal shunting(9). In the same cohort of infants, episodes of low SVC flow were linked to subsequent PVH(10), and SVC flow was shown to be a stronger predictor of PVH than cerebral artery flow(234). Long-term neurodevelopmental outcomes have now been published for this cohort of infants(167). When adjusted for confounding variables low SVC flow was found to be significantly associated with adverse outcome, and was found to be a stronger predictor of poor outcome than hypotension(167).

SVC diameter can be assessed from the parasternal long axis view, with the ultrasound beam rotated into a true sagittal plane and angled to the right of the ascending aorta(9). SVC flow velocity may be assessed from a low subcostal view, with the ultrasound beam angled anteriorly to visualise the SVC as it enters the right atrium(9).

Up to 80% of blood returning to the heart in the SVC is said to come from the brain(122), which would give SVC flow assessment potential value as a specific marker of cerebral perfusion(9). However, as discussed in section 1.4.1 absolute values of cerebral perfusion based on an assumption that 80% of SVC flow comes from the brain are considerably higher than values obtained by xenon, PET and NIRS techniques. Even if a much smaller proportion of SVC flow is derived from the brain this need not diminish the technique's value if its repeatability and clinical associations are confirmed.

No other reports are available assessing the reliability of this technique, or its association with significant neonatal outcomes.

1.6.8 Echo assessment of descending aorta flow

The pattern of diastolic flow in the descending aorta (DAo) has been studied as a marker of ductal shunt volume. The DAo is imaged from either the high parasternal or

subcostal view, and PW Doppler used to assess direction of postductal diastolic blood flow(202).

Retrograde DAo flow is associated with increased ductal size as assessed by colour Doppler diameter(48). Retrograde DAo flow can be seen as early as 7 hours postnatal age in ventilated preterm infants(48). In the presence of minimal inter-atrial shunt, retrograde diastolic DAo flow is associated with decreased RVO:LVO, suggesting increased ductal shunt volume, when compared to infants with absent or antegrade diastolic DAo flow(202). Indeed the degree of reversal of flow (expressed as a fraction of measured forward systolic DAo flow) also correlates with shunt volume in infants and children(235). Reversal of diastolic DAo flow has been shown to be associated with reversal of diastolic flow in the cerebral(62) and renal(236) arteries.

The volume as opposed to pattern of descending aortic flow has been studied in older children(237) and adults(238). DAo flow volume correlates with LVO in children(239), though results show a significant degree of scatter. Continuous monitoring of DAo flow by transoesophageal Doppler has clinical value in children(11, 237) and adults(12), and is an intriguing candidate for a mode of haemodynamic monitoring in the sick preterm neonate should appropriately sized Doppler probes be available(240).

The volume of descending aortic flow is less well studied in neonates, though there are normative data for volume of flow in healthy term infants(241). During recruitment for our current study volume of descending aortic flow in preterm infants was reported by a group of Japanese workers(198). These results provide valuable insight into the pattern of ductal shunting in preterm infants. However only small numbers of infants were studied and infants were recruited over a prolonged period. In addition, neither precise

methodology of DAo flow volume quantification nor repeatability of the technique are described in detail.

Normative data of descending aorta diameter have been obtained in infants using both echocardiography(226, 241) and angiography(242). The repeatability of descending aortic diameter measurement at the level of the diaphragm is poor in infants and children(226), though this study used a subcostal sagittal view, with cross-sectional rather than M mode echo.

1.6.9 Summary of echocardiography in the assessment of neonatal haemodynamics

Echocardiography may have considerable value in the non-invasive assessment of systemic perfusion in neonates. Measures of LVO and RVO have proven repeatability, but are confounded by fetal shunt pathways. The repeatability and clinical utility of assessment of volume of SVC flow is very promising, but has only been assessed by one research group to date. Assessment of DAo flow volume remains relatively poorly studied in neonates.

1.7 Summary of literature review

The risk of adverse neurodevelopmental outcome following preterm birth remains high. Adverse outcome is likely to be at least in part causally related to abnormalities in cerebral perfusion. Currently available methods of routinely monitoring systemic and specifically cerebral perfusion at the cotside in preterm infants are inadequate. A method of accurately (and preferably continuously) monitoring cerebral blood flow at the cotside could undoubtedly aid neuroprotective care.

The potential role for Doppler echocardiography in the assessment of haemodynamic status in sick preterm infants is clear. By measuring a group of variables in each infant one can build up a picture of overall haemodynamic status and avoid over-interpretation of individual measures(228) and help reduce the impact of measurement variability(243). Novel markers of systemic perfusion such as superior vena cava and descending aorta flow volumes, if shown to be reproducible and to be linked with clinically relevant outcomes, would be welcome additions to the current range of Doppler assessments available.

The hypothesis of this thesis is that echocardiographic assessments of superior vena cava and descending aorta flow volume are measurable and are clinically relevant markers of systemic perfusion.

The specific aims of this thesis are:

- To confirm that the process of transthoracic echocardiography does not in itself cause alterations in cardiorespiratory status which could affect haemodynamic markers
- To assess the repeatability, both within and between observers, of echocardiographic measurement of SVC and DAo flow

- To define reference ranges for SVC and DAo flow volumes in healthy term and preterm infants
- To assess the feasibility of carrying out repeated assessments of these new markers of systemic perfusion in the first week of postnatal life in sick preterm infants
- To examine whether patterns of blood flow volume differ between sick and relatively well preterm infants
- To examine the correlation between systemic blood pressure and markers of systemic perfusion in the early postnatal period
- To examine the relationship between ductal patency and markers of systemic perfusion in the early postnatal period
- To examine the relationship between markers of systemic perfusion and clinical outcomes, and in particular whether monitoring of flow adds to the haemodynamic information currently obtained by continuous monitoring of blood pressure in routine neonatal practice

It is hoped that further examination of SVC and DAo flow in preterm infants will increase knowledge of the events occurring during the transitional circulation, provide researchers with clinical tools to aid the assessment of interventions in neonatal care designed to support the circulation, and provide clinicians with reliable cotside tools to assess systemic perfusion in individual infants.

In the future it is hoped that improving circulatory monitoring and support in sick preterm infants will lead to improvements in neurodevelopmental outcome.

2 Methods

2.1 Recruitment

Infants of less than 31 weeks completed gestation who were inborn at National Women's Hospital, Auckland between 1st December 2002 and 1st May 2004 were eligible to be recruited to the study. Infants were excluded if significant structural congenital heart disease was present, if parental consent was refused, or if no operator was available to carry out the ultrasound scans.

In addition to this cohort of preterm infants, a cohort of healthy term infants, who did not require admission to NICU or have evidence of respiratory or circulatory compromise, were recruited.

Parents were approached antenatally where possible, and informed written consent was obtained. Ethical approval for the study was obtained from the local ethics committee.

The prime focus of this study was on markers of systemic blood flow in preterm infants in the first 48 hours of postnatal life. We elected to recruit infants of less than 31 weeks completed gestation as this is the cohort of infants most at risk of adverse neurodevelopmental sequelae following preterm birth(16), and is similar to gestational cohorts assessed in prior studies of SVC flow volume(9). Within this cohort, infants who did not require circulatory support and required less than 48 hours respiratory support were prospectively deemed 'healthy' and it was felt that their flow patterns would represent the best estimate of a 'normal' reference range in preterm infants.

Historically around 120 infants of less than 31 weeks gestation are admitted to National Women's Hospital NICU each year. With a >50% recruitment rate, over an 18 month recruitment period, an estimated 100 preterm infants could be studied.

Gestation-based rather than birth weight-based eligibility criteria were used so that recruitment could take place antenatally wherever possible. Women with threatened preterm delivery were approached for recruitment to the study if obstetric staff had sufficient concern to administer antenatal steroids. In cases where preterm birth was sudden and unexpected or antenatal consent was not possible, approach occurred postnatally.

Following a general discussion of the events surrounding preterm birth, parents were invited to enrol their infant/s in the study. Written information was supplied (Appendix 1). Antenatal ward midwifery staff and NICU nursing staff were briefed on the background of the study so that they could discuss study entry with parents if required. Infants were recruited to the study if the parents were able to give informed consent.

2.2 Data collection

Details of maternal health, pregnancy and delivery were recorded, as were demographic details of the infant (Appendix 2).

At the time of each scan details of ventilatory support, blood pressure, circulatory support, urine output, feed tolerance and blood gas analyses were recorded (Appendix 2).

Mode of ventilatory support was recorded as high frequency oscillation ventilation (HFOV)/synchronised intermittent mandatory ventilaton (SIMV)/pressure support ventilation (PSV)/ synchronised intermittent positive pressure ventilation (SIPPV)/nasal

continuous positive airway pressure (NCPAP)/low flow oxygen/nil. Documented mean airway pressure and fraction of inspired oxygen (FiO_2) were recorded from the ventilator/CPAP circuit.

Blood pressure was recorded invasively if the infant had an indwelling arterial catheter. Systolic, diastolic and mean blood pressures were recorded. In many cases infants with invasive blood pressure monitoring had recordings of blood pressure downloaded every 60 seconds using Marquette Solar 8000 monitors (GE medical systems, Wisconsin, USA) and Bedmaster V1.3 software (Excel Medical Electronics Inc, Florida, USA) as part of their routine NICU monitoring. This recorded blood pressure value is an average of the blood pressure monitored over the previous 6 seconds. If available these measures of blood pressure were then averaged over the duration of the scan. Intermittent blood pressure measures were only included if invasive blood pressure readings were not available and if blood pressure readings were taken within an hour of the echocardiogram.

The type and dose of inotropes/vasopressors used were recorded. Requirement for fluid boluses was recorded, along with the time and type of fluid administered and volume in ml/kg.

The level of ventilatory and circulatory support was dictated by the attending clinicians, who were not aware of the results of the echocardiograms. Our unit guideline is to use fluid boluses and inotropes to maintain mean arterial blood pressure greater than gestational age in weeks(80).

Urine output was recorded for the first 24 hours of postnatal life. The results of blood gas analysis were included if taken within 2 hours of echocardiography. Serum

potassium, pCO_2, glucose, base deficit and bicarbonate were recorded. The highest creatinine level in the first week of life was also recorded if measured.

At time of discharge or death further details on the postnatal clinical course were acquired, including date, corrected gestational age and growth parameters at discharge/death (Appendix 2). The principle clinical outcomes assessed were survival, periventricular haemorrhage, periventricular leukomalacia, necrotising enterocolitis/isolated intestinal perforation, presence of chronic lung disease and requirement for supplementary home oxygen, and time taken to tolerate full enteral feeds.

2.2.1 Outcome Definitions

Preterm infants were deemed 'healthy' if they required less than 48 hours respiratory support and did not receive fluid resuscitation or inotropic support in the first week of life.

Cranial ultrasonography was performed as part of the study protocol in the first 24 hours of postnatal life wherever possible to exclude early intracranial anomaly. Thereafter all infants <31 weeks admitted to the NICU routinely receive cranial ultrasound scans at 5-7 and 28 days postnatal age, and at 36 weeks corrected gestational age. From each of these scans the presence and grade of PVH (according to Papile (244)) and presence of intraparenchymal abnormalities were recorded.

Necrotising enterocolitis and isolated intestinal perforation were assessed by radiographic criteria, requirement for surgical intervention and results of histological examination.

Chronic lung disease was defined as requirement for oxygen or any respiratory support at 36 weeks corrected gestation. Requirement for supplementary home oxygen support was assessed according to standard unit criteria. Infants who failed to maintain oxygen saturations above 90% for more than 80% of the duration of an 8 hour saturation run were discharged with supplementary oxygen.

Full feeds were said to have been reached when infants tolerated >150ml/kg/day of milk for 3 consecutive days.

Similar maternal and demographic details were collected from the cohort of term infants. Postnatal clinical details for term infants were not recorded as all infants were required to have uneventful postnatal courses to permit inclusion in the study.

2.3 Echocardiography techniques

2.3.1 General approach

Echocardiography in preterm infants was performed as close as possible to 5, 12, 24 and 48 hours postnatal age, and again on the 7th postnatal day. Infants were nursed in incubators or on overhead heat tables to help maintain body temperature. In all cases echocardiography was performed immediately before or after routine nursing cares to minimise the number of discrete handling episodes encountered by each infant.

Echocardiography in each term infant was performed at the mother's bedside. Scans were performed as close as possible to 5, 12, 24 and 48 hours postnatal age. Scans at 24 and 48 hours were not performed if infants had been discharged home prior to this time.

In all cases warmed coupling gel was used. In some cases infants were swaddled to prevent undue distress. The exact echocardiographic views used will be described

below. The scan sequence was designed to allow images to be obtained in the most efficient manner, with subcostal views performed at the end of the series in case abdominal pressure caused distress to the infants (Appendix 3).

Where possible infants were placed slightly in the left lateral position to optimise echocardiographic windows, and the neck was placed slightly in extension to aid acquisition of high parasternal views.

Infants were examined when they were sleeping or quietly awake wherever possible. Infants who became distressed during the procedure were settled by containment holding/swaddling as necessary until they settled.

The start and finish times of each scan were recorded from the bedside monitors, along with estimates of the state of arousal of the infant.

All scans were performed with an ATL 3000 ultrasound scanner (Advanced Technological Laboratories, Bothell, Washington, USA) equipped with a 7MHz probe capable of 2D, M mode, colour Doppler and continuous and pulsed wave Doppler imaging. In all cases scans were recorded onto video tape and analysed away from the cotside to minimise duration of handling. In most cases when optimal images were obtained during the scan the screen image was frozen and recorded for ease of analysis. In most cases the infant's electrocardiogram (ECG) trace was simultaneously recorded on the ultrasound monitor.

Structural normality of the heart was confirmed at the 48 hour ultrasound scan when an estimate of ductal significance based on ductal diameter, ductal shunt pattern, direction of diastolic DAo flow and LA:Ao ratio was produced for the attending clinicians according to our current unit policy.

To analyse the intra- and inter-observer repeatability of the ultrasound techniques a cohort of infants was re-examined within 10 minutes of the initial scan by either the same or a different examiner. Images were recorded onto a separate video tape, and analysed independently of the initial scan.

Analysis of scans was carried out using in-built cardiac analysis software on the ultrasound machine. All measures were averaged over 5 consecutive cardiac cycles(227) wherever possible, unless image quality was unsatisfactory. In the case of SVC flow velocity, measures were averaged over 10 consecutive cycles to minimise the impact of marked respiratory variations in flow. In all cases heart rate was calculated from the beat-to-beat interval on the Doppler recording, averaged over 5 consecutive cardiac cycles.

Data from each scan were recorded on a pre-printed form at the time of analysis (Appendix 4)

Volume of flow (expressed as ml/kg/min) was calculated from:

$$\frac{\text{VTI} \times \text{heart rate} \times (\text{vessel diameter})^2 \times \pi}{4 \times \text{birth weight}}$$

where VTI = velocity time integral in cm, π = 3.14159, vessel diameter in cm, birth weight in kg

2.3.2 Aortic diameter

Aortic root diameter was assessed immediately distal to the valve orifice at end systole using the M mode trailing edge-leading edge technique from the parasternal long axis view. Care was taken to ensure that the transducer was positioned such that the long

axis of the aorta was exactly perpendicular to the ultrasound beam. High definition zoom was employed to optimise the view before M mode echocardiography was used to give maximum discrimination of vessel walls(226). Trailing edge-leading edge end-systolic diameters at the level of the aortic root have been shown to be the most reproducible measures of aortic size(217, 219, 224, 226).

The mean of measures taken from five consecutive cardiac cycles was taken at each scan. Changes in aortic diameter in the first week of life(222, 225) have been shown to be considerably less than the measurement error of the technique(226). Therefore the mean of each infant's systolic aortic diameters from the 5 scans in the first week of life was used for all calculations of LVO.

2.3.3 Aortic Flow Velocity

Aortic velocity time integral (VTI) was assessed using pulsed wave Doppler, with the range gate placed at the level of the tips of the aortic valve leaflets when viewed from the apical view modified to visualise the ascending aorta(245). The velocity time integral is the area under a velocity-time trace for a single cardiac cycle(246). VTI is equivalent to the mean distance travelled by the column of blood leaving the heart in a single cardiac cycle and is also known as the stroke distance(237).

To minimise any angle of insonation between the ultrasound beam and aortic blood flow the view was optimised by moving the probe laterally, caudally and rotating clockwise to image along the ascending aorta. Angle correction was not used.

While the suprasternal site has been shown to yield more reproducible estimates of LVO than the apical site in infants and children, this site may be inaccessible in infants requiring respiratory support(217). Since the suprasternal site would be inaccessible in many infants, and apical and suprasternal sites produce consistently different measures

of LVO in newborn infants(217) we elected to measure aortic flow velocity from the apical 5 chamber view in all cases. Although continuous wave Doppler has been shown to be slightly more reproducible than pulsed wave Doppler in estimating neonatal LVO(217), this effect is minimal in terms of intra-observer repeatability. We felt that a consistent approach of using PW for estimation of flow at all points in the circulation was more important than the marginal repeatability benefit obtained from using CW in this single site.

2.3.4 Pulmonary diameter

Right ventricular outflow diameter was assessed at the hinge-points of the pulmonary valve from either the parasternal short axis or tilted parasternal long axis views during cardiac systole. Valve diameter was assessed by frame-by-frame analysis of the 2D image, as it is not possible to place an M mode beam perpendicular to the valve orifice.

In general the tilted parasternal long axis view was used in preference, resorting to the short axis if visualisation was poor. When satisfactory views were obtained the image was frozen and scrolled back slowly through five cardiac cycles for ease of measurement. Since the pulmonary artery may change in size significantly during the first week of life(225), we elected to re-measure pulmonary valve diameter at each scan.

2.3.5 Pulmonary Flow Velocity

Pulmonary VTI was assessed using pulsed wave Doppler, with the range gate placed at the level of the tips of the pulmonary valve leaflets when viewed from the parasternal short axis view(224). This has been shown to be the most reproducible method for estimation of neonatal right ventricular output(224).

2.3.6 SVC diameter

Superior vena cava diameter was assessed at the point at which the vessel begins to open up into the right atrium. M mode trailing edge-leading edge technique from a high, parasternal long axis view was used. The transducer head was placed as close to the midline as possible in an attempt to acquire directly antero-posterior views of the SVC (figures 2.1 and 2.2, reproduced with parental consent). The ultrasound transducer was rotated such that it was almost in the true sagittal plane when it was in line with the long axis of the SVC. Care was taken to ensure that transducer was positioned such that the SVC was exactly perpendicular to the line of the ultrasound beam. Both maximum and minimum SVC diameter was assessed for each cardiac cycle, and the mean of these used to quantify volume of flow. While SVC flow velocity is maximal at the time of cardiac systole, which is also the time of maximal vessel diameter, there is some flow throughout the cardiac cycle. Using mean vessel has been shown by others to be a repeatable technique(9), we elected to use the same method.

These previous studies have also used the high parasternal long axis view to assess SVC diameter as the vessel begins to open up into the right atrium(9). However these studies used 2D rather than M mode echocardiography. We elected to use M mode echocardiography as it improves vessel wall definition at fast heart rates and has been shown to give better repeatability of other vessel diameter measures in infants and children(226).

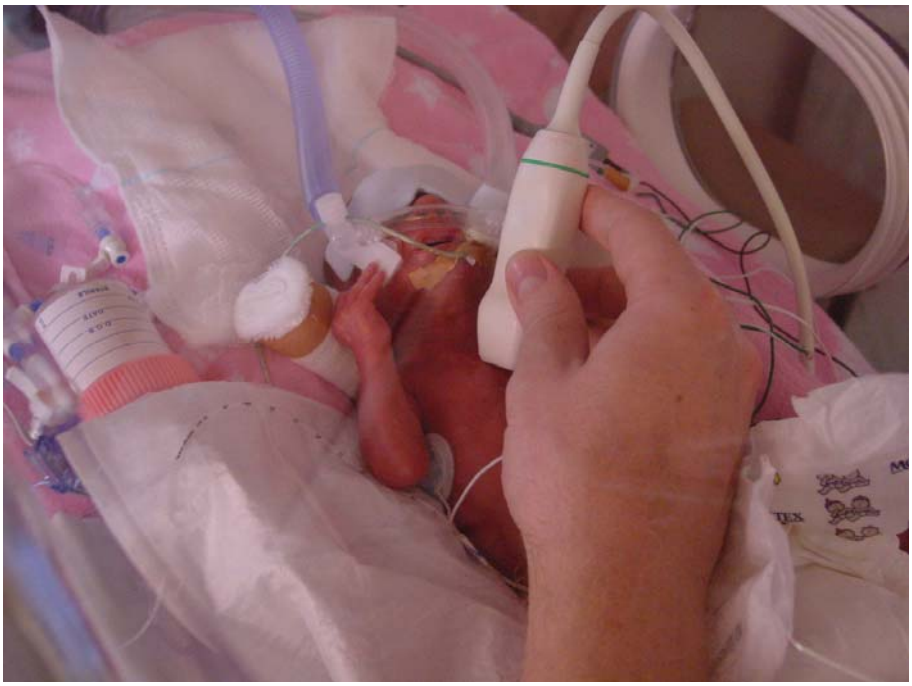

Figure 2.1 - Transducer position for SVC diameter measurement

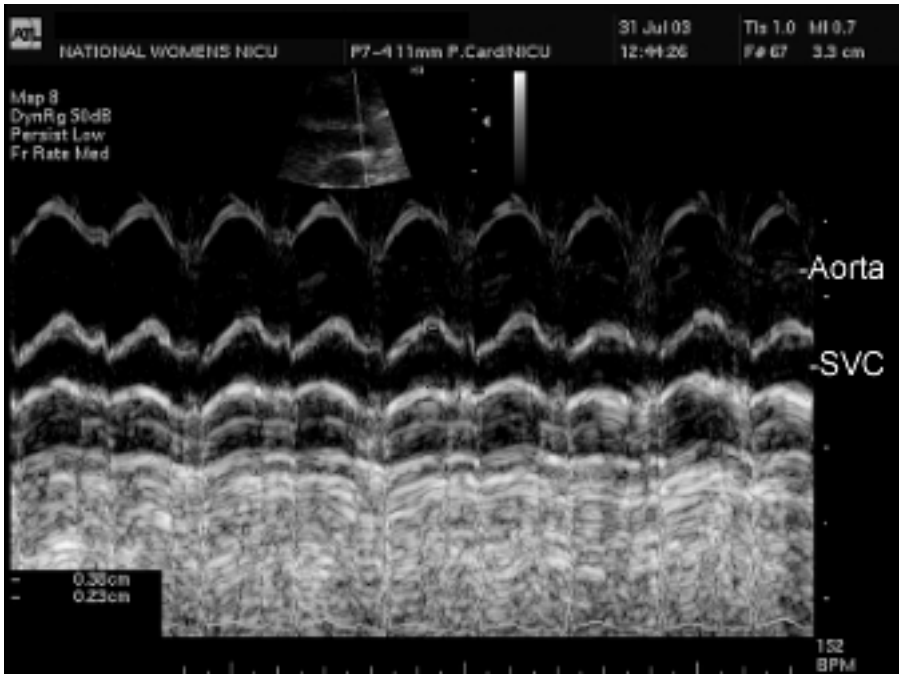

Figure 2.2 - M mode imaging for SVC diameter measurement

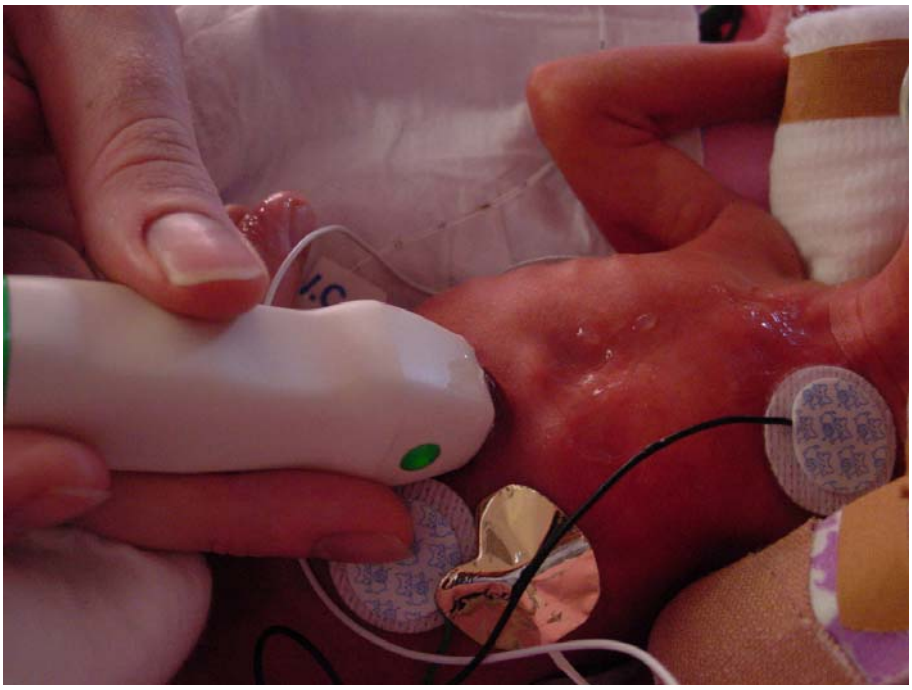

Figure 2.3 - Transducer position for SVC flow velocity measurement

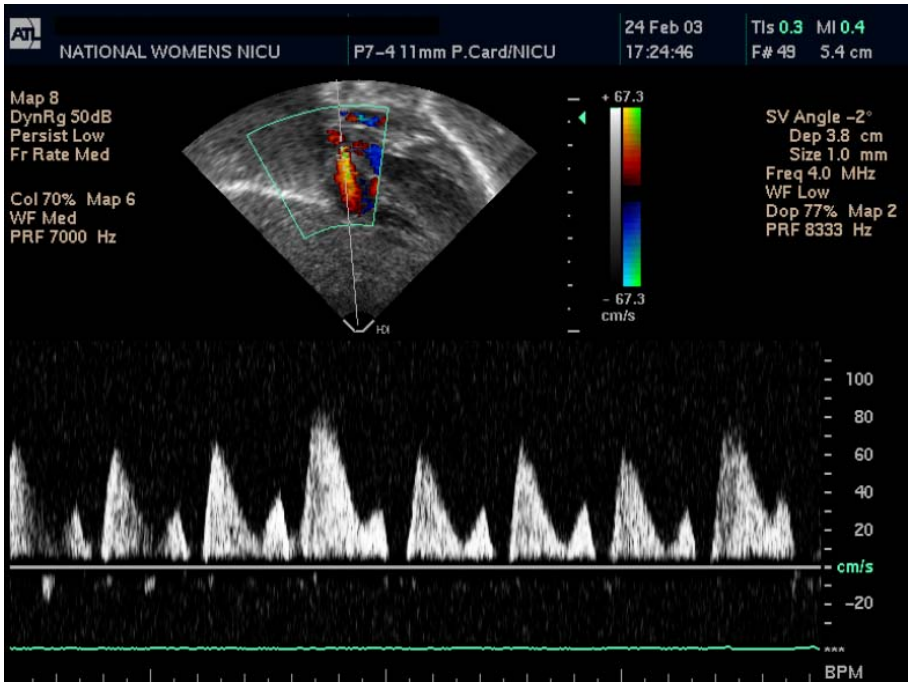

Figure 2.4 - Pulsed wave Doppler imaging for SVC flow velocity measurement

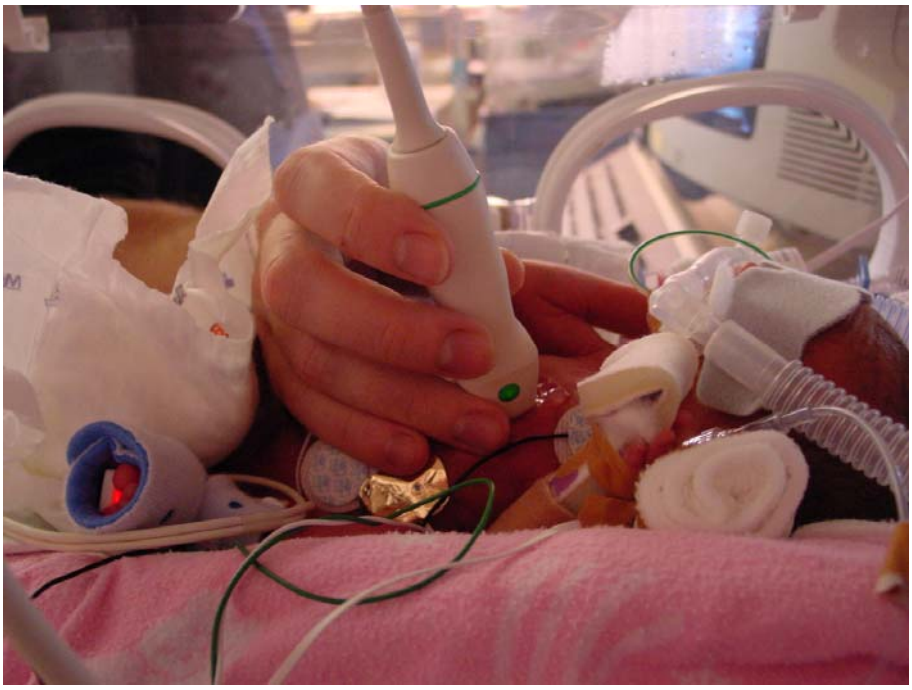

Figure 2.5 - Transducer position for DAo diameter measurement

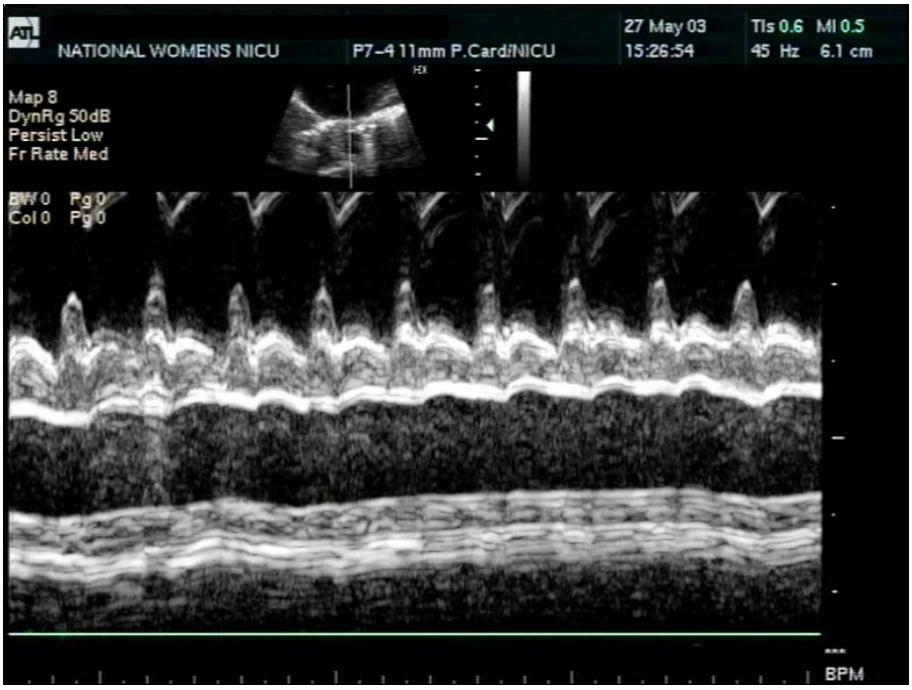

Figure 2.6 - M mode imaging for DAo diameter measurement

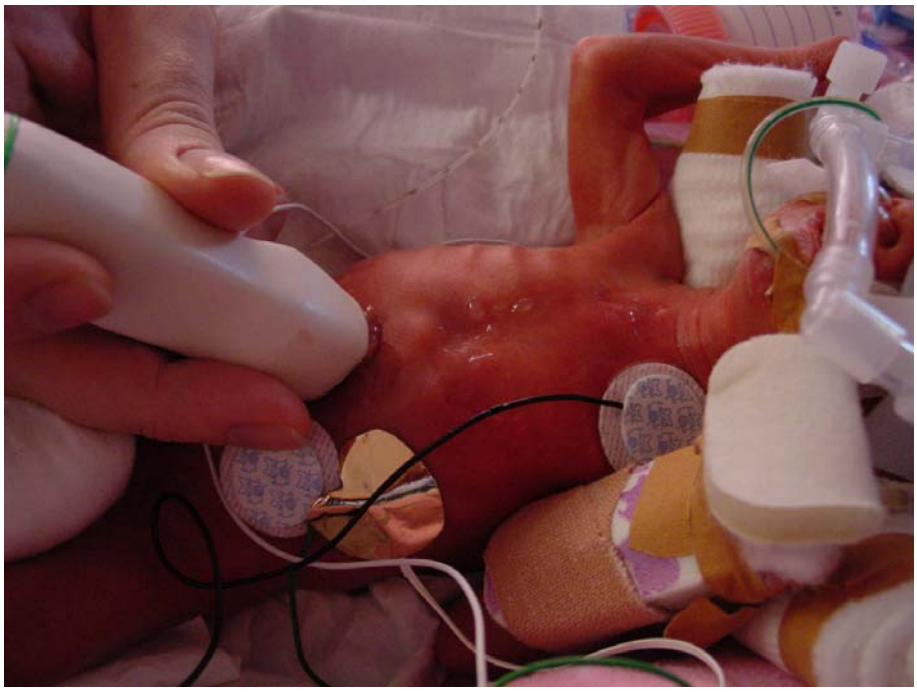

Figure 2.7 - Transducer position for subcostal DAo flow velocity measurement

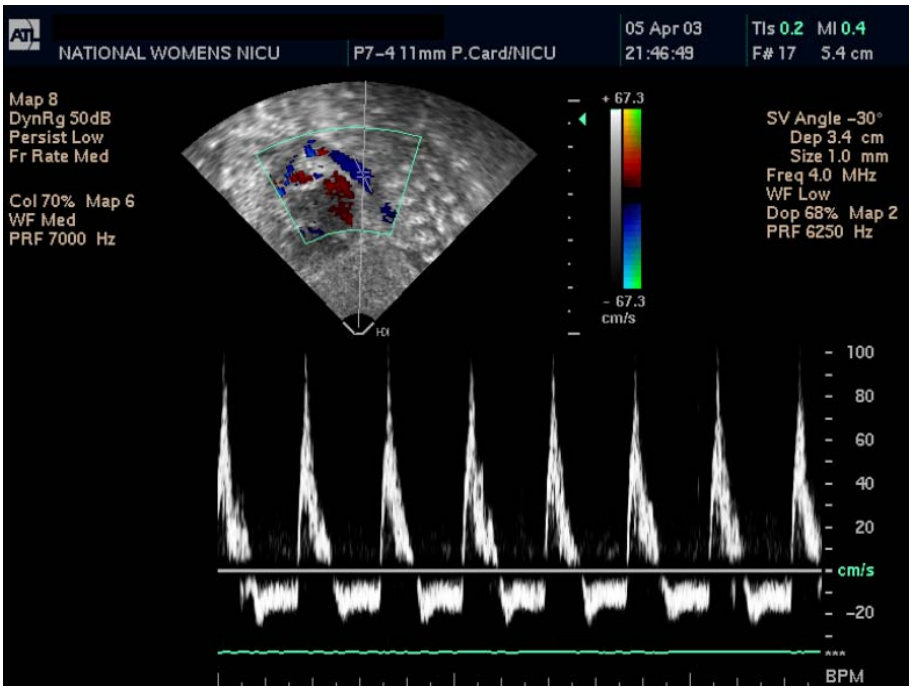

Figure 2.8 - Pulsed wave Doppler imaging for subcostal DAo flow velocity measurement

Figure 2.9 - Transducer position for suprasternal DAo flow velocity measurement

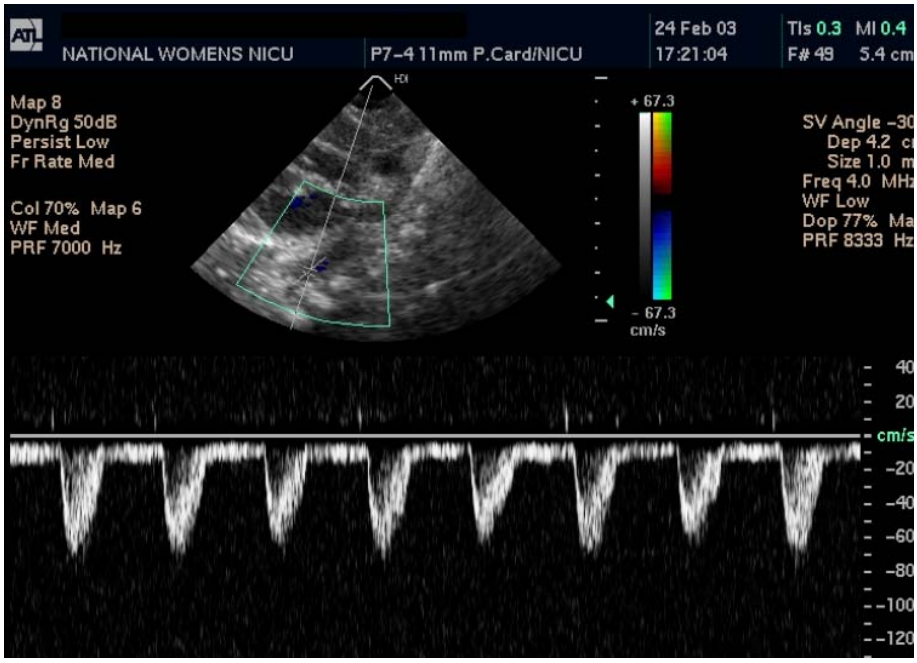

Figure 2.10 - Pulsed wave Doppler imaging for suprasternal DAo flow velocity measurement

While the transducer head was placed as close to the midline as possible in an attempt to acquire directly antero-posterior views of the SVC, in some cases shadowing by overlying lung meant that optimal images could only be obtained by sliding the transducer to the patient's left side and angling back to the patient's right. Imaging the SVC from this more lateral position may lead to increased diameter measures due to the oval cross-section of the SVC. The SVC could be seen entering the right atrium directly to the right of and posterior to the ascending aorta. Recognition of the vessel was frequently aided by the presence of 'spontaneous contrast' which is felt to be due to slow-moving red blood cell aggregates in the venous circulation(247).

2.3.7 SVC flow velocity

SVC VTI was assessed using pulsed wave Doppler, with the range gate placed at the point at which the vessel begins to open up into the right atrium, when viewed from a low subcostal view. This view is identical to that used in previous studies(9). As in these studies, the transducer head often needed to be manoeuvred close to the umbilicus so that the SVC could clearly be seen to be directly in line with the ultrasound beam (figures 2.3 and 2.4).

Measures of SVC flow velocity were averaged over 10 consecutive cardiac cycles to minimise the impact of variations in flow with respiration. Any reversal of flow in the SVC was also quantified and deducted from the measured forward flow to give an integrated VTI.

2.3.8 DAo diameter

Thoracic descending aorta (DAo) diameter was assessed as close as possible to the plane of the aortic valve when seen in the parasternal short axis view. At this point the DAo can be seen descending directly posterior to the left atrium. Systolic and diastolic

DAo diameter was assessed in five consecutive cardiac cycles by the trailing edge-leading edge technique (figure 2.5 and 2.6).

When manoeuvring the transducer to maximise the image quality care was taken to ensure that the ultrasound beam remained directly perpendicular to the vessel. Any angulation in line with the long axis of the vessel would result in an overestimation of diameter due to the oblique view obtained. When imaging in M mode a 2D image remains on display such that the vessel could be seen to be circular rather than oval in cross-section.

In general the pulsatile descending aorta was easily identifiable, but in some cases identification was confirmed either by the presence of an in-dwelling umbilical arterial catheter or by rotating the ultrasound transducer through 90 degrees and confirming pulsatile arterial flow towards the abdomen.

We used the mean of systolic and diastolic DAo diameters in the calculation of flow volumes as a significant proportion of flow volume occurs in diastole and since using the maximal number of readings would potentially decrease the impact of errors in diameter measurement.

DAo diameter has been shown to change by up to 15% (31% change in area) with alteration of blood pressure in adults(248). Since degree of pulsatility decreases with age(249) it is likely that the diameter of the newborn DAo is particularly dependent on arterial blood pressure. Therefore, despite the scope for errors in measurement of DAo diameter, we elected to re-measure DAo diameter at each scan.

2.3.9 DAo flow velocity

Velocity of DAo was assessed at two points during each scan. From a low subcostal sagittal view flow was assessed immediately distal to the ductus arteriosus (figures 2.7 and 2.8).

From a high parasternal ('ductal') view flow was assessed at approximately the level of the diaphragm (figures 2.9 and 2.10).

In both cases the DAo was initially viewed by colour Doppler, and the ultrasound transducer manoeuvred to obtain optimal images with minimum angle of insonation between the ultrasound beam and the direction of blood flow. Pulsed wave Doppler was used with appropriate angle correction. Wall thump filters were set to their minimum level to aid detection of low velocity diastolic DAo flow.

DAo flow profiles were specifically interrogated at the time of the scan to maximise detection of diastolic flow patterns which were felt to be crucial to this study. In all cases VTIs of forward and reversed flow were measured separately and only integrated for the final assessment of flow volume. Diastolic flow pattern was defined as 'forward' or 'reversed' if this pattern was seen throughout the cardiac cycle. Specifically a brief period of reversed flow in early diastole is seen in the absence of ductal shunting(250) and is postulated to be due to backward movement of the aortic valve cusps at valve closure(62). If present, this transient early diastolic retrograde flow was not labelled as reversed diastolic flow and was not deducted from the measured forward flow in the calculation of integrated VTI. Flow was deemed 'absent' if no clear pattern of diastolic flow was seen. In some cases reversed diastolic flow was only seen during cardiac cycles which corresponded with inspiration, with flow being

absent at other times. These infants were classified as having reversed diastolic DAo flow as VTIs of these intermittently reversed flows could often be large.

It is not clear whether reversed diastolic DAo flow in preterm neonates is laminar, and therefore whether it is appropriate to estimate blood flow volume from the area under a velocity-time curve. However studies in adults with aortic valve insufficiency have suggested that including the component of diastolic flow reversal does not lead to inaccuracies when comparing Doppler assessed flow to Fick measurements(238). While the proportion of reversed to forward diastolic DAo flow has been quantified in the newborn period(250), there are no published studies which quantify the volume of diastolic DAo flow in neonates.

We felt that measurement of DAo flow velocity both close to the duct and more distally in the vessel was important since diastolic flow patterns have been shown to be different at these two sites(251). In addition, oesophageal monitoring studies in adults have suggested that blood flow immediately below the aortic arch may not be evenly distributed across the vessel area.

Measurement of DAo flow velocity at a separate location from the diameter measure was not felt to be a problem since the thoracic descending aorta has a fairly uniform diameter(238).

2.3.10 Duct diameter

Duct diameter was assessed by colour Doppler from the high left parasternal ('ductal') view(202). Maximum velocity of the colour Doppler scale was generally kept at 67 cm/second, though it was occasionally increased in the presence of high velocity ductal flow. Colour gain was reduced to the point at which no interference was seen outside the duct or the aorta and pulmonary arteries.

Ductal diameter at the point of maximal constriction was assessed by frame-by-frame analysis of the 2D image. Maximal ductal diameter at this point of maximal constriction was measured once in each of 5 cardiac cycles. We chose not to select a single phase in the cardiac cycle at which to make the measurement since the phase of maximal diameter was dependent on the pattern of ductal flow. Infants with pure left to right flow generally had maximal duct diameter during systole. Infants with bidirectional shunting were often noted to have maximal ductal diameter during diastole.

2.3.11 Ductal flow velocity

The pattern and velocity of ductal shunt were assessed using continuous wave Doppler from the high left parasternal view. When ductal shunting was bidirectional the proportion of the cardiac cycle where flow was left-right was assessed(202). When left-right flow was present throughout the cardiac cycle the maximum and minimum flow velocities were assessed(85).

2.3.12 Fractional shortening

Left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) were assessed by M mode trailing edge-leading edge technique from the parasternal short axis view(118). The exact point of end-systole and end-diastole were obtained from simultaneously recorded ECG trace.

Fractional shortening (FS) was calculated as:

$$\frac{(\text{LVEDD} - \text{LVESD}) \times 100\%}{\text{LVEDD}}$$

2.3.13 Left atrial:Aortic root ratio

LA:Ao ratio was assessed by M mode leading edge-leading edge technique from the parasternal long axis view(231). With the ultrasound beam perpendicular to the aortic root the M mode cursor was dropped through the aortic cusps into the left atrium(118). Left atrial and aortic root diameter were both assessed at end-systole, and LA:Ao ratio calculated.

2.3.14 Inter-atrial shunt size

Atrial shunt diameter was assessed by colour Doppler from the subcostal 4 chamber view(228). Maximum velocity of the colour Doppler was generally kept at 67 cm/second, though it was occasionally decreased to aid discrimination of atrial shunt boundaries. Colour gain was reduced to the point at which no interference was seen outside the cardiac chambers or the SVC and pulmonary veins. Atrial shunt diameter at the level of the atrial septum was assessed by frame-by-frame analysis of the 2D image once in each of five cardiac cycles.

Direction of atrial shunting was assessed by pulsed wave Doppler from the subcostal 4 chamber view. When atrial shunting was bidirectional the proportion of the cardiac cycle where flow was left-right was assessed.

If no clear interatrial shunt could be seen on colour Doppler (irrespective of the impression of a patent foramen ovale on 2D imaging) this was classified as no shunt(228). Shunt volume was not quantified.

2.3.15 Cranial ultrasound

Cranial ultrasonography was performed with an 8 MHz sector transducer in the first 24 hours of life in the majority of infants. Sagittal and coronal sweep views were obtained, and, if present, periventricular haemorrhage was classified according to Papile(244).

Cranial ultrasound images were stored on videotape to compare with later images to allow estimation of progression of PVH.

2.4 Statistical analysis

All data were analysed using Statview software (SAS Institute, Cary, NC, USA). Statistical methods used are discussed further in the relevant chapters of this thesis.

In summary, normally distributed data are summarised by mean and standard deviation, and comparisons between groups made by paired and unpaired t tests. Non-parametric data are summarised by median and range, and comparisons between groups made by Wilcoxon signed rank test (for paired data) and Mann-Whitney test (for unpaired data).

Relations between continuous data were analysed using simple and multiple linear regression. Multivariate comparison was performed for categorical outcomes using logistic regression.

The predictive value of measures of flow was assessed by receiver operating characteristic (ROC) analysis(252).

Repeatability of individual echocardiographic measures were assessed by the Bland Altman method(215). Median variability, repeatability index and confidence step(207) analysis were also performed to allow comparison to other published studies and methods.

In all cases statistical significance was taken where $p < 0.05$.

3 Cardiorespiratory Stability during Echocardiography in Preterm Infants

3.1 Introduction

Echocardiography is being used increasingly in neonatal units, and has been shown to have a high yield for structural abnormalities(214). Repeated examinations in the preterm infant may also provide information on haemodynamic status of practical clinical value(83). The hypothesis of this thesis is that echocardiographic assessments of superior vena cava and descending aorta flow volume provide measurable and clinically relevant markers of systemic perfusion in preterm infants. It is hoped that these measures will enhance our ability to monitor and support the transitional circulation, and so achieve improvements in neurodevelopmental outcomes in preterm infants.

An assessment of whether or not these echocardiographic measures can be recommended for use in the haemodynamic assessment of sick preterm infants also requires some assessment of potential risks to the infant when carrying out the measures. The risks of ultrasound as an energy source, such as heat transfer and cavitation are acknowledged(118), though most authorities agree that ultrasound scanning is without hazard "if used prudently" in neonates(118). Controversy persists over the potential risk to infants from incorrect diagnosis of structural congenital heart disease by non-cardiologists(211, 212, 214) but this is outside the scope of this thesis.

There are no published data on the potential adverse effects of the handling involved in performing echocardiography on the cardiorespiratory status of preterm infants. Handling episodes such as nursing care procedures(253, 254), venous cannulation(255), excessive noise(253), chest radiographs(256, 257) and even nappy changes(256, 257) have been shown to impact on oxygen saturation or blood pressure in neonates. While studies designed to limit number and duration of handling procedures in preterm infants have failed to show significant benefits(169), concerns over the impact of handling have led to the widespread acceptance of principles of minimal handling in preterm infants(256).

Any clinically significant impact of echocardiography on cardiorespiratory status would lead to concerns over the risks to the infant of performing the procedure. Any potential benefit to the infant from the haemodynamic information acquired by echocardiography would have to outweigh the risks of the procedure itself(255). Altered cardiorespiratory status during echocardiography could also mean that the haemodynamic status assessed during the scan did not accurately reflect an infant's status at other times, limiting the value of the technique. Therefore the aim of this study was to examine whether cardiorespiratory status was disturbed during carefully performed echocardiography in preterm infants.

3.2 Methods

Infants were assessed for cardiorespiratory stability during echocardiography if they had continuous intra-arterial blood pressure recordings and had an undisturbed control period immediately prior to or following their echocardiogram. Efforts were made to keep infants' cardiorespiratory status stable during echocardiography, including using warm coupling gel, minimising study duration, and using swaddling where necessary.

Echocardiograms were performed immediately before or after routine nursing cares to minimise the number of discreet handling episodes. Infants were not muscle-relaxed or sedated during the study period.

Recordings of blood pressure, heart rate and oxygen saturations (SpO2) were downloaded every 60 seconds during both the echocardiogram and the undisturbed control period using Marquette Solar 8000 monitors (GE Medical Systems, Wisconsin, USA) and Bedmaster V1.3 software (Excel Medical Electronics Inc, Florida, USA). Absolute values were averaged separately over the echocardiogram and control periods. Mean values during echocardiography and during control rest periods were compared using a paired t-test. Stability within each study was assessed by coefficient of variation (standard deviation/mean). Coefficients of variation were compared with a Wilcoxon signed rank test. Impact of demographic and postnatal factors on stability was assessed by univariate analysis.

3.3 Results

Seventeen infants with a median gestation of 27 (range 25-29) weeks, and median birth weight of 880 (range 510-1430) grams were studied for cardiorespiratory stability during echocardiography. A median of two (range 1-4) echocardiograms per infant had continuous intra-arterial blood pressure monitoring and an adjacent rest period, such that 40 consecutive paired recordings were available. In 28 recordings infants were intubated and mechanically ventilated, the remainder were supported by CPAP. Median echo duration was 10 (range 7-19) minutes, and a control period of the same duration was studied in each case. Since data were downloaded every 60 seconds a median of 10 (range 7-19) readings were compared for each variable in each echo and control group, creating a total of 870 readings per variable.

3.3.1 Impact of echocardiography on absolute blood pressure, heart rate and oxygen saturation

During echocardiography there were no significant differences in absolute blood pressure when compared with the control periods. Absolute heart rate was slightly higher and oxygen saturation slightly lower during echocardiography (Table 3.1).

	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	Heart Rate (beats/min)	SpO2 (%)
Echo Mean (SD)	46.3 (7.2)	27.9 (5.2)	35.9 (5.5)	152.0 (11.9)	92.0 (3.6)
Rest Mean (SD)	46.4 (6.5)	27.4 (4.6)	35.6 (5.0)	147.9 (11.9)	93.1 (3.6)
Mean Difference	-0.1	0.5	0.3	4.1	-1.1
p value	0.82	0.25	0.56	<0.0001	0.0033

Table 3.1 - Systolic, diastolic and mean blood pressure (BP), heart rate and oxygen saturation during scan and control periods for 40 scans in 17 infants. There were no significant differences in blood pressure between scan and control periods. Heart rate was slightly higher and oxygen saturation (SpO2) slightly lower during echocardiography

3.3.2 Impact of echocardiography on stability of blood pressure, heart rate and oxygen saturation

Coefficients of variation (CV) of systolic, diastolic and mean blood pressure, heart rate and oxygen saturation within the periods of echocardiography were slightly higher than during the matched rest periods. The maximum CVs were not increased for blood pressure but were increased for heart rate and oxygen saturation (Table 3.2).

	Systolic BP Median (Range)	Diastolic BP Median (Range)	Mean BP Median (Range)	Heart Rate Median (Range)	SpO2 Median (Range)
Echo CV	5.5% (1.9-11.3%)	6.3% (2.0-13.5%)	5.2% (1.5-12.0%)	3.4% (0.9-12.4%)	2.1% (0.0-11.9%)
Control CV	4.5% (2.0-12.8%)	4.8% (2.1-14.0%)	4.1% (1.5-12.3%)	2.4% (0.4-8.2%)	1.2% (0.0-8.9%)
Median Difference	1.0%	1.5%	1.1%	1.0%	0.9%
p value	0.02	0.006	0.009	0.006	0.005

Table 3.2 - Coefficients of variation for systolic, diastolic and mean blood pressure (BP), heart rate and oxygen saturation (SpO2) during scan and control periods for 40 scans in 17 infants. Median coefficient of variation was slightly higher during echocardiography for all measures

Univariate analysis showed that CVs for blood pressure during echocardiography was not significantly affected by birth weight, mode of ventilation, postnatal age at scan or whether echocardiography occurred before or after the control rest period. Infants ≤26 weeks gestation had significantly increased coefficient of variation for blood pressure during echocardiography when compared to infants >26 weeks gestation (CVs: Systolic blood pressure - 6.9% vs 5.1%, p=0.01; Diastolic blood pressure - 7.9% vs 5.4%, p=0.01; Mean blood pressure - 6.6% vs 4.9%, p=0.03).

3.4 Discussion

As the use of echocardiography on the neonatal unit increases it is important that the handling involved in the technique neither worsens outcomes for infants nor alters haemodynamic status so as to produce measurements which do not reflect the infant's true condition.

Echocardiography was associated with a statistically significant decrease in mean oxygen saturation (mean difference -1.1%) and increase in mean heart rate (mean difference 4.1 beats/minute). However, the absolute differences in heart rate and oxygen saturation during echocardiography were only a third of the standard deviations of values seen during control rest periods - absolute differences in these measures during echocardiography are therefore considerably less than spontaneous fluctuations seen at rest in preterm infants. The changes in oxygen saturation and heart rate seen during echocardiography in our study were also less than those seen during routine noise exposure in the neonatal unit in a previously studied cohort of preterm infants(253). Despite showing statistically significant differences in absolute heart rate and oxygen saturation, echocardiography has no clinically significant impact on these variables in preterm infants.

Of particular importance to this study is the observation that no discernible effect of echocardiography on absolute blood pressure was seen. The minor increase in CV of blood pressure is also unlikely to be of clinical significance. Bada et al have previously measured mean arterial blood pressure variability on a minute-to-minute basis over 15 minute periods in 72 very low birth weight infants who did not develop periventricular haemorrhage (PVH) and found a mean CV of 7.8%(37). The average mean blood pressure CV of 5.8% during echocardiography in our cohort compares favourably with this, and no infant in our cohort had a mean blood pressure CV above 13% which Bada and colleagues suggested may be associated with PVH. Although the least mature infants in our study had the highest variability in blood pressure during echocardiography, these infants also tended to have higher variability during rest periods, and it is again reassuring that all values were within ranges which have previously been suggested not to be associated with PVH.

Previous studies have shown that BP may undergo a biphasic change during care procedures with an initial fall followed by a rise to higher than pre-handling values(254). If such a pattern were seen in this study, absolute BP could be subject to significant changes which are masked when measures are averaged over the course of the exam. This seems unlikely since the duration of the initial fall in BP in previous studies was generally longer than the complete duration of echocardiography in our study(254) and although increases in coefficient of variation in BP during echocardiography were statistically significant, they were unlikely to be large enough to account for a large biphasic swing in blood pressure during the echo period.

While it is not possible to study the impact of handling on measures of blood flow without a separate continuous non-invasive measure of flow, these data at least confirm that echocardiography can be performed in preterm infants without disrupting parameters of cardiorespiratory status which may impact on flow.

3.5 Conclusion

It is possible to perform echocardiography in the preterm infant to gain potentially valuable haemodynamic information without significantly disturbing cardiorespiratory status.

4 Repeatability of Measurement of Flow Volume in the Superior Vena Cava and Descending Aorta

4.1 Introduction

The importance of fully assessing the error inherent in any new measurement technique cannot be over-stated(207). Without an appreciation of the repeatability of a technique, clinicians cannot gauge the significance of apparent changes in a measure over time. Doppler echocardiographic techniques are perceived as being particularly useful for assessing trends rather than absolute values of cardiac output (216). Therefore assessment of repeatability of measurement is especially important.

Repeatability is the degree of variation seen in repeated measurements taken in the same subject. Repeatability can be assessed for a single observer (intra-observer) or between two observers (inter-observer). In a true steady state, repeatability is purely an assessment of inherent error in measurement technique. In clinical practice it also includes an element of random fluctuation in the measured variable(258).

Bland and Altman(215) originally suggested a statistical technique which is now widely accepted as the most robust assessment of measurement error(216). Two measures for a variable are taken in each subject and the difference between the measures plotted against the mean of the measures to confirm that degree of error is not related to mean

value. The mean of the differences between the two readings should be zero (unless the process of measurement alters the variable)(215). The standard deviation of the differences is then calculated.

The repeatability coefficient of a measure is defined as twice the standard deviation of the differences between repeated measures in the same individual(215).

$$\text{Repeatability coefficient} = 2 \times SD$$

(Where SD = the standard deviation of the differences of 2 measures)

In other words the repeatability coefficient is the difference in a variable one would have to witness to have a 95% probability that it did not occur due to chance alone. Repeatability coefficient is therefore high with techniques of poor repeatability and low with techniques of good repeatability. To assess the applicability of the measure to other populations the confidence intervals of the repeatability coefficient can also be calculated(259).

$$CI = t \times (\sqrt{3SD^2/n})$$

(Where CI = confidence interval of the repeatability coefficient, t = the critical value for a 5% two-sided test drawn from tables of t distribution with n-1 degrees of freedom, SD = standard deviation of the differences of two measures and n = sample size)

The repeatability coefficient can be divided by the mean of all the measures to obtain a repeatability index(207).

$$\text{Repeatability index} = \frac{\text{Repeatability coefficient}}{\text{Mean}}$$

(Where mean is the average of absolute measures)

This allows comparison of repeatability of measures with different units. The clinical utility of a technique depends not only on its repeatability but also on the population variation witnessed in the recorded measure(207). The number of "confidence steps" a technique provides can be calculated by detecting the population range by the repeatability coefficient.

$$\text{Confidence steps} = \frac{\text{Range}}{\text{Repeatability coefficient}}$$

(Where range is the population range of the variable)

Techniques with a larger number of confidence steps have a greater ability to detect clinically relevant trends in measures(207). Variability can also be calculated as the difference between two measures expressed as a percentage of the mean of the two measures(9). The median and range of the variability for a population can then be expressed.

Superior vena cava flow volume has previously been suggested to be a relatively repeatable measure of systemic perfusion in the ventilated preterm infant(9). However this study expressed repeatability in terms of median and range of variability, rather than repeatability coefficient. To our knowledge there are no published data on the repeatability of quantification of volume of descending aorta flow in the neonate.

The aim of this study was to fully assess the repeatability of SVC and DAo flow volume measurement by Doppler echocardiography in the preterm neonate.

4.2 Methods

SVC and DAo flow volumes were assessed by Doppler echocardiography as described in section 2.3. Repeatability of SVC and DAo flow volumes were assessed in infants where satisfactory views were obtained and where infants slept or were quietly awake

for the duration of the echocardiogram. Infants were re-examined within 10 minutes of the initial scan by either the same or a different examiner. Scans were recorded onto separate video tapes and analysed on separate occasions by a single examiner. Infants were not muscle-relaxed or sedated during the study period.

The repeatability coefficient was calculated as described by Bland and Altman(215). The repeatability index, confidence steps and median and range of variability were calculated as described above.

In some infants recordings of invasively monitored arterial blood pressure were downloaded every 60 seconds using Marquette Solar 8000 monitors (GE medical systems, Wisconsin, USA) and Bedmaster V1.3 software (Excel Medical Electronics Inc, Florida, USA) as part of routine NICU monitoring. This blood pressure value is an average of the mean blood pressure over the previous 6 seconds. The repeatability coefficient for arterial blood pressure was calculated from two single measures taken 10 minutes apart for infants in whom recordings were available. The repeatability coefficient of invasively monitored arterial blood pressure was also calculated during echo and rest periods for the cohort of infants described in chapter 3.

4.3 Results

Intra-observer repeatability was assessed from 18 scans performed on 13 preterm infants in the first week of life. Infants had a median gestation of 29 weeks (range 27-30 weeks) and median birth weight of 1235 grams (range 850-1900 grams). Three infants were intubated, 12 were on CPAP and three were receiving no respiratory support.

Inter-observer repeatability was assessed from 11 scans performed on eight preterm infants in the first week of life. Infants had a median gestation of 28 weeks (range 27-

30 weeks) and median birth weight of 1250 grams (range 910-1900 grams). Five infants were intubated, five were on CPAP and one was receiving no respiratory support.

4.3.1 Superior vena cava flow

4.3.1.1 Intra-observer repeatability for SVC flow

There was no significant difference between the mean SVC flow measured at the first and second scans (95 vs 97 ml/kg/min, p=0.60). A Bland-Altman plot of the repeatability data is shown in figure 4.1.

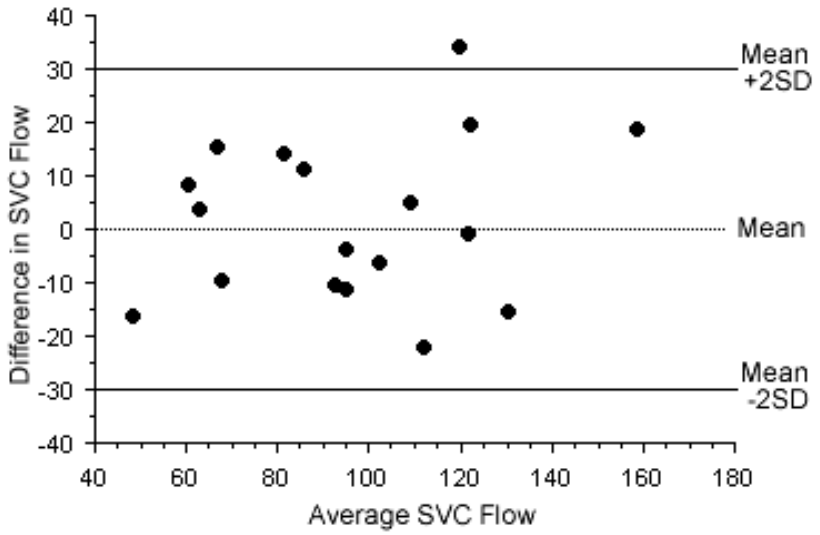

Figure 4.1 - Bland-Altman plot of intra-observer difference between two measures of SVC flow for 18 scans in 13 infants

The repeatability coefficient of intra-observer measurement of SVC flow was 29.6 ml/kg/min (95% confidence intervals of 16.6-42.6 ml/kg/min). There was a non-significant trend for the scatter of the differences in SVC flow to be less at lower averaged SVC flows (p=0.25).

Variability in SVC flow volume measurement is dependent on variability in vessel diameter, VTI and heart rate measurement. Variability in VTI was the most significant of these (Table 4.1).

	Median Variability	Variability Range	Repeatability Coefficient	Confidence steps	Repeatability Index
Mean Diameter	2.7%	0-17.7%	0.37mm	-	11.8%
Velocity Time Integral	10.2%	1.1-48.4%	3.09 cm	-	29.3%
Heart Rate	2.0%	0-9.5%	11.7 beats/minute	-	7.9%
SVC Flow Volume	12.5%	0.5-33.6%	29.6 ml/kg/min	7.0	30.7%

Table 4.1 - Intra-observer repeatability for SVC diameter, velocity time integral, heart rate and flow volume for 18 scans in 13 preterm infants

4.3.1.2 Inter-observer repeatability for SVC flow

There was no significant difference between the mean SVC flow measured by the two observers (86 vs 78 ml/kg/min, p=0.56). A Bland-Altman plot of the repeatability data is shown in figure 4.2.

The repeatability coefficient of inter-observer measurement of SVC flow was 85.4 ml/kg/min (95% confidence intervals of 35.3-135.6 ml/kg/min). There was no clear trend for the scatter of the differences in SVC flow to be less at lower averaged SVC flows (p=0.84).

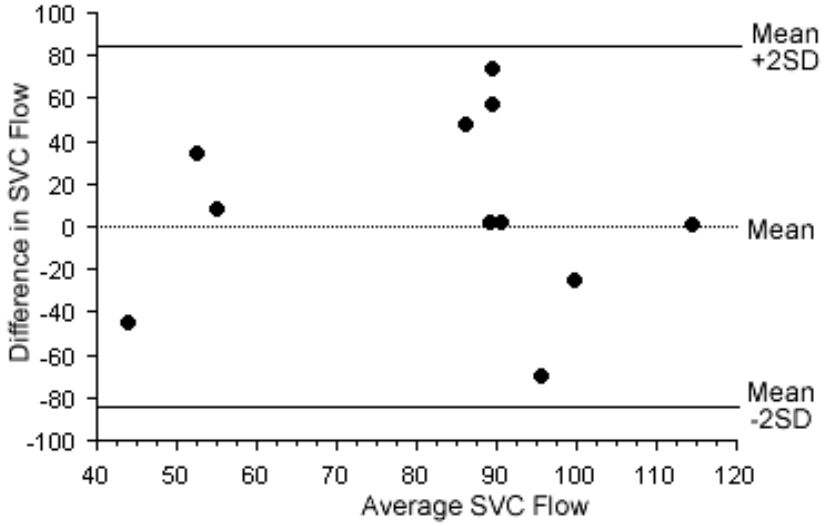

Figure 4.2 - Bland-Altman plot of inter-observer difference between two measures of SVC flow for 11 scans in eight preterm infants

Variability in diameter measurement between observers was responsible for the majority of the variability in SVC flow assessment (Table 4.2). As flow depends on area (diameter^2/4) any discrepancies in diameter measurement are compounded in the calculation of flow.

	Median Variability	Variability Range	Repeatability Coefficient	Confidence steps	Repeatability Index
Mean Diameter	25.3%	2.4-43.3%	1.54 mm	-	49.7%
Velocity Time Integral	4.8%	1.1-68.2%	2.08 cm	-	20.7%
Heart Rate	2.5%	0.0-10.7%	14.5 beats/minute	-	10.0%
SVC Flow Volume	55.8%	0.8-101.5%	85.5 ml/kg/min	2.4	104%

Table 4.2 - Inter-observer repeatability for SVC diameter, velocity time integral, heart rate and flow volume for 11 scans in eight preterm infants

4.3.2 Descending aorta flow

Both intra- and inter-observer repeatability were significantly poorer for DAo flow volume when assessed from the suprasternal as opposed to the subcostal view. All subsequent repeatability data therefore refer only to DAo flow volume with flow velocity assessed from the subcostal view.

4.3.2.1 Intra-observer repeatability for DAo flow

There was no significant difference between the mean DAo flow measured at the first and second scans (135 vs 139 ml/kg/min, p=0.63). A Bland-Altman plot of the repeatability data is shown in figure 4.3.

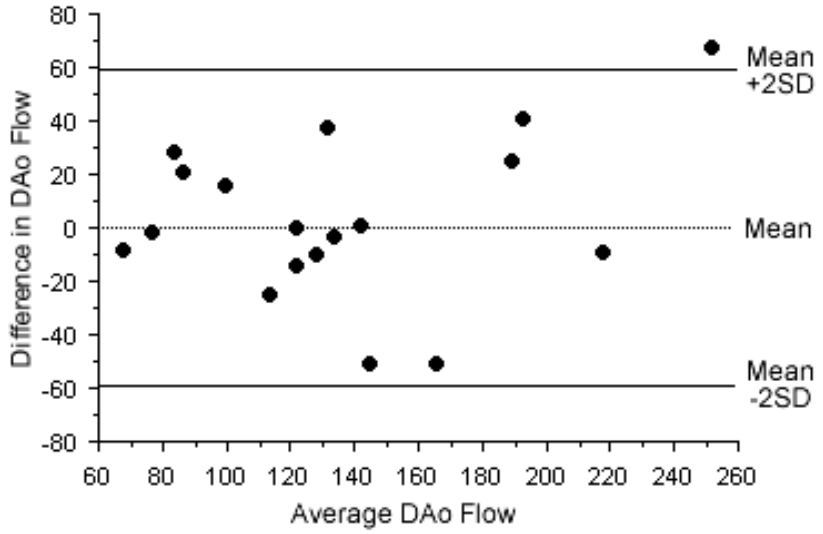

Figure 4.3 - Bland-Altman plot of intra-observer difference between two measures of DAo flow for 18 scans in 13 preterm infants

The repeatability coefficient of intra-observer measurement of DAo flow was 59.7 ml/kg/min (95% confidence intervals of 33.5-85.9 ml/kg/min). Scatter of the

differences in DAo flow was significantly less at lower averaged DAo flows (p=0.02)(Figure 4.3).

Variability in both diameter and VTI measurement contributed significantly to the variability in DAo flow assessment (Table 4.3).

	Median Variability	Variability Range	Repeatability Coefficient	Confidence steps	Repeatability Index
Mean Diameter	4.0%	1.0-24.7%	0.76 mm	-	18.7%
Velocity Time Integral	14.4%	0.9-27.9%	2.15 cm	-	23.8%
Heart Rate	1.7%	0-4.3%	6.4 beats/minute	-	4.3%
DAo Flow Volume	14.4%	0.3-35.2%	59.7 ml/kg/min	5.1	44.4%

Table 4.3 - Intra-observer repeatability data for DAo diameter, velocity time integral, heart rate and flow volume for 18 scans in 13 preterm infants

Intra-observer assessment of direction of diastolic DAo flow (forward/absent/reversed) was highly reproducible, with agreement seen in 17 of 18 cases. In the remaining case diastolic DAo flow was classified as forward in one scan and absent in the other. Only four of 18 scans showed reversal of diastolic DAo flow. The degree of reversal of diastolic DAo flow was quantified as the reversed VTI/forward VTI x 100%. In these four scans the range of difference in degree of reversal of flow assessed in the repeat scans was 10-33%.

4.3.2.2 Inter-observer repeatability for DAo flow

There was no significant difference between the mean DAo flow measured by the two observers (141 vs 141 ml/kg/min, p=0.99). A Bland-Altman plot of the repeatability data is shown in figure 4.4.

The repeatability coefficient of inter-observer measurement of DAo flow was 80.2 ml/kg/min (95% confidence intervals of 33.1-127.3 ml/kg/min). There was no trend for the scatter of the differences in DAo flow to be less at lower averaged DAo flows (p=0.98).

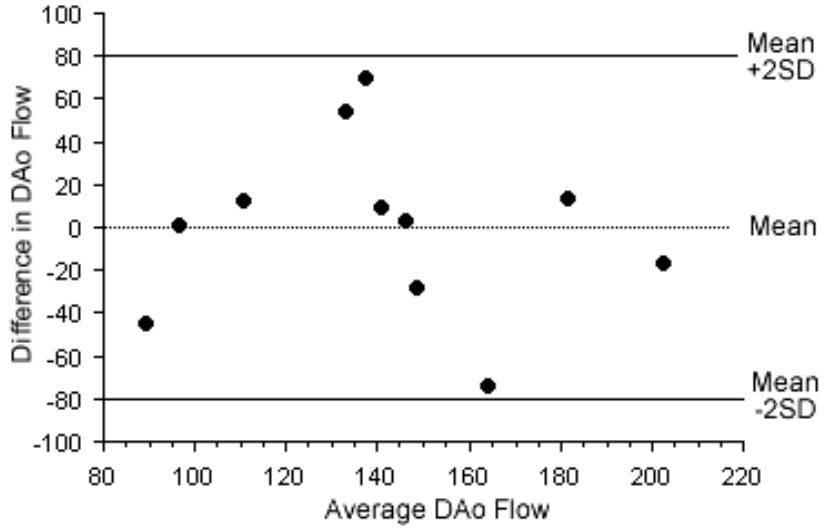

Figure 4.4 - Bland-Altman plot of inter-observer difference between two measures of DAo flow for 11 scans in eight preterm infants

Variability in both diameter and VTI measurement between observers contributed significantly to the variability in DAo flow assessment (Table 4.4).

	Median Variability	Variability Range	Repeatability Coefficient	Confidence steps	Repeatability Index
Mean Diameter	7.0%	0.2-40.5%	1.42 mm	-	30.5%
Velocity Time Integral	7.9%	0.0-51.4%	4.15 cm	-	56.5%
Heart Rate	3.6%	0.0-8.8%	12.4 beats/minute	-	8.6%
DAo Flow Volume	11.1%	0.9-51.0%	80.2 ml/kg/min	3.8	56.9%

Table 4.4 - Inter-observer repeatability data for DAo diameter, velocity time integral, heart rate and flow volume for 11 scans in eight preterm infants

Inter-observer assessment of direction of diastolic DAo flow was relatively reproducible, with agreement seen in nine of 11 cases. In no case was flow direction felt to be forward in one scan and reversed in the other. Only two of 11 scans showed reversal of diastolic DAo flow. The degree of reversal of diastolic DAo flow was quantified as the reversed VTI/forward VTI x 100%. In these two scans the differences in degree of reversal of flow assessed in the repeat scans were 8% and 24%.

4.3.3 'Spontaneous' variations in heart rate and arterial blood pressure

The repeatability index for heart rate (taken from the beat-to-beat interval at flow velocity measurement) ranged from 4-10% (Tables 4.1-4.4).

Continuous recordings of invasively-monitored arterial blood pressure were available from eight of the infants in whom repeatability of echocardiography was assessed. When comparing two measures of mean blood pressure taken 10 minutes apart during echocardiography the repeatability index for mean blood pressure was 10.7%.

When re-analysing the data from the 40 infants with continuously monitored blood pressure in chapter 3, the repeatability index for mean blood pressure with readings taken 10 minutes apart was 16.4% at rest and 20.4% during echocardiography.

4.4 Discussion

Fully assessing the error inherent in any new measurement technique is critical in determining the technique's applicability.

We found the repeatability coefficient for SVC flow, when assessed by a single observer in preterm infants, to be 29.6 ml/kg/min. This is the difference in SVC flow volume one would have to witness to have a 95% probability that it had not occurred due to chance alone. This equates to a repeatability index (the percentage difference required to suggest genuine change) of 30.7%. While these values are high the wide range of flow volumes seen in preterm infants gives the technique seven "confidence steps". This compares favourably with a number of other accepted echocardiographic techniques, including pulmonary artery stroke distance (207). This suggests that quantification of SVC flow volume may be a moderately sensitive technique for detecting haemodynamic change in the clinical setting.

Neither the repeatability coefficient nor the repeatability index were quoted in the only previous study of repeatability of SVC flow in preterm infants(9). The median intra-observer variability for SVC flow volume in their cohort was slightly lower than the 12.5% found in our study. However the range of variability seen in both our and their cohorts was 1-33%. Given that the maximum variability in the 35 scans in Kluckow et al's cohort was 33%, the repeatability index was likely to have been 25-30%.

Infants in the cohort reported by Kluckow et al were all supported by mechanical ventilation, and many of these infants received sedative medication (Personal

communication, Dr Martin Kluckow). Sedative use has been shown to decrease the spontaneous fluctuation in haemodynamic variables(260). None of our cohort received sedation in the first week of life. This difference in sedative use may explain the higher median variability seen in our cohort.

Errors in cross-sectional area measurement are generally felt to contribute more than errors in velocity measures to inaccuracies in cardiac output estimation(216). However in our cohort, even when potential error in diameter is squared for calculation of vessel area, variability in VTI contributed more to overall variability in intra-observer assessment of SVC flow volume than SVC diameter (Table 4.1).

Inter-observer repeatability for measurement of SVC flow volume in our cohort was very poor. This was almost entirely due to differences in diameter measurement (Table 4.2). This highlights the importance of a careful and consistent approach to the measurement of SVC diameter. That there was no significant difference in mean SVC flow volume assessed by the two observers in our cohort, suggests that there was no systematic difference in the diameter measures. Median inter-observer variability was considerably higher than that reported by Kluckow et al(9).

The 95% confidence limits of the inter-observer repeatability coefficient were wide, and small numbers of infants were studied, so there is a possibility that we have over-estimated the repeatability coefficient. However even if one accepts the lower limit of the 95% confidence intervals, the repeatability coefficient for SVC flow volume between observers is still 35 ml/kg/min. This must relate to differences in technique, and may be due at least in part to the operators gaining proficiency in what is a novel technique. Further work in the area is likely to improve imaging technique. Nevertheless it is clear that the technique is relatively difficult to learn, and that

standardisation is critical. Until inter-observer repeatability is significantly improved we would recommend that measures of SVC flow volume obtained by one observer should not be compared to those obtained by another.

The repeatability coefficient for DAo flow from the subcostal view when assessed by a single observer in preterm infants is 59.7 ml/kg/min. This is the difference in DAo flow volume one would have to witness to have a 95% probability that it had not occurred due to chance alone. Such poor repeatability is disappointing. There was a significant trend for the scatter of differences to be lower at lower averaged flows. This means that the calculated repeatability coefficient will be inappropriately large for low flow states and inappropriately low for high flow states(215). Conversely repeatability indexes for DAo flow will be inappropriately low for low flow states and inappropriately high for high flow states. While such deficiencies could be overcome by using a logarithmic transformation(215) the relation between mean value and error is not strong in our subjects, and the use of a simple repeatability coefficient for the raw flow volume seems a reasonable, pragmatic approach(258).

In practical terms that repeatability coefficient will be inappropriately large for low flow states means that a smaller absolute volume change in a DAo flow measure is more likely to be significant when the measured flow volume is low. Since episodes of low systemic blood flow are of particular clinical concern it may be that the technique is more sensitive to changes in flow when volume of flow is critical.

Errors in diameter measurement contributed more than errors in flow velocity to inaccuracies in DAo flow volume estimation. The repeatability index for VTI measurement in the descending aorta was 23.8% which is comparable to published repeatability for systemic(217) and pulmonary(207) stroke distances in neonates. The

descending aortic diameter can be assumed to be constant when arterial blood pressure is constant. Therefore the good intra-observer repeatability of DAo flow velocity measurement does provide scope for the technique to be used to monitor trends in flow volume over time.

Inter-observer repeatability for measurement of DAo flow volume in our cohort of preterm neonates was also poor, again predominantly due to differences in diameter measurement. There was no significant difference in mean DAo flow volume assessed by the two observers, suggesting that there was no systematic difference in the diameter measures. The 95% confidence limits of the repeatability coefficient were wide, and small numbers of infants were studied, so there is again a possibility that we have over-estimated repeatability coefficient. However even if one accepts the lower limit of the 95% confidence intervals, the repeatability coefficient for DAo flow volume between observers is still 33 ml/kg/min. Until inter-observer repeatability is significantly improved we would recommend that measures of DAo flow volume obtained by one observer should not be compared to those obtained by another.

The repeatability of assessment of direction of diastolic DAo flow was good both within and between observers. In no case were forward and reversed diastolic DAo flow felt to have been present in the same infant.

Despite the wide confidence intervals for the repeatability coefficients reported in our cohort of infants, these data provide estimates of the changes in SVC and DAo flow volumes which would have to be seen clinically by a single observer to be likely to represent significant changes in systemic perfusion. Trends in flow volume in the descending aorta could also be observed by assuming a constant vessel diameter in the presence of constant blood pressure, and recording serial VTI measures alone.

By assessing flow volume at multiple points in the circulation it may be possible to minimise the impact of measurement error and aid overall haemodynamic assessment(261). Assessing SVC and DAo flow volume in conjunction with traditional measures such as left and right ventricular output is likely to increase the value of an infant's echocardiographic haemodynamic assessment.

Repeatability for SVC and DAo volume quantification between observers was particularly poor, and is of concern. Given how new these techniques are, the poor inter-observer repeatability may not be surprising. However at present these techniques should not be used for flow assessment unless measures can be taken by the same individual for all scans in a single infant.

Published repeatability indices for left and right ventricular output volumes in neonates are around 15-25%(207, 217). These studies generally used a single measure of vessel diameter for the aorta and pulmonary artery. This is a reasonable approach since these vessel diameters change little in the first week of life(222, 225). However SVC diameter is more prone to acute changes in diameter with changes in cardiac function and circulating volume than the aortic or pulmonary roots. Therefore it would be unwise to assume constant diameter of this vessel for calculation of flow volume and an approach of combined diameter and flow velocity measurement is still required.

In the presence of a constant blood pressure it is probably reasonable to assume constant diameter of the descending aorta between scans and therefore to interpret changes in flow velocity in the presence of constant blood pressure as representing changes in blood flow volume. Whether descending aortic diameter changes in a predictable manner with changes in blood pressure has not yet been studied.

Heart rate and arterial blood pressure fluctuate considerably on a minute-to-minute basis in preterm infants. When expressed in terms of repeatability index, heart rate had a repeatability index of 4-10% in our cohort, and mean arterial blood pressure had a repeatability index of 10-20%. These fluctuations in heart rate and blood pressure are unlikely to be due to measurement error. While changes in heart rate and blood pressure are not directly related to changes in volume of blood flow in preterm infants, it could be argued that if spontaneous fluctuations of 10-20% occur in these haemodynamic parameters, similar spontaneous fluctuations may also be seen in volumes of blood flow. It is likely then that a significant proportion of the variability in echocardiographic measures of systemic perfusion in preterm infants is due to true variability in flow rather than purely measurement error.

In addition to assessing its repeatability, any new measurement technique should ideally be validated against an accepted gold standard(259). There is no gold standard for assessing SVC and DAo flow volumes in the preterm neonate, so such a validation is not possible. However if measures show reasonable repeatability and have clinical significance they will still have value as markers of blood flow.

4.5 Conclusion

The repeatability of estimation of superior vena cava flow volume by echocardiography is similar to that for other echocardiographic measures in the preterm infant. Estimation of descending aorta flow volume is less repeatable. DAo VTI shows reasonable repeatability as does direction of diastolic DAo flow.

Changes of more than 30% in SVC flow volume, or of more than 45% in DAo flow volume, when assessed by a single observer in the preterm infant, are likely to represent

true alterations in systemic blood flow. Given the range of flow volumes seen in the preterm population such changes may occur relatively frequently.

Inter-observer repeatability for quantification of SVC and DAo flow is poor such that measures obtained by one observer should not be compared to those obtained by another.

Given the degree of spontaneous fluctuation seen in other, accurately measurable, haemodynamic variables, a proportion of the discrepancy between two estimates of flow may be due to true differences in flow rather than purely measurement error.

5 Reference Ranges of Flow Volume in the Superior Vena Cava and Descending Aorta in Healthy Preterm and Term Neonates

5.1 Introduction

Episodes of low systemic perfusion are associated with adverse outcomes in preterm(143) and term(243) infants. To be able to conclude that perfusion in any individual is 'low' obviously depends on the prior establishment of a 'normal' population range. Establishing a normal range for a physiological variable is relatively straightforward in the term infant. Since prematurity itself is a pathological state, what constitutes a 'normal' range for any variable in preterm infants is less clear. However preterm infants who require minimal respiratory support in the first postnatal days have been shown to have a near-normal postnatal circulatory adaptation(204, 205). A cohort of infants requiring minimal respiratory support and with persistently normal cranial ultrasound scans is therefore likely to display a range of blood flow volumes in the transitional circulation which are compatible with a positive outcome following preterm birth.

The aim of this study was to establish reference ranges for SVC and DAo flow volumes in healthy preterm and term infants.

5.2 Methods

Preterm infants were considered healthy if they required less than 48 hours respiratory support, did not receive fluid resuscitation or inotropic support in the first week of postnatal life and had persistently normal cranial ultrasound scans. Infants were excluded if they had any structural cardiac abnormality or if they subsequently required treatment for a persistently patent ductus arteriosus. Infants were also excluded if they developed signs of sepsis or necrotising enterocolitis in the first week of postnatal life. Infants showing signs of sepsis or necrotising enterocolitis after day 7 of postnatal life were not excluded.

Term infants were excluded if they showed signs of cardiorespiratory distress or required admission to the neonatal intensive care unit.

SVC and DAo flow were assessed as close as possible to 5, 12, 24 and 48 hours postnatal age as described in chapter 2. Preterm infants were also assessed on the 7^{th} postnatal day. Results are expressed as median and range. Flow volumes at different postnatal ages were compared using the Wilcoxon Signed Rank Test. Tendency of flow volumes to change consistently over time was examined by repeated measures ANOVA using Fisher's post-hoc analysis.

5.3 Results in term infants

Thirteen healthy term infants were studied with median birth weight 3410 grams (range 2880-4330 g) and median gestation 39 weeks (range 38-42 weeks). Seven infants were delivered vaginally and six by caesarean section.

5.3.1 SVC flow in term infants

Median SVC flow was 68, 87, 89 and 60 ml/kg/min at 5, 12, 24 and 48 hours postnatal age respectively. The lowest recorded SVC flow at each of these times was 32, 53, 54 and 41 ml/kg/min respectively (Table 5.1). One infant had the lowest measured flow at both 24 and 48 hours, two other infants had the lowest measured flow at either 5 or 12 hours. There was no significant increase in SVC flow over time (p=0.24). The 5th percentile for SVC flow volume in term infants at any time in the first 48 hours was 44 ml/kg/min.

	5 hours	12 hours	24 hours	48 hours
SVC Flow (ml/kg/min)	68 32-166	87 53-127	89 54-167	60 41-167
SVC Velocity Time Integral (cm)	10.3 7.1-19.6	13.2 11.0-16.3	13.6 10.1-20.1	10.4 9.6-15.6
SVC Maximum Diameter (mm)	5.6 4.5-6.7	5.4 4.2-7.1	5.4 4.5-6.5	5.7 4.4-6.7
SVC Minimum Diameter (mm)	4.4 2.6-5.3	4.3 3.1-5.5	4.1 3.5-5.0	4.3 2.7-4.7

Table 5.1 - Values for SVC studies in 13 healthy term infants. Values are median and range

5.3.2 DAo flow in term infants

When assessed from the subcostal view, median DAo flow was 123, 171, 133 and 154 ml/kg/min at 5, 12, 24 and 48 hours postnatal age respectively. The lowest recorded DAo flow at each of these times was 115, 118, 108 and 136 ml/kg/min respectively (Table 5.2). No infant had the lowest measured flow at more than one time point. There was a significant increase in DAo flow after 5 hours postnatal age (p=0.048), but

there were no significant difference in flows between the 12, 24 and 48 hour scans. The 5th percentile for DAo flow volume in term infants at any time in the first 48 hours was 116 ml/kg/min.

	5 hours	12 hours	24 hours	48 hours
DAo Flow (ml/kg/min)	123 115-190	171 118-236	133 108-305	154 136-235
DAo Velocity Time Integral (cm)	12.2 8.6-15.9	13.8 11.0-18.3	12.1 7.9-19.4	15.3 11.2-17.6
DAo Systolic Diameter (mm)	7.1 5.9-8.2	7.2 6.2-8.5	6.6 6.0-8.2	6.5 6.0-8.1
DAo Diastolic Diameter (mm)	6.3 4.9-7.6	6.1 5.5-7.7	6.0 5.4-7.6	5.8 5.4-7.2

Table 5.2 - Values for DAo studies in 13 healthy term infants. Values are median and range

5.3.3 Left and right ventricular outputs in term infants

Median (range) left ventricular output (LVO) in the first 48 hours of life in healthy term infants was 263 (184 - 518) ml/kg/min. Median (range) right ventricular output (RVO) in the first 48 hours of life was 301 (209 - 432) ml/kg/min.

5.4 Results in preterm infants

Fourteen healthy preterm infants were studied with median birth weight 1320 grams (range 780-1850 g) and median gestation 29 weeks (range 28-30 weeks). Eleven infants received respiratory support with nasal continuous positive airway pressure in the first 48 hours of postnatal life. One infant developed sepsis at 3 weeks postnatal

age, but had an uneventful early postnatal course. One infant developed necrotising enterocolitis on day 8 of postnatal life. This infant was well at the time of the 7 day echo, with LVO, RVO and SVC and DAo flow volumes within the range of values seen in the other healthy preterm infants. Echo showed a small PDA (1.3mm), but with forward diastolic flow in the descending aorta. The PDA later closed without treatment. The infant showed no signs of clinical instability or feed intolerance at the time of the 7 day echo.

5.4.1 SVC flow in preterm infants

Median SVC flow was 90, 101, 112, 113 and 105 ml/kg/min at 5, 12, 24, 48 and 168 hours postnatal age respectively. The lowest recorded SVC flow at each of these times was 41, 40, 64, 82 and 63 ml/kg/min respectively (Table 5.3). One infant had the lowest measured flow at both 24 and 168 hours, three other infants had the lowest measured flow at one of 5, 12 or 48 hours.

SVC flow volume at 48 hours was significantly higher than at 5 hours ($p=0.03$). SVC flow volume at 7 days was also significantly higher than at 5 hours ($p=0.03$). However there were no other significant differences in SVC flow volumes at different postnatal ages. In addition, analysis by repeated measures of ANOVA suggested that the trend for SVC flow to gradually increase between 5 and 48 hours postnatal age was not statistically significant ($p=0.18$).

The 5^{th} percentile for SVC flow volume in preterm infants at any time in the first 48 hours was 56 ml/kg/min.

	5 hours	12 hours	24 hours	48 hours	7 days
SVC Flow (ml/kg/min)	90 41-132	101 40-183	112 64-193	113 82-179	105 63-154
SVC Velocity Time Integral (cm)	10.0 5.5-12.2	9.0 5.0-15.4	11.0 7.6-16.6	12.6 8.0-17.3	12.2 8.8-17.0
SVC Maximum Diameter (mm)	3.6 2.9-4.6	4.0 2.9-4.8	4.0 3.1-4.7	3.8 3.1-4.1	3.6 3.0-3.9
SVC Minimum Diameter (mm)	2.6 2.0-3.7	3.3 2.2-4.3	2.8 2.3-3.9	2.7 2.0-3.0	2.4 2.0-3.0

Table 5.3 - Values for SVC studies in 14 healthy preterm infants. Values are median and range

5.4.2 DAo flow in preterm infants

When assessed from the subcostal view, median DAo flow was 133, 134, 180, 161 and 177 ml/kg/min at 5, 12, 24, 48 and 168 hours postnatal age respectively. The lowest recorded DAo flow at each of these times was 78, 81, 93, 87 and 143 ml/kg/min respectively (Table 5.4). One infant had the lowest measured flow at both 5 and 12 hours and one infant had the lowest measured flow at both 24 and 168 hours.

DAo flow volume at 24 hours was significantly higher than at 5 hours ($p=0.04$). DAo flow volume at 7 days was also significantly higher than at 5 hours ($p=0.002$), 12 hours ($p=0.004$) and 48 hours ($p=0.046$). However there were no other significant differences in DAo flow volumes at different postnatal ages. In addition, analysis by repeated measures of ANOVA suggested that the trend for DAo flow to gradually increase between 5 and 48 hours postnatal age was not statistically significant ($p=0.21$).

The 5th percentile for DAo flow volume in preterm infants at any time in the first 48 hours was 89 ml/kg/min.

	5 hours	12 hours	24 hours	48 hours	7 days
DAo Flow (ml/kg/min)	133 78-186	134 81-193	180 93-233	161 87-242	177 143-261
DAo Velocity Time Integral (cm)	7.2 4.7-15.0	8.4 4.3-13.7	9.6 4.0-14.7	9.3 2.8-15.1	10.7 7.9-14.0
DAo Systolic Diameter (mm)	4.6 3.8-5.7	4.5 3.8-5.5	4.7 3.9-5.3	4.8 3.6-5.5	4.4 3.8-5.0
DAo Diastolic Diameter (mm)	3.9 3.2-5.3	4.0 3.1-4.9	4.3 3.5-4.9	4.3 3.2-5.2	3.9 3.3-4.6

Table 5.4 - Values for DAo studies in 14 healthy preterm infants. Values are median and range

5.4.3 Left and right ventricular outputs in healthy preterm infants

Median (range) LVO in the first 48 hours of life in healthy preterm infants was 257 (155-483) ml/kg/min. The 5th percentile for LVO in preterm infants at any time in the first 48 hours was 185 ml/kg/min. Median (range) RVO in the first 48 hours of life was 347 (184-758) ml/kg/min. The 5th percentile for RVO in preterm infants at any time in the first 48 hours was 242 ml/kg/min.

5.4.4 Blood pressure in healthy preterm infants

Median mean arterial blood pressure was 44, 43, 43, 52 and 61 mmHg at 5, 12, 24, 48 and 168 hours postnatal age respectively. No infant had a mean arterial blood pressure below 30 mmHg at the time of echocardiography. No infant received fluid volume or inotropic support for hypotension in the first week of postnatal life.

5.5 Discussion

These data establish reference ranges for SVC and DAo flow in the first 48 hours of postnatal life in term and preterm infants.

The term infants studied were all healthy. A large proportion of the infants we studied were delivered by caesarean section, as the mothers of these infants are more likely to stay in hospital for at least 48 hours after delivery than mothers who deliver vaginally. While caesarean delivery is associated with altered postnatal respiratory function(262) it has not been shown to be associated with altered cardiac output(263) or cerebral perfusion(264) postnatally. The ranges established here are likely to be applicable to infants born by either normal vaginal delivery or caesarean section.

The range of LVO reported here is very similar to that previously reported in term infants when assessed by the same method(184, 217). The range of RVO reported here is slightly higher than that previously reported in term infants(224). However this previous study calculated RVO from diameter measured at the level of the pulmonary trunk rather than the pulmonary valve which has been shown to produce lower estimates of flow volume(224). In addition the majority of measures of pulmonary valve diameter in our study were taken in the long axis view, which produces higher readings than the short axis view(224).

The range of SVC flow and diameter reported here is very similar to that previously reported in term infants by Kluckow and Evans(9). The range of DAo flow volume reported here in term infants is also very similar to that reported by Walther et al(241) and Ewert et al(251).

The preterm infants studied were all healthy at the time of echocardiographic examination. Eleven infants received respiratory support with continuous positive

airway pressure in the first 2 days of postnatal life, but none of the infants were ventilated and none required any specific circulatory support.

This definition of 'healthy' used here is similar to that used in other studies(9, 205, 223). Preterm infants requiring minimal respiratory support have been shown to have a similar postnatal circulatory adaptation to healthy term infants(204, 205). While flow volumes measured in the pathological circumstance of preterm delivery can not be considered 'normal', they can at least provide a reference range for infants who experience an uncomplicated passage through the transitional circulation.

The range of LVO reported here is very similar to that previously reported in preterm infants when assessed by the same method(265). The range of RVO reported here is higher than that previously reported in preterm infants. This could be due to inaccuracies in measurement of the pulmonary diameter, as the pulmonary valve leaflets are parallel to the ultrasound beams; the lateral resolution of ultrasound being poorer than axial resolution(118). However the discrepancy could also be due to methodological differences(224). Higher RVO in our cohort may also be due to increased surfactant use when compared to historical cohorts, leading to reductions in pulmonary vascular resistance, or to increased CPAP use which is likely to produce less haemodynamic compromise than mechanical ventilation.

No infant in the cohort reported here had either LVO or RVO below 150 ml/kg/min, a level which has previously been associated with increased severity of respiratory disease(228).

The median SVC flow in our cohort of healthy preterm infants is approximately 30% higher than that previously reported by Kluckow and Evans(9). The discrepancy

between our and their cohorts is entirely due to differences in assessment of SVC diameter, with very similar medians of SVC flow velocity being reported.

This discrepancy in SVC diameter assessment is significant and is of concern. The absolute discrepancy in mean SVC diameter measurement is only 0.3-0.4 mm, but when this diameter measure is squared to calculate vessel area the impact on volume of flow is considerable.

There are a number of potential explanations for this discrepancy. Our cohort included infants of up to 31 completed weeks gestation rather than the 30 weeks used by Kluckow. However the median birth weights in the two cohorts were very similar (1320 vs 1250 grams) and excluding the five infants of more than 30 weeks completed gestation from our cohort did not alter the median SVC flow volume.

Kluckow and Evans used two-dimensional rather than M mode echocardiography to assess SVC diameter. We opted to use M mode echocardiography as it has been shown to have improved reproducibility in discriminating vessel diameters in infants and children(226). However the data produced here and previously published by Kluckow and Evans do not suggest that two-dimensional echocardiography was less accurate, since the intra-observer repeatability of the two techniques in the two cohorts was almost identical.

M mode echocardiography may overestimate SVC diameter if the M mode cursor is not placed directly perpendicular to the long axis of the vessel. However care was taken to avoid such errors in our study.

It is possible that the differences in SVC diameters and flow volumes are real, reflecting differences between our population and that studied by Kluckow et al. One potential

cause could be an increase in circulating blood volume in our infants from placental transfusion due to delayed clamping of the umbilical cord at delivery(266). However there is no current policy to deliberately delay cord clamping at the deliveries of preterm infants in our institution (Associate Prof. Lesley McCowan, personal communication). No infant received any specific therapy aimed at increasing either circulating volume or systemic perfusion in this part of the study.

As the cross-sectional shape of the SVC is oval rather than circular throughout the cardiac cycle (see chapter 2) it is recognised that imaging the vessel other than in the true antero-posterior plane will lead to higher measures of diameter (section 2.3.6)(9). It is also appreciated that a more lateral echo window must be used in some infants to image the vessel past overlying lung tissue(9). While efforts were made to ensure imaging of the vessel in the antero-posterior plane in our study it is possible that the echo window used was more lateral to that used by Kluckow et al. Placing the ultrasound probe even slightly more laterally on the chest would produce higher measures of SVC diameter (though both are still 'true' diameters). This is perhaps the most plausible explanation for the discrepancy in SVC diameter measures between our and Kluckow's cohorts. It may also account for the lack of discrepancy in SVC diameter measurement in term infants, where hyperinflation of lung tissue is much less of a hindrance to echocardiography.

It is not possible to compare the normal ranges of SVC flow volume in our and the previous cohort with an accepted gold standard. However since the SVC is clearly oval in cross-section, neither study is attempting to quantify 'true' SVC flow volume, as to do so would require diameter measurement in two planes rather than one. In addition, an estimate of 'true' SVC flow volume would require flow velocity through the cardiac

cycle to be integrated against vessel diameter through the cardiac cycle. Current calculations assume a mean velocity of flow at a mean vessel diameter throughout the cardiac cycle. Since flow velocity and vessel diameter are both maximal during cardiac systole such an assumption will tend to underestimate 'true' flow.

However the quantification of 'true' flow, though desirable, is not the objective of this study. A measure need only be reproducible and be related to outcome to have clinical utility.

Estimates of DAo flow volume are similar to those previously reported using an intra-aortic Doppler probe in a small cohort of preterm infants(251). Mean volume of flow was virtually identical when assessed from both the subcostal and suprasternal views. Since repeatability of the subcostal technique is superior (chapter 4) we have only reported subcostal values.

SVC and DAo flow volume both tended to increase with increasing postnatal age in both term and preterm infants in our cohort. Such a pattern has been described previously for SVC flow(9) and cerebral blood flow(103) in preterm infants. However an increase in flow volume with increasing postnatal age was not seen consistently in our cohort, and the trend to increasing flow in preterm infants was not statistically significant when assessed by repeated measures of ANOVA.

When defining a reference range for flow volume in the SVC and DAo in the first 48 hours of postnatal life we elected to establish a single range for flow throughout the first 48 hours. If 'normal' flow does tend to increase throughout the first 48 hours of postnatal life this reference range may overestimate 'normal' flow at 5-12 hours, and underestimate at 24-48 hours. However the change in median flow is relatively small over the first 48 hours of life, so that any over- or under-estimation would be minimal.

Combining measures of flow from all four time points studied allowed the reference range produced to be based on a greater number of samples thereby providing a more robust assessment of normal flow(267).

We elected to define the 5th percentile for flow volumes witnessed at any time in the first 48 hours of postnatal life as the lower limit of our reference range. The lower limit of our reference range for SVC flow volume in preterm infants is therefore higher than that reported by Kluckow and Evans due both to discrepancies in diameter measurement and to the different statistical approaches taken to define normality.

The reference ranges reported here have a number of limitations. The small number of infants studied prevents the data from truly defining a 'normal range'; the study of a larger cohort may have revealed a wider range of flow volumes. To attempt to define 'normality' in the pathological state of preterm birth is to some extent a contradiction in terms. However we can at least describe a pattern of flow volume conducive to a favourable outcome. The selection of a single reference range for SVC and DAo flow over the first 48 hours of postnatal life may lead to some infants being labelled as having 'low flow' in the first 12 postnatal hours when their flow volume in fact falls within the normal range. Finally the large number of term infants delivered by Caesarean section could theoretically have a different postnatal circulatory adaptation than those delivered vaginally, though this has not been reported previously(263).

5.6 Conclusion

Reference ranges for volume of SVC and DAo flow in term infants are similar to those previously reported, as are ranges of DAo flow volume in preterm infants.

Reference range of SVC flow in preterm infants is higher than that previously reported, the discrepancy likely being due to slight differences in the echo window used. This

disparity, along with the poor inter-observer repeatability reported in chapter 4, suggests that all assessments of SVC flow volume in a single infant should be carried out by a single operator who has previously established their own reference range of flows.

In our cohort of healthy preterm infants, individuals were unlikely to experience SVC flow volumes of less than 55 ml/kg/minute or DAo flow volumes of less than 90 ml/kg/minute in the first 48 hours of postnatal life. Flow volumes lower than this, particularly when seen in combination, or with low ventricular outputs, are likely to represent systemic hypoperfusion.

6 Flow Volume in the Superior Vena Cava and Descending Aorta in Sick Preterm Neonates

6.1 Introduction

As discussed in chapter 1, there are often significant long-term adverse sequelae of extremely preterm birth. While advances in care such as antenatal steroids and surfactant have led to dramatic reductions in mortality(17), rates of neurologic and developmental morbidity remain high(268).

Despite evidence that circulatory factors play a prominent role in the pathophysiology of the two strongest predictors of neurodevelopmental morbidity(23), periventricular haemorrhage and periventricular leukomalacia(39), circulatory monitoring techniques in neonatal intensive care remain limited(4). Arterial blood pressure remains the most frequently monitored indicator of neonatal circulatory status(80) despite its uncertain relation to blood flow(4). Other commonly used clinical markers of circulatory status such as capillary refill time and central-peripheral temperature difference have also been shown to have at best only a weak correlation with systemic perfusion(79).

Echocardiography is becoming an integral component of adequate assessment and management in the critically ill newborn(211). However the techniques used to assess haemodynamic status in older patients such as left and right ventricular outputs are confounded by the persistent fetal shunt pathways seen in the early postnatal period(9).

A variety of new echocardiographic and other techniques are therefore being assessed in the hope that they will become clinically valuable methods of monitoring blood flow in end organs, particularly the brain, in sick preterm infants(9, 109, 116)

This thesis examines the use of echocardiography to assess two relatively new markers of systemic perfusion: superior vena caval (SVC) and descending aortic (DAo) blood flow. Chapter 3 has discussed the ability of the preterm infant to safely tolerate the process of echocardiography. Chapter 4 examined the repeatability of measurement of these new markers of systemic perfusion, while chapter 5 established reference ranges of flow volumes in a small population of healthy preterm infants.

The specific aim of this section of the thesis is to describe the feasibility of making these measurements in sick preterm infants, and to examine whether values seen in sick preterm infants differ from those seen in healthy infants. Chapters 7-9 will go on to discuss the relation of flow measures to arterial blood pressure, the impact of ductal shunting on systemic perfusion in early postnatal life, and the association of flow measures to outcome.

6.2 Methods

Methods are described in detail in chapter 2 of this thesis. In summary, infants of less than 31 weeks completed gestation were studied at the National Women's Hospital, Auckland between 1st December 2002 and 1st May 2004. Left ventricular output (LVO), right ventricular output (RVO), SVC and DAo flow volumes and ductal diameter were assessed as close as possible to 5, 12, 24, 48 and 168 hours postnatal age.

Infants were considered 'healthy' if they required less than 48 hours respiratory support, did not receive fluid resuscitation or inotropic support in the first week of postnatal life, had repeated normal cranial ultrasound scans and did not receive

treatment for a patent ductus arteriosus in the course of their NICU admission. Infants developing illness such as sepsis or necrotising enterocolitis after the 7th day of postnatal life were still considered 'healthy' during the first week of life if they met the above criteria.

For the purposes of this chapter of the thesis, all infants that did not meet the criteria for 'healthy' outlined above were designated as 'sick'.

Outcome data in this chapter are expressed as absolute number and percentage of the entire cohort examined. Values are expressed as median and range. Comparisons between groups were made by the Mann-Whitney test. In all cases statistical significance was accepted as $p < 0.05$.

6.3 Results

Between 1st December 2002 and 1st May 2004, 166 infants of <31 weeks gestation were inborn at National Women's Hospital. Informed parental consent was declined or not available prior to 6 hours postnatal age in 28 (17%). No operator was available to carry out echocardiography in 58 (35%). The median gestation (27 weeks) and birth weight (1022 grams) of non-enrolled infants were not significantly different from enrolled infants ($p = 0.25$ and 0.99 respectively). No infants were excluded due to the presence of significant structural congenital heart disease. The remaining 80 (48%) infants were studied. These 80 infants include the 14 healthy preterm infants described in chapter 5.

The characteristics of infants included in the study are shown in table 6.1. Sick infants had significantly lower gestation and birth weight, and were significantly less likely to have received at least one complete course of antenatal steroids than healthy infants (Table 6.1).

	Sick Infants (n=66)	Healthy Infants (n=14)	p value
Gestation (completed weeks)	28 (24-30)	29 (28-30)	0.004
Birth weight (grams)	1000 (510-1900)	1320 (780-1850)	0.02
Male gender	32 (48%)	8 (57%)	0.77
Exposure to antenatal steroids	62 (94%)	14 (100%)	0.99
At least one complete course of antenatal steroids	39 (59%)	13 (93%)	0.02
Ethnicity:			
Caucasian	33 (50%)	6 (43%)	0.77
Maori	11 (17%)	3 (21%)	0.70
Asian	10 (15%)	1 (7%)	0.68
Pacific Island	6 (9%)	4 (29%)	0.07
Other	6 (9%)	0 (0%)	0.58
Delivery Method:			
LSCS	48 (73%)	10 (71%)	0.99
Vaginal delivery	16 (24%)	4 (29%)	0.74
Assisted delivery	2 (3%)	0 (0%)	0.99
Primary reason for delivery:			
Preterm labour	33 (50%)	3 (21%)	0.07
Pre-eclampsia or abnormal Doppler flows	20 (30%)	5 (36%)	0.75
Chorioamnionitis	5 (8%)	6 (43%)	0.003
Antepartum haemorrhage	8 (12%)	0 (0%)	0.34

Table 6.1 - Characteristics of included infants. Results are median (range) or number (percentage)

6.3.1 Outcomes

6.3.1.1 Requirement for respiratory support

Forty three (54%) infants required mechanical ventilation, 34 (42%) infants received CPAP only, and three (4%) infants required no respiratory support. Sixty six (83%) infants received more than 48 hours respiratory support.

6.3.1.2 Requirement for circulatory support

Twenty six (32%) infants received at least one bolus of normal saline in the first postnatal week to support the circulation. Nine (11%) infants received inotropic support in the first postnatal week.

6.3.1.3 Treatment for patent ductus arteriosus

Twenty nine (36%) infants received treatment for a patent ductus arteriosus. In one infant indomethacin was given prior to the 48 hour echocardiogram as the infant developed a pulmonary haemorrhage in association with a large ductus arteriosus. In all other cases treatment was given after the 48 hour echocardiogram, between days 2 and 11 of life. Six (8%) infants received more than one course of indomethacin. Four (5%) infants subsequently underwent surgical ligation of a patent ductus arteriosus.

6.3.1.4 Periventricular haemorrhage

Thirteen (16%) infants developed periventricular haemorrhage (PVH). Two infants developed new, severe (grade 3 or 4) PVH in the first week of life after a normal scan in the first 24 hours. Five infants had a small PVH present on the first cranial ultrasound scan at 4-12 hours postnatal age. Three infants were seen to have grade 1 or 2 PVH at day 5-7 of life, but had not had a prior cranial ultrasound to determine whether PVH occurred antenatally or postnatally. Three infants developed new PVH late in their postnatal course, with normal scans at day 7 of life.

6.3.1.5 Necrotising enterocolitis

Four (5%) infants were diagnosed with necrotising enterocolitis, of whom three required surgical intervention.

6.3.1.6 Chronic lung disease

Fifteen (19%) infants developed chronic lung disease (requirement for oxygen or any respiratory support at 36 weeks corrected gestation). Eight (10%) infants were discharged home with a requirement for supplemental oxygen.

6.3.1.7 Survival

Six (8%) infants died prior to hospital discharge, between days 10 and 110 of postnatal life.

6.3.2 Postnatal age at echocardiography

All 80 infants were scanned five times in the first week of postnatal life. Postnatal age at echocardiography for the 80 infants is shown in table 6.2.

	Median	Range
Age at scan 1 (Hours)	5.2	4.0 - 6.5
Age at scan 2 (Hours)	11.9	10.6 - 14.0
Age at scan 3 (Hours)	23.7	21.2 - 26.8
Age at scan 4 (Hours)	47.8	42.9 - 54.7
Age at scan 5 (Hours)	169	141 - 200

Table 6.2 - Postnatal age at echocardiography in 80 preterm infants

6.3.3 Acquisition of images

All echocardiograms were performed by one of two operators; 326 (82%) by the author and 74 (18%) by Dr Carl Kuschel, Consultant Neonatologist at National Women's Hospital. Overall 97% were satisfactory for calculation of haemodynamic variables (Table 6.3).

Measurement	Number of satisfactory scans	Percentage of satisfactory scans
LVO	397	99%
RVO	380	95%
SVC flow	382	96%
DAo flow	388	97%

Table 6.3 - Scans in which satisfactory images were gained at echocardiography in 80 preterm infants

6.3.4 Structural congenital cardiac anomalies in the cohort

One infant was found to have a small (1.5mm) restrictive muscular ventricular septal defect (VSD) at the first echocardiogram. This subsequently closed spontaneously by the third week of postnatal life. This infant was not excluded from the analysis as it was considered unlikely that the defect would have any significant haemodynamic impact.

One infant was found to have a mildly dysplastic pulmonary valve at the 48 hour echocardiogram. While there was no evidence of turbulent flow across the valve this infant's right ventricular outflow measures were not included in the final analysis but all other haemodynamic measures were included in the analysis.

No other structural abnormalities were detected.

6.3.5 SVC flow in sick preterm infants

Median volume of SVC flow in sick preterm infants at 5 and 12 hours postnatal age was not different from that seen in healthy infants. However eight (12%) sick infants at 5 hours, and 10 (15%) sick infants at 12 hours had SVC flow volumes below the 56 ml/kg/min previously defined as the 5th centile for SVC flow in healthy preterm infants. At both 24 and 48 hours sick infants had significantly lower median volume of SVC

flow (Table 6.4), with 10 (15%) and 5 (8%) sick infants respectively having SVC flow below 56 ml/kg/min (figure 6.1).

	SVC Flow 5h (ml/kg/min)	SVC Flow 12h (ml/kg/min)	SVC Flow 24h (ml/kg/min)	SVC Flow 48h (ml/kg/min)
Healthy Infants n=14	90 41-132	101 40-183	112 64-193	113 82-179
Sick Infants n=66	87 19-225	89 21-209	89 35-168	97 29-176
p value	0.88	0.17	0.02	0.04

Table 6.4 - Values for SVC flow at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants. Values are median and range.

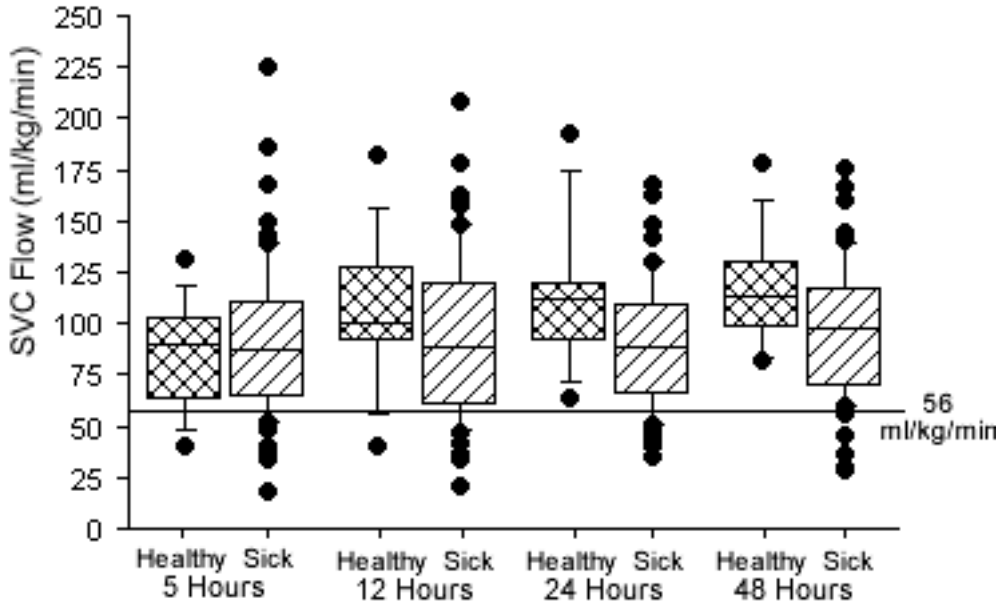

Figure 6.1 - Box and whisker plot of SVC flow at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants (10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} centiles, with outliers marked as circles). 56 ml/kg/min is the 5^{th} percentile for SVC flow in healthy preterm infants in the first 48 hours of postnatal life

6.3.6 DAo flow in sick preterm infants

	DAo Flow 5h (ml/kg/min)	DAo Flow 12h (ml/kg/min)	DAo Flow 24h (ml/kg/min)	DAo Flow 48h (ml/kg/min)
Healthy Infants n=14	133 78 - 186	134 81 - 193	180 93 - 233	161 87 - 242
Sick Infants n=66	117 54-335	121 46-266	144 29-255	146 43-261
p value	0.31	0.40	0.10	0.62

Table 6.5 - Values for DAo flow at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants. Values are median and range.

Median volume of DAo flow in sick preterm infants at 5, 12, 24 and 48 hours postnatal age was not different from that seen in healthy infants (Table 6.5). However the range of flow volume seen was wide. Of the 66 sick infants studied, 18 (27%), 13 (20%), seven (11%) and six (9%) infants at 5, 12, 24 and 48 hours respectively had DAo flow volumes below the 89 ml/kg/min previously defined as the 5th centile for DAo flow in healthy preterm infants (Figure 6.2).

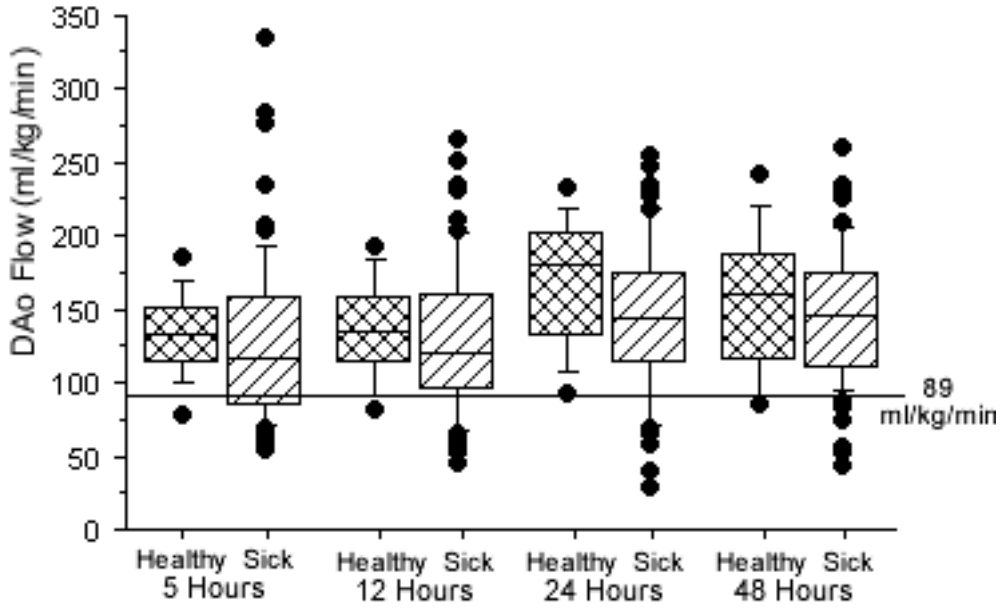

Figure 6.2 - Box and whisker plot of DAo flow at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants (10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} centiles, with outliers marked as circles). 89 ml/kg/min is the 5th percentile for DAo flow in healthy preterm infants in the first 48 hours of postnatal life

6.3.7 LVO in sick preterm infants

Median left ventricular output in sick preterm infants at 5 hours postnatal age was not different from that seen in healthy infants. At 12, 24 and 48 hours sick infants had significantly higher LVO (Table 6.6).

	LVO 5h (ml/kg/min)	LVO 12h (ml/kg/min)	LVO 24h (ml/kg/min)	LVO 48h (ml/kg/min)
Healthy Infants n=14	287 202-405	248 180-388	251 201-483	253 155-378
Sick Infants n=66	330 159-500	295 151-548	328 177-655	345 173-689
p value	0.13	0.04	0.02	0.003

Table 6.6 - Values for LVO at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants. Values are median and range.

A wide range of LVO was seen in sick preterm infants (Figure 6.3).

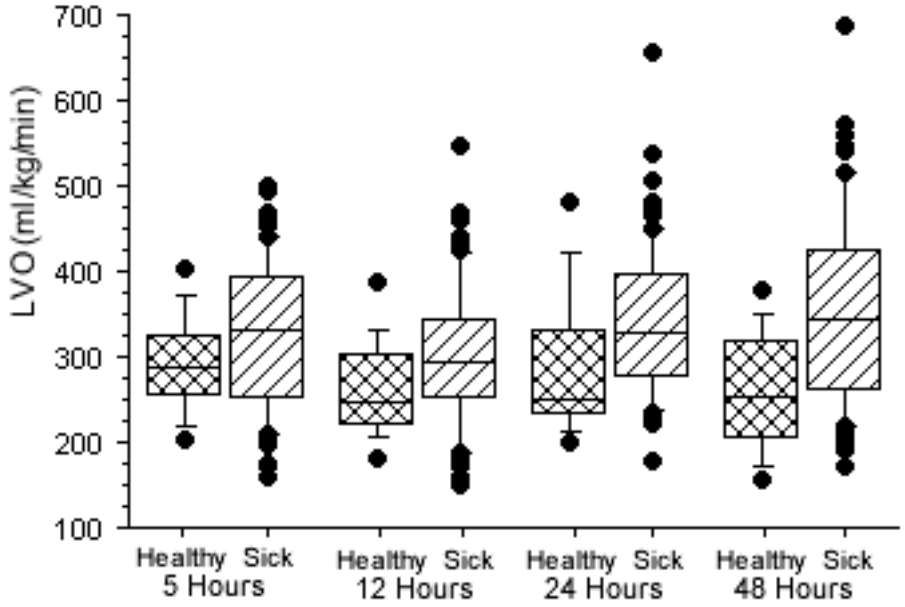

Figure 6.3 - Box and whisker plot of LVO at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants (10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} centiles, with outliers marked as circles).

6.3.8 RVO in sick preterm infants

Median right ventricular output in sick preterm infants at 5, 12 and 48 hours postnatal age was not different from that seen in healthy infants. At 24 hours sick infants had significantly higher volume of RVO (Table 6.7).

	RVO 5h (ml/kg/min)	RVO 12h (ml/kg/min)	RVO 24h (ml/kg/min)	RVO 48h (ml/kg/min)
Healthy Infants n=14	283 184-616	352 255-499	369 241-758	391 269-594
Sick Infants n=66	338 100-598	360 160-627	430 175-650	427 135-974
p value	0.42	0.79	0.02	0.28

Table 6.7 - Values for RVO at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants. Values are median and range.

A wide range of RVO was seen in sick preterm infants (Figure 6.4).

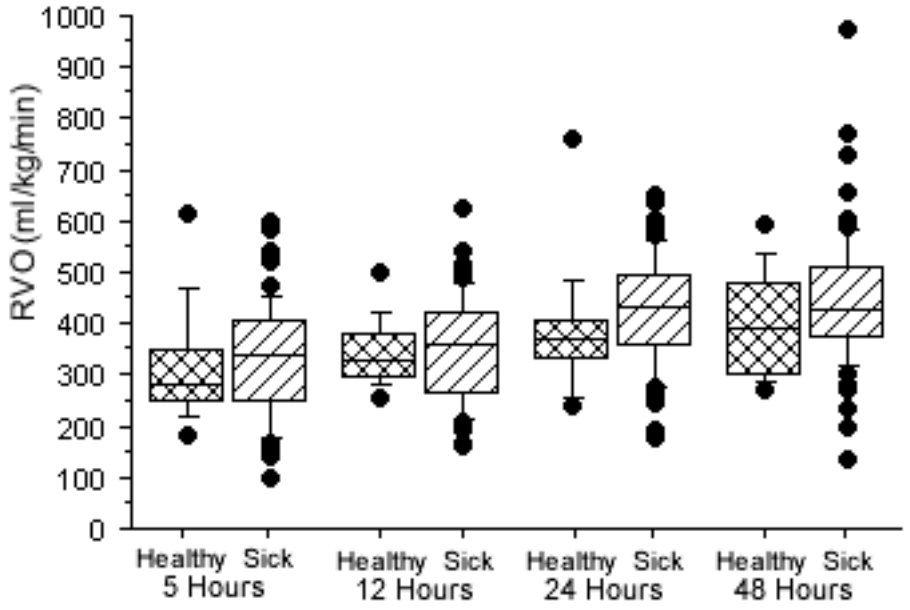

Figure 6.4 - Box and whisker plot of RVO at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants (10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} centiles, with outliers marked as circles).

6.3.9 Ductus arteriosus

Median diameter of the ductus arteriosus in sick preterm infants at 5 hours postnatal age was not different from that seen in healthy infants. At 12 hours sick infants tended to have larger ducts. At 24 and 48 hours this difference was more marked and was statistically significant (Table 6.8).

	Duct diameter 5h (mm)	Duct diameter 12h (mm)	Duct diameter 24h (mm)	Duct diameter 48h (mm)
Healthy Infants	1.8	1.4	0.6	0.0
n=14	1.3-3.0	0.0-2.6	0.0-2.0	0.0-1.3
Sick Infants	1.9	1.7	1.8	1.6
n=66	1.1-3.9	0.0-3.3	0.0-3.4	0.0-3.1
p value	0.54	0.06	0.001	0.0003

Table 6.8 - Ductal diameter at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants. Values are median and range.

6.3.10 Arterial blood pressure in sick preterms

At 5, 12, 24 and 48 hours postnatal age sick infants had lower mean arterial blood pressure than healthy infants (Table 6.9).

	Mean arterial BP 5h (mm Hg)	Mean arterial BP 12h (mm Hg)	Mean arterial BP 24h (mm Hg)	Mean arterial BP 48h (mm Hg)
Healthy Infants n=14	44 33-55	43 36-61	43 35-55	52 41-62
Sick Infants n=66	34 24-49	35 22-53	36 25-63	39 26-66
p value	0.01	0.002	0.004	0.009

Table 6.9 - Mean arterial blood pressure at 5-48 hours postnatal age in 14 healthy and 66 sick preterm infants. Values are median and range.

6.4 Discussion

To confirm the feasibility of making repeated measures of SVC and DAo flow in early postnatal life in preterm infants we aimed to recruit a relatively large cohort of infants (100 subjects) <31 weeks gestation.

We studied 80 preterm infants over a 17 month period. While this fell short of our target of 100 infants, due both to lack of operator availability and declined parental consent, the cohort size is similar to that used in other studies of echocardiographic techniques(48, 68, 192). While only around 50% of eligible infants were studied in this cohort, these infants did not differ from non-enrolled infants in terms of gestation or birth weight. The 80 infants in our cohort also had remarkably similar demographics to a large national cohort of infants reported by the Australian and New Zealand Neonatal Network (ANZNN)(16). This ANZNN cohort were virtually identical to our own in terms of rates of survival, chronic lung disease, home oxygen therapy, periventricular haemorrhage and necrotising enterocolitis(16).

It therefore seems reasonable to assume that the cohort reported in our study is representative of infants delivered in New Zealand as a whole under current obstetric and neonatal care practices.

A single operator (AG) performed the majority of scans in this study. This consistency of operator should strengthen the interpretation of results by limiting the scope for inter-observer variability. Inter-observer repeatability in individual subjects was poor in our study (chapter 4), suggesting that a single operator should ideally perform repeated measures in a single subject(9). However no systematic bias between observers was found in measurement of either SVC or DAo flow (chapter 4); therefore we would still expect the measures produced by one observer in a population of infants to be comparable to those produced by another.

The feasibility of making repeated echocardiographic measures in preterm infants depends on both the safety of the scans and on the ability of operators to acquire satisfactory images to allow haemodynamic interpretation. The results presented in chapter 3 of this thesis have shown that echocardiography can be performed without significantly disturbing cardiorespiratory status. Satisfactory image quality for haemodynamic measurement was obtained for 96% of measures of SVC flow and 97% of measures of DAo flow. While increased difficulty in obtaining adequate views of SVC diameter with advanced postnatal age has been described(9), incidences of failure in acquiring images are not generally reported in echocardiographic studies of newborn infants.

No infant was excluded from this study due to significant structural congenital cardiac anomaly. One infant was found to have a small restrictive VSD. This infant was not excluded from the analysis as it was considered that the small size of the VSD, along

with its restrictive flow pattern, meant that the volume of shunt across the defect was unlikely to be significant. Any impact of the shunt was likely to have been considerably less than that of shunting through the persistent atrial and ductal communications. One infant in this cohort was found to have a mildly dysplastic pulmonary valve. This infant was excluded from analysis of right ventricular output measures as any mild acceleration of flow at the pulmonary valve would produce falsely high RVO. As there was no evidence of significant obstruction to flow this infant's LVO and SVC and DAo flow measures were not excluded from the analysis. No infant in this cohort was found to have a persistent left SVC, a relatively rare normal variant(269) which would lead to significant underestimation of SVC flow volume(9).

Repeated measurements of SVC and DAo flow are feasible in newborn preterm infants. The clinical utility of these measures depends on whether patterns of postnatal flow provide new insights into pathophysiology (by distinct groups of infants showing consistently diverse patterns of flow) or predict outcome (by flow patterns in individual infants being associated with adverse events). Interpretation of this cohort's results is then best approached by looking at both group and individual changes.

Superior vena caval flow is significantly lower in sick than in healthy preterm infants at 24 and 48 hours but not at 5 and 12 hours. This does not appear to be because SVC flow falls in sick infants after the first 12 hours, but rather because it fails to increase to the extent that it does in healthy infants (Chapter 5). At first glance this observation of a lower SVC flow in sick infants at 24 and 48 hours appears different from the pattern suggested by previous studies that low SVC flow in the first 24 hours was predictive of PVH(10) and adverse long-term outcome(167). However the *range* of SVC flows seen in our cohort of sick infants may provide the answer to this apparent contradiction,

since the lowest flows were seen in individual infants at 5 and 12 hours. Very low SVC flow volumes (around 20 ml/kg/min) have previously been associated with subsequent development of periventricular leukomalacia(10). The clinical outcomes associated with very low flow in our cohort will be discussed in chapter 9.

A large number of sick infants also had SVC flows which were clearly above the 'normal' range. It appears that individual haemodynamic responses in sick preterm infants vary greatly, with infants having both high and low SVC flows so that there was no significant difference in median flow between the sick and well infants in this study.

Descending aortic flow volume in sick infants was not significantly lower than that seen in healthy infants at 5, 12, 24 or 48 hours. However, once again, the wide range of flow volumes seen was striking, with some low DAo flow volumes seen at each postnatal age studied (Chapter 9).

Left ventricular output (LVO) was significantly higher in sick than in healthy preterm infants at 12, 24 and 48 hours postnatal age. The tendency for sick infants to have high LVO has been reported previously(82, 186). In sick infants this high LVO is due to the increased ductal diameter and volume of left-to-right ductal shunting seen in sick infants compared to healthy infants who make a smooth cardiorespiratory transition to extrauterine life(204, 205). This is supported in our cohort, where sick infants had significantly increased ductal diameter at 12, 24 and 48 hours. The finding of higher LVO in sicker infants serves to underscore the importance of assessing systemic perfusion at points in the circulation not confounded by fetal shunt pathways(9). The role of ductal shunting in production of high LVO, and specifically whether ductal shunting is causative of the low SVC flow seen at 24 and 48 hours in sick infants will be discussed in chapter 8.

The range of LVO seen in sick infants was again wide, though no infant in our cohort had an LVO below the value of 150 ml/kg/min which others have associated with adverse respiratory outcome(228). The more striking aspect of the range of LVO seen in sick infants was the number of infants with grossly elevated LVO, emphasising how significant volume of ductal shunting can be, even in early postnatal life(46)(chapter 8).

Right ventricular output (RVO) in sick infants was not significantly different from that seen in healthy infants at 5, 12, 24 or 48 hours. However, once again, the wide range of flow volumes seen was striking. Two infants had RVO below 150 ml/kg/min, a level which previously has been associated with adverse outcome(228). Our method of calculation of pulmonary diameter (at the valve leaflets) may tend to overestimate RVO compared to measures based on pulmonary trunk diameter(224). It may be that incidence of low RVO is higher than suggested by our cohort due to this higher estimation of flow using this technique. However the technique used here has been shown to be the most repeatable method of RVO determination(224). At 24 and 48 hours low RVO became less common, which may in part be due to an increase in intra-atrial shunting(228). The relatively small volume of intra-atrial shunting in the early postnatal period had led to RVO being suggested as a more reliable indicator of systemic perfusion than LVO at this time(228).

Arterial blood pressure was lower in sick compared to healthy preterm infants at 5, 12, 24 and 48 hours (table 6.8). The relationship between blood pressure and systemic perfusion is not straightforward(80, 270), and will be discussed in the next chapter.

The sick preterm infants had significantly lower median birth weight and gestation than the healthy infants in this cohort. That the sick infants were smaller and less mature may account for some of the differences seen in blood flow patterns and blood

pressures between the groups. However relationships between flow volume and birth weight or gestation across the cohort were weak (data not shown), and would not have accounted for the magnitude of differences seen between the groups. Similarly, differences in blood pressure between the groups can not be accounted for by differences in maturity alone. The normal systolic blood pressure has been shown to increase by 1-2mmHg per week gestation between 28 and 32 weeks gestation in healthy survivors of preterm birth(206), so the median difference of one week gestation between the sick and healthy infants described in our cohort could not account for the 7-13mmHg difference in mean BP. In addition, differences in flow volumes between the groups were not consistent, but rather varied with postnatal age, suggesting that sick infants may have an altered postnatal circulatory adaptation(205). An altered course of postnatal circulatory adaptation in sick infants is supported by the finding that sick and healthy preterm infants show no difference in ductal diameter at 5 hours, but that at 24 and 48 hours ductal diameter is significantly greater in sick infants.

This study has a number of limitations, particularly the low recruitment which to some extent limits the generalisability of the data. The outcome division between 'sick' and 'healthy' is very broad, with many 'sick' infants going on to have a good outcome. Outcomes to hospital discharge will be discussed in chapter 9. The crucial outcome in any neonatal study is long-term neurodevelopmental outcome. This data is not yet available for this complete cohort, but data collection continues. The interpretation of individual infant flow values and patterns, and also of the range of values seen must be guarded in view of the inherent variability in the measures examined (chapter 4). Some of the wide range of values seen in sick infants may be due to measurement error rather than true differences in measured values.

The three crucial issues of relation of flow volume to arterial blood pressure, impact of the duct on systemic perfusion in the early postnatal period and association between systemic perfusion and outcome will be discussed in detail in the next three chapters.

6.5 Conclusion

Repeated measurements of SVC and DAo flow are feasible in newborn preterm infants. LVO tends to be higher and SVC flow lower in sick preterm infants. The ranges of SVC and DAo flow, and LVO and RVO are considerably wider in sick than in healthy preterm infants. A significant proportion of sick preterm infants have systemic flow volumes below the normal range described in healthy preterm infants.

7 Relationship between Blood Pressure and Blood Flow in Sick Preterm Neonates

7.1 Introduction

The definition, incidence and clinical significance of hypotension in the newborn period have recently been reviewed(80, 270). Despite an association between hypotension and adverse outcomes in preterm infants(26, 37, 140, 271), there is no evidence that treating hypotension improves outcome(270).

The relationship between blood pressure and blood flow is not straightforward. While blood flow is likely to be the more clinically important variable(4), ability to measure flow is limited. Blood pressure therefore remains the more frequently monitored indicator of neonatal circulatory status(80).

Blood pressure is the product of flow and resistance. A high blood pressure may then be due to high flow, high resistance, or both. High pressure in the pulmonary circulation is strongly associated with low pulmonary flow, yet for many years a normal or high systemic blood pressure has been taken to indicate a normal systemic flow(4, 243).

Studies in neonates have variously suggested weakly positive(81), weakly negative(82) or no(83, 84) association between arterial blood pressure and left ventricular output (LVO). Measures of cerebral blood flow (CBF) have similarly been suggested to have positive(179), negative(143), or no(180) association with arterial blood pressure.

Volume of SVC flow has been suggested to have a weakly positive association with blood pressure(10). There are no published data on the association between blood pressure and right ventricular output (RVO) which may be a more reliable marker of systemic perfusion than LVO in the presence of ductal shunting(228), though is itself confounded by atrial shunting.

While systemic arterial blood pressure remains a frequently monitored marker of circulatory status in neonates receiving intensive care further study of blood pressure's relationship with volume of blood flow in the transitional circulation is crucial.

The aim of this study was to examine the association between arterial blood pressure and four measures of systemic perfusion, LVO, RVO and SVC and DAo flow, in the first 48 hours of postnatal life in sick preterm neonates.

7.2 Methods

Only the infants defined as 'sick' in chapter 6 of this thesis were examined. The study was limited to those infants who had continuous recordings of invasively monitored blood pressure downloaded over the precise duration of the echocardiogram (section 2.2) to maximise the reliability of blood pressure values studied.

The level of circulatory support provided to the infants was dictated by the attending clinicians, who were not aware of the results of the echocardiograms. In general, clinicians followed our unit guideline of using fluid boluses and inotropes to maintain mean arterial blood pressure greater than gestational age in weeks(80).

Left and right ventricular outputs, SVC flow, DAo flow and ductal diameter were assessed by echocardiography as described in section 2.3.

At each scan left and right ventricular outputs, SVC flow and DAo flow were each compared with mean blood pressure using univariate analysis. The impact of potential confounding factors such as birth weight and gestation was examined by adjusting for these variables using multiple linear regression analysis. In all cases statistical significance was taken where $p<0.05$.

7.3 Results

Of the 66 sick preterm infants described in chapter 6, 34 (52%) had readings of invasively monitored arterial BP downloaded every 60 seconds during echocardiography.

These 34 infants had a median gestation of 27 (range 24-29) weeks and median birth weight of 885 (range 510-1430) grams. Sixteen (47%) were male. The mothers of 32 (94%) infants received antenatal corticosteroids, with the mothers of 23 (68%) infants receiving at least one complete course. Thirty-one (91%) infants required mechanical ventilation, three (9%) required CPAP only. Twenty (59%) infants received at least one bolus of intravenous fluid for circulatory support, of whom 17 (50%) received a bolus prior to their first echocardiogram. Seven (21%) infants received inotropic support with dopamine; no other inotropic agents were used. Two (6%) infants were receiving dopamine at the time of the 5 hour scan, six (18%) were receiving dopamine at each of the 12 and 24 hour scans and three (9%) were receiving dopamine at the 48 hour scan. Seven (21%) infants developed PVH and four (12%) died prior to hospital discharge.

7.3.1 5 Hour Scan

At 5 hours postnatal age there was a weak but significant inverse correlation between volume of SVC flow and mean arterial blood pressure ($R^2=0.141$, $p=0.04$) (Figure 7.1).

For each 1mm Hg increase in mean BP, SVC flow decreased on average by 2.1 ml/kg/min.

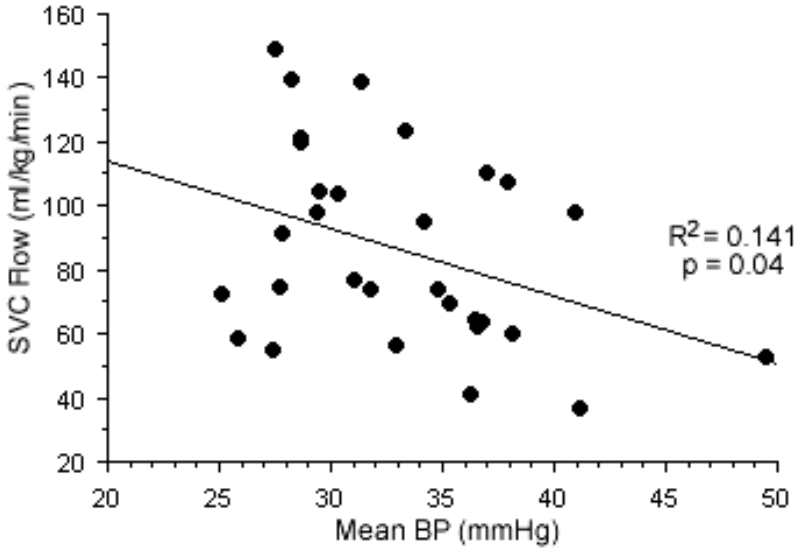

Figure 7.1 - Inverse relationship between SVC flow and continuously monitored mean blood pressure in 30 preterm infants at 5 hours postnatal age.

The inverse correlation between mean blood pressure and SVC flow volume persisted when correcting for either birth weight (p=0.04) or gestation (p=0.03) on multiple regression analysis. The correlation also persisted when the two infants receiving inotropic support at the time of echocardiography were excluded from the analysis (R^2=0.168, p=0.02).

Mean blood pressure was not significantly associated with volume of DAo flow (p=0.23), LVO (p=0.27), RVO (p=0.15) or ductal diameter (p=0.86) at 5 hours postnatal age.

7.3.2 12 Hour Scan

At 12 hours postnatal age volume of SVC flow tended to decrease as mean blood pressure increased (Figure 7.2). However this association was not statistically significant (R^2=0.113, p=0.06).

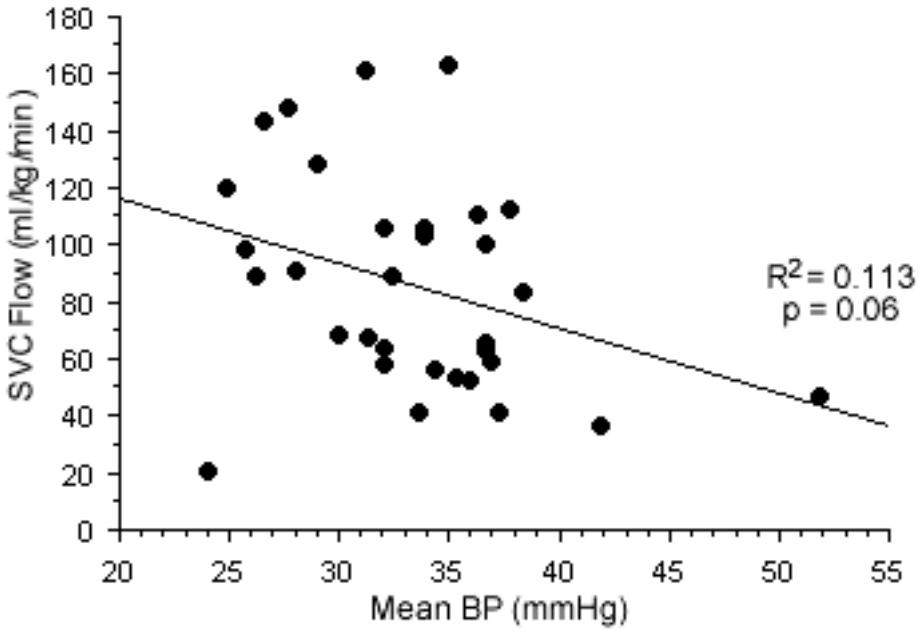

Figure 7.2 - Inverse relationship between SVC flow and continuously monitored mean blood pressure in 32 preterm infants at 12 hours postnatal age

The inverse correlation between blood pressure and volume of SVC flow became significant when the six infants receiving inotropic support at the time of echocardiography were excluded from the analysis (R^2=0.180, p=0.03).

Mean blood pressure was not significantly associated with volume of DAo flow (p=0.21), LVO (p=0.12), RVO (p=0.22) or ductal diameter (p=0.77) at 12 hours postnatal age.

7.3.3 24 Hour Scan

Mean blood pressure was not significantly associated with volume of SVC flow (p=0.46), DAo flow (p=0.82), LVO (p=0.75), RVO (p=0.47) or ductal diameter (p=0.34) at 24 hours postnatal age.

7.3.4 48 Hour scan

Mean blood pressure was not significantly associated with volume of SVC flow (p=0.22), DAo flow (p=0.59), LVO (p=0.23), RVO (p=0.08) or ductal diameter (p=0.27) at 48 hours postnatal age.

7.4 Discussion

The most striking feature of the results presented here is that at no time in the first 48 hours of postnatal life was there evidence of a positive association between blood pressure and volume of blood flow at any of the four sites studied in preterm infants.

It is widely acknowledged in reviews of hypotension in the neonate that the relationship between blood pressure and blood flow in neonates is weak(8, 80, 270). Blood pressure is determined by a complex interplay of circulating blood volume, myocardial contractility, systemic vascular resistance (SVR) and, in the presence of a patent ductus arteriosus, pulmonary vascular resistance(8). Myocardial contractility may be impaired in the immediate postnatal period(84, 194) and some studies have suggested that SVR is the principle determinant of blood pressure in preterm infants(80, 82, 83). Given this complex interplay of factors, and the dominance of SVR in governing blood pressure, it is perhaps not surprising that the relationship between blood pressure and blood flow is so variable in published reports(10, 81-84, 143, 179, 180).

We included only blood pressure readings taken from indwelling arterial lines, and downloaded over the exact duration of echocardiography - minimising the possibility that inaccuracies in blood pressure measurement could be masking a true positive association. While the limited repeatability of the echocardiographic estimates of volume of blood flow could potentially mask an association, the repeatability of SVC

measurement has been shown to be adequate both in this thesis and elsewhere(9). Repeatability of LVO and RVO estimation by the methods used here has also been thoroughly assessed(217, 224).

While it is impossible to exclude a positive association between pressure and flow in our cohort, the results presented here suggest that should there be any positive association between pressure and flow, it must be a weak one.

The only previous study of SVC flow volume in preterm infants did show a weakly positive association between blood pressure and SVC flow at 5 hours postnatal age(10). Similar techniques were used to assess both blood pressure and flow in the two studies, so the disparity in results is unlikely to be due to methodological differences.

It is more likely that the disparity seen is due to differences in the cohorts of infants studied. We elected to examine the relationship between blood pressure and blood flow in sick preterm infants only, whereas the previous study looked at all preterm infants. Healthy preterm infants have a more normal circulatory adaptation(205), and tend to have both higher blood pressure and higher SVC flow in early extra uterine life, with sicker infants tending to have both lower blood pressure and lower flow (chapter 6). This population effect would produce a positive association between blood pressure and flow when all infants are analysed together, but this association may disappear on analysis of a sub-group of sick infants. We found no association between blood pressure and SVC flow in our cohort when both sick and healthy infants were examined together (data not shown). However these data are of limited value as healthy infants did not tend to have blood pressure monitored invasively or continuously in our study.

The discrepancy in the relationships between blood pressure and flow in the two studies may also be due to differences in clinical management, including use of volume support, inotropes and indomethacin (79).

The fact that there may be an inverse relationship between SVC flow volume and arterial blood pressure at 5 hours in our cohort is intriguing. Others have found an inverse relationship between blood pressure and echocardiographic measures of flow in the first postnatal day in preterm infants(82). If SVR is the dominant factor in governing blood pressure in the neonate an inverse relationship between blood pressure and flow would be to be expected, with infants with the highest blood pressure also having the highest SVR, hindering flow.

An inverse relationship between pressure and flow in early postnatal life is also potentially of some importance as systemic hypotension occurs most commonly in the immediate postnatal period(270), and the use of inotropes is usually triggered by this(272). Circulatory support could in fact then be being given to infants with relatively higher levels of blood flow.

Interpretation of the data linking low SVC flow to high blood pressure must be cautious since the association, though statistically significant, was weak. The association was no longer statistically significant at 12 hours postnatal age, and associations between low DAo flow, LVO or RVO and high blood pressure were not seen at any time in the first 48 hours.

Studies using near infrared spectroscopy to look at cerebral blood flow have also variously shown a positive(179), negative(143), or no(180) association with blood pressure in populations of preterm infants. It has been clearly demonstrated however that at least a proportion of sick preterm infants have limited cerebral autoregulation

such that increases in blood pressure are associated with increases in cerebral blood flow in individual infants(157, 179). In one study by Munro et al infants with low blood pressure were shown to have low CBF, and crucially inotropic support to raise blood pressure also raised CBF(179). While some of the correlation of blood pressure and CBF in this study could be accounted for by repeated measurements being taken from infants at a range of postnatal ages (both blood pressure and CBF tend to increase with time), and while the exact association between blood pressure and flow was not consistent in some infants, the findings of this study are important.

The discrepancy between the results from Munro's study and those from our current cohort can potentially be explained in two ways - by the characteristics of longitudinal and cross sectional studies, or by potential differences in vascular reactivity between the cerebral circulation and the remainder of the peripheral vasculature.

Our study describes the relation between blood pressure and flow on a cross-sectional basis, whereas Munro's study has a principally longitudinal focus. While it is clear that some infants will show an increase in CBF with increasing blood pressure on a longitudinal basis, it does not necessarily follow that high blood pressure will be predictive of high CBF in a cross-sectional study.

The apparent inverse relation between flow and blood pressure in our cohort seems counter-intuitive given that a significant proportion of the infants studied would be expected to have deficient cerebrovascular autoregulation and therefore to show increases in CBF with increasing blood pressure, at least on a second-to-second basis(157, 173). The apparent contradiction that flow seems to increase with increasing blood pressure over time in individual infants, but may decrease with increasing blood

pressure across a population may be explained by the interplay of factors controlling blood pressure and the mechanisms involved in autoregulation.

Many studies of cerebrovascular autoregulation are based on the response of CBF to transient changes in blood pressure (172). Since these changes precede any alterations in peripheral vascular tone it seems sensible to conclude that they are due to changes in cardiac output (i.e. increased preload or contractility) rather than resistance. If intact, cerebral autoregulation then occurs by altering the vascular tone to compensate for this change in blood pressure (and presumably cardiac output) to return flow to its previous level. Intact autoregulation therefore describes the ability of the brain to maintain steady CBF on a short term basis. Infants with absent autoregulation may show increases in CBF with increases in blood pressure on a second-to-second or minute-to-minute basis, but abnormal functioning of their peripheral vascular tone may be less predictable. An infant with broadly low vascular tone will tend to have low blood pressure (and perhaps high flow), while an infant with broadly high vascular tone will tend to have high blood pressure (and perhaps low flow). Both infants could still have impaired autoregulation and therefore show increases in flow with transient, myocardial contractility induced, changes in blood pressure.

Another explanation for the discrepancy between studies suggesting a positive correlation between CBF and blood pressure and our own apparent inverse relation between SVC flow and blood pressure is more concerning to our technique, and involves potential differences in the vascular reactivity between the cerebral circulation and the remainder of the peripheral vasculature. If a significant proportion of flow in the SVC comes from the peripheral rather than the cerebral vasculature (section 1.4.1), and if the cerebral vasculature has a different pattern of vasoreactivity to the peripheral

circulation, it would be possible for blood pressure to be positively correlated with CBF in sick preterm infants, but not with SVC flow.

Since the brain receives a relatively small proportion of the cardiac output(273) changes in peripheral vasculature resistance will have more impact than cerebral vasculature resistance on blood pressure. An infant with relatively high peripheral vascular resistance and low cerebral resistance could potentially have low SVC flow, but high CBF. An infant with relatively low peripheral vascular resistance and high cerebral resistance may have high SVC flow, but low CBF. Changes in the proportion of SVC flow coming from the brain due to changes in relative resistance of the cerebral and peripheral circulations could not be detected by our method.

It is however by no means clear that the cerebral vasculature does behave significantly differently to the peripheral vasculature. Cerebrovascular autoregulation acts by intrinsic contraction and relaxation of vessel musculature in the same way as the rest of the circulation, and cerebral vessels have been shown to respond to dopamine with vasoconstriction(274) in a similar way to the peripheral vasculature.

The potential for alterations in the proportion of SVC flow coming from the brain with changes in blood pressure does provide scope for results of studies of SVC flow to be misinterpreted. However to dismiss SVC flow as a marker of CBF on this basis seems misguided as the proportion of SVC flow coming from the brain remains unclear, the relative vasoactive properties of the cerebral and peripheral vasculatures are unknown, and a consistent positive relation between blood pressure and CBF is far from proven(180).

Our study has a number of limitations, some of which are described above. It is important to appreciate that echocardiographic measures of cardiac output are prone to

a degree of variability(217, 224), such that true associations can be masked(215). However variability in measurement techniques may also produce erroneous associations. This raises the possibility that the inverse relation of SVC flow and blood pressure seen in our cohort is a chance association.

That an inverse relation between blood pressure and SVC flow was no longer seen at 24 and 48 hours postnatal age could lend support to the earlier inverse relation being a chance finding. However the transitional circulation of the preterm infant undergoes vast changes in the first postnatal days. Blood pressure and flow change, as do volumes of shunt through the fetal channels and possibly inherent myocardial contractility. It is perhaps not surprising then that the associations between blood pressure and blood flow in this cohort changed over time.

Further study of the relationship between blood pressure and blood flow in the preterm neonate is clearly required. A quantitative simultaneous study of cerebral blood flow and SVC flow could demonstrate the relative specificity of SVC for cerebral perfusion, and also help clarify the vasoactive properties of the cerebral and peripheral circulations.

Whether or not the putative negative relationship between blood flow and blood pressure described in this chapter is substantiated by further research, it is evident that no positive correlation between blood pressure and perfusion was seen in our cohort of sick preterm infants in the first 48 hours of life. Monitoring for hypotension is therefore not a good population screening tool, as on a cross-sectional basis hypotension does not predict hypo-perfusion. There is clearly a huge scatter in flow volumes at any given blood pressure. A sick preterm infant with a mean arterial blood pressure of between 30 and 40 mmHg may have an SVC flow of greater than 140ml/kg/min or less than

40ml/kg/min. Infants with high blood pressure in the first postnatal hours may be the exact same ones with low flow, leaving infants with critically low systemic perfusion without the circulatory support they need.

Efforts targeted at improving neurodevelopmental outcome via improvements in cerebral and systemic perfusion in the early postnatal period need to focus on the monitoring and maintenance of indices of blood flow rather than blood pressure.

7.5 Conclusions

Low blood pressure is not predictive of low flow in the first 48 hours of postnatal life. Superior vena cava flow volume may be inversely related to arterial blood pressure at 5 hours postnatal age. Monitoring blood pressure is not a substitute for monitoring blood flow.

8 Impact of the Ductus Arteriosus on Systemic Perfusion in Preterm Infants in the First 48 Hours of Postnatal Life

8.1 Introduction

Patency of the ductus arteriosus is common in the first 48 hours of postnatal life in healthy term and preterm infants(85). Patency tends to be prolonged in preterm infants with respiratory distress syndrome(85). Patency of the ductus after the first 48 hours of life is associated with significant volume of systemic to pulmonary shunting, leading to worsening of respiratory status(52), and perhaps systemic hypoperfusion due to 'steal' of blood flow from the systemic circulation(59, 62). Prolonged ductal patency is associated with a variety of adverse outcomes in preterm infants, though a causative association has not been established(48, 275).

Previous studies of ductal flow in the first postnatal day have suggested that pulmonary and systemic pressures remain balanced, leading to the belief that shunt through a patent ductus may not be significant at this time(276). However even with relatively balanced pulmonary and systemic pressures ductal shunting is still predominantly left to right(47).

There is now substantial evidence that pulmonary pressure in some infants, especially those treated with surfactant(208, 209), may fall well below systemic levels in the first

day of extra-uterine life(85). High volume shunt through the duct, evidenced by reversal of blood flow during diastole in the descending aorta, has been seen as early as 7 hours after birth(48). Decreased SVC flow has been associated with increased ductal size at 5 hours(10). High volumes of ductal shunt may occur days before clinical signs of shunting appear(85).

Left ventricular output has been shown to increase with increased ductal shunting in preterm infants(228), but the immature myocardium may have a limited ability to respond to systemic to pulmonary shunt in the first postnatal day(194). In this case, if significant volume of ductal steal occurs, systemic perfusion may be impaired.

The optimal approach to treating ductal patency in preterm infants remains controversial(46, 56, 277). Prophylactic treatment with indomethacin significantly decreases the risk of periventricular haemorrhage in extremely low birth weight infants(57), but does not improve neurodevelopmental outcome at 18 months or reduce the incidence of chronic lung disease(69). Early indomethacin targeted towards at-risk infants decreases ductal diameter 2 hours after administration, but does not improve systemic perfusion(170). Even in infants with symptomatic PDA and high volume shunting, there is no evidence that closure of the duct improves long-term outcome(56). Further trials are required to establish benefit of any intervention(277).

Ductal patency is associated with adverse outcome, yet therapy to close the duct doesn't appear to improve outcome. This leaves the possibility that the association between ductal patency and adverse outcome is due to confounders, that it is casual rather than causal (section 1.2).

Significant volume of ductal shunt may occur even on the first day of extra uterine life. Left ventricular output has been shown to increase in the presence of ductal shunting,

but myocardial function may be impaired in the early postnatal period. It is possible then that ductal shunt at this time may not be adequately compensated for by increases in left ventricular output and that impaired systemic perfusion could result. This would provide a potential causal link between ductal shunt and adverse outcome.

The aim of this study was to investigate the patterns of ductal shunting in the first 48 hours of postnatal life and to attempt to clarify whether systemic perfusion is compromised by any shunting that does occur.

8.2 Methods

Left and right ventricular outputs, SVC flow, DAo flow and ductal diameter were assessed by echocardiography as described in section 2.3. The proportion of reversed DAo flow was assessed from the velocity time integrals (VTIs) as reversed VTI/forward VTI.

Changes in duct diameter over time were assessed using a Wilcoxon signed rank test.

At each time point the relationship between duct diameter and measurements of systemic perfusion (SVC and DAo flow) and cardiac output (LVO and RVO) were assessed using linear regression. Adjustment for potential confounding variables was carried out using multiple linear regression. Variables included in this analysis were birth weight, intra-uterine growth restriction (assessed by birth weight z score), antenatal steroid use and severity of respiratory disease (assessed by mean airway pressure (MAP), fraction of inspired oxygen (FiO_2) and pCO_2).

At each scan infants were divided into two groups according to whether duct size was larger or smaller than the median of the whole cohort at that time. SVC flow, DAo flow, LVO and RVO were compared between these two groups using a Mann-Whitney

test. Within the group of infants with duct diameter above the median, flow volumes were compared between infants with and without diastolic DAo reversal using a Mann-Whitney test. Within the group of infants with a duct diameter greater than the median and reversal of diastolic DAo flow, degree of flow reversal was compared with volume of flow on univariate analysis.

8.3 Results

The 80 infants described in chapter 6, with median (range) birth weight 1060 (510-1900) grams and gestation 28 (24-30) weeks, were examined at 5, 12, 24 and 48 hours postnatal age.

The median ductal diameter and direction of ductal shunting at each time point are shown in table 8.1. Median duct diameter was significantly larger at 5 hours than at 12, 24 and 48 hours ($p<0.0001$ for each time point). Duct diameter at 12 hours was not different from at 24 hours ($p=0.12$), but was larger than at 48 hours ($p=0.0004$). Duct diameter at 24 hours was significantly larger than at 48 hours ($p=0.01$). The proportion of infants with bidirectional ductal shunting decreased with increasing postnatal age (Table 8.1).

	5 Hours	12 Hours	24 Hours	48 Hours
Median Duct Diameter (Range)	1.9mm (1.1-3.9)	1.5mm (0-3.3)	1.7mm (0-3.4)	1.4mm (0-3.1)
Left to Right	53 (66%)	63 (79%)	55 (69%)	54 (68%)
Bidirectional	24 (30%)	11 (14%)	7 (9%)	3 (4%)
Right to Left	3 (4%)	0 (0%)	0 (0%)	0 (%)
Duct Closed	0 (0%)	6 (7%)	18 (22%)	23 (28%)

Table 8.1 - Duct diameter and direction of shunt in 80 preterm infants at 5, 12, 24 and 48 hours postnatal age

8.3.1 Impact of duct at 5 hours

At 5 hours postnatal age duct size was not associated with SVC flow, DAo flow, LVO or SVC on univariate analysis. There were no differences in any of the measurements of flow in infants with duct diameter >1.9 mm when compared to infants with smaller ducts.

Of the 40 infants with duct diameter >1.9 mm at 5 hours, 19 (48%) had reversal of diastolic DAo flow. These 19 infants showed a trend towards increased LVO (median (range) 351 (209-500) vs 284 (173-454) ml/kg/min, p=0.053) when compared to infants with large ducts without reversal of diastolic DAo flow.

However in these 40 infants with large ducts there was no difference in SVC flow (p=0.28), DAo flow (p=0.15) or RVO (p=0.80) between infants with and without reversal of diastolic DAo flow.

Degree of reversal of diastolic flow was not associated with SVC flow volume (p=0.55) or LVO (p=0.80) on univariate analysis. DAo flow volume was inversely associated with degree of reversal of diastolic flow on univariate analysis (R^2=0.23, p=0.03).

8.3.2 Impact of duct at 12 hours

Duct diameter was inversely related to SVC flow volume at 12 hours (R^2=0.095, p=0.006). Duct diameter was not associated with volume of DAo flow, LVO or RVO on univariate analysis. On multivariate analysis the association between duct diameter and SVC flow remained significant when correcting for birth weight, birth weight z score and antenatal steroid use (p=0.001). When additional correction for respiratory status was included the association between duct diameter and SVC flow remained significant when correcting for MAP and FiO_2 (p=0.004). When correcting for pCO_2 the association between duct size and SVC flow volume was no longer significant (p=0.24). However, only 44 infants had pCO_2 levels measured at the time of the echocardiogram, limiting the power of this analysis.

At 12 hours postnatal age the 44 infants with duct diameter greater than the median (1.5 mm) had lower SVC flow than infants with smaller ducts (median (range) 83 (34-179) vs 105 (21-209) ml/kg/min, p=0.02). There were no differences in DAo flow, LVO or RVO between the groups.

Of the 44 infants with duct diameter >1.5 mm at 12 hours, 15 (34%) had reversal of diastolic DAo flow. These 15 infants had increased LVO (median (range) 346 (237-548) vs 268 (151-461) ml/kg/min, p=0.0007) when compared to infants with large ducts without reversal of diastolic DAo flow (Figure 8.1).

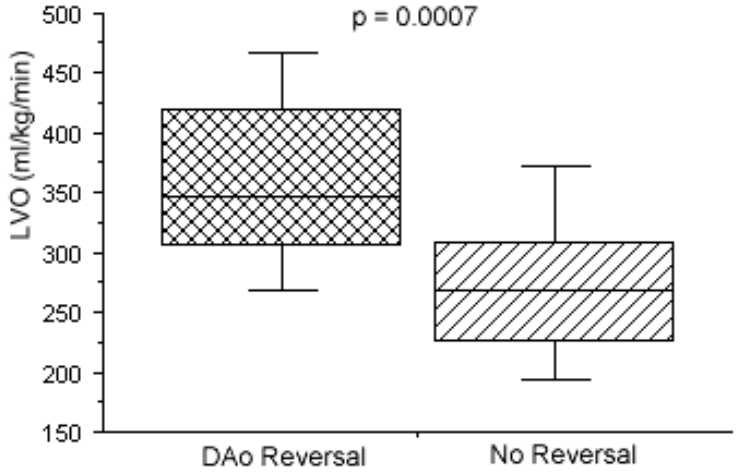

Figure 8.1 - Presence of reversal of diastolic DAo flow is associated with increased LVO in infants with duct diameter >1.5 mm at 12 Hours

However in these 44 infants with large ducts there was no difference in SVC flow (p=0.76) or RVO (p=0.94) between infants with and without reversal of diastolic DAo flow. Infants with large ducts and reversed diastolic DAo flow tended to have lower DAo flow volume when compared to infants with large ducts without reversal of diastolic DAo flow, but this association did not reach statistical significance (median (range) 115 (46-172) vs 132 (53-266) ml/kg/min, p=0.06).

Degree of reversal of diastolic flow was not associated with LVO (p=0.35) or SVC flow volume on univariate analysis (Figure 8.2).

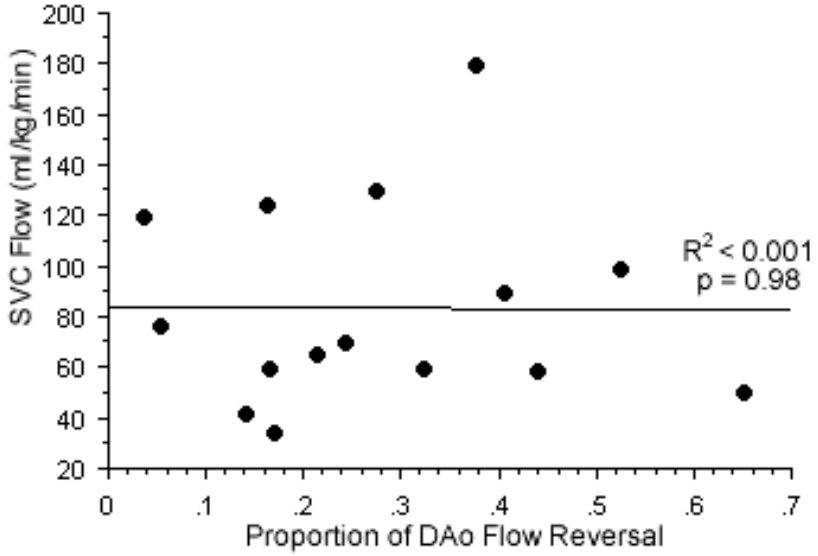

Figure 8.2 - Degree of reversal of diastolic DAo flow is not associated with SVC flow in infants with duct diameter >1.5 mm at 12 Hours. Proportion of DAO reversal is expressed as volume of reversed flow/volume of forward flow.

DAo flow volume was inversely associated with degree of reversal of diastolic flow on univariate analysis (Figure 8.3).

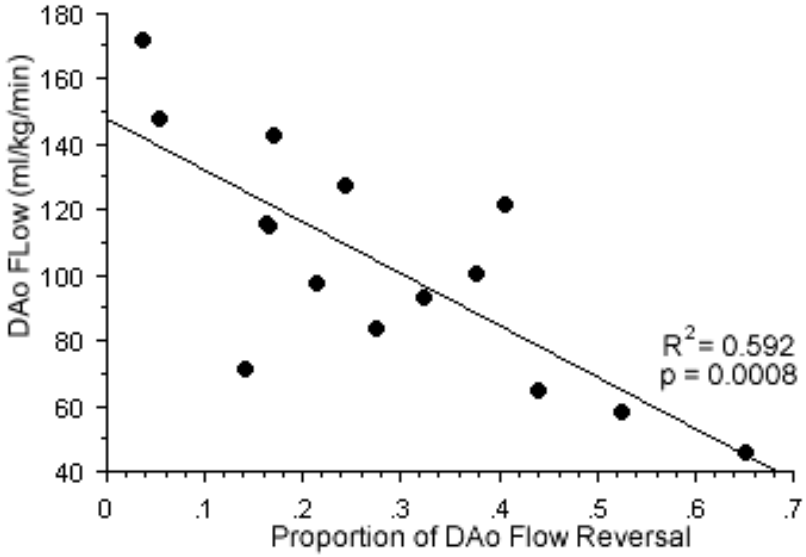

Figure 8.3 - Increased degree of reversal of diastolic DAo flow is associated with decreased DAo flow in infants with duct diameter >1.5 mm at 12 Hours. Proportion of DAO reversal is expressed as volume of reversed flow/volume of forward flow.

8.3.3 Impact of duct at 24 hours

Duct diameter was inversely related to SVC flow volume at 24 hours (R^2=0.113, p=0.003). Duct diameter was positively related to LVO at 24 hours (R^2=0.135, p=0.008). Duct diameter was not associated with volume of DAo flow or RVO on univariate analysis. On multivariate analysis the association between duct diameter and SVC flow remained significant when correcting for birth weight, birth weight z score and antenatal steroid use (p=0.001). When additional correction for respiratory status was included the association between duct diameter and SVC flow remained significant when correcting for MAP and FiO_2 (p=0.003). When correcting for pCO_2 the association between duct size and SVC flow volume was no longer significant (p=0.11). However, only 28 infants had pCO_2 levels measured at the time of the echocardiogram, limiting the power of this analysis.

At 24 hours postnatal age the 37 infants with duct diameter greater than the median (1.7 mm) had lower SVC flow than infants with smaller ducts (median (range) 84 (35-163) vs 96 (48-193) ml/kg/min, p=0.04). Infants with large ducts also had lower DAo flow (median (range) 135 (29-248) vs 150 (65-255), p=0.02) despite higher LVO (median (range) 348 (239-655) vs 288 (177-483), p=0.01) when compared to infants with smaller ducts. There was no difference in RVO between the groups.

Of the 37 infants with duct diameter >1.7 mm at 24 hours, 17 (46%) had reversal of diastolic DAo flow. These 17 infants had increased LVO (median (range) 398 (259-655) vs 312 (151-461) ml/kg/min, p=0.01) and decreased DAo flow volume (median (range) 107 (29-156) vs 165 (98-248) ml/kg/min, p=0.0001) when compared to infants with large ducts without reversal of diastolic DAo flow.

However in these 37 infants with large ducts there was no difference in SVC flow (p=0.68) or RVO (p=0.22) between infants with and without reversal of diastolic DAo flow.

Degree of reversal of diastolic flow was not associated with LVO (p=0.77) or SVC flow volume (p=0.14) on univariate analysis. DAo flow was inversely related to degree of reversal of diastolic flow on univariate analysis (R^2=0.713, p<0.0001).

8.3.4 Impact of duct at 48 hours

Duct diameter was inversely related to SVC flow volume (R^2=0.075, p=0.01) and DAo flow volume (R^2=0.079, p=0.01) at 48 hours. Duct diameter was positively related to LVO (R^2=0.354, p<0.0001) and RVO (R^2=0.060, p=0.03) at 48 hours. On multivariate analysis the association between duct diameter and SVC flow remained significant when correcting for birth weight, birth weight z score and antenatal steroid use (p=0.03). When additional correction for respiratory status was included the association between duct diameter and SVC flow remained significant when correcting for MAP and FiO_2 (p=0.03). When correcting for pCO_2 the association between duct size and SVC flow volume was no longer significant (p=0.17). However, only 28 infants had pCO_2 levels measured at the time of the echocardiogram, limiting the power of this analysis.

At 48 hours postnatal age the 40 infants with duct diameter greater than the median (1.4 mm) had lower SVC flow than infants with smaller ducts (median (range) 91 (29-176) vs 110 (64-179) ml/kg/min, p=0.004). Infants with large ducts had higher LVO (median (range) 386 (218-689) vs 262 (155-437), p<0.0001) and higher RVO (median (range) 448 (135-974) vs 389 (200-594), p=0.006) when compared to infants with smaller ducts. There was no difference in DAo flow volume between the groups

Of the 40 infants with duct diameter >1.4 mm at 48 hours, 18 (45%) had reversal of diastolic DAo flow. These 18 infants had increased LVO (median (range) 450 (305-689) vs 350 (218-517) ml/kg/min, p=0.002) and decreased DAo flow volume (median (range) 104 (43-194) vs 163 (75-261) ml/kg/min, p=0.002) when compared to infants with large ducts without reversal of diastolic DAo flow.

However in these 40 infants with large ducts there was no difference in SVC flow (p=0.33) or RVO (p=0.88) between infants with and without reversal of diastolic DAo flow.

Degree of reversal of diastolic flow was not associated with LVO (p=0.78) or SVC flow volume (p=0.64) on univariate analysis. DAo flow was inversely related to degree of reversal of diastolic flow on univariate analysis (R^2=0.494, p=0.002).

8.3.5 Summary of results

	5 Hours	12 Hours	24 Hours	48 Hours
duct size, univariate analysis	No association	↓ SVC flow	↓ SVC flow LVO	↓ SVC flow ↓ DAo flow LVO RVO
duct size, correcting for weight, z score, steroids, MAP and FiO_2	No association	↓ SVC flow	↓ SVC flow	↓ SVC flow
Duct size > median	No association	↓ SVC flow	↓ SVC flow ↓ DAo flow LVO	↓ SVC flow LVO RVO
Of infants with duct > median, presence of DAo reversal	LVO (p=0.053)	LVO	LVO ↓ DAo flow	LVO ↓ DAo flow
Of infants with DAo reversal, degree of reversal	↓ DAo flow	↓ DAo flow	↓ DAo flow	↓ DAo flow

Table 8.2 - Associations of ductal size and DAo flow reversal at 5, 12, 24 and 48 hours postnatal age in 80 preterm infants. $p<0.05$ unless stated otherwise. MAP - mean airway pressure, FiO_2 - fraction of inspired oxygen.

8.4 Discussion

This study suggests that increased ductal diameter is associated with decreased SVC flow volume at 12, 24 and 48 hours postnatal age, and that this association persists when correcting for a number of confounding variable, but may not persist when correcting for pCO_2. This may suggest that SVC flow volume is related more to severity of respiratory disease than to volume of ductal flow. Increased ductal diameter also tends to be associated with decreased DAo flow volume and increased LVO and RVO. Presence of reversal of diastolic DAo flow is associated with decreased DAo

flow, and increased LVO and RVO, but is not associated with decreased SVC flow volume.

The haemodynamic impact of ductal shunting appears to be more marked at 24 and 48 hours postnatal age than in the first 12 hours of extra uterine life.

Patency of the ductus arteriosus is common in the first 48 hours of postnatal life. The volume and direction of shunting through the duct at this time will depend on the size of the duct and the pressure gradient between the systemic and pulmonary circulations.

Postnatal constriction of the duct varies greatly between infants and within individual infants over time(48). Many relatively healthy preterm infants achieve ductal constriction and closure over a similar time period to term infants(204). Some sick preterm infants achieve a degree of early ductal constriction followed by an increase in size(48), whilst other infants have persistently broadly patent ducts throughout early postnatal life(48). Duct diameter assessed by colour Doppler echocardiography has been shown to be a strong predictor of shunt volume(202).

Volume of ductal shunt also depends on the systemic to pulmonary pressure gradient. Postnatal changes in pulmonary pressure have been studied extensively in preterm infants(205, 210). Postnatal fall in pulmonary pressure occurs more slowly in preterm than term infants such that systemic and pulmonary pressures tend to be balanced, producing bidirectional shunting across the duct in some infants(6). While in some cases this may mean that shunt volume is low, this need not necessarily be the case. Bidirectional shunting predominantly occurs because pulmonary systolic pressure falls more slowly than diastolic(210). The pressure wave from the right ventricle also reaches the duct slightly earlier than that from the left ventricle, encouraging right to left shunt in early systole(278). However the delay between pulmonary and systemic

pressure waves reaching the duct is only 10-20 milliseconds in preterm infants, and only accounts for a small proportion of right to left shunting(278). Right to left shunting occurs during systole (around a quarter of the cardiac cycle(202)) and left to right shunting occurs during diastole (around three quarters of the cardiac cycle(202)). Direction of flow is therefore predominantly left to right. Studies have shown that the net volume of systemic to pulmonary shunting when bidirectional ductal flow is present is up to 50% of that seen during pure left to right shunting(47).

Pulmonary pressure falls progressively through the first days of life in preterm infants(6). Current neonatal respiratory care with antenatal steroid and surfactant administration may mean that pulmonary pressures drop more quickly than suggested by earlier studies(209). The incidence of bidirectional ductal shunt at 12-24 hours in our cohort is considerably less than that seen in earlier studies(6). In the presence of pure left to right ductal shunt (seen in 70-80% of our infants at 12-24 hours) net direction of ductal steal must be away from the systemic circulation. It is not unusual for this shunt to account for up to 50% of the left ventricular output in the first postnatal days(46), and volume of shunt has been quantified as 100-200 ml/kg/min(47, 279).

Presence of reversal of diastolic DAo flow (as blood in the descending aorta is 'sucked' upwards and through the duct) has been shown to be a reliable marker of high volume ductal shunt(202). We have already shown that detection of diastolic flow reversal is repeatable in preterm infants (Chapter 4). Degree of reversal of diastolic flow (expressed as a proportion of forward flow) is also associated with volume of shunt(235). In our cohort of infants diastolic DAo reversal occurred as early as 4 hours after birth, and was seen in 34 and 46% of infants with large ducts at 12 and 24 hours respectively.

Our data show that ductal shunting in the first 48 hours of postnatal life in preterm infants is predominantly systemic to pulmonary and that significant volumes of blood may be shunted at this time.

Systemic to pulmonary shunting causes increased pulmonary perfusion and may be associated with occurrence of pulmonary haemorrhage(52). Further discussion of the respiratory sequelae of ductal shunting is outside the scope of this thesis.

The impact of ductal shunting on the systemic circulation is less clear(277). In the presence of high volume left to right ductal shunt left ventricular output needs to increase by an equivalent volume to maintain the same tissue perfusion. If this occurs despite 'haemodynamically significant' ductal shunt, systemic perfusion will be preserved. Compared to term infants, myocardial contractility in preterm is impaired(194, 196), and may be especially prone to failure in the presence of high afterload(195). This raises the possibility that even relatively small volumes of ductal steal in the first postnatal day may lead to impairment of systemic perfusion.

However ductal shunting leads to increased venous return to the left atrium and therefore increased preload to the left ventricle. This allows increased cardiac output by the Frank-Starling mechanism and does not require an increase in inherent contractility(181). In addition, high volume ductal shunting can only occur into a pulmonary circulation with relatively low resistance. If pulmonary resistance is low then afterload is low, and animal studies suggest that even the immature myocardium can increase left ventricular output considerably in the presence of both high preload and low afterload(197).

The myocardium of the immature infant may similarly be able to increase left ventricular output to maintain systemic perfusion in the first postnatal days. In our

cohort of infants, increased ductal size on colour Doppler was significantly associated with increased LVO on univariate analysis at 24 and 48 hours, and volume of ductal steal as assessed by presence of reversed diastolic DAo flow was significantly associated with increased LVO at 12, 24 and 48 hours.

Increasing duct size at 12, 24 and 48 hours postnatal age was also associated with decreased SVC flow volume on univariate analysis in our cohort of infants. An association between increased duct size and decreased perfusion in early extra-uterine life has been suggested previously(10). However an association between duct size and low flow states does not necessarily imply a causal relationship, since many confounders exist. While this previously published study suggested that low SVC flow is associated with large duct diameter when correcting for gestation and respiratory disease as confounders at 5 hours, there was no significant association at 12 and 24 hours(10).

At 12, 24 and 48 hours postnatal age in our study, increasing duct diameter was related to decreasing SVC flow volume when correcting for birth weight, intra-uterine growth restriction, antenatal steroid use and some markers of severity of respiratory disease. While the relationship was not significant at any time point when correcting for pCO_2, this lack of significance may be due to the low numbers of infants in whom pCO_2 levels were measured around the time of echocardiography.

Multiple regression analysis assesses strength of association between variables, and is not able to assess causation. Increased ductal diameter is associated with worsened respiratory disease(48, 280). However it is not clear whether ductal patency worsens respiratory status (by lowering lung compliance due to pulmonary congestion), whether respiratory disease itself may induce ductal patency, or whether a common factor (eg

immaturity) causes both worsened respiratory disease and increased ductal patency. In other words, in most infants, ductal size, and the increase in ductal shunting may be 'bystanders' to the respiratory illness, and not of great import with respect to cerebral blood flow.

If ductal patency worsens respiratory disease by shunting blood away from the systemic circulation then correcting for respiratory status when assessing the relation between ductal size and systemic perfusion could potentially mask a 'true' association. However if respiratory disease is itself causative of ductal patency, and is also causative of low systemic perfusion, or if a common separate factor such as immaturity causes both worsened respiratory disease and ductal patency, then correcting for respiratory status when assessing the relation between duct diameter and systemic perfusion is essential.

It is not possible to say which respiratory variables should be included in multiple regression analysis, so the technique cannot reliably demonstrate whether ductal diameter is independently associated with, and causative of, low systemic perfusion.

In addition there may be multiple other confounding factors, not corrected for, which could produce an artefactual correlation between SVC flow and ductal size.

To try to clarify this issue we looked at SVC flow volumes in infants with large ducts, in the presence and absence of reversal of diastolic DAo flow, which has been shown to be a strong marker of high volume ductal shunting(202).

In our cohort, infants with DAo reversal had increased LVO at 12, 24 and 48 hours, but did not have decreased SVC flow volume at any time. Given that increased ductal size has a strong relationship with increased LVO, and that increased LVO was seen in infants with large ducts in the presence of diastolic DAo reversal, it seems likely that

preterm infants in general can increase LVO to respond to systemic to pulmonary ductal shunting(281). Reversal of diastolic DAo flow in infants with large ducts was not associated with decreased volume of SVC flow. Degree of reversal of DAo flow, which has also been shown to be a marker of shunt volume(235), also showed no association with volume of SVC flow. These data suggest that, in general, preterm infants in the first 48 hours of life are able to increase LVO to compensate for ductal shunt and therefore maintain upper body perfusion(281).

Infants with large ducts and reversal of diastolic DAo flow at 12 hours showed a trend towards decreased absolute volume of descending aorta blood flow, and this association was significant at 24 and 48 hours. This decrease in DAo flow was seen despite significant increases in LVO. Degree of reversal of DAo flow was also associated with low DAo flow volume.

That increased LVO in the presence of significant ductal steal maintains SVC flow but does not maintain DAo flow could be explained by ductal steal occurring preferentially from the lower body.

The ductus arteriosus does not join the aorta at a right angle, but is oriented towards the descending limb, leaving an acute angle between the duct and the ascending aorta (282, 283). In the case of left to right ductal shunt, more flow may occur from the descending portion of the vessel because of the duct's orientation. The greater capacitance of the descending aorta may also induce preferential ductal shunting away from the lower body (197). The early arrival of the right ventricular pressure wave at the ductus may also increase upper body perfusion by transiently increasing the pressure in the descending aorta and augmenting distribution of left ventricular output to the lower resistance upper body vessels(278). The orientation, capacity and pressure profiles seen

in the duct and descending aorta provide plausible mechanisms by which ductal steal could occur preferentially from the lower body.

The anterior cerebral and renal arteries in preterm infants show similar patterns of systolic and diastolic flow velocities when the ductus is closed(198). However in the presence of significant ductal steal the renal arteries show increased pulsatility and are more likely to have reversed diastolic flow than the cerebral vessels(198, 281). Studies in the newborn lamb have also shown that abdominal organs are more affected by high volume ductal shunt than the brain(197).

It seems likely that systemic to pulmonary ductal steal occurs preferentially from the lower body. Our data suggest that, in most infants, the increase in LVO induced by the increased venous return to the left side of the heart is sufficient to maintain upper body perfusion, but is not sufficient to maintain lower body perfusion, potentially making infants more vulnerable to intestinal and renal hypoperfusion.

Nevertheless, given the increases in LVO seen in infants with large ducts, it is easy to understand how decreased myocardial contractility or hypovolaemia might result in impaired upper body perfusion in individual infants.

This study has a number of limitations. The accuracy of assessment of ductal diameter by colour Doppler is not known. While repeatability of measures of diameter taken from a single video recording has been shown to be reasonable (coefficient of variation 12%)(48), the duct is a dynamic structure and an isolated measure of diameter may not be representative of duct size over longer time periods. Precision of colour Doppler measures is limited by pixel size(118), and while the technique has proven accuracy in estimating intracardiac shunts of 4-12 mm(284) its precision may not be sufficient for assessing diameters of ducts measuring 1-3mm. The repeatability of quantification of

degree of reversal of DAo flow is also unknown. However assessment of presence or absence of reversal of DAo flow appears to be repeatable (chapter 4).

It is possible that we have overestimated the fall in DAo flow in the presence of a significant degree of flow reversal since we made no correction for the diastolic diameter of the vessel (when reversed flow occurs) being smaller than the systolic diameter. In addition, reversed flow appeared to be more turbulent than forward flow, such that the VTI for reversed flow may be overestimated.

The low number of blood gas readings available for analysis in this study is also a significant limitation. These limited numbers prevented us from drawing any firm conclusions as to whether or not the degree of respiratory disease and pCO_2 level are significant confounders on the association between ductal size and low SVC flow. However the study did not set out to address this issue, and consent was not obtained for additional blood gas testing at the time of echocardiography such that only gases required clinically could be analysed.

It may not be appropriate to simply consider duct diameter as 'large' or 'small' within a cohort of infants of such varied birth weight. This simplistic approach to duct diameter may be masking an association between ductal constriction and systemic perfusion. While the diameter of other blood vessels can be predicted from birth weight in the perinatal period, defining 'normality' of ductal size is not possible as size is considerably affected by degree of constriction. Duct size did not show a significant relation to birth weight in our cohort of infants (data not shown). It may be that infants of lower birth weights have less constricted ducts postnatally such that these relatively unconstricted ducts have the same absolute diameters as the relatively constricted ducts in larger infants. Such unconstricted ducts may have a relatively greater impact on

systemic flow in a small infant than a constricted duct of the same diameter in a larger infant. The inaccuracy in assessment of ductal constriction in our study could potentially be masking a stronger association between ductal status and flow volume.

Findings from this study can not be extrapolated to assessing the impact of ductal shunting after 48 hours of age. After this time ductal shunt has increased impact on blood pressure(44, 50) and patterns of cerebral perfusion(59, 62), and it may be that a greater proportion of preterm infants develop impaired systemic perfusion as a result of increased ductal steal with decreasing pulmonary pressure.

Despite these limitations this study suggests that in the majority of infants systemic to pulmonary shunting in the first 48 hours of postnatal life does not significantly impair upper body perfusion.

The newborn heart can increase stroke volume when presented with increased preload (section 1.5.1 of this thesis), and is likely to be particularly capable of raising ejection fraction in the presence of low afterload (section 1.5.2). Shunting of blood through the duct to the pulmonary circulation increases left ventricular preload. High volume shunting will only occur when there is a significant pressure drop between the systemic and pulmonary circulations, i.e. when the pulmonary circulation is providing a low afterload run-off. It may be that high volume ductal shunting will only occur in circumstances which automatically act to enable an increase in left ventricular output, so maintaining upper body perfusion.

The conclusion that upper body perfusion is maintained in the presence of ductal shunt is drawn from mean values seen across a large cohort of preterm infants, and it is clear that the transitional circulation varies greatly between individuals(10, 279). It may be that a sub-group of infants with high volume ductal steal and low systemic flow may

benefit from ductal closure in the first days of extra-uterine life. However our data do not support the need for therapeutic trials of ductal closure to support the cerebral circulation in the first 48 hours of life in preterm infants.

8.5 Conclusion

Even in early postnatal life ductal shunting is predominantly systemic to pulmonary, and significant volumes of shunt may occur.

However left ventricular output increases in the presence of ductal shunt. Increased ductal size may not be independently associated with decreased upper body perfusion in the first 48 hours of postnatal life when correcting for severity of respiratory disease. Increased volume of ductal shunt as assessed by reversal of descending aortic flow is not associated with decreased upper body perfusion in the first 48 hours. Increased ductal shunt volume may be associated with decreased lower body perfusion at 24 and 48 hours postnatal age.

In general, in preterm infants in the first 48 hours of life, as volume of ductal shunt increases, left ventricular output increases appropriately, maintaining upper body perfusion.

9 Relationship between Markers of Systemic Blood Flow and Clinical Outcome in Preterm Neonates

9.1 Introduction

As discussed in chapters 1 and 6 of this thesis, extremely preterm delivery is associated with a significant risk of long-term adverse consequences, and rates of neurologic and developmental morbidity do not appear to be improving significantly with time(17).

There is considerable evidence that circulatory factors play a role in the pathogenesis of cerebral injury in the preterm neonate(39), yet arterial blood pressure, which has an uncertain relationship with cerebral perfusion(180), is commonly the most frequently monitored indicator of neonatal circulatory status(80).

Low superior vena caval blood flow has been associated with subsequent periventricular haemorrhage(10) and adverse long-term neurodevelopmental outcome(167). The same research group that initially showed this association have since suggested that low SVC flow may be a stronger predictor of adverse outcome than either arterial blood pressure(167) or intracranial Doppler assessment of cerebral perfusion(234).

Other organ dysfunction may also occur secondary to poor systemic output. A link has been demonstrated between poor systemic perfusion when assessed

echocardiographically and poor renal function as assessed by urine output and rise in serum potassium(95). While an association between hypoperfusion and intestinal injury is less directly studied in human neonates, evidence suggests that circulatory factors play a significant role in the pathophysiology of both necrotising enterocolitis(285, 286) and isolated intestinal perforation(287).

Preceding chapters of this thesis have shown that it is possible to perform echocardiography without significantly disturbing cardiorespiratory status (chapter 3), that repeated echocardiographic measures are feasible (chapter 6), and that estimation of SVC flow volume has repeatability similar to other echocardiographic measurements in preterm infants (chapter 4). In addition our data suggest that SVC flow volume tends to be lower in sick compared to healthy preterm infants (chapter 6), and that low blood pressure is not predictive of low flow in the first 48 hours of postnatal life (chapter 7).

The specific aim of this section of the thesis is to examine the relationship between low systemic perfusion and adverse outcomes. Particular attention will be paid to whether the current practice of monitoring arterial blood pressure is sufficient to detect haemodynamic compromise in those infants who will develop an adverse outcome. In addition the potential role for monitoring flow volume by echocardiography in aiding detection of infants with circulatory failure who may be at risk of adverse outcome will be discussed.

9.2 Methods

Left and right ventricular outputs, SVC flow, and DAo flow were assessed by echocardiography as described in section 2.3. Flow volumes were averaged from the 5, 12 and 24 hour scans to give a mean flow volume for the first 24 hours of postnatal life

at each point in the circulation(167). In addition individual flow patterns were examined to detect shorter periods of abnormal flow.

Blood pressure was recorded as described in section 2.2. Mean arterial blood pressure values were averaged from readings taken at the 5, 12 and 24 hour scans to give an average mean blood pressure for the first 24 hours of postnatal life. Mean blood pressure was corrected for gestation by subtracting each infants' weeks of gestation from the average mean blood pressure, such that a 24 week gestation infant with a mean blood pressure of 25mmHg would have a corrected blood pressure of +1mmHg (288).

Urine output was recorded for the first 24 hours of postnatal life. The highest creatinine level in the first week of life was also recorded if measured. Full feeds were deemed to have been reached when infants tolerated >150ml/kg/day of milk for three consecutive days.

Cranial ultrasonography was performed as part of the study protocol in the first 12 hours of postnatal life wherever possible to exclude early intracranial abnormalities. Thereafter all infants <31 weeks admitted to the NICU routinely received cranial ultrasound scans at 5-7 and 28 days postnatal age, and at 36 weeks corrected gestational age. From each of these scans the presence and grade of PVH (244) was recorded.

Necrotising enterocolitis (NEC) and isolated intestinal perforations (IIP) were assessed by radiographic changes, requirement for surgical intervention, findings at laparotomy and results of histological examination.

Chronic lung disease (CLD) was defined as requirement for oxygen or any respiratory support at 36 weeks corrected gestation. Infants who failed to maintain oxygen saturations above 90% for more than 80% of the duration of an 8 hour saturation run

were maintained on supplementary oxygen. Requirement for supplementary home oxygen support was assessed according to the same criteria.

Mental development index and psychomotor development index were assessed for surviving infants at 9 and 18 months after birth. This neurodevelopmental follow up remains on-going, and results are not included in this thesis.

Comparisons between groups of infants were made by the Mann-Whitney U test. Multivariate comparison was performed using logistic regression. Comparisons for continuous variables were made using univariate analysis. Outcomes were also compared between infants who did and did not experience flow volumes below the 5th centile for healthy preterm infants as described in chapter 5. The incidence of adverse outcome in the presence or absence of low flow was made using Fisher's exact test. In all cases statistical significance was taken where $p<0.05$. The ability of measures of flow to discriminate between infants who would subsequently have good and poor outcomes was assessed by receiver operating characteristic (ROC) analysis(252).

9.3 Results

The 80 infants described in chapters 6 and 8, with median (range) birth weight 1060 (510-1900) grams and gestation 28 (24-30) weeks, were studied.

9.3.1 Survival

Six (8%) infants died prior to discharge home, at 10, 12, 16, 26, 100 and 110 days postnatal age. Causes of death were respiratory insufficiency in three infants, sepsis in one infant, necrotising enterocolitis in one infant and sudden infant death in one infant. The six infants who died tended to have lower gestations than surviving infants (median gestation 27 vs 28 weeks, $p=0.05$), but did not differ from the surviving infants in birth weight ($p=0.18$). There were no significant differences between infants who died and

surviving infants in mean SVC flow (p=0.21), mean DAo flow (p=0.60), mean LVO (p=0.69), mean RVO (p=0.76), mean blood pressure (p=0.17) or mean blood pressure relative to gestation (p=0.43) in the first 24 hours.

When only the four neonatal deaths were included in the analysis (death at <28 days) there were no significant differences between infants who died and surviving infants in gestation (p=0.16), birth weight (p=0.56), mean SVC flow (p=0.48), mean DAo flow (p=0.71), mean LVO (p=0.49), mean RVO (p=0.70), mean blood pressure (p=0.14) or mean blood pressure relative to gestation (p=0.27) in the first 24 hours.

9.3.2 Periventricular Haemorrhage

Thirteen (16%) infants had periventricular haemorrhage (PVH) (Table 9.1). Eight infants had abnormal findings at their first cranial ultrasound scan, five developed PVH during their neonatal course. Two infants (numbers 32 and 75, table 9.1) developed severe (grade 3 or 4) PVH in the first week of life after a normal scan in the first 12 hours. Five infants had a PVH (3 grade 1, 1 grade 2, 1 grade 3) present on the first cranial ultrasound scan at less than 12 hours postnatal age. None of these infants subsequently had an extension of the haemorrhage. Three infants (numbers 14, 30 and 76, table 9.1) were seen to have grade 1 or 2 PVH at day 5-7 of life, but did not have a prior cranial ultrasound to determine whether PVH occurred antenatally or postnatally. Three infants developed new PVH late in their postnatal course, with normal scans at day 7 of life.

The 13 infants who had a PVH tended to have lower gestations than infants with persistently normal head scans (median gestation 27 vs 28 weeks, p=0.09), but did not differ from these infants in birth weight (p=0.64). There were no significant differences

Infant No.	Initial Scan	Day 5-7 Scan	Late Scan	Classification	Max Grade	Any low SVC flow?	Any low DAo flow?
6	Not done	Normal	Abnormal	PVH after 7 days	Grade 1	Low flow 52ml/kg/min	Normal flow
14	*Not done*	*Abnormal*	*Abnormal*	*Uncertain timing of PVH*	*Grade 1*	*Normal flow*	*Normal flow*
30	*Not done*	*Abnormal*	*Abnormal*	*Uncertain timing of PVH*	*Grade 2*	*Normal flow*	*Low flow 85ml/kg/min*
32	**Normal**	**Abnormal**	**Abnormal**	**New PVH in week 1**	**Grade 4**	**Low flow 34ml/kg/min**	**Low flow 29ml/kg/min**
42	Normal	Normal	Abnormal	PVH after 7 days	Grade 1	Low flow 41ml/kg/min	Low flow 63ml/kg/min
49	Abnormal	Abnormal	Abnormal	PVH before 12 hours	Grade 1	Normal flow	Normal flow
54	Abnormal	Abnormal	Abnormal	PVH before 5 hours	Grade 2	Normal flow	Normal flow
56	Normal	Normal	Abnormal	PVH after 7 days	Grade 4	Normal flow	Normal flow
61	Abnormal	Abnormal	Not done	PVH before 12 hours	Grade 1	Normal flow	Low flow 55ml/kg/min
62	Abnormal	Abnormal	Not done	PVH before 12 hours	Grade 1	Normal flow	Normal flow
75	**Normal**	**Abnormal**	**Not done**	**New PVH in week 1**	**Grade 3**	**Low flow 19ml/kg/min**	**Low flow 53ml/kg/min**
76	*Not done*	*Abnormal*	*Abnormal*	*Uncertain timing of PVH*	*Grade 1*	*Normal flow*	*Low flow 69ml/kg/min*
80	Abnormal	Abnormal	Abnormal	PVH before 5 hours	Grade 3	Normal flow	Low flow 76ml/kg/min

Table 9.1 - Timing of periventricular haemorrhage and occurrence of low blood flow in the first 24 hours in the 13 preterm infants of the 80 studied. Infants with new severe PVH are shown in bold. Infants with uncertain timing of PVH are shown in italics

between the groups of infants who did and did not have PVH in mean SVC flow (p=0.19), mean DAo flow (p=0.56), mean LVO (p=0.98), or mean RVO (p=0.61) in the first 24 hours. However infants who had a PVH had significantly lower mean blood pressure in the first 24 hours than infants with normal head scans (32 vs 37 mmHg, p=0.005). Infants who had a PVH also had significantly lower mean blood pressure relative to their gestation than infants with normal head scans (p=0.005).

However the association between gestation corrected blood pressure and PVH was no longer significant when correcting for birth weight z score, antenatal steroid use and severity of respiratory disease (as assessed by mean airway pressure and fraction of inspired oxygen) using logistic regression.

When looking at individual flow patterns in infants with PVH, the two infants with new PVH in the first week of life (infant numbers 32 and 75) had markedly low SVC flow volume at 5 hours (34 and 19 ml/kg/minute respectively). These were the lowest volumes of SVC flow seen in the entire cohort at the 5 hour scan. Only three other infants had lower volumes of SVC flow at any time in the first 48 hours. Of these three, one infant developed necrotising enterocolitis and subsequently died, and another infant developed necrotising enterocolitis, required prolonged mechanical ventilation (38 days) and was discharged home in supplementary oxygen. The remaining infant (28 weeks gestation, birth weight 1270 grams) remained healthy (Table 9.3).

In addition three infants developed late PVH with a previously normal scan at 5-7 days. Infant number 56 had normal SVC flow perinatally but developed PVH on day 17 of life after a collapse secondary to sepsis. The other two infants with late PVH had low SVC flow (<55 ml/kg/min) in the first 24 hours of postnatal life. Thus four of five

infants developing PVH during their neonatal course had low SVC flow in the first 24 hours. This compares to 19 of 67 infants who never had PVH (p=0.03).

In the two infants (numbers 32 and 75) with new PVH in the first week of life, there were other abnormal haemodynamic markers of a low flow state in addition to low SVC flow volume. Both infants also had markedly low DAo flow volume at 5 hours - 56 and 59 ml/kg/minute respectively.

Infant number 32 (24 weeks gestation, birth weight 785 grams, SVC flow 34 ml/kg/min at 5 hours) developed a grade 4 bleed in the first postnatal week and had markedly low LVO (159ml/kg/minute) and RVO (100ml/kg/minute) at 5 hours postnatal age. Despite this pattern of global circulatory failure the infant's arterial blood pressure was normal at 5 hours postnatal age (mean blood pressure 25 mmHg) and the infant was not receiving inotropes at this time. Diameter of the ductus arteriosus was 1.8 mm, with bidirectional shunt present, and there was no reversal of diastolic descending aortic flow.

Infant number 75 (27 weeks gestation, birth weight 1075 grams, SVC flow 19 ml/kg/min at 5 hours) developed a grade 3 bleed in the first postnatal week and had normal LVO (344 ml/kg/minute) and RVO (338 ml/kg/minute) at 5 hours postnatal age. However there was a borderline low blood pressure (mean blood pressure 27 mmHg) and the infant was receiving inotropic support with dopamine and adrenaline at the time of the 5 hour scan. Diameter of the ductus arteriosus was 2.1 mm, with bidirectional shunt present, and there was no apparent reversal of diastolic descending aortic flow.

The three infants with PVH at day 5-7 of life, but no prior cranial ultrasound to rule out antenatal or immediately postnatal haemorrhage, had SVC flow volumes greater than 55 ml/kg/minute at all scans. Two infants had low DAo flow volumes (69 and 86

ml/kg/minute respectively) at 5 hours, but normal DAo flow at all other times. Ten infants who did not show PVH in the first week of life had similarly low volumes of DAo flow at 5 hours postnatal age.

9.3.3 Periventricular Leukomalacia

No infants had periventricular leukomalacia (PVL) detected on any of the late cranial ultrasound scans.

9.3.4 Necrotising Enterocolitis and Isolated Intestinal Perforation

Five (6%) infants developed necrotising enterocolitis (NEC) or isolated intestinal perforation (IIP) (Table 9.2). In three cases the diagnosis was confirmed at surgery. In one case disease progression was rapid and death occurred prior to planned surgery. In the fifth case a diagnosis of probable NEC was made due to clinical presentation and presence of air in the bowel wall on abdominal radiographs. Symptoms settled with 7 days medical treatment.

Infant number	Diagnosis	Postnatal age (days)	Outcome	Any low SVC flow?	Any low DAo flow?
34	NEC	7	Surgery	Normal flow	Normal flow
53	NEC	16	Medical treatment	Low flow 21ml/kg/min	Low flow 55ml/kg/min
63	NEC	25	Death	Low flow 52ml/kg/min	Normal flow
71	IIP	3	Surgery	Low flow 51ml/kg/min	Normal flow
73	IIP	4	Surgery	Normal flow	Low flow 65ml/kg/min

Table 9.2 - Timing of symptom onset and occurrence of low blood flow in the first 24 hours in the 5 preterm infants with abdominal pathology of the 80 studied. (NEC = necrotising enterocolitis, IIP = isolated intestinal perforation)

The five infants with NEC did not differ from infants without NEC in gestation (p=0.47), birth weight (p=0.36), mean SVC flow (p=0.21), mean DAo flow (p=0.79), mean LVO (p=0.93), mean RVO (0.13), mean blood pressure (p=0.26) or mean blood pressure relative to gestation (p=0.22) in the first 24 hours.

When looking at individual flow patterns in infants with NEC/IIP, four of the five infants had at least one episode of low flow in either the SVC (<55 ml/kg/min) or DAo (<90 ml/kg/min) in the first 24 hours of postnatal life, compared to 37 of 75 infants who never had NEC/IIP (p=0.36). Three of five (60%) infants with NEC/IIP had an episode of low SVC flow in the first 24 hours of postnatal life, compared to 20 of 75 (27%) infants who did not develop NEC/IIP (p=0.14). Two of five (40%) infants with NEC/IIP had an episode of low DAo flow in the first 24 hours of postnatal life, compared to 28 of 75 (37%) infants who did not develop NEC/IIP (p=0.99).

Infant number 53 (24 weeks gestation, birth weight 705 grams) had markedly low SVC flow (21 ml/kg/min) at 12 hours postnatal age. This was associated with low DAo flow (55 ml/kg/min) and relatively low LVO (185 ml/kg/min). RVO was normal at this time (282 ml/kg/min). At the time of the 12 hour scan the infant was receiving inotropic support with dopamine due to prior hypotension, and mean blood pressure (24 mmHg) was relatively normal at 12 hours.

Infant number 71 (27 weeks gestation, birth weight 910 grams) had borderline SVC flow at 24 hours (51 ml/kg/min), but markedly low flow at 48 hours (29 ml/kg/min). DAo flow, LVO, RVO and mean blood pressure were normal throughout the study, and no inotropic support was given.

Infant number 73 (29 weeks gestation, birth weight 700 grams) had a single episode of low DAo flow at 24 hours (65 ml/kg/min), which was associated with normal SVC

flow, LVO and RVO. Invasive blood pressure recordings were not available from the infant at the time of low DAo flow, but the infant was not hypotensive at any other time during admission, and no inotropic support was given.

Infant number 63 (27 weeks gestation, birth weight 1060 grams) had only mildly low SVC flow (52ml/kg/min) at 24 hours, and otherwise normal SVC and DAo flow volumes. LVO, RVO and mean blood pressure were normal throughout the study, and no inotropic support was given.

Infant number 34 (29 weeks gestation, birth weight 1500 grams) had normal flow patterns in the first 24 hours, and had only mildly low DAo flow at 48 hours postnatal age (87 ml/kg/min) which was associated with low LVO (155 ml/kg/min) but normal RVO. No blood pressure recording was available from the infant at the time of low DAo flow, but the infant was not hypotensive at any other time during admission, and no inotropic support was given prior to the development of NEC.

9.3.5 Chronic Lung Disease

Of the 74 infants who survived to discharge 14 (19%) developed chronic lung disease (CLD). Of these 14 infants, eight were discharged home in supplementary oxygen. Infants with CLD were significantly less mature (25 vs 28 weeks, $p<0.0001$) and lighter (713 vs 1163 grams, $p<0.0001$) than infants without CLD. There were no significant differences between infants with and without CLD in mean SVC flow ($p=0.99$), mean DAo flow ($p=0.51$), LVO ($p=0.91$), or RVO ($p=0.78$) in the first 24 hours. However infants who developed CLD had significantly lower mean blood pressure in the first 24 hours than infants with normal respiratory outcomes (31 vs 38 mmHg, $p=0.0002$). Infants who developed CLD also had significantly lower mean blood pressure relative to their gestation than infants without CLD ($p=0.012$).

The association between gestation corrected blood pressure and CLD was no longer significant when correcting for birth weight z score, antenatal steroid use and severity of respiratory disease (as assessed by mean airway pressure and fraction of inspired oxygen) using logistic regression.

There were no significant differences between infants with and without a requirement for home oxygen in mean SVC flow (p=0.55), mean DAo flow (p=0.39), LVO (p=0.47), or RVO (p=0.45) in the first 24 hours. However infants who required home oxygen had significantly lower mean blood pressure in the first 24 hours than infants with normal respiratory outcomes (29 vs 38 mmHg, p<0.0001). Infants who required home oxygen also had significantly lower mean blood pressure relative to their gestations than infants without home oxygen (p=0.026).

The association between gestation corrected blood pressure and requirement for home oxygen did remain significant (p=0.005) when correcting for birth weight z score, antenatal steroid use and severity of respiratory disease (as assessed by mean airway pressure and fraction of inspired oxygen) using logistic regression.

Four of the 14 (29%) infants who developed CLD had an episode of SVC flow <55 ml/kg/min in the first 24 hours of postnatal life, compared to 19 of 66 (29%) infants who did not develop CLD (p=1.0). Seven of the 14 (50%) infants who developed CLD had an episode of DAo flow <90 ml/kg/min in the first 24 hours of postnatal life, compared to 23 of 66 (35%) infants who did not develop CLD (p=0.36).

9.3.6 Feed tolerance

Median (range) time to full enteral feeds (defined as >150ml/kg/day of milk for three consecutive days) in the entire cohort was 9 (3-47) days. Larger birth weight was significantly but weakly associated with decreased time taken to reach full enteral feeds

(R^2=0.049, p=0.05). On univariate analysis time to reach full feeds was not significantly associated with gestation (p=0.12). When mean volumes of blood flow in the first 24 hours were compared to feed tolerance on univariate analysis there was no significant association between time to full feeds and mean SVC flow (p=0.78), DAo flow (p=0.62), LVO (p=0.12), or RVO (p=0.23).

Infants with higher mean blood pressure in the first 24 hours took significantly less time to reach full enteral feeds, though again this association was weak (R^2=0.06, p=0.04). However this association was no longer significant when blood pressure was corrected for gestation (p=0.13).

9.3.7 Renal function

Median (range) urine output in the entire cohort was 61 (30-141) ml/kg/day in the first 24 hours. On univariate analysis urine output in the first 24 hours was not significantly associated with either birth weight (p=0.52) or gestation (p=0.61). When mean volumes of blood flow in the first 24 hours were compared to urine output on univariate analysis there was no significant association between urine output in the first 24 hours and mean SVC flow (p=0.85), DAo flow (p=0.97), LVO (p=0.10), or RVO (p=0.73) on univariate regression analysis.

As the association between low LVO and low urine output was approaching statistical significance we compared infants in the lowest quartile for mean LVO in the first 24 hours with the remainder of the cohort. Infants with a mean LVO in the lowest quartile (mean LVO <251 ml/kg/min in the first 24 hours) had significantly lower urine output in the first 24 hours (45 vs 65 ml/kg/day, p=0.049) when compared to the remainder of the cohort.

There was no association between urine output in the first 24 hours and mean blood pressure (p=0.28) or mean blood pressure relative to gestation (p=0.18) in the first 24 hours. However infants with a gestation-corrected mean BP in the lowest quartile in the first 24 hours had significantly lower urine output in the first 24 hours (44 vs 67 ml/kg/day, p=0.016) when compared to the remainder of the cohort.

Median (range) highest creatinine level in the entire cohort was 0.08 (0.04-0.11) mmol/L in the first postnatal week. On univariate analysis highest creatinine level in the first week was not significantly associated with either birth weight (p=0.68) or gestation (p=0.10). When mean volumes of blood flow in the first 24 hours were compared to highest creatinine level in the first week on univariate analysis there was no significant association between creatinine level and mean SVC flow (p=0.27), DAo flow (p=0.18), LVO (p=0.26), or RVO (p=0.88). There was also no association between creatinine level and mean blood pressure (p=0.21) or mean blood pressure relative to gestation (p=0.39) in the first 24 hours.

Highest serum potassium level in the first 24 hours in the entire cohort had a median (range) value of 5.0 (3.5-7.7) mmol/L. Infants of larger birth weight had significantly *higher* potassium levels (R^2=0.24, p<0.0001). Infants of higher gestation also had significantly higher potassium levels (R^2=0.156, p=0.0008). While sampling site (arterial, venous or capillary) for blood taken for estimation of potassium levels was not recorded, our unit policy is that infants with arterial lines in situ would have all blood specimens sampled from these lines. When only infants with arterial lines were included in the analysis the association between high potassium level and larger birth weight remained significant (R^2=0.21, p=0.0034). However the association between higher potassium level and higher gestation was no longer significant (p=0.64).

When mean volumes of blood flow in the first 24 hours were compared to highest potassium level in the first 24 hours on univariate analysis there was no significant association between potassium level and mean SVC flow (p=0.71), DAo flow (p=0.09), LVO (p=0.95), or RVO (p=0.54). When only infants with arterial lines were included there were still no significant associations between potassium level and volume of flow. Infants with a mean DAo flow in the lowest quartile (mean DAo flow <102 ml/kg/min in the first 24 hours) did not have significantly higher potassium levels in the first 24 hours when compared to the remainder of the cohort.

Infants with higher mean blood pressure in the first 24 hours had significantly higher maximum potassium levels in the first 24 hours (R^2=0.16, p=0.0015). This association remained significant when blood pressure was corrected for gestation (R^2=0.11, p=0.011). However when only infants with arterial lines were included the association between higher potassium level and higher blood pressure was no longer significant (p=0.53), suggesting that the infants without arterial lines may have had higher mean blood pressure, and some haemolysis of their blood samples.

9.3.8 Combined outcomes

9.3.8.1 Death or PVH

Eighteen (22%) infants either died or developed PVH, and were thus designated as having a 'poor outcome'. These 18 infants with poor outcome had lower gestations than infants with good outcome (median gestation 27 vs 28 weeks, p=0.018), but did not differ from these infants in birth weight (p=0.19). There were no significant differences between infants with good and poor outcomes in mean SVC flow (p=0.22), mean DAo flow (p=0.74), mean LVO (p=0.64) or mean RVO (p=0.64) in the first 24 hours. However infants with poor outcome had significantly lower mean blood pressure in the

first 24 hours than infants with good outcome (33 vs 38 mmHg, p=0.005). Infants with poor outcome also had significantly lower mean blood pressure relative to their gestation than infants with good outcome (p=0.019).

The association between gestation corrected blood pressure and poor outcome was no longer significant when correcting for birth weight z score, antenatal steroid use and severity of respiratory disease (as assessed by mean airway pressure and fraction of inspired oxygen) using logistic regression.

Six of the 18 (33%) infants with a poor outcome had at least one episode of low SVC flow in the first 24 hours, compared to 17 of 62 (27%) infants with good outcome (p=0.77). Seven of the 18 (39%) infants with a poor outcome had at least one episode of low DAo flow in the first 24 hours, compared to 23 of 62 (37%) infants with a good outcome (p=1.0).

9.3.8.2 Death, PVH, NEC or CLD

If the definition of 'poor outcome' was broadened to include NEC/IIP or CLD a total of 32 (40%) infants were designated as having poor outcome. These 32 infants with poor outcome had lower gestations (median gestation 27 vs 29 weeks, p=0.0001), and lower birth weights (median birth weight 850 vs 1165 grams, p=0.002) than infants with good outcomes. There were no significant differences between infants with good and poor outcomes in mean SVC flow (p=0.67), mean DAo flow (p=0.73), mean LVO (p=0.95) or mean RVO (p=0.53) in the first 24 hours. However infants with poor outcome had significantly lower mean BP in the first 24 hours than infants with good outcome (33 vs 39 mmHg, p<0.001). Infants with poor outcome also had significantly lower mean BP relative to their gestation than infants with good outcome (p=0.0003).

The association between gestation corrected blood pressure and this broader poor outcome did remain significant (p=0.03) when correcting for birth weight z score, antenatal steroid use and severity of respiratory disease (as assessed by mean airway pressure and fraction of inspired oxygen) using logistic regression.

Nine of the 32 (28%) infants with a poor outcome had at least one episode of low SVC flow in the first 24 hours, compared to 14 of 48 (29%) infants with good outcome (p=1.0). Fourteen of the 32 (44%) infants with a poor outcome had at least one episode of low DAo flow in the first 24 hours, compared to 16 of 48 (33%) infants with a good outcome (p=0.36).

9.3.9 Predictive value of episodes of low systemic perfusion

9.3.9.1 Low SVC flow

The 5th centile for SVC flow volume in the first 48 hours of postnatal life in the 14 healthy infants described in chapter 5 of this thesis was approximately 55 ml/kg/minute.

Twenty-three infants (29%) had at least one episode of SVC flow <55 ml/kg/min at one of the three scans in the first 24 hours of postnatal life. Nine of the 23 (39%) infants with low SVC flow had a poor outcome (Death/PVH/NEC/CLD), compared to 23 of 57 (40%) infants with consistently normal SVC flow (p=1.0). The sensitivity for SVC flow <55 ml/kg/min in predicting this combined adverse outcome was only 28%, with a specificity of 71%.

Due to the poor sensitivity and specificity of SVC flow <55ml/kg/min in predicting adverse outcome we elected to repeat the analysis both with a lower minimum threshold

for SVC flow, and for a narrower range of adverse outcomes which we considered had a stronger vascular component to their pathophysiology.

SVC flow volume of <35 ml/kg/min (ie approximately 65% of the 5th centile range described in healthy preterm infants) was seen in only four (5%) infants in the first 24 hours of postnatal life. Of these four infants, three (75%) had a poor outcome (Death/PVH/NEC/CLD). Of the remaining 76 infants with SVC flow volume consistently >35 ml/kg/min, 29 (38%) had a poor outcome (p=0.30).

When poor outcome is restricted only to confirmed new PVH or NEC/IIP, five of the 23 infants with SVC flow <55 ml/kg/min had poor outcome, but 55 of the 57 infants with normal flow had normal outcome (p=0.02). Therefore the sensitivity for SVC flow <55 ml/kg/min in predicting this narrower combined outcome of new PVH/NEC/IIP was 71%, with a specificity of 75%. However the positive predictive value was only 22%. Of the four infants with SVC flow <35 ml/kg/min, three (75%) had a poor outcome (new PVH or NEC/IIP). Of the remaining 76 infants with SVC flow volume consistently >35 ml/kg/min, only four (5%) had a poor outcome (p=0.001).

Lowest SVC flow (ml/kg/min)	Infant number	Gestation (weeks)	Birthweight (grams)	Time of lowest flow	Inotropes at time of low flow?	Outcome
19	75	27	1075	5 hours	Inotropes	New PVH, Died
21	53	24	705	12 hours	Inotropes	NEC, CLD
29	71	27	910	48 hours	No inotropes	IIP, Died
30	69	28	1270	48 hours	No inotropes	Healthy
34	32	24	785	5 hours	No inotropes	New PVH, CLD
34	72	29	1080	12 hours	No inotropes	Healthy
35	29	26	880	24 hours	No inotropes	Healthy
37	22	25	640	12 hours	No inotropes	CLD
37	79	27	890	5 hours	No inotropes	Healthy
40	17	28	1435	12 hours	No inotropes	Healthy

Table 9.3 - Outcomes in ten premature infants with very low SVC flow (<41 ml/kg/min) in the first 48 hours (PVH - periventricular haemorrhage, CLD - chronic lung disease, NEC - necrotising enterocolitis, IIP - isolated intestinal perforation)

9.3.9.2 Low DAo flow

The 5th centile for DAo flow volume in the first 48 hours of postnatal life in the 14 healthy infants described in chapter 5 of this thesis was approximately 90 ml/kg/minute.

Thirty infants (38%) had at least one episode of DAo flow <90 ml/kg/min at one of the three scans in the first 24 hours of postnatal life. Fourteen of the 30 (47%) infants with low DAo flow had a poor outcome (Death/PVH/NEC/CLD), compared to 18 of 50 (36%) infants with consistently normal DAo flow (p=0.36). Therefore the sensitivity for DAo flow <90 ml/kg/min in predicting this combined adverse outcome was only 44%, with a specificity of 67%.

DAo flow volume of <60 ml/kg/min (ie approximately 65% of the 5th centile range described in healthy preterm infants) was seen in only eight (10%) infants in the first 24 hours of postnatal life. Of these eight infants, five (63%) had a poor outcome

(Death/PVH/NEC/CLD), compared to 27 of 72 (38%) infants with DAo flow volume consistently >60 ml/kg/min (p=0.26).

When poor outcome is restricted only to confirmed new PVH or NEC/IIP, four (13%) of the 30 infants with DAo flow <90ml/kg/min had poor outcome, but 47 (94%) of the 50 infants with flow consistently >90ml/kg/min had normal outcome (p=0.41). Therefore the sensitivity for DAo flow <90 ml/kg/min in predicting this narrower combined outcome of new PVH/NEC/IIP was 57%, with a specificity of 64%. The positive predictive value is only 13%. Of the eight infants with DAo flow <60 ml/kg/min, three (38%) had a poor outcome (new PVH or NEC/IIP) compared to 4 of 72 (6%) of infants with DAo flow volume consistently >60 ml/kg/min (p=0.02).

Lowest DAo flow (ml/kg/min)	Infant number	Gestation (weeks)	Birthweight (grams)	Time of lowest flow	Inotropes at time of low flow?	Outcome
29	32	24	785	24 hours	Inotropes	New PVH, CLD
41	59	30	1500	24 hours	No inotropes	Healthy
43	19	24	700	48 hours	Inotropes	CLD
46	8	30	1230	12 hours	No inotropes	Healthy
53	75	27	1075	12 hours	Inotropes	New PVH, Died
54	29	26	880	48 hours	No inotropes	Healthy
55	61	27	750	5 hours	No inotropes	PVH
55	53	24	705	12 hours	Inotropes	NEC, CLD
60	79	27	890	5 hours	No inotropes	Healthy
63	42	28	1250	12 hours	No inotropes	PVH

Table 9.4 - Outcomes in ten premature infants with very low DAo flow in the first 48 hours (PVH - periventricular haemorrhage, CLD - chronic lung disease, NEC - necrotising enterocolitis, IIP - isolated intestinal perforation)

9.3.9.3 Low LVO

The 5th centile for LVO in the first 48 hours of postnatal life in the 14 healthy infants described in chapter 5 of this thesis was approximately 185 ml/kg/minute.

Ten (13%) infants had at least one episode of LVO <185 ml/kg/min at one of the three scans in the first 24 hours of postnatal life. Four of the 10 (40%) infants with low LVO had a poor outcome (Death/PVH/NEC/CLD), compared to 28 of 70 (40%) infants with consistently normal LVO (p=1.0).

LVO of <120 ml/kg/min (ie approximately 65% of the 5th centile range described in healthy preterm infants) was not seen in any infant in the cohort.

When poor outcome is restricted only to confirmed new PVH or NEC/IIP, two (20%) of the 10 infants with LVO <185 ml/kg/min had poor outcome, compared to five of 70 (7%) infants with flow consistently >185 ml/kg/min (p=0.21). Therefore the sensitivity for LVO <185 ml/kg/min in predicting this narrower combined outcome of new PVH/NEC/IIP was 29%, with a specificity of 89%.

9.3.9.4 Low RVO

The 5th centile for RVO in the first 48 hours of postnatal life in the 14 healthy infants described in chapter 5 of this thesis was approximately 242 ml/kg/minute.

Twenty-four (30%) infants had at least one episode of RVO <242 ml/kg/min at one of the three scans in the first 24 hours of postnatal life.

Eleven of the 24 (46%) infants with low RVO had a poor outcome (Death/PVH/NEC/CLD), compared to 21 of 56 (38%) infants with consistently normal RVO (p=0.62).

RVO of <160 ml/kg/min (ie approximately 65% of the 5th centile range described in healthy preterm infants) was seen in only three (4%) infants in the cohort. Of these three infants, two (67%) had a poor outcome (Death/PVH/NEC/CLD). Of the

remaining 77 infants with RVO consistently >160 ml/kg/min, 30 (39%) had a poor outcome (p=0.56).

When poor outcome is restricted only to confirmed new PVH or NEC/IIP, three (13%) of the 24 infants with RVO <242 ml/kg/min had poor outcome. Four (7%) of the 56 infants with flow consistently >242 ml/kg/min had poor outcome (p=0.42). Therefore the sensitivity for RVO <242 ml/kg/min in predicting this narrower combined outcome of new PVH/NEC/IIP was 43%, with a specificity of 71%.

9.3.10 Summary of Results

Development of new PVH during the neonatal period was associated with episodes of low SVC flow in the first 24 hours of postnatal life. Four of five infants with new PVH had prior episodes of low SVC flow, and both infants with new PVH by day 7 showed strikingly low volumes of SVC flow at 5 hours. Importantly neither of these infants was consistently identified as having circulatory failure at the time of low flow. As a group, infants with PVH had significantly reduced blood pressure. However the fact that this association did not persist when correcting for other markers of illness severity with logistic regression analysis suggests that blood pressure may be a surrogate marker of disease severity itself.

The cohort of infants who developed necrotising enterocolitis or isolated intestinal perforation did not have significantly decreased systemic perfusion or mean blood pressure in the first 24 hours of postnatal life. However again strikingly low flow volumes were seen in some infants with NEC/IIP, and these infants were not consistently recognised as having circulatory failure at the time of low flow.

Episodes of low SVC flow in the first 24 hours of postnatal life were associated with the combined outcome of new PVH and NEC/IIP. Episodes of very low DAo flow were also associated with this combined outcome.

The groups of infants who developed chronic lung disease, were discharged home with supplementary oxygen, or who took longer to establish full enteral feeds did not have significantly decreased systemic perfusion in the first 24 hours of postnatal life overall. However these infants did have significantly lower arterial blood pressure in the first 24 hours of postnatal life. The association between lower gestation-corrected blood pressure and chronic lung disease did not persist when correcting for other markers of illness severity. The association between lower gestation-corrected blood pressure and discharge home in supplemental oxygen did persist under logistic regression.

Infant mortality was not demonstrably associated with decreased systemic perfusion or mean blood pressure in the first 24 hours of postnatal life.

There were no significant associations of decreased systemic perfusion or mean blood pressure in the first 24 hours of postnatal life with biochemical indices of renal impairment or with urine production.

The performance of the measures of flow volume in predicting outcome are summarised below.

The sensitivity, specificity, positive predictive value and negative predictive value of a single episode of each of low SVC or DAo flow, LVO or RVO for predicting the combined outcomes of Death/PVH/NEC/CLD is shown in table 9.5. The area under a receiver operating characteristic (ROC) curve for the lowest level of flow in the first 24 hours for each parameter as a predictor of adverse outcome is also shown.

The equivalent data for measures of flow predicting the narrower combined outcomes of new PVH or NEC/IIP is shown in table 9.6.

	SVC	DAo	LVO	RVO
Sensitivity	28%	44%	13%	34%
Specificity	71%	67%	88%	73%
Positive Predictive Value	39%	47%	40%	46%
Negative Predictive Value	60%	64%	60%	63%
Area Under ROC Curve	0.55	0.57	0.50	0.50

Table 9.5 - Sensitivity, specificity, positive predictive value and negative predictive value of a single episode of each of low SVC or DAo flow, LVO or RVO for predicting the combined outcomes of death/PVH/NEC/CLD in 80 preterm infants. (PVH=periventricular haemorrhage, NEC=necrotising enterocolitits, CLD=chronic lung disease, ROC=receiver operating characteristic)

	SVC	DAo	LVO	RVO
Sensitivity	71%	57%	29%	43%
Specificity	75%	64%	89%	71%
Positive Predictive Value	22%	13%	20%	13%
Negative Predictive Value	96%	92%	93%	93%
Area Under ROC Curve	0.77	0.70	0.57	0.53

Table 9.6 - Sensitivity, specificity, positive predictive value and negative predictive value of a single episode of each of low SVC or DAo flow, LVO or RVO for predicting the narrower combined outcomes of new PVH or NEC in 80 preterm infants. (PVH=periventricular haemorrhage, NEC=necrotising enterocolitis, ROC=receiver operating characteristic)

9.4 Discussion

Very low levels of blood flow were associated with increased risk of PVH in our cohort, yet we found no statistically significant association between mean levels of markers of systemic perfusion (SVC or DAo flow) or cardiac output (LVO or RVO) in the first 24 hours of postnatal life and clinical outcomes. This observation would suggest that there may be a threshold below which homeostasis is disturbed to a degree where there is a high risk of damage relating to circulatory failure(243).

The observation that low blood pressure in the early postnatal period is linked to later respiratory disease is interesting, especially since the association with prolonged oxygen dependency persists when correcting for other markers of disease severity. Perhaps this relates primarily to pulmonary hypertension, but it may also reflect

mechanical ventilation impeding venous return, while at the same time compounding respiratory damage.

At first inspection the findings that hypotension is associated with adverse outcomes, and that systemic perfusion below the range seen in healthy preterm infants is not associated with overall adverse outcome would appear to support current clinical practice of careful monitoring of arterial blood pressure. These findings could also be taken to argue against the need for monitoring of blood flow volumes in preterm infants as standard practice.

However such conclusions would ignore three significant factors. Firstly, that the associations between most adverse outcomes and hypotension in our cohort were no longer significant when correcting for other markers of illness severity using logistic regression. Hypotension may be a feature of delayed circulatory adaptation, and therefore may be associated with increased ductal patency and relative pulmonary hypertension. As discussed in chapter 8 of this thesis, such factors may be markers of disease severity rather than causative of adverse outcome. Multiple regression analysis is not able to distinguish between causation and association. Hypotension in the early postnatal period could even be a marker of antenatal insult; five of the infants with PVH in our study already had PVH at the time of the first cranial ultrasound at 5-12 hours of age.

Secondly that, despite current practice of monitoring and supporting arterial blood pressure, long-term developmental outcome is not improving following extremely preterm birth. A central concept of this thesis is the need to find new additional approaches to aid neuroprotection through the detection and improved circulatory support of “at risk” infants.

Thirdly, while arterial hypotension has been associated with a variety of adverse outcomes in previous studies there is little evidence that intervention to increase blood pressure leads to improved outcome(80). Since 'the aim of treating hypotension is to preserve adequate organ perfusion'(270), the relationship between pressure and flow is crucial. As discussed in chapter 7 of this thesis, higher arterial blood pressure is not significantly associated with improved tissue perfusion in preterm infants and, indeed, in the first 5-12 hours of postnatal life higher blood pressure may even be associated with lower levels of blood flow.

The results presented in this chapter suggest that volume of SVC or DAo flow below the range in healthy preterm infants described in chapter 5 is neither sensitive nor specific in predicting adverse outcome. However, as shown in tables 9.3 and 9.4, very low values of SVC or DAo flow do appear to be associated with subsequent adverse outcome. While such low flow values may only affect a small proportion of infants, the associated outcomes themselves are significant; including severe PVH and NEC, and the patterns of low flow seen may provide an opportunity to intervene to support a failing circulation.

A prime stimulus to carrying out our current study was the publication by Kluckow and Evans of a study suggesting a strong association between low SVC flow and subsequent PVH in preterm infants(10). These investigators prospectively followed with cranial ultrasound scans and echocardiography at 5, 12, 24 and 48 hours postnatal age a cohort of 126 infants born at <30 weeks gestation. They defined a normal range of SVC flow as the lowest value seen in a sub-cohort of uncomplicated preterm infants at each time point. In total 27 (21%) infants developed PVH. In 21 infants this PVH either occurred or extended after 5 hours postnatal age when the first cranial ultrasound scan was

performed. Eighteen of these 21 infants had an episode of low SVC flow prior to the development or extension of PVH. In contrast, only nine infants in their cohort had SVC flow below the normal range without subsequent PVH or PVL. The infants with low flow without apparent adverse effect were significantly more mature than those who developed PVH or PVL.

When examining the predictive value of episodes of low SVC flow these investigators found that any episode of flow below 30 ml/kg/day (the lowest level seen at any time in uncomplicated preterm infants) had an 80% sensitivity for late cranial ultrasound changes (new PVH, PVL, or extension of early PVH), with a specificity of 90%.

These sensitivity and specificity levels are clearly very different from those seen in our cohort. This difference could be due to a number of factors:

Firstly the outcomes examined in our and their cohorts are different. In Kluckow's cohort a combined 'late cerebral ultrasound changes' outcome included new PVH, PVL and extension of early PVH. In our cohort there were no cases of PVL or extension of pre-existing PVH. Only two definite cases of new PVH in the first week were seen, both of which had preceding episodes of low flow. While this would in theory give a sensitivity of 100% in our cohort, it is clearly not appropriate to express the results of only two cases as a percentage figure. Our data are limited by the three infants who were seen to have PVH at 5 to 7 postnatal days, but in whom no previous cranial scan had been performed. However since none of these three infants had any episode of SVC flow <55 ml/kg/min, whatever the timing of the haemorrhage, the flow patterns in these infants would not strengthen the association between SVC flow and PVH in our cohort.

We elected to broaden our outcome data to include death, any PVH, NEC/IIP and CLD. If these additional outcomes are less dependent on perfusion it would be expected that

sensitivity and specificity would fall. By narrowing our outcome criteria to only confirmed new PVH (by assuming that the three infants mentioned would have had PVH present at a 5 hour scan) and NEC/IIP it appeared that the sensitivity and specificity for both low SVC flow and low DAo flow improved, though again the numbers of affected infants were small.

Receiver operating characteristic (ROC) analysis provides a further assessment of the predictive value of a test(289). The area under the ROC curve is an indicator of how well a test performs; an area of 0.50 being be obtained by a test that performed no better than tossing a coin, and an area of 1.0 representing a perfect test(290). As shown in tables 9.5 and 9.6, when looking at the value of SVC flow in predicting only new PVH and NEC/IIP the area under the curve approached 0.80, which is generally appreciated as defining a test that performs well(252). Volume of DAo flow also showed better performance in predicting this narrower range of adverse outcomes. However LVO and RVO continued to perform poorly.

A second potential cause of discrepancy between our results and those presented by Kluckow et al is that the levels of flow considered normal and abnormal for the analyses of predictive value differed between the cohorts. Kluckow et al considered the lowest flow seen in a cohort of uncomplicated infants (30 ml/kg/min) as the cut-off for abnormal flow. We elected to use the 5^{th} centile to define normality. If we use the lowest flow seen in our healthy cohort (40 ml/kg/min) the predictive value of low SVC flow in detecting the narrower combined outcome of new PVH/NEC/IIP improves, sensitivity 57%, specificity 92%, PPV 40% (raw data not shown). However using an isolated 'lowest flow seen' to define the normal range potentially allows for the lower limit of normal to be artificially decreased by an out-lying low flow value in the healthy

infants. Whilst the outlying value may be a true value, it may be exaggerated by the potential for bias introduced by the limited repeatability of the scans (chapter 4).

There are a number of other potential explanations for the apparently poorer predictive value of low SVC flow in our cohort. Kluckow and Evans recruited some 85% of eligible infants to their cohort, compared to 48% in our cohort. While the gestations and birth weights of recruited and non-recruited infants did not differ significantly in our cohort (chapter 6) this low recruitment rate in our cohort raises the possibility that we have examined a biased sub-group of infants who potentially could have had different circulatory changes and risk factors for PVH than the cohort as a whole. In addition, even without our selective recruitment, it is possible that the pattern of PVH in our neonatal unit may differ from that seen in the two units in which Kluckow's cohort were based(291).

The lower rate of new postnatal PVH or extension of PVH in our cohort compared to that of Kluckow et al could be due to selective recruitment in our cohort, or could potentially be due to differences in management between the centres. Whatever the explanation, perhaps the combined finding of a lower proportion of infants with very low SVC flow in our cohort and a lower incidence of new PVH is significant.

While the predictive value of low SVC flow in our study was poor, and only limited numbers of infants with new PVH were studied, the finding that the two infants with new PVH in the first week had very low SVC flow at 5 hours would support Kluckow et al's hypothesis that very low SVC flow is associated with subsequent PVH. In addition, our study suggests that very low values of either SVC flow or DAo flow tend to be associated with a range of adverse outcomes (tables 9.3 and 9.4).

The observation that these episodes of low flow may not be associated with hypotension, may not be detected clinically, and may not prompt interventions to support the failing circulation is critical.

Four infants in our cohort had episodes of very low SVC flow (<35 ml/kg/min) and subsequent adverse outcome (table 9.3). Of these four, two infants were receiving inotropic support at the time of low flow. However infant numbers 32 and 71 had very low values of both SVC and DAo flow, but blood pressure values were within normal limits, and no inotropic support was being given. These infants developed new grade 4 PVH and isolated intestinal perforation requiring surgery respectively. One of the infants subsequently died.

Such cases of unrecognised circulatory failure with subsequent adverse outcome, along with the weak relation between blood pressure and blood flow demonstrated in chapter 7 of this thesis and reported widely in the literature(10, 83, 180) present a strong argument for the need for monitoring of blood flow as well as blood pressure in sick preterm infants.

Furthermore, in a number of instances in our cohort, circulatory failure (or at least hypotension) was recognised and inotropic support instituted by attending clinicians, but levels of flow remained very low. Infant numbers 75 and 53 had been noted to have prior hypotension and were both receiving infusions of inotropes at the time of their lowest levels of SVC flow. Despite this their SVC flow volumes were the two lowest seen in the entire cohort at any time, and the infants subsequently developed severe PVH and NEC respectively.

While some published reports suggest that inotropic intervention to maintain blood pressure is associated with improvements in flow volumes in preterm infants(179), such

findings are not universal(190, 199). Some reports have also suggested that dopamine (which has significant vasoconstrictor activity) may reduce blood flow volume despite increasing blood pressure(201).

It must be appreciated that there are no data to show that monitoring and intervening to support blood flow (as opposed to blood pressure) improves outcomes. However it appears that there are some infants in our cohort in whom inotropic support is being given to support blood pressure, but where blood flow volume is still critically low. That adverse outcomes follow in some of these infants provides further support for the need to monitor blood flow as well as blood pressure in sick preterm infants.

Some extremely preterm infants appear to maintain both blood pressure and blood flow in the early postnatal period. Conversely, some infants have hypotension associated with low flow states. However in some infants blood pressure is maintained but flow is low, and in yet others flow is maintained but blood pressure is low. The results presented in chapter 7 of this thesis clearly demonstrate the wide range of blood flow volumes that can be present at any given level of blood pressure. This variability in the haemodynamic adaptation to extremely preterm birth reinforces the notion that multiple techniques to monitor the course of circulatory adaptation should be utilised to help detect those infants most at risk of adverse outcome.

This study has a number of limitations, some of which are alluded to above. Most significant of these is that the small numbers of infants recruited limits the power of the study to detect clinically important differences between groups, or associations between flow, blood pressure and outcomes of interest.

No clear confirmation of the findings published by Kluckow and Evans can be made since only two infants suffered confirmed new PVH in the early postnatal period. A

higher recruitment rate of eligible infants would have increased the number of cases of new PVH, and carrying out an early cranial ultrasound scan in all cases would have removed any doubt over timing of PVH.

Echocardiography was performed at 5, 12, 24 and 48 hours postnatal age in our study to replicate the work of others(10) and because low systemic perfusion has been shown to be common at this time(68). However information from isolated scans obviously only represents 'snapshots' of what has happened in the early postnatal period, and is not necessarily representative of haemodynamic status in early postnatal life. Continuous measurement of flow volume would be better, were it possible.

The limited repeatability of individual measures of flow (chapter 4) also means that any individual flow measure must be interpreted with caution. However very low flow volumes are unlikely to have been due to measurement error alone. Averaging of flow volumes over the first 24 hours will have eliminated some measurement error, and averaged flow volumes were not associated with the crude adverse outcome indices available to us. This paucity of association between flow volume and outcome in cohorts of infants shows that multiple factors, rather than circulatory factors alone, are involved in dictating outcome in any one infant. Longer term neurodevelopmental follow up may reveal some other significant findings, particularly given the evidence of concurrent EEG abnormalities with low flow(292).

9.5 Conclusion

Whilst only small numbers of infants with new PVH were observed, very low levels of flow appeared to be associated with a range of adverse outcomes. Our finding that extremely low levels of SVC flow were seen in the two infants who subsequently

developed PVH in the first postnatal week would support the hypothesis that low SVC flow may be associated with subsequent PVH(10).

As a population screening tool, SVC or DAo flow volume below the range seen in healthy preterm infants described in chapter 5 is neither sensitive nor specific in predicting adverse outcome. Mean arterial blood pressure seems to have a more powerful relationship with outcome, particularly CLD. However in most cases the associations of blood pressure with adverse outcomes were no longer significant when correcting for other markers of illness severity using logistic regression, meaning that intervention for low blood pressure is unlikely to affect outcome.

In some infants low levels of systemic perfusion were not associated with low arterial blood pressure and no circulatory support was given. In some cases these episodes of low systemic perfusion were associated with adverse outcome. This finding supports the suggestion that monitoring measures of flow with echocardiography could add to the haemodynamic information supplied by continuous blood pressure monitoring in routine neonatal practice. However, further work is required to evaluate the clinical settings in which these investigations may be reliable and have good predictive value for identifying infants at risk of sequelae related to their haemodynamic state.

10 Conclusions and Future Directions

The central aim of this thesis was to increase understanding of the pathophysiology of the transitional circulation of the preterm neonate using echocardiography, and in particular assessment of SVC and DAo flow. Our hope is that this and future research will lead to improved cardiovascular monitoring and support in preterm infants with the goal of improving long term neurodevelopmental outcomes via improved systemic and cerebral perfusion.

This study has produced significant insights into the practice of echocardiography in newborn preterm infants. Trans-thoracic echocardiography, when performed with due care, does not significantly disturb cardiorespiratory status in preterm infants. It seems reasonable to conclude that the handling involved in the process of echocardiography is unlikely to increase the risk of adverse outcomes, nor to influence the reliability of echocardiographic measures of haemodynamic status. In addition, repeated measurements of flow are feasible in newborn preterm infants, with image acquisition satisfactory for calculation of flow in 97% of cases.

We found that the repeatability of assessment of volume of SVC flow by a single observer was similar to that reported previously, and similar to the repeatability of assessment of left and right ventricular outputs in neonates. A change in SVC flow volume in a preterm infant of more than 30%, when assessed by a single observer, is likely to represent a true alteration in systemic blood flow. The repeatability of assessment of volume of DAo flow by a single observer was poorer, though assessment

of velocity of flow was again similar to aortic and pulmonary velocities. Repeatability between two observers was poor for volumes of flow in both SVC and DAo. Measurements obtained by one observer could not reliably be compared to those made by another.

We have described reference ranges for SVC and DAo flow volume in healthy term and preterm infants. The disparity between our reference range for SVC volume and that previously described in preterm infants is most likely due to slight differences in imaging window. This highlights the importance of each operator optimising their imaging technique and establishing their own reference range. Further work in standardising imaging techniques seems essential before useful comparison can be made between different operators for research or clinical management.

In general, mean volume of blood flow did not differ significantly between sick and healthy preterm infants, demonstrating that despite the adverse circumstances of prematurity and lung immaturity, most infants can maintain an adequate cardiac output. However sicker infants tended to have a wider range of flow volumes. In addition a significant proportion of sick infants had systemic flow volumes below the reference range described in healthy infants.

This study has also provided insights into the transitional circulation. Most importantly, we found no positive association between arterial blood pressure and volume of blood flow at any of the four sites in the circulation studied. This lack of association is probably due to the dominance of systemic vascular resistance over volume of flow in determining arterial blood pressure in early extra-uterine life. Indeed our data suggest that this dominance may even produce an inverse association between blood pressure and SVC flow volume at 5 hours postnatal age, with infants with lower blood pressure

actually having higher volumes of systemic perfusion. Although this apparent inverse relation between blood pressure and blood flow could be due to a chance finding in our cohort, and requires confirmation, it is clear that low blood pressure is not predictive of low flow in the first 48 hours of postnatal life in preterm infants.

Findings from this study have also provided further evidence of the physiological importance of ductal shunting, challenging the conventional belief that volume of blood shunted across the ductus arteriosus is low in the early postnatal period. We found ductal shunting to be predominantly systemic to pulmonary, even at 5 hours postnatal age when the volume of shunted blood may be high enough to produce detectable reversal of diastolic blood flow in the descending aorta. However while increased ductal size was associated with decreased volume of SVC flow, reversal of DAo flow in infants with large ducts was not associated with decreased SVC flow; another finding that challenges conventional beliefs. It seems that reversal of DAo flow only occurs when a large ventricular output is achieved. Thus, while being a marker of high volume left to right ductal shunt, it may actually not be a sign of impending circulatory compromise. We found that in most preterm infants in the first 48 hours of life, including those with respiratory disease, left ventricular output increases appropriately as volume of ductal shunt increases, thereby maintaining upper body perfusion. However it is possible that if ductal steal occurs preferentially from the lower body circulation, renal and intestinal perfusion may be compromised more than the cerebral circulation.

Episodes of low systemic perfusion in the first 48 hours did not, in general, predict later adverse outcomes. On a population basis, flow below the reference range described in healthy preterm infants did not predict adverse outcomes, particularly chronic lung

disease. However flow measurements were more strongly predictive of an adverse outcome where the outcomes in question more obviously have a strong circulatory component to their pathophysiology, such as new PVH and NEC. While obviously limited by the small numbers of affected infants, our finding that the two lowest levels of SVC flow at 5 hours of age were seen in the two infants who subsequently developed new severe PVH would support the hypothesis that low SVC flow may be associated with subsequent PVH. The low recruitment rate in our cohort raises the possibility that we have studied a relatively well sub-group of infants, where low levels of systemic perfusion are well tolerated. It seems likely that in infants with an adverse perinatal course, low systemic perfusion will be more predictive of adverse outcome.

It is clear that echocardiography can be used clinically to add to information about the circulatory status of preterm infants currently gained by assessment of blood pressure, capillary refill time, lactate levels and urine output. Frequent echocardiographic examinations are feasible given the rapidity of measurement and the lack of disturbance to cardiorespiratory status with due care. By using multiple haemodynamic measurements at each scan a single clinician can gain a global picture of cardiac function and minimise the potential impact of measurement error from a single variable.

In the future, further research may help refine current clinical approaches to circulatory support by enhancing assessment of preload, contractility, afterload and volume of flow through the fetal shunt pathways. By examining each of these factors individually clinicians may gain an understanding of the relevant pathophysiology and thus be in a position to treat the underlying causes of circulatory failure in individual infants. Echocardiographic techniques may aid such an approach through assessment of left

atrial filling (as a marker of preload), volume of flow relative to blood pressure (as a measure of afterload) as well as volume of ductal shunt using currently available techniques. Assessment of cardiac contractility appears more challenging, but may be possible with the advent of cardiac MRI in preterm infants, or with more complex echocardiographic techniques.

A continuous measure of systemic perfusion would undoubtedly help the clinician with appropriate circulatory monitoring and support in the sick neonate. However even in adult intensive care this goal remains elusive. Given our findings of reasonable repeatability of assessment of DAo flow velocity, a useful next step would be to establish whether DAo diameter changes in a predictable way with changes in blood pressure. If so, monitoring of DAo flow volume via a transoesophageal probe would allow continuous estimation of systemic perfusion should sufficiently small devices become available.

An important immediate role of echocardiography in preterm infants is the use of measures of flow as short-term end-points in clinical trials of circulatory support. Such studies have already demonstrated the potential advantage of dobutamine over dopamine in improving systemic perfusion(190). A randomised trial of prophylactic milrinone as a means of reducing afterload to prevent low systemic perfusion in at risk preterm infants is already underway(168).

By increasing the use of multiple measurements of circulatory status in clinical practice we may be able to improve identification of at-risk infants. Clinical trials using measurements of flow as outcomes could enhance our ability to support the circulation when it is identified as failing. This may in turn provide opportunities for achieving enhanced neurodevelopmental outcome in preterm infants.

11 Appendices

APPENDIX 1 - PARENTAL INFORMATION SHEET

A Study of Blood Flow in Newborn Preterm Babies

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You are invited to enrol your baby in a study. We hope to examine how much blood babies can pump around their circulations to different parts of the body, and at different ages so that we can understand more fully how best to look after sick newborn babies.

Background to this study

When babies are born their hearts and circulations need to make important changes to the way in which they work to adapt to life outside the womb. After birth, the heart needs to pump much more blood to the lungs to collect oxygen, and also needs to pump blood around the rest of the body against higher pressures than were present before birth.

The hearts of sick newborn babies - especially those born prematurely - can find this difficult. We know that sometimes a baby's heart struggles to cope with the necessary work, and blood supply to these important areas may fall. This can make breathing more difficult, and may cause problems in a baby's health and long-term development.

At present we use ultrasound scans in preterm babies to help assess how well they are coping with the demands put on their circulations. As a newborn baby's circulation is quite complex, and blood flows through connections not seen in older children and adults it is sometimes difficult to be sure how much blood is actually reaching the tissues or the lungs.

Advances in technology mean we can use ultrasound scans to study blood flow in more detail than ever before. We want to study two relatively new measurements of blood flow – blood being pumped towards the internal organs in the abdomen (descending aorta) and blood returning to the heart from the brain (superior vena cava). We hope these two measurements will help us understand the changes that take place in babies circulations after they are born, what goes wrong when they fail, and will later help us study how best to help babies cope with the demands put on their circulations if they are unwell.

What we propose

We hope to look at around 100 babies born before 31 completed weeks gestation. If you agree to your baby taking part in this study, we would carry out ultrasound scans four times in the first two days of life, with a further scan at one week of age. One of the scans would be quite

detailed, to check that the heart has a normal structure, and will take around 20 minutes. Other scans will only be to measure blood flow, and will only take 5-10 minutes.
The ultrasound scans are very similar to those that you will have had or seen during pregnancy. The scans themselves are very safe, with no side effects. We will make every effort to do the scans as quickly possible, and at the time most convenient for your baby, as we want to let your baby rest as much as possible.

How this will affect your baby

Information gained in this study may not be of any direct benefit to you or your baby but it may help us decide how best to support babies in the future. We will not automatically tell the doctors caring for your baby what the scan showed, unless your doctor feels the information would help in making decisions about your baby's care.

Your decision to have your baby participate in this study is entirely voluntary (your choice). Your baby does not have to take part in the study and if you choose not to, this will not affect the care provided to your baby in any way. If you do agree to have your baby take part, you are free to withdraw him or her from the study at any time without giving a reason and this will in no way affect his or her future care. Participation in this study will be stopped if your doctor feels that it is not in your baby's best interest to continue.

Further information

If you want any further information about this study, you may ask the investigators whose contact details are at the top of Page One, or the nurse or doctor looking after your baby. If you need an interpreter, we'll be pleased to provide one.

If you have any queries or concerns about your rights as a participant in this research, you may contact the Health Advocate Trust (Phone 0800 555 050 Northland to Franklin)

You may also contact the Auckland District Health Board Maori Health Services, Mata Forbes RGON, Co-ordinator/Advisor, Auckland Hospital, Grafton. Mobile 021 348432, tel. 3074949 extn. 7292.

No material which could personally identify you or your baby will be used in any reports in this study. Any information about your baby will be kept strictly confidential. If you wish to be sent a summary of the results of this study, please indicate this by marking the appropriate box on the Consent Form. We do not expect the results of this study to be available before August 2004.

Compensation

In the unlikely event of a physical injury as a result of your baby's participation in this study, he or she will be covered by the Accident Compensation Legislation with its limitations. If you have any questions about ACC, please feel free to ask the researcher for more information before you agree to take part in this trial.

This study has received ethical approval from the Auckland Ethics Committee. The Manager and Clinical Director of Newborn Services at National Women's Hospital have given permission for this study to be carried out.

Please feel free to contact the researchers if you have any questions about this study.

APPENDIX 2 - DEMOGRAPHIC AND MONITORING DATA COLLECTION

Baby Name - Baby NHI -
Parents Names - Address -
Baby D.O.B - Time- Sex - M / F
Ethnicity -
Birth Weight - _____ grams (Centile) Gestation - _____weeks _____ days
Birth Length - Birth Head Circumference -
Delivery – SVD / Assisted / Elective CS / Urgent CS
Maternal NHI –
Maternal Illness – Hypertension / GPH / Diabetes / Other:
Maternal Medication Steroids – Y / N Date Completed –
Mag Sulph – Y / N Date Completed –
Other –
ROM - (date) (time) Maternal pyrexia? – Y / N
Prime stimulus for delivery - Highest RI on antenatal Doppler -
Normal anatomy confirmed at scan? Y / N

	4-6 hours	12 hours	24 hours	48 hours	7 days
HFOV / SIMV PSV/ SIPPV/ Cpap Low flow / Nil					
MAP					
FiO2					
Systolic BP					
Mean BP					
Diastolic BP					
BP method					
On inotropes? (Drug and dose mcg/kg/min)					
Volume given since last scan?					
Indomethacin? (date commenced)					
Urine output (ml/kg/day)	N / A	N / A			
Serum K+ (date + time)					
Base deficit					
Bicarbonate					
Glucose					
CO2					
Feed tolerated? (ml/kg/day)	N / A	N / A			

NEC? – None / Possible / Probable / Definite / Surgical Intervention Time to full feeds -
Highest creatinine week 1 - (Date -)
Cranial ultrasound early (date) Left side - (Subarach -)
Right side- (Subarach -)
Cranial ultrasound at 36 weeks (date) Left side- (Subarach -)
Right side- (Subarach -)
Days of ventilation- Days on CPAP- Duration of O2-
Date of discharge- Home oxygen – Y / N CLD - Y / N
Discharge weight - Discharge Length - Discharge Head Circ -
Indomethacin doses - Ligation - Y / N Ligation date -
Age at death- Cause of death -

APPENDIX 3 - SEQUENCE OF IMAGE ACQUISITION

Parasternal Long Axis
1 - Aortic root diameter
2 - LA:Ao ratio

Short axis
1 - Fractional shortening
2 - Descending aorta diameter
3 - Pulmonary root diameter
4 - Pulmonary VTI

Ductal view
1 - Ductal dimension
2 - Ductal flow CW
3 - Descending aorta VTI
4 - SVC diameter

Apical
1 - Aortic VTI

Subcostal
1 - SVC VTI
2 - Descending aorta VTI
3 - Atrial shunt diameter
4 - Atrial shunt flow on CW

APPENDIX 4 - ECHOCARDIOGRAPHIC DATA COLLECTION FORM

Scan 1 – 4 - 6 hours **Baby NHI -** **AG / CK** **Tape-**
Date____ Time start_____finish___ Time since last feed – **Variability study Y / N**

State of Arousal – Sleeping / Quiet awake / Restless / Crying
Aortic root (lead-lead) - Left atrium (lead-lead) -

Aortic root dimension (mm)-	/	/	/	/
Aortic Peak Velocity (m/s)-	/	/	/	/
Aortic VTI -	/	/	/	/

Heart rate during LVO (5 cycles)-

Pulmonary root dimension (mm)-	/	/	/	/
Pulmonary Peak Velocity (m/s)-	/	/	/	/
Pulmonary VTI -	/	/	/	/

Heart rate during RVO (5 cycles)-

Desc Aorta end-syst dimension (mm)-	/	/	/	/
Desc Aorta end-diast dimension (mm)-	/	/	/	/

DA Peak velocity (**Suprastern**) (m/s)-	/	/	/	/
DA Forward VTI (**Suprastern**)-	/	/	/	/
DA Reversed VTI (**Suprastern**)-	/	/	/	/

Heart rate during **Suprastern** (5 cycles)-
Angle correction used - ^{o} Diastolic flow - Forward / Absent / Reversed

DA Peak velocity (**Subcostal**) (m/s)-	/	/	/	/
DA Forward VTI (**Subcostal**)-	/	/	/	/
DA Reversed VTI (**Subcostal**)-	/	/	/	/

Heart rate during **Subcostal** (5 cycles)-
Angle correction used - ^{o} Diastolic flow - Forward / Absent / Reversed

SVC max dimension (mm)-	/	/	/	/
SVC min dimension (mm)-	/	/	/	/
SVC Forward VTI -	/	/	/	/
SVC Forward VTI -	/	/	/	/
SVC Reversed VTI -	/	/	/	/
SVC Reversed VTI -	/	/	/	/

Heart rate during SVC (5 cycles)-

Minimum **Ductal** diameter (mm)-
Maximum velocity (m/s)- Minimum velocity (m/s)–
Shunt Direction – L-R / Bidirectional / R – L If Bidirectional, proportion as L – R

Atrial shunt diameter (mm)-	/	/	/	/

Shunt Direction – L-R / Bidirectional / R – L / No flow
If Bidirectional, proportion as L – R

LV EDD (mm)-	/	/	/	/
LV ESD (mm)-	/	/	/	/
LV fractional shortening % -	/	/	/	/

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