# END ORGAN EFFECTS OF PAEDIATRIC CARDIOPULMONARY BYPASS

**Tony Vassalos**

M.B.Ch.B, MRCS (Glasg)

A Thesis Submitted to the University of Glasgow in Fulfilment of the Requirements for the Degree of Doctor of Medicine

Based on Work Conducted in the Department of Paediatric Cardiac Surgery
Royal Hospital for Sick Children
Glasgow, G3 8SJ

February 2011

**“Grasp the subject, the words will follow.”**

**Cato the Elder**

***Roman orator & politician (234 BC - 149 BC)***

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## ABSTRACT

Despite the scientific, technological and surgical improvements of the past 50 years organ dysfunction following elective paediatric cardiac surgery utilising cardiopulmonary bypass continues to account for increased complications, often leading to a protracted course in hospital with a longer stay in intensive care and the potential for irreversible organ damage long term. Furthermore, paediatric cardiac surgeons are routinely undertaking more complex operations with a shift from palliation to early correction. This has resulted in younger children being subjected to longer periods on the bypass machine with increased effects on vital organs.

This thesis describes two clinical studies designed to further assess and characterise peri-operative cardiac, renal and pulmonary function in children undergoing elective cardiac repair at a tertiary referral centre in Scotland, UK. In the first instance a prospective, observational study was undertaken in forty-five children to examine the use of tissue Doppler imaging in the assessment of peri-operative cardiac function, its relationship to myocardial injury and clinical outcome. Tissue Doppler parameters were obtained using a Vivid 7 ultrasound scanner with a 7-MHz probe pre-operatively, on admission to paediatric intensive care and on day one. Myocardial injury was assessed using Troponin-I on the first post-operative day by a commercially available chemiluminescent immunoassay.

In twenty children within this group peri-operative renal function was also investigated using standard estimates of glomerular filtration rate, namely creatinine clearance measured by the kinetic Jaffe method during the first and second twelve hour post-operative periods, in comparison to serum creatinine and the novel biomarker cystatin C. Routine plasma retained pre-operatively and on days 0, 1, 2 and 3 post-operatively was used to measure serum cystatin C and creatinine using a particle-enhanced nephelometric

immunoassay and the Roche Creatinine Plus enzymatic assay respectively. The association between cystatin C and recorded perfusion parameters including bypass duration, pump flow, haematocrit, oxygen delivery and Troponin-I was investigated.

Peri-operative pulmonary function was evaluated through a phase IV, randomised, double-blind, placebo controlled trial. In total, twenty four children were randomised to receive oral sildenafil or equivalent volume placebo four times the day before surgery. Blood samples were collected peri-operatively to measure serum cyclic guanosine monophosphate with a commercially available competitive enzyme immunoassay. Haemodynamic data and echocardiography were acquired at two and twenty four hours post-operatively including pulmonary vascular resistance index and bi-ventricular contractility. Post-operative oxygenation was also determined at the same time by oxygen delivery and oxygenation index.

In Chapter 2, peri-operative cardiac function as assessed by tissue Doppler imaging was examined. The results of this study demonstrated that pre-operatively, bi-ventricular systolic function in the study group was reduced compared with normal controls, displaying a significant step-wise decrease with increasing complexity of lesion. This picture persisted post-operatively predominantly in the right ventricle and was significantly associated with the extent of myocardial injury. Impaired peri-operative left ventricular function correlated with clinical outcomes.

In Chapter 3, peri-operative renal function as assessed by cystatin C and its association with parameters of perfusion was examined. The results of this study demonstrated that in comparison to serum creatinine, cystatin C had a superior correlation with glomerular filtration rate in the early post-operative period. An elevated level of this biomarker was

significantly associated with bypass duration, minimum pump flow and post-operative myocardial injury. Haematocrit was not directly linked to renal dysfunction in this study although evidence of a critical dysoxic threshold within the kidney was suggested indirectly through oxygen delivery calculations.

In Chapter 4, peri-operative pulmonary function and vascular reactivity in association with the pre-operative administration of oral sildenafil (0.5mg/kg, six hourly) was examined. The results of this trial demonstrated that compared to placebo, pre-operative sildenafil resulted in modest elevations of serum cyclic guanosine monophosphate, limited effects on pulmonary vascular resistance index, significant reductions in peri-operative bi-ventricular contractility, significant reductions in post-operative oxygen delivery and a trend for increasing ventilatory support.

In summary, the current thesis has demonstrated that in children undergoing corrective cardiac surgery peri-operative bi-ventricular function can be accurately assessed by tissue Doppler imaging which to date has had limited use in this patient group. With regards to renal function, cystatin C was shown to be a better estimate of glomerular filtration rate and a more sensitive marker of early renal dysfunction in children after surgery. Furthermore, cystatin C identified a transient post-operative renal impairment, the magnitude of which was associated with duration of bypass, pump flow and myocardial injury. In relation to pulmonary function, this research identified that pre-operative administration of oral sildenafil to children undergoing cardiac surgery produced limited effects on pulmonary vascular resistance but was associated with reduced ventricular contractility and post-operative oxygenation raising significant concerns over its routine clinical use.

## ACKNOWLEDGEMENTS

I would, without any hesitation, like to formally thank my supervisors:

Professor Fiona Lyall — Department of Medical Genetics, Royal Hospital for Sick Children, Yorkhill Division, Glasgow, UK

Mr Mark H Danton — Department of Paediatric Cardiac Surgery, Royal Hospital for Sick Children, Yorkhill Division, Glasgow, UK

For initially believing in me, giving me the opportunity to realise my ambitions, supporting me both in and out of the working environment, their time, effort, friendship and continued patience throughout this project. I hope we have the opportunity to continue our work together in the future.

I would also like to thank all the staff in the Department of Cardiology at Yorkhill Hospital whose constant support and friendship is the source of many happy memories. In particular, I would like to say a special thank you to Stuart Lilley, a truly brilliant echocardiographer whose reliable input was imperative to both studies, and Lorraine Leask for putting up with me for a year and a half – it would have been a very dull existence without her company in the office.

I would also like to thank Anne Theriault for her help with amongst other things all my laboratory orders, Dr Peter Galloway (Department of Biochemistry, Yorkhill Hospital) for his valuable advice, Staff at the Clinical Departments of Biochemistry (Victoria Infirmary,

Glasgow and Kent and Canterbury Hospital, Canterbury), Dr Alastair Gracie (University of Glasgow) for taking the time in the laboratory and Sarah Casey for her help and support during the sildenafil trial.

I am especially grateful to Professor Lawrence Weaver, Mr Jim Pollock, Mr Kenneth MacArthur, all the Cardiac Anaesthetists, Intensivists and theatre staff for their invaluable input throughout this project. Thank you also to Dr David Young for his statistical advice and friendship.

Finally, I would like to thank my colleague Dr Edward Peng, without whose help, this project would at times not have been possible.

## AUTHOR'S DECLARATION

The work presented in this thesis was performed entirely by the author except as acknowledged below. This thesis has not been previously submitted for a degree or diploma at this or any other institution.

Routine blood sampling was performed by the medical staff of the Paediatric Intensive Care Unit, Yorkhill Hospital, Glasgow.

Laboratory analysis of cardiac Troponin-I was performed by staff at the UK National External Quality Assessment Service, Clinical Department of Biochemistry, Victoria Infirmary, Glasgow.

Routine laboratory analysis of serum Creatinine and urinary Creatinine Clearance was performed by staff at the Department of Biochemistry, Yorkhill Hospital, Glasgow.

Laboratory analysis of cystatin C was performed by staff at the Department of Clinical Biochemistry, Kent and Canterbury Hospital, Canterbury, UK.

All echocardiography pertaining to this thesis was performed by the same experienced paediatric echocardiographer, Mr Stuart Lilley at the Department of Paediatric Cardiac Surgery, Yorkhill Hospital, Glasgow.

Statistical analysis was performed with the assistance of Dr David Young, Department of Mathematics and Statistics, University of Strathclyde, Glasgow.

Tony Vassalos, May 2010.

## PUBLICATIONS

The work presented in this thesis has resulted in the following publications:

Vassalos A, Peng E, Young D, Walker S, MacArthur K, Pollock J, Lyall F, Danton MH. Pre-operative sildenafil and pulmonary endothelial related complications following cardiopulmonary bypass: a randomised trial in children undergoing surgical repair. Accepted for publication, Anaesthesia, January 2011.

Vassalos A, Lilley S, Young D, Peng E, MacArthur K, Pollock J, Lyall F, Danton MH. Tissue Doppler imaging following paediatric cardiac surgery: early patterns of change and relationship to outcome. Interact Cardiovasc Thorac Surg 2009;9:173-177.

Vassalos A, Young D, MacArthur K, Pollock J, Lyall F, Danton MHD. Serum cystatin C: A sensitive marker of early renal injury after paediatric cardiac surgery. Heart Surgery Forum 2008, OP-936.

## PRESENTATIONS

The work presented in this thesis has resulted in the following presentations:

Vassalos A, Peng E, Young D, MacArthur K, Pollock J, Lyall F, Danton MHD. Does pre-operative Sildenafil protect against pulmonary related complications following cardiopulmonary bypass? A randomised trial in children undergoing cardiac surgical repair. Oral Presentation, 90th Annual Meeting of the American Association for Thoracic Surgery, Metro Toronto Convention Centre, Toronto, ON, Canada, May 2010.

Vassalos A, Peng E, Young D, MacArthur K, Pollock J, Lyall F, Danton MHD. Pulmonary hypertension and pre-operative Sildenafil: A randomised trial in children undergoing cardiac surgical repair. Oral presentation, 37th Annual Scientific Joint meeting of the Association of Paediatric Anaesthetists of Great Britain and Ireland with the Canadian Pediatric Anesthesia Society, Glasgow, UK, May 2010.

Vassalos A, Young D, MacArthur K, Pollock J, Lyall F, Danton MHD. Serum cystatin C: A sensitive marker of early renal injury after paediatric cardiac surgery. Oral presentation, 18th World Congress, World Society of Cardio-thoracic Surgeons, Kos Island, Greece, May 2008.

Vassalos A, Young D, MacArthur K, Pollock J, Lyall F, Danton MHD. Critical oxygen delivery during cardiopulmonary bypass and early (<24hr) renal function after paediatric cardiac surgery. Oral and Poster presentations, Society for Cardiothoracic Surgery in Great Britain and Ireland, Annual Meeting, Edinburgh, March 2008.

Vassalos A, Lilley S, Young D, Knight B, MacArthur K, Pollock J, Lyall F, Danton MHD. Patterns and potential value of tissue Doppler imaging after paediatric cardiac surgery. 5th European Association for Cardiothoracic Surgery (EACTS) / European Society of Thoracic Surgeons (ESTS) Joint Meeting, Stockholm, Sweden, September 2006.

## DEDICATION

Dedicated to my wife Rhonda, my son Connor, my mum Marilena and my dad Dracos for their unquestionable love and support.

## LIST OF ABBREVIATIONS

*(In order of appearance*)

- CPB : cardiopulmonary bypass
- IVOX : intravascular oxygenator
- IMO : intravascular membrane oxygenator
- CHD : congenital heart disease
- SIRS : systemic inflammatory response syndrome
- MODS : multiple organ dysfunction syndrome
- TDI : tissue Doppler imaging
- AVSD : atrioventricular defect
- VSD : ventricular septal defect
- ASD : atrial septal defect
- PICU : paediatric intensive care unit
- RV : right ventricle
- LV : left ventricle
- $Sa_{RV}$ : peak right ventricular systolic myocardial velocity
- $Sa_{LV}$ : peak left ventricular systolic myocardial velocity
- $Sa_{S}$ : peak septal myocardial velocity
- $IVV_{LV}$ : peak left ventricular velocity during isovolumic contraction
- $IVV_{RV}$ : peak right ventricular velocity during isovolumic contraction
- $IVV_{S}$ : peak septal myocardial velocity during isovolumic contraction
- $IVA_{LV}$ : left ventricular myocardial acceleration during isovolumic contraction
- $IVA_{RV}$ : right ventricular myocardial acceleration during isovolumic contraction
- $IVA_{S}$ : septal myocardial acceleration during isovolumic contraction
- PreOp : pre-operative
- PostOp : post-operative
- ITU : intensive therapy unit
- cTnI : cardiac Troponin-I
- IVV : peak myocardial isovolumic velocity
- IVA : myocardial acceleration during isovolumetric contraction
- ANOVA : analysis of variance
- IVC : isovolumetric contraction
- Emax : maximum elastance

- PRSW : preload-recruitable stroke work
- MRI : magnetic resonance imaging
- CT : computerised tomography
- $Q_P$ : pulmonary blood flow
- $Q_S$ : systemic blood flow
- GFR : glomerular filtration rate
- cysC : serum cystatin C
- Cr : serum creatinine
- CrCl : creatinine clearance
- $Q_{min}$ : lowest pump flow on bypass
- $Hct_{min}$ : lowest haematocrit on bypass
- $DO_{2min}$ : oxygen delivery at lowest haematocrit on bypass
- ARF : acute renal failure
- RRT : renal replacement therapy
- PENIA : particle-enhanced nephelometric immunoassay
- Q : pump flow on bypass
- ROC : receiver operating characteristics
- XC : cross-clamp time
- BP : cardiopulmonary bypass time
- cGMP : cyclic guanosine monophosphate
- SIL : sildenafil patient group
- PLA : placebo patient group
- $FiO_2$ : inspired oxygen concentration
- $DO_2$ : oxygen delivery
- PVRI : pulmonary vascular resistance index
- $WU.m^2$ : woods units per metre squared body surface area
- PHT : pulmonary hypertension
- PED : pulmonary endothelial dysfunction
- PVR : pulmonary vascular resistance
- NO : nitric oxide
- PDE5 : phosphodiesterase-5
- iNO : inhaled nitric oxide
- MHRA : medicines and healthcare products regulation agency
- MAP : mean arterial pressure
- LA : left atrium

- PA : pulmonary artery
- BSA : body surface area
- OI : oxygenation index
- PDE6 : phosphodiesterase-6

# CHAPTER 1: INTRODUCTION

*"A physician at the bedside of a child dying of an intracardiac malformation as recently as 1952 could only pray for a recovery! Today with the heart-lung machine, correction is routine. To bypass the heart, one needs a basic understanding of physiology of the circulation, a method of preventing the blood from clotting, a mechanism to pump blood, and finally, a method to ventilate the blood."*

*Lillehei (1993)*

## 1.1 The history of cardiopulmonary bypass

Cardiopulmonary bypass (CPB) is the process by which a patient is sustained by diverting the circulation through synthetic devices designed to replace the heart and lungs. Over the past 50 years it has evolved from an extremely hazardous procedure into a safe, systematic process that is practiced daily in thousands of centres worldwide. The developmental advances in CPB can be broadly divided chronologically into: (1) a conceptual period, pre-1950 (2) a technological period, 1950-1970 and (3) a refinement period, 1970 to present.

### 1.1.1 Conceptual period (Pre- 1950)

The goal of CPB has always been to deliver nutritive solutions to the body, namely oxygen, with sufficient drive as to maintain physiological homeostasis. As early as 1812 Julien-Jean Cesar le Gallois demonstrated that blood flow through a circuit located outside the body (extracorporeal circulation) was feasible and put forward the original idea for resuscitating decapitated heads through the use of blood transfusions. [1] Brown-Sequard in 1858 arterialised desaturated blood by whipping it and then perfused the head of a dog with moderate success. [2] In 1869 Ludwig and Schmidt produced the first report on artificial oxygenation when they enriched blood with oxygen by shaking it with gas in a balloon. [3] They additionally highlighted the importance of anti-coagulation in enabling

flow during extracorporeal circulation. The earliest uses of 'bubble' and 'film' oxygenator devices that combined blood with oxygen were respectively reported in 1882 by von Schroeder and by von Frey and Gruber in 1885. [4, 5] In 1915, Hooker reported a type of film oxygenator that utilised a rotating disc assembly within an inverted bell jar. Hooker's device became the forerunner for the disc oxygenator later developed to be used in many of the early perfusions in humans in the 1950s. [6]

Other key medical developments occurring alongside advances in blood-gas exchange systems that strongly influenced progress towards extracorporeal flow came in 1916 when Jay McLean, a medical student working in the laboratory of W. H. Howell at the Johns Hopkins University at Baltimore, discovered 'heparin' and its anticoagulating properties. [7] Some 20yrs later Chargaff and Olson discovered 'protamine' and its antagonistic actions to heparin while exploring a means of extending heparin's anticoagulating effects. [8]

In an attempt to mimic the normal circulation, Dale and Schuster in 1928 created a valved pumping device that delivered a pulsatile wave. This prototype became the predominant device used for extracorporeal perfusions. [9] In 1934, DeBakey modified the twin roller pump previously described for blood transfusions to create a roller pump that worked on the principle of occluding collapsible tubing along a rigid metal plate. This pump became the standard by which all positive displacement pumps used today for extracorporeal circulation have been modelled. [10]

The culmination of these developments came in 1937 when Dr John H Gibbon, Jr. reported the first successful total CPB of an animal (cat) with the use of heparin, a Dale-Schuster-type pump and vertical rotating cylinder oxygenator. [11]

### 1.1.2 Technological period (1950 – 1970)

Despite Gibbon's success the ability of blood-gas exchange devices to replace the entire human circulation was not achieved for some 15 years. On April 5 1951, Dennis and colleagues performed the first total CPB in a 6-year-old patient with an atrial septal defect.[12] Although their heart-lung machine functioned well, the patient could not be separated from CPB and died on the table. After World War II Gibbon and his wife Mary, whom many considered to be the first cardiac perfusionist, continued to refine their work on CPB and on May 6 1953 at the Thomas Jefferson Hospital in Philadelphia successfully closed an atrial septal defect in an 18 year old college girl. The time on the heart-lung machine was 26 minutes. [13] Unfortunately, Gibbon was unable to repeat this success and with the death of his next 4 patients gave up his pursuit of open heart surgery with the heart-lung machine. Other surgeons however utilised techniques that Gibbon had developed to successfully treat children with congenital heart disease. It is for this reason that Gibbon is considered by most as the 'father of cardiopulmonary bypass'. [13, 14]

Kirklin et al. at the Mayo Clinic in Rochester Minnesota further developed Gibbon's oxygenator into the Mayo-Gibbon pump-oxygenator apparatus which became commercially available and led to a pioneering series of successful human intra-cardiac operations in March 1955. [15, 16] Around the same time, Kay and Cross and their colleagues in 1956 modified the previously described Bjork oxygenator for clinical use. [17, 18] Despite satisfactory results from both devices worldwide, by the mid to late 1950s numerous problems associated with the film-type blood-gas exchange systems were being reported. Their complex, bulky, non-disposable nature, the large quantity of blood and saline required to prime the machine and their inefficient gas exchange led to their eventual removal from clinical practice. [19, 20]

In 1950, Clark and colleagues expanded on von Schroeder's concept and demonstrated that oxygen bubbled through a sintered-glass placed directly into a column of venous blood, created foam whereby gas exchange occurred. [21] In 1956 Dewall and Lillehei devised a bubble oxygenator that was simple and made from readily available hospital equipment. [22] In the same year Rygg and Kyvsgaard devised an oxygenator that combined the same three basic components (bubbling chamber, settling chamber and arterial reservoir) into a single disposable polyethylene bag. [23] Bubble-type blood-gas exchange systems were inherently simpler, highly efficient, disposable, required smaller (potentially blood free) priming volumes and were easier to assemble. [19] As a result, within a short time of being introduced bubble oxygenators were commercially available and became a major driving force behind the expansion of CPB and open-heart surgery. Despite their success, concerns emerged regarding the increasing rates of blood haemolysis, coagulation disorders, activation of white blood cells, acidosis and progressive organ failure seen when CPB lasted more than four hours. [19] This was thought to be due to the direct contact of blood with air surfaces and the plastic and metal components of the pump oxygenator circuit. [19, 24] These disadvantages were initially accepted and counteracted by the relatively short duration of open-heart surgery and through the use of profound hypothermia. By cooling the body, with the aid of a heat-exchanger, to a core temperature of 10-12$^{o}$C CPB could be turned off for up to one hour while prolonged intra-cardiac surgery was performed. [25, 26]

Meanwhile, attention turned to the idea of a protective membrane existing between the blood and air to reduce trauma inherent with direct-contact oxygenators (bubble and film). Kolff and Berk in 1944, whilst developing an artificial kidney, observed that blood passing through the cellophane membrane of their haemodialysis machine became oxygenated by the aerated dialysates through the process of passive diffusion. [27] Although their device

was incapable of exchanging gas other than at very low flows, Kloff and Berks' finding sparked the search for a suitable inert material that could best mimic the endogenous alveolar capillary membrane. [27] In 1957, Clowes and colleagues were the first to report the clinical use of a 'membrane' oxygenator. The surface area of the teflon membrane they used however was excessive ($25m^2$) and led to blood protein denaturation and coagulopathies. [28] In 1963, Kolobow and Bowman designed a silicone membrane oxygenator whose biocompatibility was especially attractive for long-term CPB. The first practical membrane oxygenators appeared in the mid to late 1960s as the Bramson lung and Lande-Edwards oxygenators. [29, 30] Despite their theoretical advantage membrane oxygenators were not widely utilised during the 1960s and 70s mainly due to their higher production costs and the lack of clinical evidence that proved their superiority to direct-contact oxygenators. They remained less efficient, cumbersome and more prone to plasma leakages and thrombotic occlusions than their direct-contact counterparts. [19]

### 1.1.3 Refinement period (1970 – present)

#### 1.1.3.1 Oxygenators

Research in the design of membrane oxygenators continued and refinements to the geometrical arrangement of the gas exchange compartment occurred. The importance of reducing blood film thickness and disrupting laminar flow characteristics within the oxygenator to optimise gas exchange became apparent. [19] Various design modifications including screens, spacers and sheets were utilised to promote 'secondary flow' which improved gas exchange and resulted in a 10-fold reduction in the membrane surface area required for clinical perfusion. [19, 31] With the use of secondary flows in membrane oxygenators, the membrane itself, once again became the limiting factor to gas exchange. Modern membrane gas exchangers are almost exclusively constructed of microporous polypropylene arranged as a bundle of hollow fibre capillaries. [32-34] Bodell and colleagues

first put the conceptual use of tubular capillary fibres for gas exchange forward in 1963. [35] The concomitant use of secondary flow and hollow-fibre technologies resulted in the highly efficient gas exchange devices used clinically during the early 1980s. [19, 32-34] By the late 1980s a variant of the above design termed inverse-flow or 'blood outside' design where blood flowed in the external chamber originally designed for gas, while gas flowed within the hollow fibres predominated. This new configuration resulted in a much larger gas exchange area, lower shear forces of blood and an overall improvement in gas exchange performance such that membrane areas were 2 to 2.5 times smaller than the conventional design. [32-34] These combined advantages of high gas exchange efficiency, low blood trauma, compactness, simplicity and low cost meant that microporous membrane devices began to replace efficient bubble oxygenators in the cardiac operating theatres in the 1980s. [32-34] Today such devices have surface areas of 2 to 3$m^2$ and are used exclusively in 95% of centres; transferring up to 400ml of oxygen while removing more than 300mls of carbon dioxide in a single pass. [32-34, 36]

The success of hollow fibre oxygenators has led to the innovative concept of 'intracorporeal oxygenation' termed the intravascular oxygenator or IVOX. [37] In IVOX a bundle of silicone coated hollow fibres is inserted into the inferior vena cava of a patient and oxygen is pumped through. [37, 38] Secondary flow occurs as blood migrates through the fibres on its way back to the heart. Further enhancements where a balloon pump is incorporated within these fibres to optimise secondary flow has been suggested and termed 'intravascular membrane oxygenator' or IMO. [37-38] At present IVOX / IMO devices can only offer partial respiratory support and have failed to gain widespread acceptance due to a lack of clinical benefit. [37-39]

### 1.1.3.2 Reservoirs

Venous blood reservoirs form an essential component of the paediatric bypass circuit in providing a safety chamber to sequester excess blood during CPB. Two types of reservoirs have traditionally been used relating to open (hard shell) and closed (collapsible bag) systems. [32, 34, 36, 40] Open systems which vent air to the atmosphere usually incorporate a cardiotomy reservoir, defoaming compartment and are made of rigid plastic. Although offering a low inlet resistance and passive removal of venous air the direct blood-air interface results in a greater inflammatory response as well as the possibility of air being pumped into the patient if the reservoir becomes completely empty. [41] The soft-shell, collapsible, closed system on the other hand requires a separate cardiotomy reservoir and active removal of any air entrained into the venous system during CPB. [36, 40] However, despite the inherent safety of the closed system the ease of use of the open system together with the option of vacuum assisted venous drainage has prevailed in 88% of centres in North America. [32]

### 1.1.3.3 Pumps

Since the advent of CPB by Gibbon in the 1950s simplistic, low cost, positive displacement roller pumps have predominantly performed the role of the artificial heart. In the 1970s however a team of engineers collaborated in the design of a new blood pump that relied on centrifugal forces. [32, 42] In the absence of any occlusive mechanism centrifugal pumps offered a theoretical reduction in the blood trauma normally seen with roller pumps. Despite this theoretical advantage centrifugal pumps have not yet gained favour in the majority of paediatric cardiac centres in Europe and Asia and usage is somewhat confined to the United States where they are used exclusively in 2-14% of centres. [32, 33]

#### 1.1.3.4 CPB circuit modification

The process of CPB necessitates the exposure of blood to the artificial surfaces of the tubing, oxygenator, reservoirs and filters in addition to other non-physiological conditions which are all implicated in blood activation ultimately expressed as a systemic inflammatory response. [32, 36, 40, 43] In relation to body surface area, compared to adults, the surface area of the CPB circuit used in infants is substantially higher and is directly related to the magnitude of inflammatory response. [44] As a result numerous attempts have been made to improve the biocompatibility of the circuit through the application of biologically active or inert substances onto the surfaces and reduction in circuit size. Heparin-bonded CPB circuits have been available for clinical use since the late 1980s and extensive studies have demonstrated consistent reductions in plasma levels of complement fragment C3a, TNF-α, IL-6, IL-8, E- and P-selectins leading to improved clinical outcomes. [45-47] Despite this it has taken some 20 years for the use of modified circuits to become routine and they currently represent 74% of circuits used in paediatric centres across North America. [32]

## 1.2 The management of perfusion during cardiopulmonary bypass

Despite the technological advances of the past 50 years there is still no generally accepted definition of optimal perfusion during CPB. This is even more difficult when one considers variations in the period of survival monitored and the extent to which organ dysfunction is investigated. As a result there is considerable variation in how CPB is conducted between centres to optimise the fundamental balance between oxygen supply and demand, limit activation of inflammatory cascades, maintain vital organ function and ensure a safe, rapid and long-lasting recovery. [32-34, 36, 40] Furthermore, important anatomical and physiological differences exist between infants and adults which have to be considered in the setting of CPB of the paediatric patient.

### 1.2.1 Paediatric anatomical and physiological considerations

In contrast to adults, infants have a high body surface area to weight ratio which when combined with their immature thermal auto-regulation makes them particularly vulnerable to rapid fluctuations in body temperature. [36] Temperature regulation and the speed with which it occurs is an important consideration of perfusion and has been linked to post-operative organ dysfunction. [48-52]

Compared to adults the immature heart has a smaller percentage of contractile proteins, a lower oxidative capacity, decreased sympathetic innervation, altered calcium handling and decreased compliance. [36, 53] As a result children have a relatively fixed stroke volume making their heart rate and rhythm the main determinants of cardiac output. [53] Additionally, the myocardium of the newborn has an increased glucose dependence when compared with the adult with episodes of hypoglycaemia potentiating heart failure. [54] Native differences are further complicated in the context of congenital heart disease (CHD) where a broad spectrum of cyanotic and acyanotic lesions exists, each with specific pathophysiological changes that can result in hypoxia, heart failure, altered pulmonary blood flow and low cardiac output. [33, 36]

Children with CHD in contrast to adults often have pre-existing abnormalities in respiratory mechanics prior to surgery. Lesion specific alterations to pulmonary blood flow, pulmonary hypertension and volume / pressure loading of the heart are associated with decreased lung compliance, atelectasis, pulmonary oedema, increased airway resistance and pulmonary endothelial dysfunction related increases in pulmonary vascular resistance manifest as episodes of pulmonary hypertension post-operatively. [55-57] Exposure to CPB exacerbates pulmonary endothelial dysfunction and is associated post-operatively

with an acute lung injury, the severity of which is directly related to the duration on bypass. [55-58]

Renal function in neonates and infants is associated with differences in electrolyte transport, decreased medullary concentrating ability, impaired tubular function, impaired excretion of free water and a lower glomerular filtration rate than adults even when corrected for body surface area. [59-61] Despite these differences, however, most children undergo CPB without developing post-operative renal failure. That being said children exposed to a period of CPB have a much greater fluid retention post-operatively compared to adults which if not adequately diuresed can result in prolonged ventilatory support. [36] The investigation of renal function in children is complicated by the superimposition of renal dysfunction on a background on continuing renal development. Moreover, the changing muscle mass of the infant influences circulating creatinine levels with resultant inaccuracies in the measurement of creatinine clearance. [62, 63]

Many factors are known to affect cerebral blood flow during CPB including temperature, cerebral metabolic rate, arterial carbon dioxide partial pressure, pump flow and perfusion pressure. [64] Retrospective studies have identified decreasing age as an incremental risk factor in the development of post-operative neuropsychological dysfunction. [51, 65] In particular it has been shown that during periods of deep hypothermia, sometimes used during repair of CHD, cerebral autoregulation and cerebral flow / metabolism coupling are lost with cerebral blood flow becoming dependent on perfusion pressure. [36, 51, 64]

The coagulation system in newborns is deficient with reduced levels of factors XII, XI, vitamin K-dependent factors and coagulation inhibitors (antithrombin III, protein C and protein S) commonly seen. [66] This is even more so in children with cyanotic congenital

heart disease who are known to have platelet dysfunction in addition to decreased levels of factors II, V, VII, VIII, and IX. [67]

### 1.2.2 Physiological cardiopulmonary bypass variables

Modalities used during CPB in adults to a moderate degree are often used in children to a much greater extent resulting in an increased physiological insult which is often remarkably well tolerated. Marked temperature changes (deep hypothermia with or without circulatory arrest), profound haemodilution (2-3 times blood volume), large foreign surface area exposure, low perfusion pressures, wide variations in pump flow rates, wide-ranging pH management and altered glucose regulation are commonplace during paediatric perfusion. [36, 40]

#### 1.2.2.1 Perfusion pressure and flow

The fundamental aim of CPB is to maintain tissue perfusion of all vital organs whilst cardiac and pulmonary function is suspended. The optimal mean arterial pressure during CPB that would guarantee satisfactory tissue perfusion is not currently known. As a consequence the lower limit used varies widely in the adult population from 50 to 80mmHg and is essentially based on data relating to cerebral autoregulation. [68] In children lower perfusion pressures are normally seen ranging from 20 to 50mmHg in association with a greater degree of hypothermia. [36] It is generally accepted that at normothermia in children a pump flow rate of 100ml/kg/min or 2.2-2.5L/min/$m^2$ together with a mixed venous saturation >75% and arterial oxygen saturation >30mmHg are suggestive of adequate perfusion. [40] However, as with mean arterial pressure the minimal safe flow rate during CPB has not yet been established.

#### 1.2.2.2 Hypothermia

A degree of hypothermia is used during CPB in virtually all cases of corrective paediatric heart surgery to provide organ protection, particularly the brain, by reducing metabolic rate and oxygen requirements during periods of altered flow. [34, 40, 69] Cerebral metabolic rate drops exponentially as temperature is reduced with minimal adverse effects on psychomotor testing seen with circulatory arrest times of up to 41 minutes at 18$^{o}$C. [52, 70] Typically the degree of hypothermia used varies directly with the complexity of the repair and ranges from mild (32 -35$^{o}$C), through moderate (25-32$^{o}$C) to deep (15-20$^{o}$C) hypothermia with or without circulatory arrest. However in recent years, with improving surgical and perfusion techniques, the use of deep hypothermia with circulatory arrest during corrective paediatric heart surgery has become more selective with some groups favouring low-flow CPB. [33, 52, 70]

#### 1.2.2.3 pH management

Arterial blood gas management may be more important in children due to the higher levels of hypothermia used during cardiac surgical repair. Cooling increases $CO_2$ solubility and results in a lower arterial $CO_2$ partial pressure and associated mild alkalosis (0.015pH units/$^{o}$C). [71] Cerebral blood flow is directly affected by arterial $CO_2$ partial pressure and becomes pressure-dependent at high levels through a loss of autoregulation. [40] This is thought to be an age-dependent process with children <1yr of age demonstrating a blunted response to elevated $CO_2$ levels. [36, 72] During periods of hypothermia acid-base balance is currently managed by 2 main strategies. The majority of centres use 'alpha-stat strategy' whereby $CO_2$ is not added to the circuit and pH is therefore not corrected to temperature. [33] Others in contrast use the 'pH-stat strategy' in which $CO_2$ is added to the system to maintain a constant pH over varying temperatures. In both adult and paediatric populations

recent reviews have been unable to show any clear benefit on clinical outcomes with either strategy leading to the use of a combined technique in some centres. [33, 36, 72-74]

#### 1.2.2.4 Haemodilution

The concept of haemodilution was popularised in the 1950s in the belief that it would reduce blood and blood product usage in addition to optimising micro-circulation through reduced blood viscosity. [34, 75, 76] Compared to adults the relative priming volume of the bypass circuit in children is significantly greater than their blood volume and often results in a 3 fold haemodilution. [36, 75, 77] The priming fluid used in children can therefore directly impact on fluid and electrolyte balance with glucose containing solutions generally avoided. [40] Furthermore, paediatric patients have an impaired ability to excrete the excess fluid load which tends to accumulate extravascularly in the tissues. [40, 59-61, 75] In time several issues emerged related to haemodilution namely reduced perfusion pressure, increased cerebral blood flow, renal dysfunction and decreased oxygen delivery all of which contributed to the increased morbidity and mortality seen with decreasing haematocrit and stimulated the debate as to the optimal pre-CPB haematocrit level. [34, 75-77]

A large contribution to this area in infants was provided by the Boston group who investigated the effects of haematocrit in children undergoing cardiac surgery on post-operative psychomotor development through a succession of randomised clinical trials and advocated a haematocrit level of at least 25% before, during and after CPB. [75, 76]

#### 1.2.2.5 Myocardial protection

The cornerstone of myocardial protection in paediatric CPB is hypothermia particularly in children with cyanotic CHD and increased non-coronary collateral flow to the heart resulting in cardioplegia washout and rewarming of the myocardium. [36] Hypothermia also generates a cushion for reducing pump flows which ultimately decreases collateral

circulation in such patients. The optimum electrolyte composition of cardioplegia for paediatric patients is not currently known but potassium, calcium, magnesium, sodium and buffers are thought to preserve myocardial function. [36] Although variations in electrolyte composition exist, blood cardioplegia is currently used by the majority of cardiac centres with a mean temperature of 8$^{o}$C and a haematocrit of 15%. [33]

### 1.2.3 The inflammatory response to cardiopulmonary bypass

The process of CPB is a non-physiological form of circulation that elicits a profound inflammatory response within the body through the activation of plasma protein systems (coagulation, contact, fibrinolytic, complement) involved in cellular immunity and protection. [78-81] This reaction is further enhanced by the activation of blood cells during CPB including platelets, neutrophils, monocytes, endothelial cells and lymphocytes culminating in the release of powerful vasoactive substances. [79-81] Clinically this systemic inflammatory response syndrome (SIRS) manifests within the first 24 hrs post-operatively as tissue oedema, thromboembolism, coagulopathy and temporary organ dysfunction particularly in the heart, lungs, brain and kidneys. [79, 80] The severity of SIRS ranges from being almost undetectable (mild pyrexia, leucocytosis, tachycardia) to the development of multiple organ dysfunction syndrome (MODS) requiring intensive supportive therapy. [80, 82] Furthermore, these sequelae of CPB can occur in the face of an apparently effective and complete cardiac repair with satisfactory haemodynamic performance. [81] Children through their immature organ systems, higher metabolic demands, greater haemodilution and comparatively larger CPB circuits appear to be more susceptible and therefore at risk of injury. [83]

Several non-biological variables are known to affect the magnitude of this response including myocardial ischaemia and reperfusion times, temperature and the biomaterials in

contact with blood. [79] Mediators released during CPB include pro-inflammatory cytokines TNF-α, interleukins (IL-1, IL-2, IL-4, IL-6, IL-8 and IL-10), complement (C3a, C5a, C5b-9) and endotoxin whose mechanisms of action are thought to involve neutrophil activation and nitric oxide induction. [78-84] The management of the SIRS response to CPB is a difficult problem, due to the complex interplay of inflammatory cascades, but includes haemofiltration, adenosine, steroids, aprotinin, leukocyte depletion and heparin-bonded circuits. [78-84] As a result it is unlikely that a single therapy will successfully inhibit the inflammatory response to CPB and current efforts are mainly centred on symptomatically treating the multi-organ system dysfunction produced.

#### 1.2.3.1 Ultrafiltration

Haemodilution, immature renal function and endothelial dysfunction relating to CPB are all implicated in the accumulation of extravascular fluid and associated end-organ injury commonly seen in children following cardiac surgery. Ultrafiltration of venous blood to reduce fluid overload, increase haematocrit and remove inflammatory mediators was first introduced as a concept in 1976 by Romanoli et al. [85] Since then the technique has been developed to be used before CPB during priming, intermittently and continuously during CPB and modified ultrafiltration post CPB to remove patient fluid and haemoconcentrate. [86] Multiple studies have demonstrated the effectiveness of modified ultrafiltration in increasing haematocrit, improving haemodynamics, reducing tissue oedema, removing inflammatory markers and optimising end organ function following paediatric CPB. [32, 36, 40, 87, 88] It is therefore not surprising that currently 98% of centres are routinely using ultrafiltration as a whole with 75% utilising the modified approach. [32]

## 1.3 Safety

If one examines heart-lung bypass machines at their advent in the early 1950s and compare their safety features to those of modern day devices it is evident that there have been limited advances in safety and overall automation of the process of extracorporeal circulation. Original designs were complex (often custom built) containing several safety features such as oxygenator blood level sensors, level floats and pressure sensors with elaborate feedback mechanisms. [89] As the process of extracorporeal circulation moved from the lab to clinical practice an increasing need emerged for simpler, cheaper machines as seen during the 1970s and early 80s when many CPB circuits were only composed of an oxygenator / heat exchanger, roller pump and tubing. [89]

However, as the risk of air and particulate embolism became more apparent added attention was given to monitoring anti-coagulation and safety devices such as blood level sensors, bubble detectors and filters were added. In time newer designs have incorporated feedback loops, from these safety devices, that automatically shut off roller pumps if any air is detected within the circuit. [89] This is of particular importance in children with CHD where intra-cardiac surgery and intra- and extra-cardiac shunts are a common occurrence. [36] Arterial line filters (20-40μm) in addition to significantly reducing both gaseous and particulate emboli have been shown to improve post-operative neurological function in adults and should be a feature of all circuits. [32, 34, 36, 40, 90]

Further developments with in-line sensing systems enable perfusionists to continuously measure arterial and venous pH, $PO_2$, $PCO_2$, mixed venous saturation, haemoglobin / haematocrit and temperature. [34, 36] With the evolution of computers within the theatre environment data acquisition, storage and analysis became readily available. However, the ultimate jump to a fully automated system has to date not been met with much success. In

recent years, pump control systems that utilise microcomputers and proportional-integral control algorithms to maintain constant intravascular volumes have been developed for CPB and applied on 15 children undergoing cardiac surgery. [91]

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## 1.5 Aims of the thesis

The objective of this research was to utilise novel investigations and treatment strategies to assess and further characterise peri-operative cardiac, renal and pulmonary function in children undergoing, present-day standard, elective cardiac surgery utilising cardiopulmonary bypass in the hope that this would ultimately increase clinical knowledge and improve outcomes. In this regard two clinical studies were undertaken:

1) A prospective observational study to assess cardiac and renal function by exploring:

- characteristic changes in peri-operative tissue Doppler imaging parameters, their relationship to myocardial injury and clinical outcome
- the use of cystatin C as a means of quantifying renal performance early after cardiac surgery and its association with perfusion parameters and peri-operative myocardial injury

2) A randomised, double-bind, placebo-controlled trial that:

- investigated the use of pre-operative oral sildenafil on haemodynamics, contractility, oxygenation and serum cyclic guanosine monophosphate levels in children at risk of acute post-operative pulmonary hypertension.

# CHAPTER 2: CARDIAC FUNCTION

## 2.1 Abstract


### 2.1.1 Background

The purpose of this study was to utilise tissue Doppler imaging (TDI) to assess changes in ventricular function following repair of congenital heart defects. The relationship between TDI indices, myocardial injury and clinical outcome was explored.

### 2.1.2 Methods

Forty-five children were studied; thirty-five undergoing elective repair (AVSD n=11, VSD n=16, ASD n=8) and ten normal controls. TDI was performed pre-operatively, on admission to PICU and day 1. Regional myocardial Doppler signals were acquired from the RV free-wall (RV), LV free-wall (LV) and basal septum (S). TDI indices were derived by off-line analysis including: (1) Peak systolic velocities [cm/sec]: $Sa_{RV}$, $Sa_{LV}$, $Sa_{S}$ (2) Isovolumic velocities [cm/sec]: $IVV_{LV}$, $IVV_{RV}$, $IVV_{S}$ and (3) Isovolumic acceleration [cm/sec$^2$]: $IVA_{LV}$, $IVA_{RV}$, $IVA_{S}$. Troponin-I was measured in study patients at 15 hours (day-1) post-operatively.

### 2.1.3 Results

Pre-operatively, bi-ventricular systolic TDI velocities in the study group were reduced compared with normal controls and displayed a significant step-wise decrease with increasing complexity of lesion. Post-operatively, RV velocities were significantly reduced and this persisted to day-1 ($Sa_{RV}$ PreOp vs. PICU and day-1: 7.7±2.2 vs. 3.4±1.0, $p<0.0001$ and 3.55±1.29, $p<0.0001$). By contrast LV velocities initially declined but recovered towards baseline by day-1 ($Sa_{LV}$ PreOp vs. PICU and day-1: 5.31±1.50 vs. 3.51±1.23, $p<0.0001$ and 4.70±1.37, $p=0.36$). Isovolumic parameters in all regions were persistently

reduced throughout the post-operative period. Increased Troponin-I release correlated with longer x-clamp times ($r=0.82$, $p<0.0001$), bypass duration ($r=0.83$, $p<0.0001$) and reduced RV velocities ($r=0.42$, $p=0.028$). Reduced pre- and post-operative LV velocities were associated with longer ventilation (PreOp: $r=0.54$, $p=0.002$; PostOp: $r=0.42$, $p=0.026$) and PICU times (PreOp: $r=0.53$, $p=0.002$; PostOp: $r=0.48$, $p=0.01$).

### 2.1.4 Conclusion

This study identified a relationship between the extent of peri-operative myocardial injury and the degree of post-operative RV contractile impairment as measured by tissue Doppler imaging. LV TDI velocities correlated with post-operative outcomes. TDI provided an assessment of bi-ventricular function that could allow optimised timing of surgical correction and post-operative therapies.

## 2.2 Introduction

Advances in surgical technique and ITU management have contributed to the improved survival of children undergoing repair of congenital heart disease. Despite this, peri-operative myocardial injury and ventricular dysfunction continue to be major contributors to morbidity and mortality. [1]

Tissue Doppler imaging (TDI), an echocardiography based technique, measures Doppler shift frequencies produced by the contraction and relaxation of longitudinal muscle fibres that run from the plane of the atrio-ventricular annuli to the heart apex. [2] The resulting Doppler spectrum and measured myocardial velocities have allowed quantification of systolic and diastolic function in many clinical and experimental studies. [3, 4] More recently myocardial acceleration and peak velocity measured during the period of isovolumetric contraction have been proposed and validated as systolic contractile indices independent of changing loading conditions. [5, 6, 7]

In children undergoing congenital heart surgery TDI may offer many potential advantages over conventional echocardiography and other ventricular imaging modalities. Of particular interest is the ability to quantify right ventricular (RV) function independent of its complex, non-geometric shape. [8] Published data on normal TDI parameter values in children with normal anatomical hearts is available. [9] To date however, there is little information on the pattern of change in TDI parameters that follows cardiac surgery in children.

The objective of this study was to investigate the variations in systolic TDI parameters of myocardial velocity and acceleration that occur before and after congenital heart surgery and explore the association between TDI change, myocardial injury and clinical outcome.

## 2.3 Methods

Both local and regional research ethical committees approved the study. Informed consent was obtained prior to study enrolment. In total, thirty-five subjects with a range of atrial and ventricular defects undergoing surgical repair and ten control subjects with structurally normal hearts were studied. Neonates and patients with cyanotic heart disease were excluded. All patients underwent elective surgery using standard techniques of cardiopulmonary bypass with blood cardioplegia. The study group comprised eight patients with ASD (5 primum, 3 secundum), sixteen patients with VSD and eleven patients with complete AVSD (Table 1). This population thus provided a study cohort with a range of ages, complexity of condition and varying duration of cardiopulmonary bypass and cross-clamp times.

Tissue Doppler imaging was performed using a Vivid 7 ultrasound scanner (GE Vingmed, Horten, Norway) with a 7-MHz probe by the same, experienced paediatric echocardiographer (SL). Measurements were obtained at the following time points: (1) Pre-operatively, in operating room under general anaesthesia (PreOp); (2) 1hr post-operatively following return to paediatric ICU (PICU); and (3) 15hrs post-operatively (Day-1). The heart was imaged from a transthoracic 4-chamber view. Colour-coded myocardial velocities were recorded from the basal septum and the lateral free walls of both ventricles immediately below (0.5cm) the insertion of the mural leaflet of the left and right AV valves as previously described. [10] Filters and gains were adjusted to allow a clear tissue signal and minimize background noise. Recordings were made simultaneously with

ECG at a sweep speed of 100mm/sec. A cine loop of at least three consecutive cardiac cycles was recorded and stored digitally for later off-line analysis. Intra-observer and intra-individual variation was assessed randomly in 10 patients.

An arterial blood sample for cardiac Troponin-I (cTnl) was taken 15 hours post-operatively (day-1). cTnI concentration was measured using a commercially available chemiluminescent immunoassay (Beckman Coulter AccuTnI, UK) with the upper limit of normal being 0.04μg/litre.

### *2.3.1 Table 1. Characteristic data of patient groups*

| Demographics | Control | ASD | VSD | AVSD |
|---|---|---|---|---|
| N | 10 | 8• | 16 | 11 |
| Male | 4 | 3 | 11 | 5 |
| Age (months) | 23.6 ± 20.9* | 37.8 ± 15.2* | 8.0 ± 5.5 | 6.1 ± 6.0 |
| Weight (kg) | | 13.2 ± 2.6* | 6.4 ± 2.5 | 5.1 ± 0.8 |
| XC (mins) | | 44.0 ± 30.1 | 50.1 ± 15.9 | 108.8 ± 19.8** |
| CPB (mins) | | 73.8 ± 38.3 | 83.56 ± 20.9 | 155.2 ± 24.9** |
| Ventilation time (hrs) | | 18.3 ± 21.9 | 61.1 ± 57.5 | 107.6 ± 57.4*** |
| PICU stay (days) | | 1.38 ± 0.7 | 3.89 ± 2.7 | 6.00 ± 2.6*** |
| cTnI (day-1, μg/L) | | 3.82 ± 3.7 | 3.83 ± 2.2 | 12.9 ± 4.3*** |

Data expressed as mean ± SD.

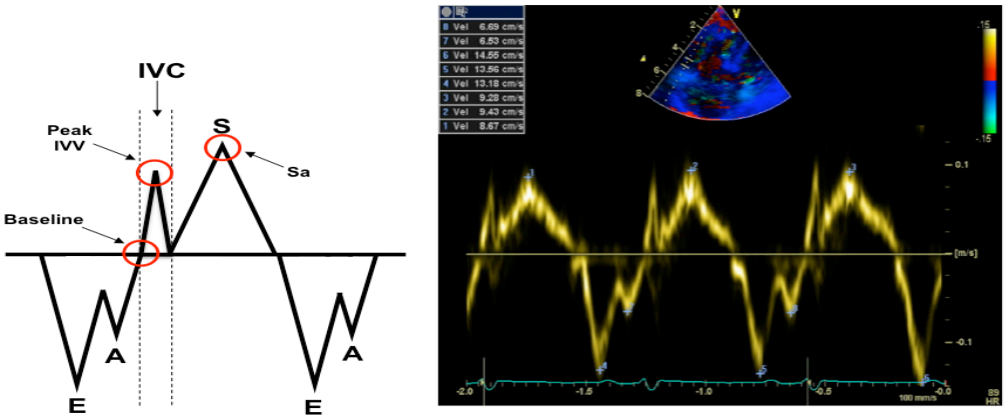
Control, structurally normal heart; ASD, atrial septal defect; VSD, ventricular septal defect; AVSD, atrio-ventricular septal defect; XC, cross-clamp; CPB, cardiopulmonary bypass; PICU, paediatric intensive care unit; cTnI, cardiac troponin-I

•5 primum, 3 secundum; *Control and ASD groups were significantly older (ASD; and heavier) than VSD and AVSD groups ($p<0.01$); **Cross-clamp and cardiopulmonary bypass duration were significantly longer for AVSD group compared to VSD and ASD groups ($p<0.0001$); ***Ventilation time, PICU stay and cTnI were significantly higher in AVSD group compared to VSD and ASD groups ($p<0.001$).

### 2.3.2 TDI Analysis

Stored TDI data was displayed (Figure. 1) and analysed using commercially available software (Echopac, GE Vingmed). Each TDI parameter was derived by analysis of the TDI annular signal and represented the functional description of corresponding ventricle i.e. tricuspid valve for right ventricle, mitral valve for left ventricle and basal septum for septum. Peak myocardial velocity (Sa) was measured by electronic callipers as the wave height during systole. Peak myocardial isovolumic velocity (IVV) was defined as the maximum velocity during isovolumetric contraction (Q wave to onset of systolic ejection). Isovolumic acceleration (IVA) was calculated by dividing the peak isovolumic velocity by the time interval from baseline to peak isovolumic velocity (Figure 1). [5] All measurements were derived from 3 consecutive cardiac cycles with the average recorded. Of note, diastolic TDI parameters were invariably more difficult to interpret due to the merging of peak early and late (E and A waves depicted below) annular velocities. Intra-observer and intra-individual variation in systolic TDI parameter analysis was found to be less than 5%.

#### *2.3.2.1 Figure 1. Normal TDI waveform depicting measured and acquired parameters*



## 2.4 Statistical Analysis

Data are expressed as means ± standard deviations. Demographic and outcome data (age, bypass and cross-clamp times, cardiac troponin-I, ventilation time, and PICU stay), TDI comparisons with controls and the change in TDI parameters with time were all analysed by ANOVA with post-hoc Bonferroni correction. Correlations were performed between quantitative variables to assess the degree of linear association using Pearson method. All analyses were done using Minitab (Version 14) with a significance level of 5%.

## 2.5 Results

Within the study group there was no mortality; all patients were discharged without significant complication. Study and control patient demographics are summarised in Table 1. Control and ASD patients were significantly older than VSD and AVSD patients. There was an incremental increase in operative duration, troponin release and ventilation/PICU times with increasing cardiac complexity.

Post-operative Troponin-I was elevated compared to the normal range. cTnI (μg/L, all patients; 6.6 ± 5.3) was significantly higher following AVSD repair compared with VSD and ASD groups (Table 1). There was a strong positive correlation between cTnl release and both cross-clamp ($r=0.82$, $p<0.0001$) and bypass duration ($r=0.83$, $p<0.0001$).

### 2.5.1 TDI Parameters

Pre-operatively in both control and study patients, all TDI parameters were greater in the RV compared with LV and septum, with the highest values in the control group (Table 2). Peak myocardial velocities demonstrated an obvious step-wise decrease with increasing

complexity of cardiac lesion. By contrast the isovolumetric parameters, particularly in the septum, tended to remain more consistent across the cardiac lesion spectrum.

LV, RV and septal peak systolic velocities were all significantly reduced in the immediate post-operative period (PICU) but demonstrated a modest trend to recovery by day-1, particularly in the LV (Figure 2a). By contrast both isovolumetric parameters, IVA (Figure 2b) and IVV (Figure 2c) decreased in the early period and continued to decline at day-1.

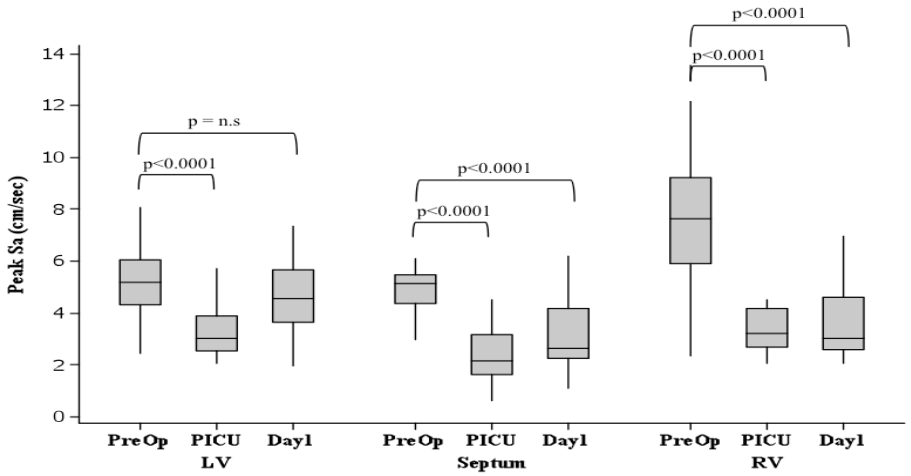
#### *2.5.1.1 Table 2. Controls versus pre-operative TDI parameters by diagnosis*

| | Sa (cm/sec) | | | IVV (cm/sec) | | | IVA (cm/sec$^2$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | LV | Sep | RV | LV | Sep | RV | LV | Sep | RV |
| Control | 7.75±1.70 | 7.43±0.46 | 12.65±0.99 | 6.20±1.56 | 7.03±0.74 | 11.86±4.93 | 300.8±62.4 | 291.8±110.8 | 470.8±189.3 |
| ASD | 6.63±1.47 | 5.21±0.68$^{\blacklozenge}$ | 8.64±1.89$^{\blacklozenge}$ | 6.37±1.20 | 7.61±0.57 | 9.80±0.83 | 230.2±153.9 | 216.4±44.5 | 263.0±93.0$^{\blacktriangle}$ |
| VSD | 5.46±1.30$^{\blacklozenge}$ | 4.92±0.98$^{\blacklozenge}$ | 7.73±2.29$^{\blacklozenge}$ | 6.86±3.97 | 7.32±2.45 | 9.04±3.68 | 220.9±132.9 | 233.6±113.7 | 274.5±116.4$^{\blacktriangle}$ |
| AVSD | 4.19±0.93$^{\blacklozenge}$ | 4.52±1.41$^{\blacklozenge}$ | 6.93±2.32$^{\blacklozenge}$ | 6.17±2.35 | 6.94±3.00 | 9.58±2.27 | 142.2±88.4$^{\blacktriangle}$ | 218.6±79.6 | 317.6±111.6$^{\blacktriangle}$ |

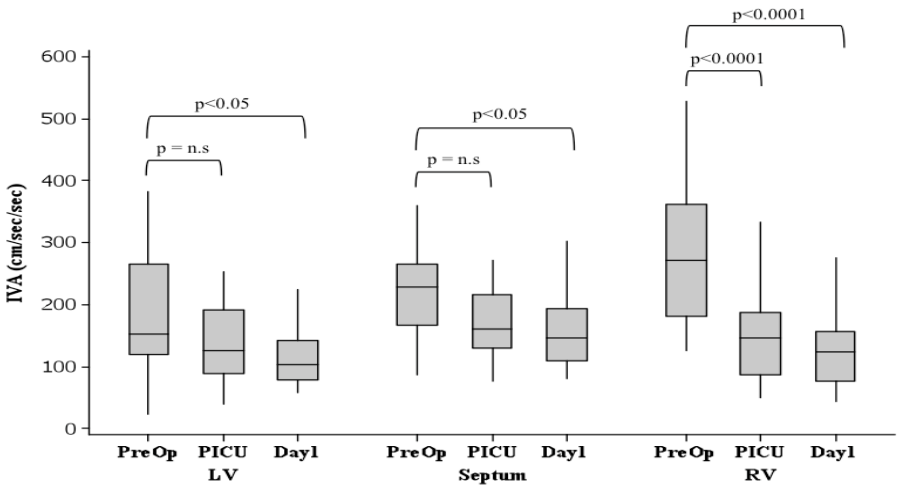
TDI data expressed as mean ± SD; (TDI velocity versus control: $p<0.05^{\blacktriangle}$, $p<0.0001^{\blacklozenge}$)

Sa, peak systolic myocardial velocity; IVV, peak velocity during isovolumetric contraction; IVA, isovolumetric acceleration; LV, left ventricle; Sep, basal septum; RV, right ventricle; Control, anatomically normal heart; ASD, atrial septal defect; VSD, ventricular septal defect; AVSD, atrio-ventricular septal defect

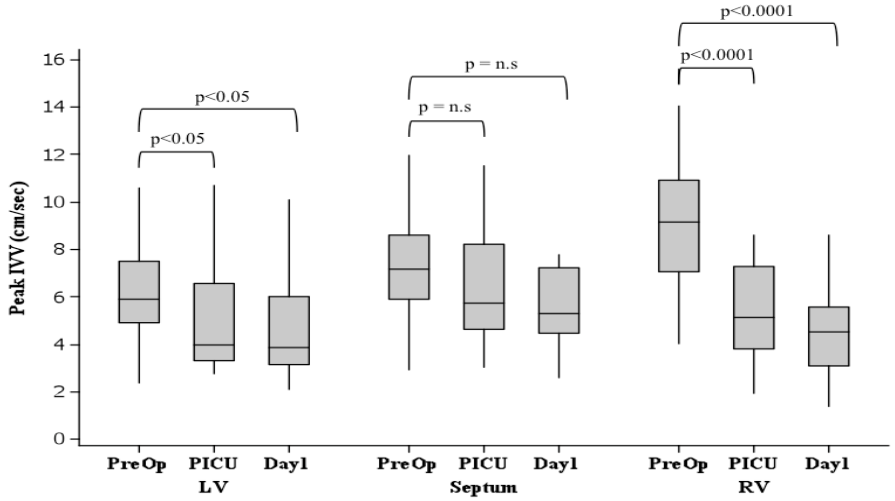
### *2.5.1.2 Figure 2a. Change in peak systolic myocardial velocity*




### *2.5.1.3 Figure 2b. Change in isovolumic acceleration*



#### *2.5.1.4 Figure 2c. Change in isovolumic velocity*

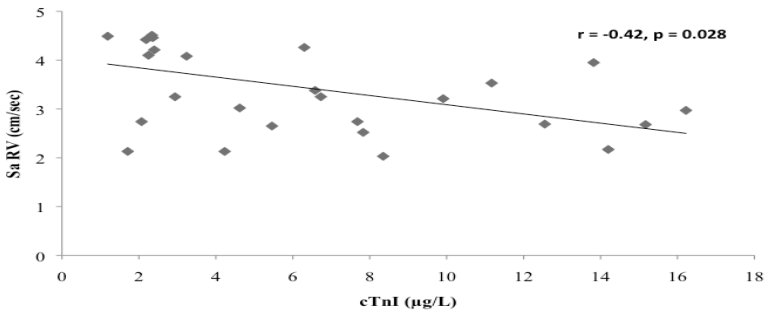



### 2.5.2 Correlation of TDI parameters with myocardial injury and outcome

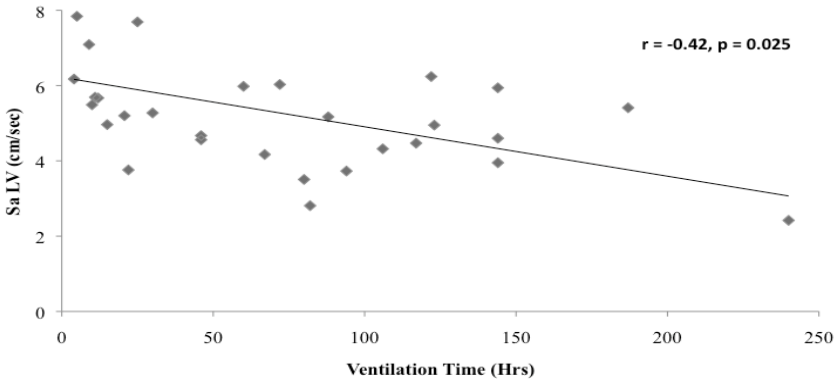
Reduced pre-operative LV systolic velocity correlated with greater cTnI release and longer ventilation (r=0.54, p=0.002) and PICU times (r=0.53, p=0.02). Post-operatively reduced RV (Figure 3a) and septal systolic and isovolumic velocities were associated with greater cTnI release. By comparison there was no correlation between post-operative LV TDI parameters and troponin-I release ($Sa_{LV}$ r=0.06, p=0.84; $IVA_{LV}$ r=0.15, p=0.75).

Post-operatively, longer ventilation and ITU times were associated with reduced LV and septal parameters (Figures 3b-c). There were no significant correlations between RV TDI parameters and ventilation time ($Sa_{RV}$ r=0.12, p=0.65; $IVA_{RV}$ r=0.30, p=0.30).

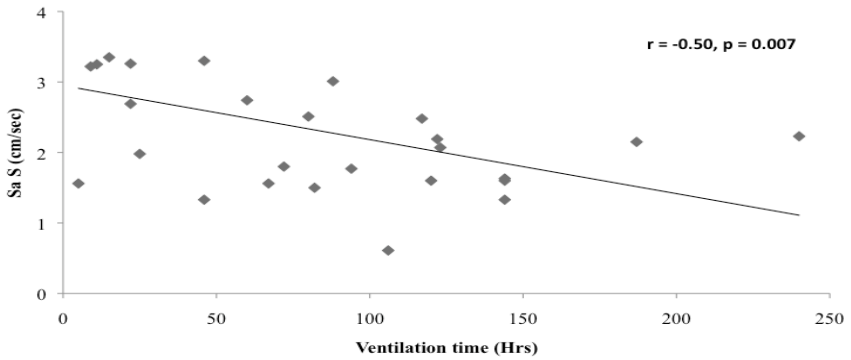
### *2.5.2.1 Figure 3a. RV systolic velocity (PICU) vs. cTnI*




### *2.5.2.2 Figure 3b. LV systolic velocity (PICU) vs. Ventilation Time*



### *2.5.2.3 Figure 3c. Septal systolic velocity (PICU) vs. Ventilation time*



## 2.6 Discussion

The principal findings in this study were; (1) In patients with congenital heart lesions pre-operative bi-ventricular systolic tissue Doppler parameters were reduced compared to normal controls (2) Following cardiac surgery there was a reversible reduction in TDI velocities with sustained decline in isovolumic indices (3) Reduced post-operative RV velocities were associated with a greater extent of myocardial injury defined by Troponin-I release and (4) Reduced pre- and post-operative LV velocities were associated with longer ventilation and PICU times.

The assessment of ventricular function following paediatric cardiac surgery is complicated by the alteration to loading conditions caused by the elimination of intra-cardiac shunting and variation in post-operative pulmonary and systemic vascular resistances. [1] The contractile indices of maximal elastance (Emax) and preload-recruitable stoke work (PRSW), derived from pressure-volume data, are considered gold standard because of their load independence. [11] However the methodology involved in ventricular volume measurement, usually by conductance catheter, is invasive and not suitable for routine clinical use.

In this study contractility was quantified using absolute (peak systolic and isovolumic velocity) and derived (isovolumic acceleration) tissue Doppler indices. Peak systolic myocardial velocities have been validated as an index of contractility in animal experiments of ischaemia-reperfusion and in studies of inotrope modulation, where velocities have correlated closely with fractional shortening measured by sonomicrometry and maximum elastance from pressure-volume data. [4, 12] In clinical studies, systolic TDI velocities have proved to be reliable indicators of LV and RV function as well as

predicting survival in patients with heterogeneous cardiac diseases. [13-15] However, the magnitude of systolic myocardial velocities has been shown to be preload- and afterload-dependent. [5, 6, 16] Recently peak isovolumic velocity (IVV) and acceleration (IVA) have been proposed and validated through animal studies as load-independent contractile indices within the physiological range expected post-operatively. [5-7, 17] The potential ability of TDI to assess ventricular function independent of load is particularly important when considering the RV which in the context of congenital heart disease is frequently characterised by pressure or volume loading. [15, 17] Although RV function can be derived from volumetric data obtained through cardiac MRI, multi-slice CT or 3D echocardiography their load-dependency, invasiveness, high cost, low availability and time consuming data analysis limits their use in children. [8] The RV has a preponderance of longitudinal muscle fibres which allows optimal alignment of the tissue Doppler signal. This permits a sensitive measure of velocity variations within the RV and thus TDI has gained favour in its functional assessment. [14, 15, 17, 18] This was confirmed in the present study where TDI indices were consistently higher in the RV compared with septum and LV for all patients, including controls.

From the pre-operative data it was found that, not only were the patients with cardiac lesions observed to have reduced velocities compared to controls but also the extent of velocity reduction was greater with increasing cardiac complexity. By contrast the variation observed in isovolumic indices, particularly IVV, was much less across the disease spectrum consistent with the load-independent character of these parameters. Of interest isovolumic acceleration appeared to be most reduced in the specific ventricle likely to be volume loaded by the cardiac lesion, i.e. RV with ASD and LV with VSD/AVSD. The study also identified that reduced pre-operative LV TDI indices were associated with longer ventilation/PICU times, confirming a clinical impression that patients in pre-

operative heart failure with VSD lesions, with large Qp:Qs and volume loaded LV, are likely to require longer post-operative recovery. Previous adult studies have also shown that LV systolic velocities may predict survival in patients with congestive heart failure. [19] Ultimately it may be that LV systolic TDI parameters will have a predictive value in determining post-operative course and can be factored into optimising the timing of surgery.

In the current study the finding of a reduction in all systolic TDI parameters measured at the septum, mitral and tricuspid annuli suggests an overall global impairment of contractility in the early post-operative period. Of particular interest was the disparity seen between RV and LV TDI parameters post-operatively. LV peak systolic velocities recovered towards baseline pre-operative values by 15 hours post-operatively and this pattern of change may reflect the nadir in cardiac index described by Wernovsky. [20] However, peak systolic velocities are an ejection-phase index and therefore load-dependent and the initial fall in LV velocities may in part result from diminished LV preload following VSD closure. [16] Similarly the observed later velocity recovery may represent a reduction in systemic vascular resistance rather than an improvement in intrinsic LV contractility per se. [10, 20] By contrast, RV and septal systolic velocities remained reduced with no significant recovery between the 2 post-operative time points. Potentially these velocities could also be reduced as a consequence of increased RV afterload by elevations in pulmonary arterial pressure common in post-operative patients with repaired VSD or canal defects. The greater and more significant reduction in RV isovolumic acceleration seen post-operatively supports the view that an intrinsic RV contractile impairment existed. Likewise the smaller reduction in post-operative LV isovolumic acceleration, although not initially statistically significant, could also indicate a contractile dysfunction within the LV. Ultimately however, there exists a complex interplay of changes in loading conditions

and myocardial contractility that are often interrelated and therefore not possible to definitively isolate.

In this study post-operative serum Troponin-I was measured to quantify myocardial injury sustained during surgery. Troponin-I has been shown to correlate well with the extent of peri-operative myocardial damage and is predictive of post-operative outcome in the paediatric population. [21, 22] In this patient group although direct retraction trauma cannot be avoided, no ventricular incisions or myocardial resection were performed and therefore myocardial injury and cTnI release should relate to the duration of ischemia-reperfusion. This was strongly evident in our study with clear correlations between cross-clamp and bypass times and cTnI levels. The significant correlations between cTnI levels and RV and septal TDI parameters would implicate myocardial injury as the principal mechanism behind the reduced contractility. Of interest, although both ventricles were exposed to the same ischemic-reperfusion insult, no significant correlations between troponin-I and post-operative LV TDI parameters were found. This may reflect a vulnerability of the RV to myocardial injury in this patient group. In the patients with VSD physiology, pre-operative exposure of the RV to systemic pressures will induce myocardial hypertrophy, which has been shown to decrease sub-endocardial blood flow and lower concentrations of high energy phosphates, factors which may render the ventricle more susceptible to injury during the cross-clamp period. [23] The RV is also vulnerable to direct surgical trauma and contusion resulting from retraction, ventriculotomy (although not performed in this study) and VSD suture placement. Post-operative conduction abnormalities, including right bundle branch block may have a more pronounced effect on RV contraction although no conduction defects were seen in these patients.

In the acute post-operative setting TDI had a predictive value for early outcome with reduced LV and septal TDI significantly correlating with longer ventilation time and PICU stay. By contrast RV TDI, the ventricle with the most marked post-operative TDI reduction, did not demonstrate a correlation with outcome. Although the reasons for this remain unclear it may be that the RV TDI was not measured beyond 15 hours post-operatively and it is possible that subsequent ventricular recovery and appropriate modulation of the contractile deficit by inotropes may confound a potential relationship. In addition other factors, including renal and pulmonary impairment, may supersede the influence of ventricular function on early outcome with respect to ventilation and PICU times.

## 2.7 Clinical implications and future considerations

Pre-operative TDI quantification of LV systolic function may prove to be a useful adjunct in determining the timing of corrective surgery. TDI also has the ability to assess contractile dysfunction in the PICU setting, allowing early and ongoing assessment of ventricular performance following cardiac surgery. This may enable appropriate therapeutic intervention prior to the onset of the systemic manifestations of low cardiac output including lactic acidosis and renal failure. Due to its particular sensitivity for RV contraction, TDI may provide better evaluation of right ventricular-pulmonary interaction post cardiac surgery and assessment of the effectiveness of nitric oxide and other intervention strategies.

## 2.8 Study limitations

Pulmonary arterial pressures were not directly measured and therefore this influence was not evaluated. Never the less, no patient required supportive measures including nitric oxide administration in the ITU management. The lower velocity changes and the more

variable and inconsistent pattern of change observed in LV post-operatively may reflect a misalignment of the Doppler signal with the predominant circular mid-wall fibres found in the left ventricle. However, the majority of published studies of TDI quantifying LV contraction used longitudinal mitral annular velocities and have satisfactorily predicted ventricular function and outcome. [3, 4, 13, 19, 24]

TDI parameters were not analysed beyond the first post-operative day and it would have been interesting to know if the RV and septal parameters returned to normal during the early post-operative period. In a previous study of post-op Fallot's patients a reduction in RV myocardial acceleration persisted late after repair. [17] However this may relate to ongoing haemodynamic lesions including chronic pulmonary regurgitation rather than a permanent myocardial injury sustained at the time of surgical repair.

## 2.9 Conclusion

This study highlights the vulnerability of the RV to ischaemia-reperfusion injury sustained during the repair of congenital heart disease, as quantified by TDI. TDI is a readily accessible, non-invasive technique that allows real-time evaluation of ventricular function after surgery enabeling the clinician to make rapid therapeutic management decisions. Through its particular sensitivity to RV contraction, TDI may provide better evaluation of the RV-pulmonary interaction after cardiac surgery and the effectiveness of nitric oxide and other intervention strategies. Pre-operative evaluation of LV systolic TDI parameters may have a role in patient risk stratification and timing of cardiac surgical correction.

## 2.10 Acknowledgements

This study was supported through a successful research grant application to 'The Association of Children with Heart Disorders'.

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# CHAPTER 3: RENAL FUNCTION

## 3.1 Abstract

### 3.1.1 Background

Cardiopulmonary bypass (CPB) and post-operative low cardiac output are important factors in acute renal injury following cardiac surgery. In patients with renal disease cystatin C has emerged as a new biomarker marker that is sensitive to changes in glomerular filtration rate (GFR) and may provide a better estimate of renal function than creatinine alone. In this study we wished to:

1. Evaluate cystatin C as a means of quantifying renal performance after cardiac surgery in children.
2. Utilize cystatin C to investigate acute changes in renal function following cardiac surgery in children and its association with cardiopulmonary bypass factors and peri-operative myocardial injury.

### 3.1.2 Methods

Twenty children, aged 4-58 months (median 5.5, 10 M: 10 F), undergoing surgery (AVSD n=7, VSD n=9, ASD n=4) were prospectively studied. Blood samples were collected pre-operatively and on days 0, 1, 2 and 3 post-operatively to measure serum cystatin C (cysC) and creatinine (Cr). GFR was quantified by creatinine clearance during the first and second 12hr post-operative periods; $CrCl_{0\text{-}12}$ and $CrCl_{12\text{-}24}$ respectively. Recorded CPB parameters included bypass duration, the lowest pump flow ($Q_{min}$), the lowest hematocrit ($Hct_{min}$) and the corresponding lowest oxygen delivery ($DO_{2\ min}$). Myocardial injury was quantified post-operatively by Troponin-I release.

### 3.1.3 Results

Serum cystatin C and creatinine increased post-operatively to peak on days 3 and 2 respectively ($cysC_{Day0}$ 0.8±0.3 vs. $cysC_{Day3}$ 1.5±0.5; p=0.02) ($Cr_{PreOp}$ 31.0±6.9 vs. $Cr_{Day2}$ 36.9±12.2; p=0.03). During the first 24hrs after surgery GFR remained unchanged ($CrCl_{0-12}$ 63.6±37.0 vs. $CrCl_{12-24}$ 65.1±27.5; p=n.s.). In comparison to creatinine, cystatin C demonstrated a superior correlation with GFR ($cysC_{Day0}$ r=0.58 p=0.018; $Cr_{Day0}$ r=0.09 p=0.74). Receiver-operator cut-off level for cystatin C >1.044mg/l exhibited a 100% sensitivity and 67% specificity for detecting renal dysfunction, defined as a GFR <55ml/min/1.73m$^2$, compared to 85% sensitivity and 88% specificity for creatinine >34μmol/l. Serum cystatin C significantly increased during the first 24hr post-operative period ($cysC_{Day0}$ 0.8±0.3 vs. $cysC_{Day1}$ 1.1±0.4; p=0.003) and was strongly associated with CPB duration ($cysC_{Day1}$ r=0.67, p=0.001), $Q_{min}$ ($cysC_{Day1}$ r=0.61, p=0.005) and greater Troponin-I release ($cysC_{Day1}$ r=0.73, p<0.001). Lowest $DO_{2\ min}$ and $Hct_{min}$ did not correlate with cystatin C.

### 3.1.4 Conclusions

This study has shown that in comparison to creatinine, cystatin C provides a better estimate of GFR and is more sensitive in detecting significant renal dysfunction in children following cardiac surgery. Cystatin C identified a transient post-operative renal impairment, the magnitude of which was associated with duration of bypass, low pump flows and the degree of myocardial injury.

## 3.2 Introduction

Acute renal failure (ARF) following cardiopulmonary bypass (CPB) in children is a well recognised and serious complication of cardiac surgery whose incidence varies widely, 1to 31%, depending on the defining criteria. [1-5] When severe enough to require renal replacement therapy (RRT) morbidity and mortality are significantly increased despite supportive measures. [2, 3]

The artificial process of CPB necessary for cardiac surgery is known to trigger multiple inflammatory cascades within the body that culminate in a capillary leak syndrome and multi-organ dysfunction. [6-8] In an attempt to maximise operating conditions, further potential stresses are often inflicted on the kidney through haemodilution, hypothermia, hypoperfusion and non-pulsatile flow. [3,9,10] Younger and smaller patients as a group are particularly vulnerable due to higher metabolic demands, complex cardiac lesions, longer CPB times, reactive pulmonary vasculature and immature organ systems with altered homeostasis.[2, 5, 7] Evidence also exists of a low cardiac output syndrome (LCOS) that occurs 6 to 18 hours after paediatric cardiac surgery which is multi-factorial in aetiology and associated with increased mortality due to secondary organ failure. [6, 11]

Glomerular filtration rate (GFR), defined as the volume of plasma completely cleared of a particular substance by the kidneys in a unit of time, is the best overall indicator of renal function. [12-14] The expensive, labour-intensive determinations of GFR by exogenous substances such as inulin remain the 'gold standard' but have been replaced clinically by more straightforward endogenous substances including creatinine. Although the measurement of serum creatinine is the most common method for estimating GFR, it often fails to identify patients with moderately reduced renal function. [12-15] Cysteine proteases are a group of enzymes that degrade polypeptides. In humans they are responsible for

apoptosis, MHC class II immune responses, proteasome and lysosomal regulation, pro-hormone processing and extracellular matrix remodeling important to bone development. [16-18] Cystatin C (cysC) is a cysteine proteinase inhibitor uniquely expressed in all nucleated cells and produced at a constant rate. [16-18] It is considered to be a sensitive marker of renal injury and unlike creatinine its levels are not influenced by height, age, sex, muscle mass or acute phase reactions highlighting the potential diagnostic importance of this novel biomarker.[12-14,16-19]

The value of cysC in quantifying early renal dysfunction following paediatric cardiac surgery in conjunction with measured GFR and serum creatinine has not been tested. This important issue has been addressed in this study as well as the association of cysC with peri-operative CPB factors, oxygen delivery and myocardial injury.

## 3.3 Methods

### 3.3.1 Patients

Both local and regional research ethics committees approved the study. Following written informed consent the first twenty children enrolled to our prospective observational cohort study designed to assess cardiac and renal function, with a range of atrial and ventricular septal defects undergoing corrective surgical repair, were prospectively recruited. Exclusion criteria included neonates, patients with cyanotic heart disease and pre-operative use of mechanical ventilation, inotropes, extracorporeal life support or pre-existing renal dysfunction.

### 3.3.2 Data Collection and Definitions

#### *3.3.2.1 Renal Function*

Serum creatinine (Cr) was measured as part of routine investigations for all patients undergoing cardiac surgery pre-operatively, on admission to paediatric intensive care (PICU) and days 1, 2 and 3 post-operatively using the Roche Creatinine Plus enzymatic assay with a normal range of 18 – 40 μmol/L.

Plasma retained from routine investigations, at the above time points, was stored at -70$^{o}$C until measurement of cysC using a particle-enhanced nephelometric immunoassay (PENIA) on a BN ProSpec analyser (Dade Behring Ltd., Milton Keynes, UK) with a normal range of 0.54 – 1.06 mg/L.

Urinary creatinine measured by the kinetic Jaffe method on an Abbott AEROSET analyser was used to estimate GFR through creatinine clearance (CrCl) during the first and second twelve-hour post-operative periods and indexed to body surface area (Normal range 54 – 86 ml/min/1.73m$^2$). Hourly urine output (ml/kg) was recorded for the first twenty-four hours after surgery.

#### *3.3.2.2 Perfusion and Myocardial Injury*

Besides CPB and cross-clamp times (min), the following operative parameters were recorded at four time points during surgery (commencement of CPB, at 30 min, at 1hr and on removal of cross-clamp): haemoglobin (g/dL), haemoglobin saturation (%) and arterial oxygen tension (mmHg) from routine arterial blood gas analysis, pump flow (ml/min/m$^2$), mean arterial pressure (mmHg) and oxygen delivery (ml/min/m$^2$). In addition the lowest

indexed pump flow ($Q_{min}$), lowest haematocrit ($Hct_{min}$) and corresponding oxygen delivery ($DO_{2\ min}$) that occurred during CPB were also noted.

Oxygen delivery ($DO_2$, ml/min/$m^2$) which is the amount of oxygen delivered to the peripheral tissues was calculated by multiplying the indexed pump flow (Q) by the arterial oxygen content according to the equation: *$DO_2$ = Q x (haemoglobin x 1.34 x haemoglobin saturation + 0.003 x arterial oxygen tension)*. [3]

An arterial blood sample for cardiac Troponin-I (cTnl) was taken 15 hours post-operatively (Day1). cTnI concentration was measured using a commercially available chemiluminescent immunoassay (Beckman Coulter AccuTnI, UK), with the upper limit of normal being 0.04μg/litre.

### 3.3.3 Cardiopulmonary Bypass

All patients underwent elective surgery by the same surgeon (MHD) using moderate hypothermic cardiopulmonary bypass (25-32$^0$C) with antegrade, cold, blood cardioplegia. The bypass circuit was primed with 4.5% albumin (150 - 200 mls), mannitol (0.5g/kg), $NaHCO_3$ (10 – 15 mmol) and heparin (3000 – 5000 iu). Packed red cells were added to the prime to achieve a minimum haematocrit of 25%. Acid base was managed with alpha stat control. Conventional ultrafiltration (Minntech HPH 400 haemofilter) during re-warming occurred in all patients.

Extracorporeal circulation was performed using a Medos 2800 (Chalice Medical, Shireoaks, Notinghamshire, UK) oxygenator, Pall AL3 (Chalice Medical, Shireoaks, Notinghamshire, UK) arterial filter and Stockert S3 roller pump (Sorin, Gloucester,

Gloucestershire, UK). Non-pulsatile perfusion was maintained at 1.0 – 1.8L/min (<12 months) and 1.8 – 3.0L/min (12 – 58 months): flow = body surface area x 2.4 L/min/$m^2$.

### 3.4 Statistical Analysis

Associations between peri-operative variables and measures of post-operative renal function ($CrCl_{12\text{-}24}$, $Cr_{Day1}$ and $cysC_{Day1}$) were initially investigated univariately. Creatinine clearance was defined as a dependent binary variable (0 to 1; using cut-off values at 55 and 85 ml/min/1.73$m^2$ to further delineate any association with severity of renal impairment) and its association with the various independent variables was explored using a logistic regression analysis with each possible predictor tested one at a time. For continuous outcomes, a general linear model was used in the same way. Significant variables from the univariate analyses were then used in multivariate models using backwards selection to determine the independent predictors of outcomes.

Parameters of renal function (Cr, cysC and CrCl) were measured over time and a repeated measures analysis of variance was performed to test for differences between time points. Where differences existed, post-hoc comparisons between time points were completed using the Bonferroni correction for multiple testing.

Receiver operating characteristics (ROC) analyses were performed to investigate the potential of $Cr_{Day1}$ and $cysC_{Day1}$ as diagnostic predictors of abnormal $CrCL_{12\text{-}24}$, coded as binary, with cut-offs at 55 and 85 ml/min/1.73$m^2$.

All analyses were done using Minitab (version 15) or SPSS (version 17) at a significance level of 5%.

## 3.5 Results

Twenty children were recruited to the study with demographic and peri-operative variables as outlined in Table 1 and Figures 1a-d. Within this group there was no surgical hospital mortality; all patients were discharged home without complication.

### *3.5.1 Table 1. Demographic characteristics between groups*

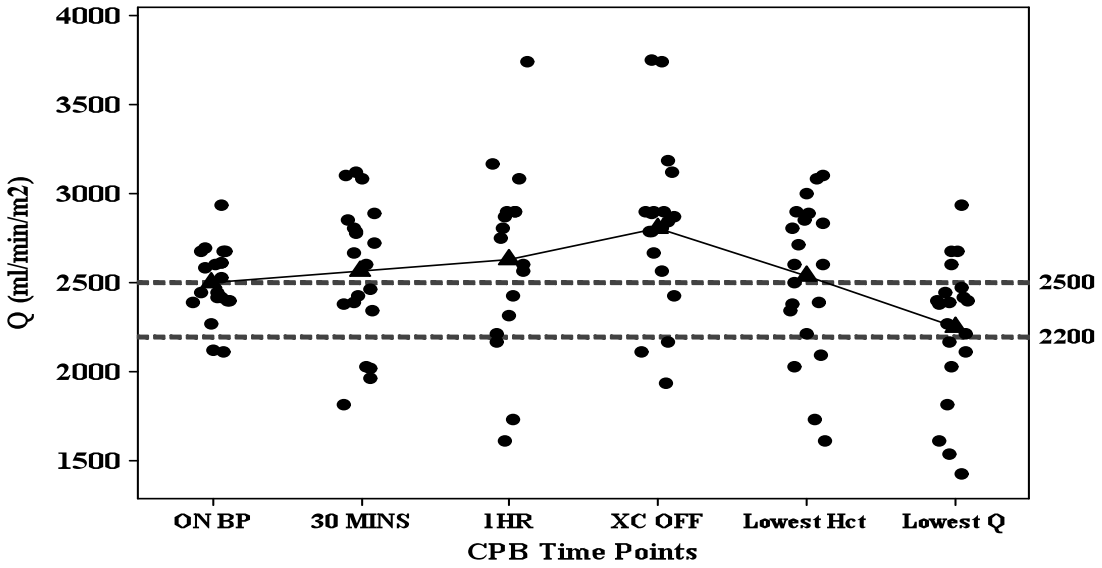
| | | AVSD (n = 7) | VSD (n = 9) | ASD (n = 4) | p-value | **All Patients (n = 20)** |
|---|---|---|---|---|---|---|
| Patient factors | Gender (Male) | 2 (29%) | 6 (67%) | 2 (50%) | 0.319 | **10 (50%)** |
| | Age (mth) | 6.5 ± 1.7 | 8.1 ± 2.1 | 41.8 ± 6.8 | 0.005 | **14.3 ± 15.9** |
| | Weight (Kg) | 6.1 ± 1.5 | 6.0 ± 2.5 | 14.0 ± 3.0 | 0.007 | **7.7 ± 3.9** |
| Operative factors | XC (min) | 106 ± 20 | 54 ± 17 | 43 ± 23 | <0.001 | **70.4 ± 33.4** |
| | BP (min) | 147 ± 25 | 86 ± 25 | 69 ± 28 | <0.001 | **100 ± 41** |
| | $Hct_{min}$ (%) | 24.14 ± 3.13 | 23.43 ± 2.88 | 22.75 ± 2.36 | 0.725 | **23.75 ± 2.73** |
| | $Q_{min}$ (ml/min/$m^2$) | 2037.4 ± 408 | 2299.8 ± 430 | 2551 ± 262 | 0.111 | **2250.5 ± 398.7** |
| | $DO_{2min}$ (ml/min/$m^2$) | 244.3 ± 47.7 | 309.9 ± 49.8 | 290.6 ± 11.4 | 0.034 | **281.0 ± 49.1** |
| Post-Op factors | $cTnI_{Day-1}$ (µg/L) | 11.6 ± 3.6 | 4.9 ± 2.4 | 2.2 ± 0.6 | <0.001 | **6.7 ± 4.8** |
| | Ventilation (hrs) | 101 ± 67.4 | 69.3 ± 66.9 | 21.3 ± 26 | 0.05 | **70.8 ± 65.4** |
| | PICU stay (days) | 5.7 ± 3.0 | 4.1 ± 3.0 | 1.3 ± 0.5 | 0.020 | **4.1 ± 3** |
| | Hospital stay (days) | 19.1 ± 18.0 | 8.8 ± 2.5 | 7.2 ± 2.2 | 0.035 | **12.1 ± 11.8** |

Data expressed as mean ± SD. (p-value: refers to between group comparisons)

AVSD, atrio-ventricular septal defect; VSD, ventricular septal defect; ASD, atrial septal defect; XC, cross-clamp time; BP, cardiopulmonary bypass time; $Hct_{min}$, lowest haematocrit during bypass; $Q_{min}$, lowest indexed pump flow during bypass; $DO_{2min}$, indexed oxygen delivery at lowest haematocrit during bypass; $cTnI_{Day-1}$ , serum cardiac troponin-I on first post-operative day; Post-Op, post-operative; PICU, paediatric intensive care unit.

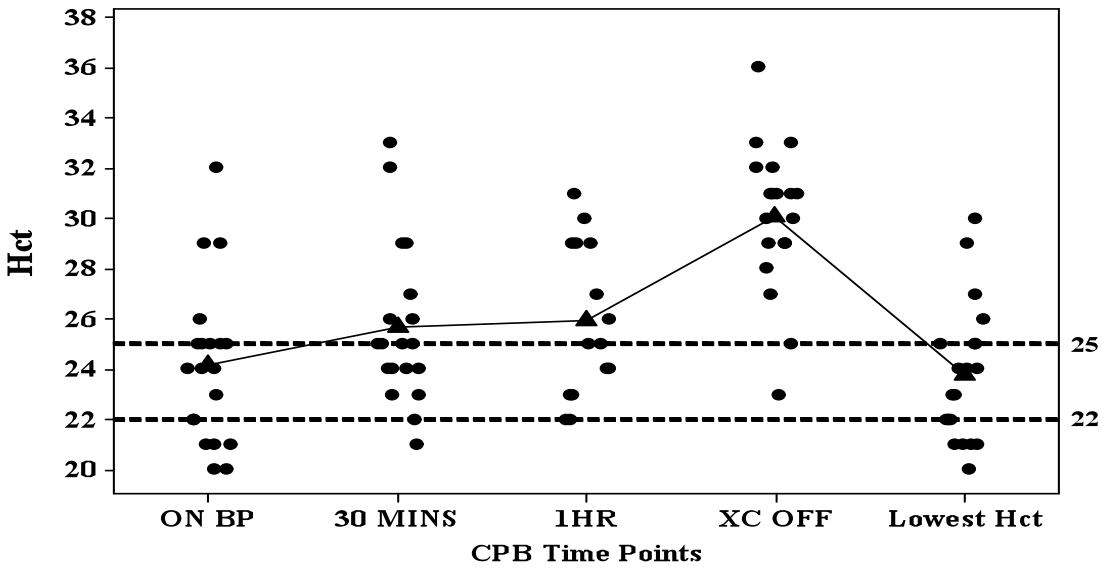
***3.5.1.1*** ***Figure 1a.*** *Peri-operative pump flow (--- ideal parameter boundaries)*

*Mean values interconnected. Wide variation in perfusion flow evident with general trend for increasing flows prior to removal of cross-clamp (XC).*



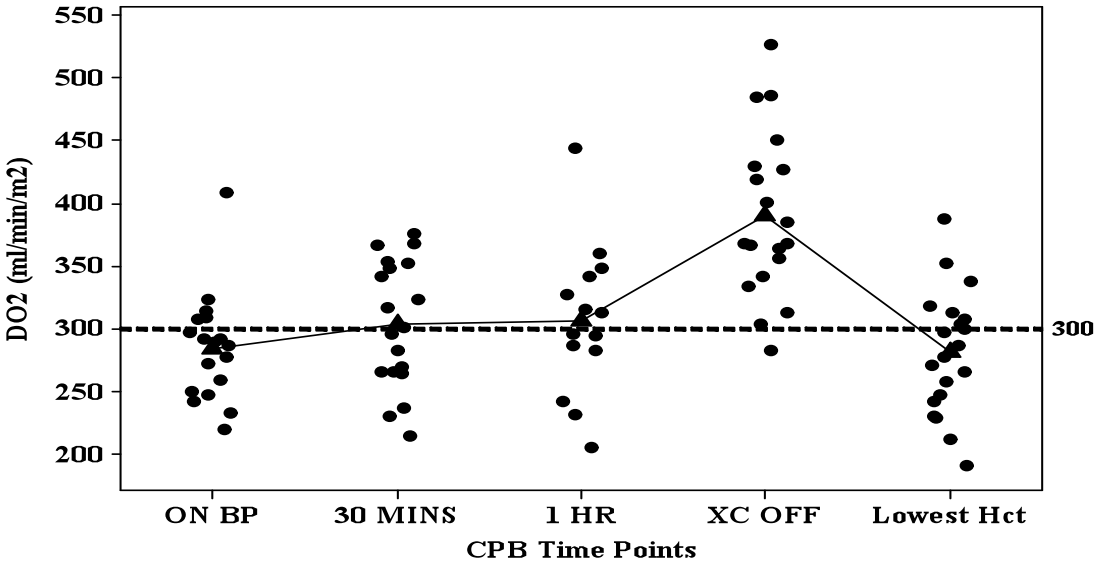

***3.5.1.2*** ***Figure 1b.*** *Peri-operative haematocrit (--- ideal parameter boundaries)*

*Mean values interconnected. Wide variation in haematocrit levels evident particularly on commencement of bypass (on BP). Increased haematocrit levels associated with ultrafiltration prior to cross-clamp removal (XC off).*



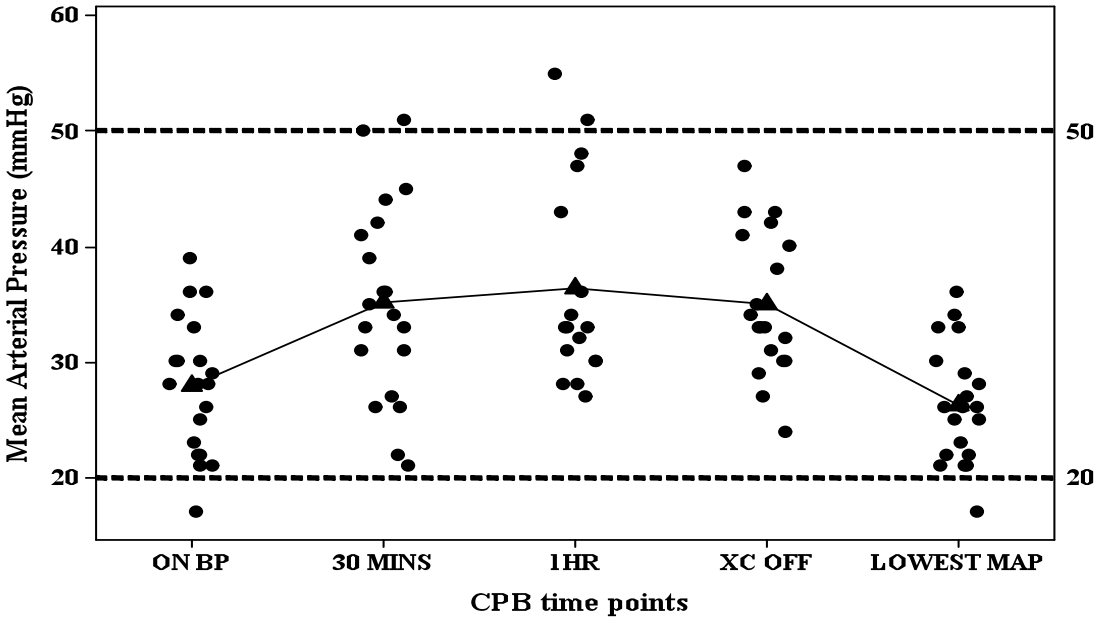
### *3.5.1.3* ***Figure 1c.*** *Peri-operative oxygen delivery (--- critical dysoxic threshold)*

*Mean values interconnected. Wide variation in oxygen delivery demonstrated with increased levels prior to cross-clamp removal (XC off) in conjunction with increasing pump flows and haematocrit.*




### *3.5.1.4* ***Figure 1d.*** *Peri-operative mean arterial pressure (--- ideal parameter boundaries)*

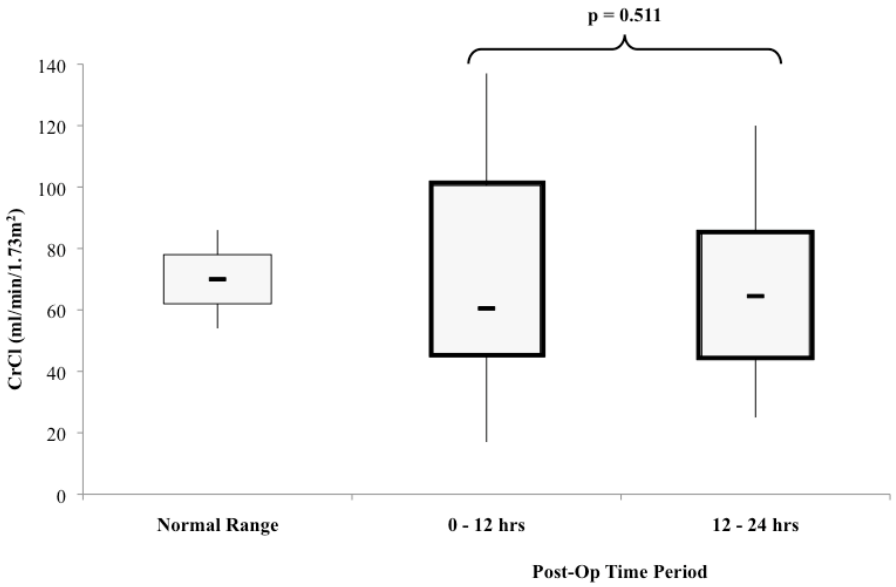
*Mean values interconnected.*



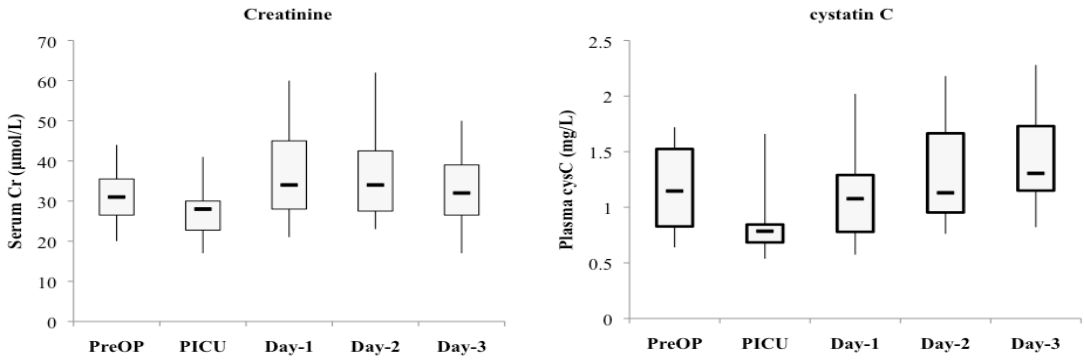
### 3.5.2 Renal Function

There was no significant change in measured GFR in the first twenty-four hours after surgery (Figure 2). Pre-operatively, serum Cr and cysC were in the normal range for all patients and in contrast to CrCl both increased significantly during the early post-operative period continuing to peak on days two and three respectively (Figure 3). Serum Cr levels returned to normal by day three. This deterioration in renal function was associated with a non-significant drop in urine output in the early post-operative period (Figure 4). In comparison to Cr, cysC demonstrated a superior correlation to measured GFR in the early post-operative period (Figure 5). No patients required post-operative renal replacement therapy.

#### *3.5.2.1 Figure 2. Indexed post-operative Creatinine Clearance*



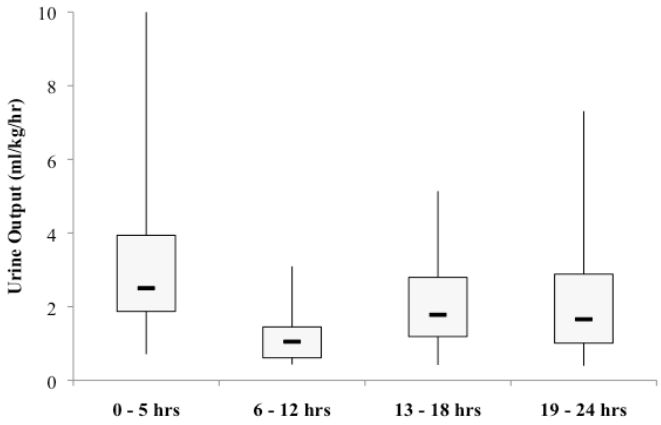
### *3.5.2.2 Figure 3. Peri-operative serum Creatinine and cystatin C*




| Comparison | p-value |
|---|---|
| PreOp vs. PICU | 0.13 |
| PICU vs. Day 1 | <0.001 |
| PICU vs. Day 2 | <0.001 |
| PICU vs. Day 3 | 0.05 |

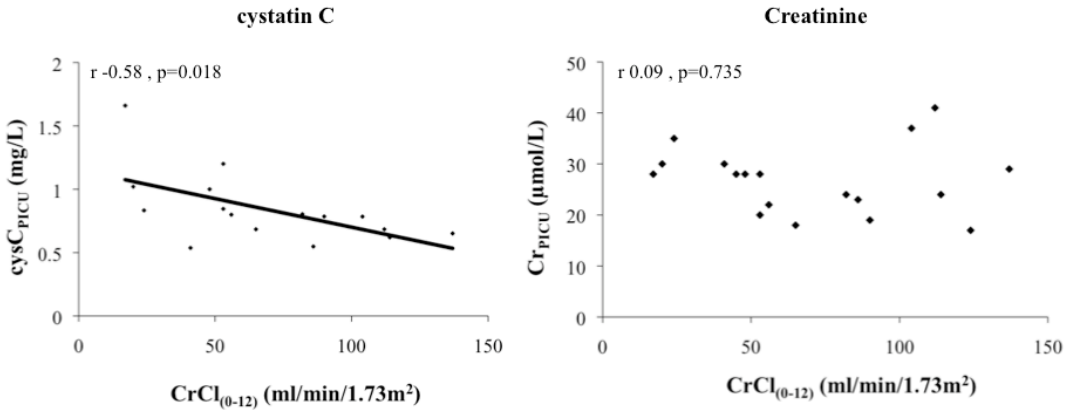
| Comparison | p-value |
|---|---|
| PreOp vs. PICU | 0.002 |
| PICU vs. Day 1 | 0.017 |
| PICU vs. Day 2 | 0.019 |
| PICU vs. Day 3 | 0.02 |

### *3.5.2.3 Figure 4. Early post-operative urine output (24hrs)*




| Comparison | p-value |
|---|---|
| 0-5 vs. 6-12 | 0.102 |
| 6-12 vs. 13-18 | 0.932 |
| 6-12 vs. 19-24 | 0.397 |

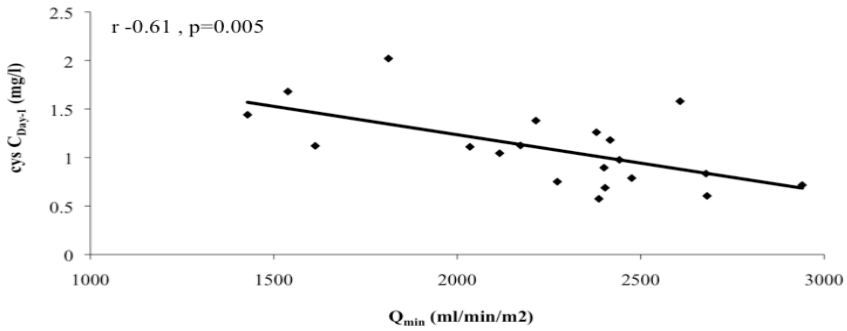
#### *3.5.2.4 Figure 5. Correlation of cystatin C and serum Creatinine with CrCl*



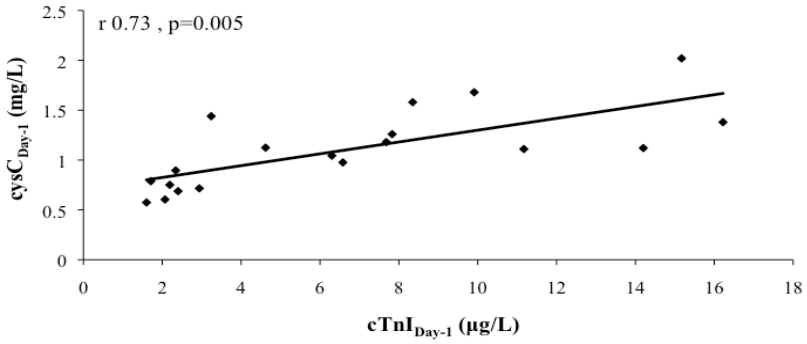

### 3.5.3 Univariate Analyses

$CrCl_{12-24}$ <85ml/min/1.73m$^2$ was not significantly associated with any of the measured variables whereas <55ml/min/1.73m$^2$ correlated with XC (p=0.033) time. $cysC_{Day-1}$ was significantly associated with $Q_{min}$ (p=0.005; Figure 6a), $cTnI_{Day-1}$ (p<0.001; Figure 6b), BP and XC times (both, p=0.001). $cysC_{Day-1}$ did not correlate significantly with $DO_{2\ min}$ (p=0.47) or $Hct_{min}$ (p=0.336). However, $DO_{2\ min}$ <300ml/min/m$^2$ demonstrated a significant association with $cysC_{Day-1}$ (r=-0.68, p=0.007).

### *3.5.3.1 Figure 6a. Correlation of cystatin C and lowest pump flow during CPB*




### *3.5.3.2 Figure 6b. Correlation of cystatin C and cTnI*



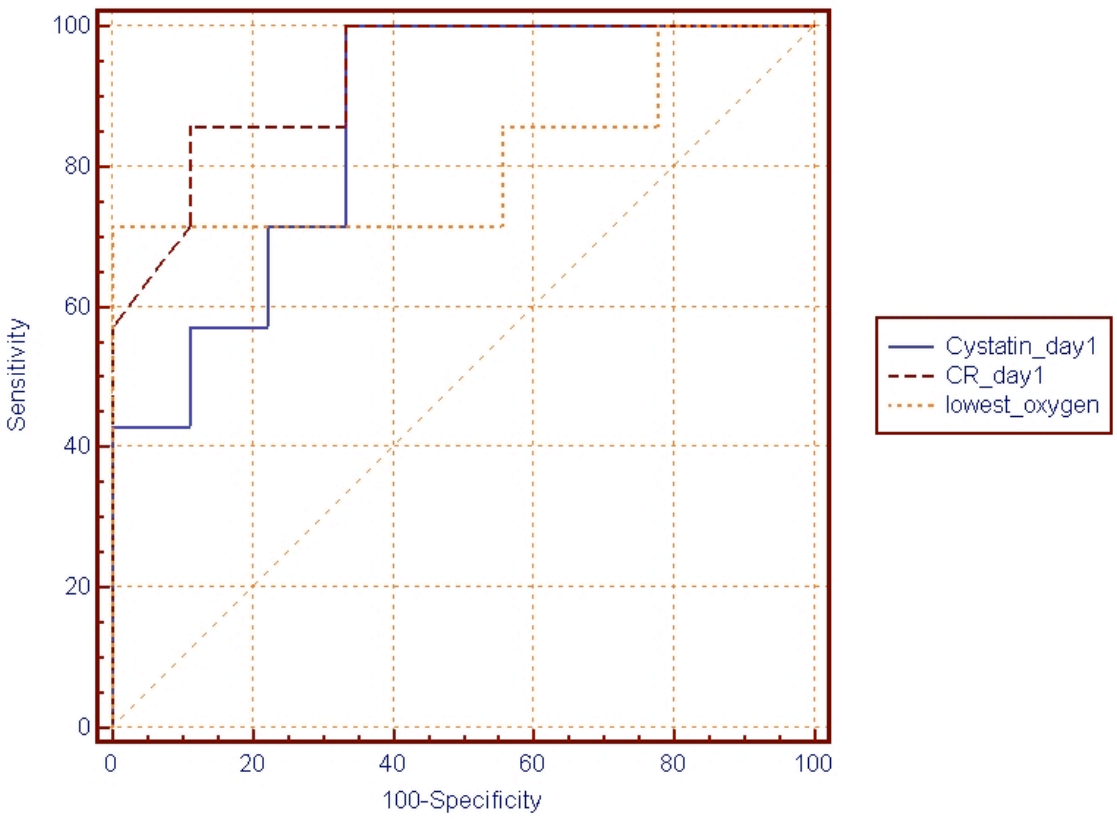
### 3.5.4 Multivariable Analyses

XC time (OR 1.07, 95% CI (1.01,1.14)) in association with $CrCl_{12\text{-}24}$ <55ml/min/1.73m$^2$ and $cTnI_{Day\text{-}1}$ in association with $cysC_{Day\text{-}1}$ (p=0.01) and $Cr_{Day\text{-}1}$ (p<0.001) were the only significant independent predictors of early post-operative renal dysfunction in this patient group.

### 3.5.5 ROC Analyses

$cysC_{Day1}$ >1.044mg/l exhibited a 100% sensitivity and 67% specificity for detecting renal dysfunction, defined as a GFR<55ml/min/1.73m$^2$, compared to 85% sensitivity and 88% specificity for $Cr_{Day1}$ >34µmol/l. $DO_{2\ min}$ <242ml/min/m$^2$ exhibited a 72% sensitivity and 100% specificity for detecting renal dysfunction, defined as a GFR<55ml/min/1.73m$^2$ (Figure. 7).

#### 3.5.5.1 Figure 7. ROC



### 3.5.6 Clinical Outcomes

$cysC_{Day1}$ was the only marker of post-operative renal injury that significantly correlated with ventilation time ($p<0.001$) and PICU stay ($p=0.002$). Compared to serum Cr, cysC demonstrated superior correlation to overall hospital stay (hospital stay vs. $Cr_{Day1}$ and $cycC_{Day1}$: $r=0.62$, $p=0.005$ and $r=0.73$, $p<0.001$ respectively).

## 3.6 Discussion

Acute renal failure is a common complication after cardiac surgery which continues to be associated with significant morbidity and in the presence of replacement therapy, mortality. [1-5] In this prospective study, the use of cystatin C to predict early renal dysfunction following paediatric cardiac surgery and the related impact of CPB parameters was investigated. Our principal findings were that: (1) cystatin C is a more sensitive marker of early renal dysfunction than serum creatinine (2) CrCl is not significantly altered in the first 24hrs after surgery (3) intra-operative ischaemia-reperfusion and myocardial injury were independent predictors of early renal dysfunction and (4) a raised cystatin C post-operatively is significantly linked to clinical outcome measures of ventilation time and PICU stay.

Serum creatinine and urine output are commonly used to define ARF whose pathophysiology involves a broad pattern of peri-operative mechanisms. [1, 4, 5] However, in addition to being influenced by age, height, sex and muscle mass creatinine is actively secreted by the proximal tubules resulting in as much as a twofold overestimation of GFR.[1, 12-18] Importantly, this disparity increases as GFR decreases further limiting the ability of creatinine to monitor moderate renal dysfunction. As a result, approximately 50% of renal function can be lost without an increase in serum creatinine. [12, 14] Urine output is a far less specific marker of renal

function and severe ARF can exist despite normal urine production. [1, 5] Changes in urine output, in the absence of diuretics, can occur long before biochemical changes are apparent making it an important consideration. According to published consensus definitions of ARF, no patient in this study developed an acute (<24hrs) post-operative renal injury or failure. [5]

Cystatin C is also freely filtered by the glomerulus but unlike creatinine is neither secreted by the renal tubules nor reabsorbed into the bloodstream making it an 'ideal' endogenous marker of renal function.[13, 14-19] In adult patients with varying chronic and acute renal pathologies, renal transplant, oncology and elderly patients cystatin C has consistently been shown to be more sensitive than creatinine in measuring small changes in GFR. [12, 13, 16-18] The independence of cystatin C on height, gender, age and muscle mass is of particular importance in the paediatric population. Pham-Huy et al demonstrated that compared to creatinine only cystatin C correlated with measured GFR ($^{99m}$Tc DTPA) in children with spina bifida who tend to be of short stature and low muscle mass. [19] Filler et al provided evidence that cystatin C is independent of age in 216 paediatric urology patients (0.8 – 18 yrs) with normal GFR determined by $^{51}$Cr-EDTA clearance. [20]

To our knowledge the evaluation of cystatin C as a marker of acute renal injury has not been previously reported in this patient group. In keeping with the current evidence this study demonstrated that cystatin C (>1.044mg/L) was more sensitive than creatinine (100% vs. 85%) in predicting early renal dysfunction defined as CrCl <55 ml/min/1.73m$^2$. Furthermore, cystatin C alone correlated significantly with CrCl after cardiac surgery allowing earlier identification of at-risk patients with potential implications for reducing post-operative morbidity and mortality. Compared to creatinine, cystatin C was more significantly associated with post-operative clinical outcomes of ventilation time and PICU stay. However serum cystatin C is not currently available on routine biochemical analysis

and remains an expensive option (£15-20/test). Future studies demonstrating significant reductions in overall hospital stay with cystatin C based perioperative management are needed to drive the clinical impetus necessary for the routine availability of this test.

In agreement with previous studies significant intra-operative correlates of early post-operative renal dysfunction included myocardial injury, pump flow, cross-clamp and CPB duration. [2-5] However, after adjusting for possible confounding influences amongst variables only cross-clamp time and myocardial injury, measured by cardiac troponin-I, were found to be significant independent predictors. Post-operative troponin-I has previously been shown to be significantly associated with the extent of peri-operative myocardial injury, manifest post-operatively as a low cardiac output, and is predictive of outcome including acute renal failure in a similar patient group. [6, 11, 21, 22] Both cystatin C and creatinine significantly correlated with troponin-I in this study highlighting the importance of maintaining cardiac output during the early post-operative period on renal dysfunction. Renal ischaemia, unlike cardiac, is generally silent with signs and symptoms unlikely to identify high risk patients making the availability of a sensitive serum biomarker an important clinical decision-making tool.

The principal objective of CPB is the delivery of oxygen to the systemic circulation during periods of cardio-pulmonary arrest necessary for cardiac surgical repair. In addition to the inflammatory cascades and low cardiac output state associated with CPB the renal medulla is particularly vulnerable to even minor reductions in oxygen delivery.[3, 5-10] Risk of peri-operative renal injury is further exacerbated in infants due to immature auto-regulatory function and reduced renal blood flow. [5, 7, 8] Current evidence suggests that a low haematocrit level (<25%) during CPB is associated with post-operative renal dysfunction, low cardiac output syndrome, lower psychomotor scores and mortality. [3, 9, 10, 23] Despite

mean haematocrit levels of 23.75% in this study only lowest pump flow during CPB was significantly linked with post-operative renal impairment as defined by cystatin C. This may imply that intra-operative oxygen delivery, which is dependent on both pump flow and haematocrit, is of greatest overall importance in preserving post-operative organ function. The significance of oxygen delivery was however not readily apparent in our study suggesting either that renal-specific oxygen delivery was maintained or oxygen extraction increased during CPB or that a 'critical threshold' exists within the renal parenchyma that has to be breached before notable damage occurs.

Indeed Boston et al demonstrated an organ-specific hierarchical distribution of oxygen delivery during CPB in pigs with oxygen delivery to muscle and visceral organs sacrificed to maintain the brain and kidneys. It was not until pump flow dropped to less than 1.4L/min/$m^2$ that a significant reduction in oxygen delivery to the kidneys was observed. [24] Interestingly in this study a mean lowest pump flow of 2.2L/min/$m^2$ during CPB was significantly associated with acute post-operative renal dysfunction. Evidence of a dysoxic threshold exists in adult critical care patients in whom an oxygen delivery of less than 325ml/min/$m^2$ results in anaerobic metabolism, lactic acidosis and eventual multi-organ dysfunction. [3, 25] This concept is further supported in this study where an oxygen delivery of less than 300ml/min/$m^2$ became significantly associated with post-operative renal impairment. In addition, ROC analyses demonstrated that a cut-off value of 242ml/min/$m^2$ exhibited a 72% sensitivity and 100% specificity for detecting renal dysfunction, defined as a GFR <55ml/min/1.73$m^2$. This value is within the range quoted in similar studies involving adult CPB. [3, 25] Our data therefore defends the hypothesis of an ischaemic insult ultimately being responsible for early renal dysfunction after paediatric cardiac surgery emphasising the need for improved categorisation and optimisation of perfusion parameters during surgery.

## 3.7 Study Limitations

Blood lactate measurements were not recorded peri-operatively in this study and we are therefore unable to demonstrate that patients with critical oxygen delivery developed an increased lactic acidosis. The relationship between haematocrit on CPB, related blood transfusion and post-operative renal dysfunction was not explored. The incidence of 'low cardiac output syndrome' which is a recognised cause of post-operative renal dysfunction in this patient group was not investigated in this study.

## 3.8 Conclusion

This study has shown that in comparison to serum creatinine, cystatin C provides a better estimate of GFR in children immediately after corrective cardiac surgery. Early post-operative renal impairment is independently predicted by ischaemia-reperfusion times and the degree of myocardial injury measured by Troponin-I. Oxygen delivery may be an important consideration for the future development of perfusion parameters.

## 3.9 Acknowledgements

This study was supported through a successful research grant application to 'The Association of Children with Heart Disorders'.

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# CHAPTER 4: PULMONARY FUNCTION

## 4.1 Abstract

### 4.1.1 Background

To investigate the acute effects of pre-operative sildenafil on haemodynamics, oxygenation and cyclic-guanosine-monophosphate (cGMP) levels in children at risk of pulmonary hypertension after cardiac surgery.

### 4.1.2 Methods

24 children (median age, 0.4 years) undergoing repair of VSD-type congenital heart disease were randomized to oral sildenafil (0.5mg/kg) or placebo 6hrly the day before surgery. Blood samples were collected peri-operatively to determine cGMP and NO production. Haemodynamic and echocardiography data were acquired at 2 and 24 hours post-operatively: (1) Mean pulmonary artery and left atrial pressures (2) Pulmonary vascular resistance index (PVRI) = transpulmonary gradient/pulmonary blood flow on spectral Doppler and (3) Bi-ventricular systolic tissue Doppler (TDI) velocities ($Sa_{LV}$, $Sa_{RV}$). Post-operative oxygenation was assessed by oxygen delivery ($DO_2$) and oxygenation index (OI) in PICU and on day-1.

### 4.1.3 Results

cGMP (pmol/ml) levels and NO (μM, total nitrate/nitrite) production trended higher in the sildenafil group (149.50 (0.5-344.80) vs. 19.30 (0-457.60), p=0.47; 0.70 (0-142.80) vs. 0.00 (0-52.70), p=0.62) whilst PVRI ($WU.m^2$) remained unchanged at 2 (2.67±0.91 vs. 2.07±1.98, p=0.43) and 24 hours (2.64±2.28 vs. 1.9±1.12, p=0.43) post-operatively. Bi-ventricular systolic TDI parameters (cm/sec) were significantly reduced in the sildenafil

group both pre- ($Sa_{LV}$ 3.78±0.94 vs. 4.55±1.08; $Sa_{RV}$ 6.93±1.47 vs. 8.09±2.25, p=0.002) and post-operatively ($Sa_{LV}$ 2.54±1.74 vs. 3.29±1.16; $Sa_{RV}$ 2.32±0.77 vs. 3.20±1.53, p=0.002). Post-operative $DO_2$ (ml/min/$m^2$) was significantly reduced in the sildenafil group (57.18±21.24 vs. 74.13±35.46, p=0.04) with OI trending higher (5.29±4.60 vs. 3.38±2.54, p=0.26).

### 4.1.4 Conclusion

This study identified that pre-operatively administered sildenafil produced a modest but not significant increase in cGMP and NO production without effect on pulmonary vascular resistance. Sildenafil was associated with reduced ventricular contractility and post-operative oxygenation.

## 4.2 Introduction

In children with ventricular septal defect physiology excessive pulmonary blood flow, at systemic pressures, can adversely affect the pulmonary endothelium evident as increased pulmonary vascular resistance (PVR) and episodes of pulmonary hypertension (PHT). [1-3] Pulmonary endothelial dysfunction (PED) can be further exacerbated by subsequent cardiopulmonary bypass (CPB) required for repair by several mechanisms including cytokine activation and ischaemia-reperfusion injury. [1] Post-operatively, these children can demonstrate an increased pulmonary vascular reactivity with acute elevations in PVR initiating a cycle of right ventricular failure and reduced cardiac output. Increased pulmonary capillary permeability augments the alveolar-arterial oxygen gradient with reduced lung compliance and decreased systemic oxygenation. This often results in prolonged ventilation and intensive care stay, that correlates with the degree of PVR reversibility. [1-3]

In the normal lung, nitric oxide (NO) is produced by the endothelium to regulate vessel tone, blood flow and optimise ventilation-perfusion coupling. PED can be defined as a failure of the pulmonary endothelium to produce adequate amounts of NO with a loss of vascular homeostasis. [2, 3] NO acts through a secondary messenger, intracellular cyclic-guanosine-monophosphate (cGMP), to vasodilate pulmonary blood vessels and is finally oxidised to form nitrate and nitrite. [2, 4] cGMP is metabolized by phosphodiesterase-5 (PDE-5) which is the predominant phosphodiesterase in normal lung and may be up regulated in patients with PED. [2, 4, 5]

Currently post-operative PED related complications, including PHT, are managed by the administration of inhaled NO (iNO). Although this has proved to be clinically effective, significant disadvantages do exist with some patients failing to respond and others developing life-threatening PHT upon withdrawal. [6,7] Recently oral sildenafil citrate (Pfizer, Sandwich, Kent, UK), a potent PDE-5 inhibitor that prevents the breakdown of cGMP, has been shown to be as effective as iNO in treating PHT. [8, 9] Oral sildenafil is now an established treatment for chronic PHT in children and is considered effective when given at therapeutic doses (0.5-2.0mg/kg; 4hrly). [10]

To date the consequences of oral sildenafil given pre-operatively to children undergoing corrective cardiac surgery has not been evaluated. We hypothesised that the administration of sildenafil prior to surgery would augment pulmonary cGMP levels attenuating CPB-induced post-operative endothelial dysfunction and the clinical consequences that ensue. A prospective, randomized, placebo-controlled, double-blind trial was performed to determine whether pre-operative oral sildenafil supplementation produces: (1) an effect on NO production and plasma cGMP (2) a reduction in PVR index and pulmonary artery pressure (3) an effect on cardiac contractility (4) an effect on ventilation and oxygen delivery and (5) clinical outcome including adverse events.

## 4.3 Methods

### 4.3.1 Patients

Study approval was obtained from local ethics committee (R&D no. 05/CH/01) and UK Medicines and Healthcare products Regulatory Agency (EudraCT No. 2005-001034-32). Eligible patients were children over 3 months of age with either ventricular or complete atrioventricular septal defects suitable for elective corrective cardiac surgery utilising CPB.

Parental consent was obtained. All children underwent elective surgery using standard techniques of CPB and blood cardioplegia.

### 4.3.2 Study Protocol

Following admission patients were randomised to either placebo or sildenafil by the clinical trials pharmacist using a computer-based stratified minimisation algorithm based on sex, cardiac diagnosis and the presence or absence of Down's syndrome (Minim, available at www.sghms.ac.uk/depts/phs/guide/randser.htm). [11] All clinicians and the child's parents were blinded to the treatment allocation.

Sildenafil (2.5mg/ml) and placebo suspensions (Ora Plus suspending vehicle, Ora sweet syrup +/- sildenafil) were prepared by the hospital pharmacy. Oral sildenafil (0.5mg/kg) or equivalent volume placebo was administered by the clinical trials pharmacist 6hrly the day before surgery. Patients were monitored pre-operatively on the ward and post-operatively in intensive care (PICU). Compliance and adverse events (systemic hypotension, post administration reactions etc.) were reported and monitored by the clinical trials pharmacist.

### 4.3.3 Data Collection

Patients returned to PICU after surgery and were sedated (morphine and/or midazolam), ventilated and haemodynamically stabilised with dopamine infusion. Residual intracardiac shunt or significant atrioventricular valve regurgitation was excluded through intra-operative epicardial and post-operative transthoracic echocardiography. All other intravenous NO-donors, PDE inhibitors and iNO were avoided. Data collection was deferred for 2 hours following admission to PICU to allow for rewarming, tracheal suctioning, adjustments to sedation and inotropic support. Mean arterial (MAP) and pulmonary artery (PA) pressures were recorded for the first 24 hours post-operatively.

Inotrope scores and blood lactate levels were documented in all patients at 2 and 24 hours post-operatively. [12] Arterial blood gas analysis was undertaken before any data collection to allow optimisation of ventilation and correction of acidosis. [12] Echocardiography and haemodynamic data collection were carried out at 2 and 24 hours post-operatively under standardised conditions (ventilated with an inspired oxygen fraction ($FiO_2$) of 0.65) to avoid variation in pulmonary vascular tone. [1, 2]

### 4.3.4 Blood samples

2mls of blood were aspirated directly from the LA and main PA before (post-heparin) and after CPB (pre-protamine). Samples were aliquoted and stored (-70$^{o}$C) until laboratory analysis. cGMP levels were measured using a commercially available competitive enzyme immunoassay (R&D Systems, Abingdon, UK) with a calibration range of 0.56 – 3.06 pmol/ml. Nitrate and nitrite levels were measured using a commercially available colorimetric assay kit (BioScience Ltd, Cambridge, UK) with a detection limit of 2.0 μM.

### 4.3.5 Echocardiography

All patients underwent transthoracic echocardiography using a Vivid 7 ultrasound scanner (GE Vingmed, Horten, Norway) with a 7-MHz probe by the same, experienced paediatric echocardiographer (1) pre-operatively, on table under anaesthesia (2) 2hrs and (3) 24hrs post-operatively. A cine loop of at least three consecutive cardiac cycles was recorded and stored digitally for later off-line analysis (Echopac, GE Vingmed). All echocardiographic measurements were repeated 3 times and then averaged.

#### *4.3.5.1 Estimated* aortic and pulmonary outflow (L/min/m$^2$)

Pulsed Doppler 2D echocardiography was used to estimate aortic ($Q_S$) and pulmonary ($Q_P$) outflow according to the formula: Q = (Stroke $Volume_{Doppler}$ x heart rate). Doppler-

estimated stroke volume was determined using the velocity time integral of flow through the left or right ventricular outflow tract x aortic or pulmonary annular cross-sectional area respectively. Both annuli were measured immediately proximal to the point of insertion of valve leaflets at maximal systolic leaflet separation (inner diameter).[13]

#### *4.3.5.2 Post-operative PVRI (Wu.m$^2$)*

Transpulmonary gradient (mean PA - LA pressure measured from respective monitoring lines placed in theatre as per study protocol) and estimated pulmonary blood flow on spectral Doppler were used to derive PVR from the equation: PVR = (transpulmonary gradient / pulmonary blood flow) which was then indexed to body surface area (PVR x BSA). [12]

#### *4.3.5.3 Tissue Doppler Imaging (TDI)*

Peak myocardial velocities (Sa) were recorded from basal septum ($Sa_S$) and lateral free walls of both ventricles immediately below (0.5cm) the insertion of the mural leaflet of the left ($Sa_{MV}$) and right ($Sa_{TV}$) AV valves. RV ($IVA_{RV}$) and LV ($IVA_{LV}$) isovolumic acceleration was calculated for respective ventricles by dividing the peak isovolumic velocity by the time interval from baseline to peak isovolumic velocity as previously described. [14]

### 4.3.6 Post-operative Oxygenation

Oxygen delivery ($DO_2$) at 2 and 24hrs post-operatively was calculated using Doppler derived systemic CO ($Q_S$) and arterial oxygen content according to the equation: $DO_2 = Q_s$ *x* [Hb x 1.34 x Hb saturation + 0.003 x $PaO_2$ (mmHg)]. Oxygenation index (OI) was calculated at the same time using: ventilator mean airway pressure (cm $H_2O$) x 100 x $FiO_2$ / $PaO_2$ (mmHg). The period of ventilation (hrs) and length of stay in PICU (hrs) were noted.

## 4.4 Statistical Analysis

The Anderson-Darling test assessed normality. Descriptive statistics are reported as means and standard deviations or medians and ranges according to distribution of data. Two-sample t-tests were used for between group comparisons in normally distributed data, otherwise Mann-Whitney tests were performed. A nested general linear model was used to examine the effects of drug and time on each outcome variable. If significant, post-hoc tests with Bonferroni correction were applied to quantify the effect. All analyses were performed using Minitab (version 15, Minitab Ltd, Coventry, Warwickshire, UK) at a significance level of 5%.

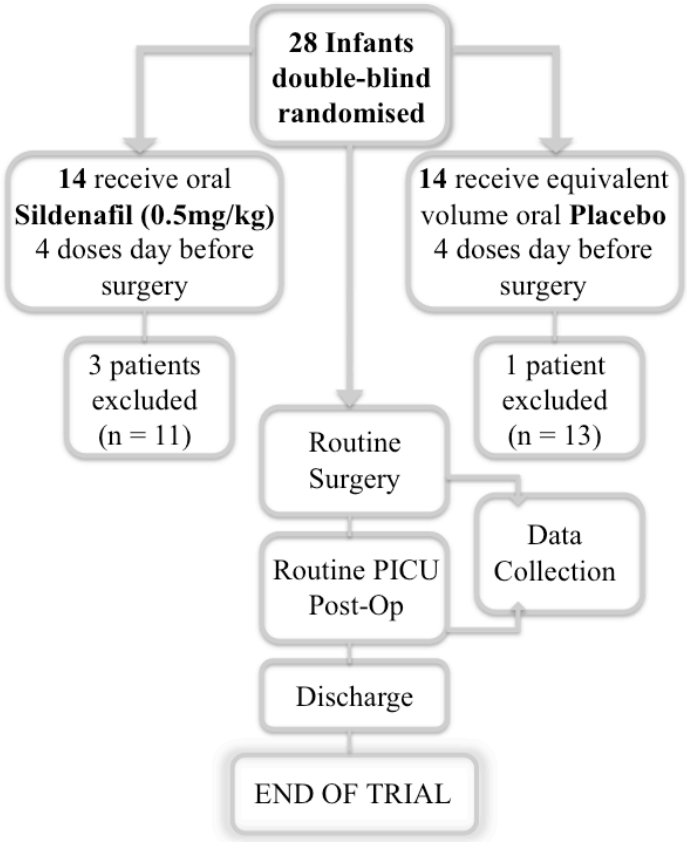
The power calculation for this study was based on data published by Schulze-Neick et al. where a mean PVRI of 8.32 $Wu.m^2$ and standard deviation of 3.464 was observed post-cardiac surgery. [2] A sample size of 12 patients in each group would have a power of 80% to detect a minimum difference in PVRI of 4.14 $WUm^2$ between oral sildenafil and placebo groups (nQuery, version 5.0, Statistical Solutions Ltd, Cork, Ireland).

## 4.5 Results

### 4.5.1 Patients

Twenty-eight children were prospectively recruited into the trial at the Royal Hospital for Sick Children, Yorkhill Division, Glasgow, UK from June 2006 to January 2008. 4 children were excluded following theatre cancellation leaving a total of 24 patients (age 0.26 to 2.26 years, median 0.4 years; mean weight 5.6kg) who completed the trial as depicted in Figure 1. Demographic characteristics of the two groups are summarised in Table 1.

#### *4.5.1.1 Figure 1. Trial Profile*




#### *4.5.1.2 Table 1. Demographic characteristics between groups*

| | | Sildenafil (n = 11) | Placebo (n = 13) | P-value |
|---|---|---|---|---|
| **Patient factors** | Gender (male) | 5 (45%) | 7 (54%) | 0.681 |
| | Age (years) | 0.62 ± 0.57 | 0.42 ± 0.17 | 0.286 |
| | Weight (kg) | 5.6 ± 1.3 | 5.7 ± 2.0 | 0.884 |
| | Trisomy 21 present | 6 (55%) | 6 (46%) | 0.681 |
| **Diagnosis** | AVSD | 4 (36%) | 7 (54%) | 0.444 |
| | VSD | 7 (64%) | 6 (46%) | 0.383 |
| **Operative factors** | XC (mins) | 75.1 ± 45.0 | 77.5 ± 36.9 | 0.889 |
| | BP (mins) | 126.7 ± 60.9 | 122.8 ± 46.7 | 0.864 |
| | Ventilation (hrs) | 93.2 ± 126.0 | 82.5 ± 56.6 | 0.799 |
| | PICU stay (hrs) | 128.3 ± 159.2 | 125.7 ± 75.4 | 0.961 |

Data expressed as mean ± SD.

AVSD, atrio-ventricular septal defect; VSD, ventricular septal defect; XC, cross-clamp time; BP, bypass time; PICU, paediatric intensive care unit

### 4.5.2 Adverse Events

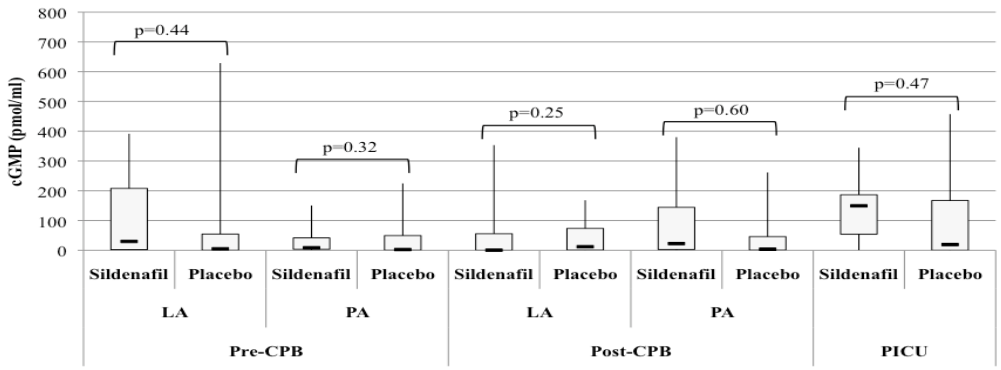
There were no post-administration drug reactions and no mortality; all patients were discharged home without significant complication. No significant difference in MAP (mmHg) existed between sildenafil and placebo groups post-operatively (58.53±3.92 vs. 57.49±1.65, p=0.15).

### 4.5.3 Plasma cGMP levels (pmol/ml)

Compared to placebo, pre-operative cGMP levels sampled from the LA and PA were higher in the sildenafil group (LA: 29.9 (0.5-391.5) vs. 5.2 (0-628.9), p=0.44 and PA: 8.7 (1.1-150.5) vs. 2.6 (0.0-224.9), p=0.32). cGMP levels were also greater in the sildenafil group post-operatively on admission to PICU (149.5 (0.5-344.8) vs. 19.3 (0-457.6), p=0.47). Overall 0.5mg/kg of oral, pre-operative sildenafil given 4 times the day before surgery raised peri-operative plasma cGMP but did not reach statistical significance (Figure 2).

#### *4.5.3.1 Figure 2. Plasma cGMP levels*

*(PICU sample acquired during routine venous blood sampling)*



### 4.5.4 Plasma Nitrate / Nitrite Levels (μM)

NO breakdown products, within LA, trended higher following CPB in the sildenafil group (pre-CPB vs. post-CPB: 0.0 (0-1.0) vs. 0.7 (0-142.8), p=0.057) compared to placebo group which remained similar (pre-CPB vs. post-CPB: 0.1 (0-41.4) vs. 0.0 (0-52.7), p=0.80) (Table 2).

#### *4.5.4.1 Table 2. Plasma nitrite / nitrate levels*

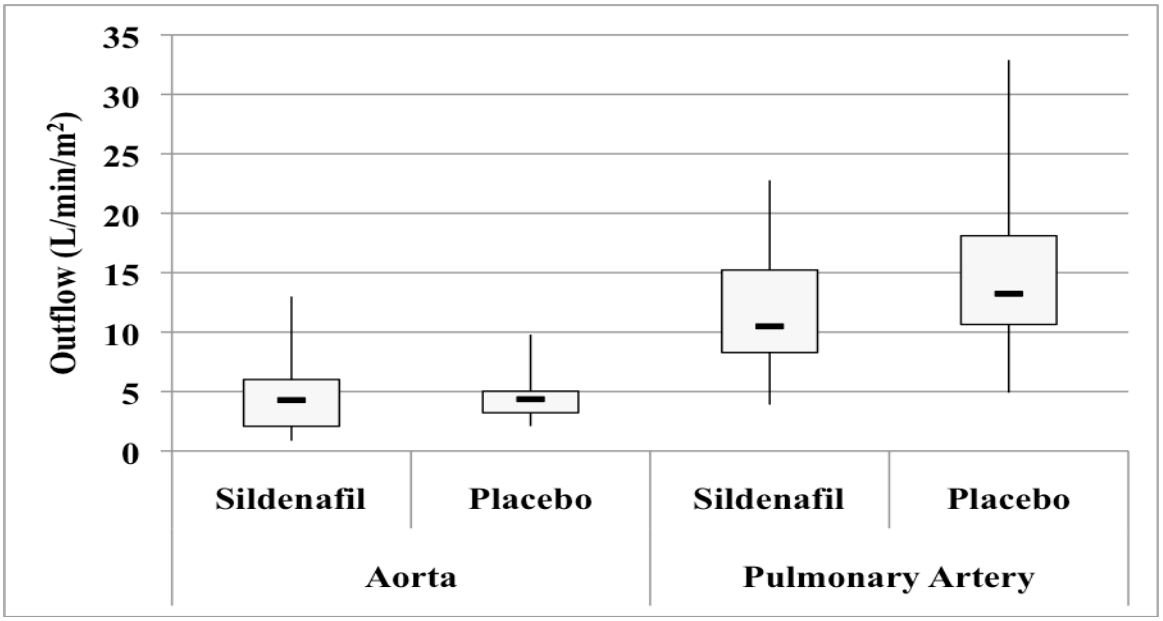
| Group | Time | Sample | Total plasma nitrite / nitrate levels (μM) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mean | StDev | Min | Median | Max |
| Placebo | Pre-CPB | LA | 3.95 | 11.34 | 0.00 | 0.09 | 41.39 |
| | | PA | 2.36 | 6.55 | 0.00 | 0.00 | 23.70 |
| | Post-CPB | LA | 5.49 | 14.34 | 0.00 | 0.00 | 52.73 |
| | | PA | 1.64 | 2.87 | 0.00 | 0.00 | 10.42 |
| Sildenafil | Pre-CPB | LA | 0.09 | 0.30 | 0.00 | 0.00 | 1.00 |
| | | PA | 0.23 | 0.75 | 0.00 | 0.00 | 2.47 |
| | Post-CPB | LA | 20.5 | 44.7 | 0.00 | 0.70 | 142.80 |
| | | PA | 6.37 | 17.31 | 0.00 | 0.73 | 58.37 |

### 4.5.5 Echocardiography

#### *4.5.5.1 Estimated pulmonary and aortic outflow (L/min/m$^2$)*

Pre-operative $Q_p$:$Q_s$ shunt fraction was similar between sildenafil and placebo groups (10.49:4.28 vs. 13.23:4.36, p=0.20) (Figure 3a). Doppler derived cardiac index was significantly reduced in the sildenafil group compared to control post-operatively in PICU (4.78±1.70 vs. 3.62±1.21, p<0.001) and day-1 (4.83±1.71 vs. 4.16±1.71, p<0.001).

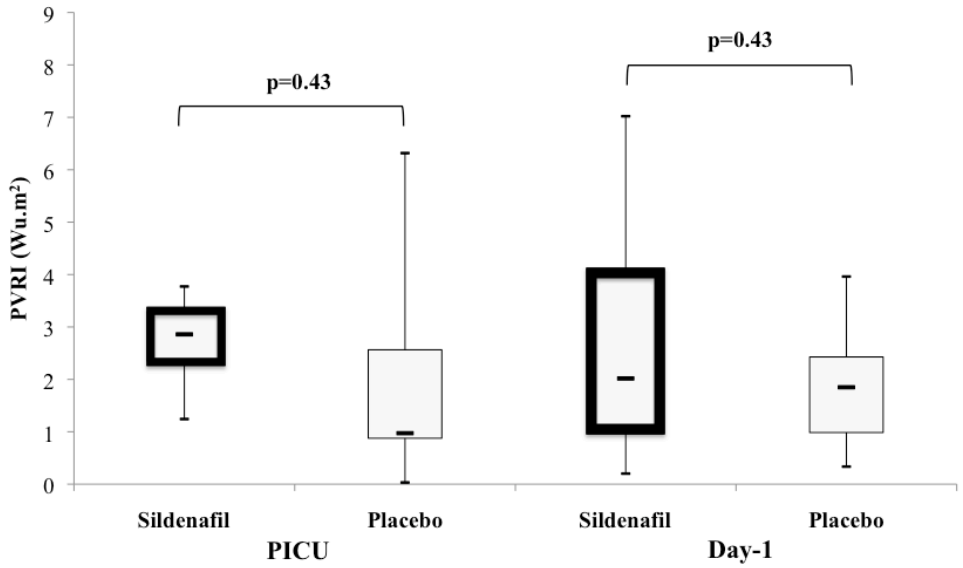
#### *4.5.5.1.1 Figure 3a. Pre-operative aortic and pulmonary outflow*




### *4.5.5.2 PVRI (Wu.m$^2$)*

Post-operatively, no significant alteration in PVRI was demonstrated in sildenafil patients at 2 (2.67±0.91 vs. 2.07±1.98) and 24 hours (2.64±2.28 vs. 1.90±1.12) (Figure 3b). Mean PA pressure (mmHg) in sildenafil and placebo groups was similar post-operatively (20.72±1.09 vs. 19.88±0.84, p=0.30).

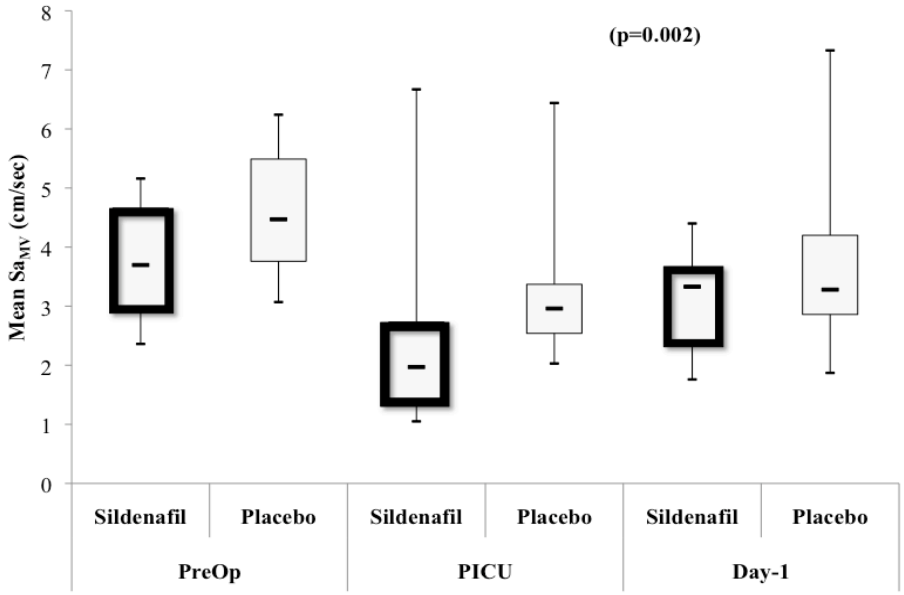
#### *4.5.5.2.1 Figure 3b. Post-operative PVRI*



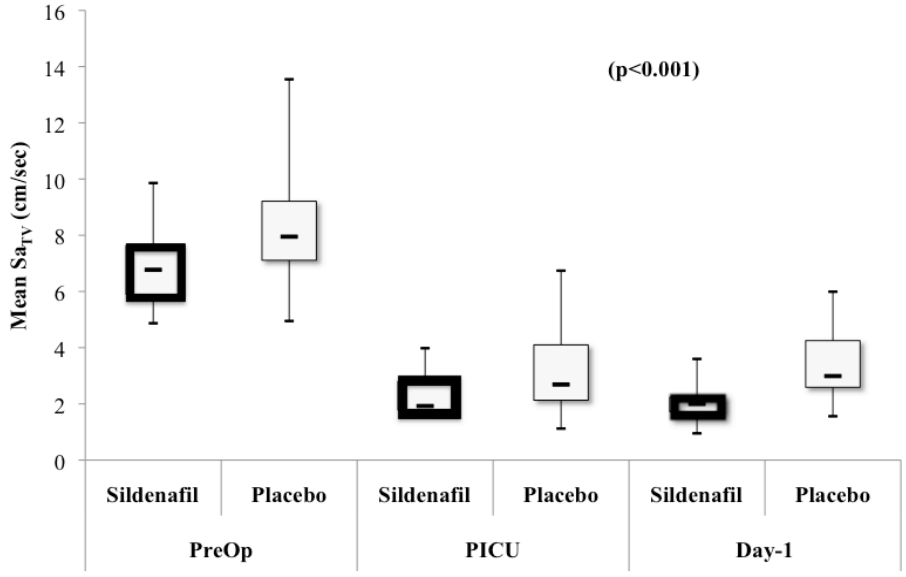

### *4.5.5.3 TDI*

LV, RV and septal peak systolic velocities and biventricular isovolumic acceleration were all significantly reduced in both groups in the immediate post-operative period. All velocities trended towards recovery by day-1, particularly in the LV. Pre- and post-operative bi-ventricular and septal systolic function were significantly reduced in the sildenafil group compared to placebo and this persisted onto day-1 (Figure 4a, b; Table 3). RV and LV isovolumic accelerations were also lower in the sildenafil group but this did not reach statistical significance (Table 4).

#### *4.5.5.3.1 Figure 4a. LV systolic function*




#### *4.5.5.3.2 Figure 4b. RV systolic function*



#### *4.5.5.3.3 Table 3. Peri-operative TDI peak systolic velocities*

| Group | Variable | Time | Peak Systolic Myocardial Velocity (cm/sec) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mean | StDev | Min | Median | Max |
| Placebo | LV | PreOp | 4.55 | 1.08 | 3.07 | 4.47 | 6.24 |
| | | PICU | 3.29 | 1.16 | 2.03 | 2.96 | 6.44 |
| | | Day-1 | 3.95 | 1.60 | 1.87 | 3.28 | 7.33 |
| | RV | PreOp | 8.09 | 2.25 | 4.95 | 7.95 | 13.55 |
| | | PICU | 3.20 | 1.53 | 1.12 | 2.69 | 6.74 |
| | | Day-1 | 3.35 | 1.38 | 1.56 | 2.99 | 5.99 |
| | Septum | PreOp | 4.70 | 1.03 | 2.39 | 4.86 | 5.8 |
| | | PICU | 2.30 | 1.13 | 0.83 | 2.09 | 4.53 |
| | | Day-1 | 2.61 | 1.26 | 0.97 | 2.45 | 4.65 |
| Sildenafil | LV | PreOp | 3.78 | 0.94 | 2.36 | 3.70 | 5.16 |
| | | PICU | 2.54 | 1.74 | 1.05 | 1.97 | 6.67 |
| | | Day-1 | 3.10 | 0.83 | 1.76 | 3.33 | 4.40 |
| | RV | PreOp | 6.93 | 1.47 | 4.87 | 6.77 | 9.86 |
| | | PICU | 2.32 | 0.77 | 1.61 | 1.93 | 3.98 |
| | | Day-1 | 2.06 | 0.75 | 0.95 | 2.00 | 3.60 |
| | Septum | PreOp | 3.91 | 0.69 | 2.96 | 3.85 | 4.97 |
| | | PICU | 1.56 | 1.11 | 0.80 | 1.065 | 4.39 |
| | | Day-1 | 2.09 | 0.98 | 0.55 | 2.00 | 3.66 |

#### *4.5.5.3.4 Table 4. Peri-operative TDI isovolumic acceleration*

| Group | Variable | Time | Isovolumic Myocardial Acceleration (cm/sec$^2$) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mean | StDev | Min | Median | Max |
| Placebo | LV | PreOp | 154.47 | 89.06 | 50.93 | 126.37 | 309.18 |
| | | PICU | 71.00 | 21.68 | 41.95 | 69.85 | 101.67 |
| | | Day-1 | 101.84 | 61.89 | 42.04 | 94.47 | 259.64 |
| | RV | PreOp | 309.55 | 107.74 | 177.34 | 282.44 | 527.17 |
| | | PICU | 133.50 | 55.75 | 50.33 | 132.63 | 227.92 |
| | | Day-1 | 103.92 | 53.28 | 42.49 | 97.70 | 200.19 |
| Sildenafil | LV | PreOp | 162.73 | 51.83 | 82.33 | 157.77 | 265.40 |
| | | PICU | 64.40 | 14.54 | 46.01 | 68.51 | 79.46 |
| | | Day-1 | 90.71 | 37.18 | 51.83 | 84.48 | 158.5 |
| | RV | PreOp | 303.88 | 84.16 | 187.66 | 290.12 | 489.43 |
| | | PICU | 116.66 | 59.20 | 63.78 | 108.22 | 229.82 |
| | | Day-1 | 135.07 | 69.45 | 55.92 | 117.07 | 290.70 |

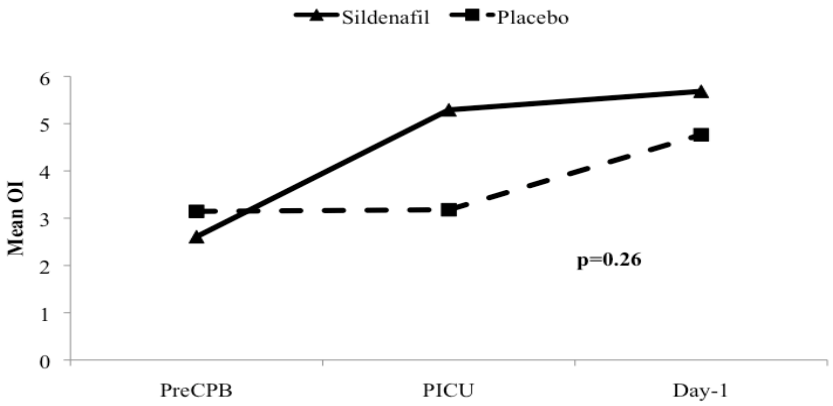
### *4.5.6 Inotrope Scores*

No significant difference in inotrope score existed between sildenafil and placebo patients at 2 (5.73±5.12 vs. 5.15±4.36, p=0.884) and 24 (5.55±4.61 vs. 5.62±5.59, p=0.884) hours post-operatively.

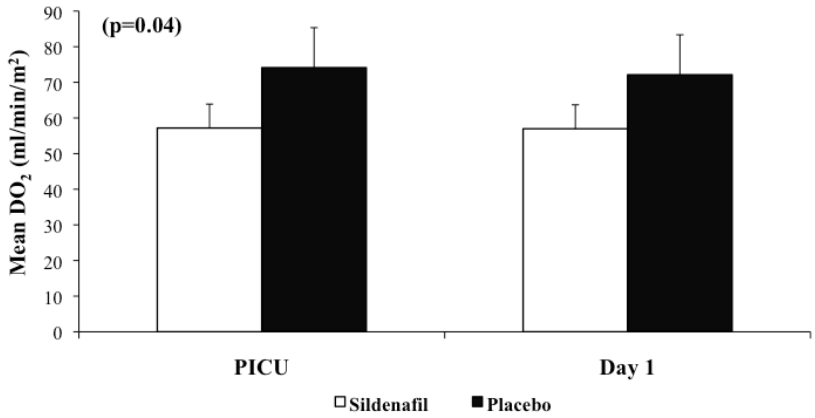
### 4.5.7 Post-operative oxygenation

OI trended higher in the sildenafil group compared with control on admission to PICU (5.3±4.6 vs. 3.4±2.5) with further increases in both groups on day-1 (5.7±4.3 vs. 4.8±3.4, Figure 4c). $DO_2$ (ml/min/$m^2$) was significantly reduced in the sildenafil group compared with control on admission to PICU (57.18±21.24 vs. 74.13±35.46) and on to day-1 (56.98±23.64 vs. 72.13±33.00, Figure 4d) (Table 5). No significant differences in ventilation time (hrs) or PICU stay (hrs) were found between sildenafil and placebo groups (Table 1). Subgroup analysis of T21 patients demonstrated no significant differences in post-operative OI (p=0.09), $DO_2$ (0.07), ventilation time (p=0.07) or PICU stay (p=0.141).

### *4.5.7.1 Figure 4c. Peri-operative Oxygenation Index*




### *4.5.7.2 Figure 4d. Post-operative Oxygen Delivery*



#### *4.5.7.3 Table 5. PaO2 values at 2 and 24 hours post-operatively*

| Group | Time | $PaO_2$ (mmHg) | | | | |
|---|---|---|---|---|---|---|
| | | Mean | StDev | Min | Median | Max |
| Placebo | 2 hrs | 178.17 | 59.03 | 90.00 | 179.25 | 321.00 |
| | 24 hrs | 93.41 | 25.88 | 32.25 | 100.50 | 126.00 |
| Sildenafil | 2 hrs | 142.57 | 49.34 | 71.25 | 141.00 | 221.60 |
| | 24 hrs | 91.53 | 28.61 | 54.75 | 86.13 | 135.00 |

### 4.5.8 Lactate

No significant differences were seen in blood lactate levels (mmol/L) between sildenafil and placebo patients at 2 (1.1±0.2 vs. 1.2±0.4, p=0.307) and 24 (1.2±0.3 vs. 1.1±0.3, p=0.307) hours post-operatively.

## 4.6 Discussion

In children with congenital heart disease post-operative pulmonary endothelial dysfunction and pulmonary hypertension continue to be an important cause of morbidity, prolonged PICU stay and in severe cases mortality.[1-5] The purpose of this study was to investigate whether administrating sildenafil prior to paediatric cardiac surgery mitigates clinical consequences of post-operative pulmonary endothelial dysfunction by maintaining cGMP levels. Our principal findings were that pre-operative sildenafil dosing resulted in: (1) modest elevations in plasma cGMP levels both pre- and post- CPB but this did not reach statistical significance (2) increased post-CPB NO production (3) no significant effects on post-operative PVRI (4) a significant reduction in peri-operative bi-ventricular contractility and (5) a significant reduction in post-operative oxygen delivery.

Although children with VSD or AVSD operated on at 6 months of age or less are unlikely to demonstrate classical clinical pulmonary hypertensive crises post-operatively the clinical consequences of pulmonary endothelial dysfunction are commonplace. [1] The endothelial injury following CPB is primarily related to ischaemia-reperfusion and cytokine activation characterized by capillary permeability leading to decreased oxygenation manifest as increased alveolar-arterial oxygen gradient and decreased lung compliance. [1-3, 5, 15] The pulmonary endothelium plays an important role in vasomotor tone, inhibiting platelet aggregation and neutrophil adhesion through the release of vasoactive factors including NO. [5, 9, 16] CPB results in PDE-5 over activity, activation of NO antagonists, reduction in NO precursors and production of vasoconstrictive factors. [1-4] Ultimately this can increase right ventricular afterload, adversely affecting right ventricular function and increasing the duration of ventilation. [1] Many centers will now liberally use iNO and sildenafil in the post-operative setting to manage these complications.

Sildenafil is a potent and selective inhibitor of 3'5'-cGMP-specific PDE-5 with substantially lower affinity for the other PDE isoenzymes. Tissue distribution of PDE-5 and hence site of action of sildenafil is well defined (corpus cavernosum, pulmonary vasculature, platelets, skeletal and visceral muscle). Sildenafil is rapidly absorbed orally with an absolute bioavailability of 40%. It undergoes hepatic metabolism with an elimination half-life of 3 to 5 hours. [17, 18] Children in the active arm of our study received 0.5mg/kg of oral sildenafil 6hrly, the day before surgery. This dose was based on our own clinical experience and current best available evidence that (0.025-0.66mg/kg) of intravenous sildenafil and (0.25-1mg/kg; 6hrly) of oral sildenafil, safely and significantly reduces PHT in this patient group. [2, 15, 16, 19]

In this study the direct effect of sildenafil on pulmonary endothelial function was assessed by measuring plasma cGMP and NO production via its breakdown products nitrates and nitrites. Our study demonstrated that although sildenafil increased cGMP levels both pre- and post- bypass, the effect was modest and variable between patients. The effects of sildenafil on plasma cGMP levels is not widely reported particularly in relation to peri-operative paediatric cardiac surgery. Atz et al demonstrated that rebound pulmonary hypertension on withdrawal of iNO following paediatric cardiac surgery was attenuated by sildenafil. In their study, post-operative oral sildenafil (0.33mg/kg), in association with iNO, resulted in cGMP levels of up to 45 pmol/ml. [7] Sildenafil infusions used pre-operatively by Schulze-Neick et al produced a non-significant increase in plasma cGMP (44±18 pmol/ml) associated with significant reductions in PVRI. [2]

In this study NO products inclined to be higher post-CPB in the sildenafil group, suggesting a greater capacity of the pulmonary endothelium in these patients to respond to the CPB mediated injury. [1-3, 5, 15] Interestingly, compared to sildenafil patients, NO

products tended to be higher in the placebo group pre-CPB. It is possible that increased cGMP levels induced by sildenafil in the treatment group down-regulated NO production pre-CPB. Again, in a similar pattern to cGMP, there was considerable variation amongst sildenafil treated patients and the NO response. These findings support the conclusion that sildenafil administered at 0.5mg/kg was associated with a biological effect at the pulmonary endothelial level.

Despite trends in the sildenafil group, to higher cGMP levels pre-CPB (108 pmol/ml) and increased NO production post-CPB no significant haemodynamic effect on PVRI or post-operative PA pressure was apparent. The reasons for this are likely to be multi-factorial. The majority of children recruited to the trial were under 6 months and therefore at low risk for post-operative PHT crises. Furthermore, none of the children recruited to the trial had pre-existing PHT by echo criteria (bi-directional flow at VSD). It is possible that in a more at-risk group (longstanding VSD with pre-operative PHT) sildenafil may have had a significant impact on PA pressures and PVRI. Alternatively, larger doses of oral sildenafil may have been required pre-operatively to produce a measureable pulmonary vasodilatory effect. Intra-individual variations in the response to oral sildenafil have been demonstrated in some patients with persistently high PVR despite increasing vasodilator therapy. [15] This supports our findings of patient variation in cGMP and NO production in the sildenafil group. However, it should be noted that other studies with a similar patient cohort have demonstrated reductions in PVR with sildenafil administrated during the post-operative phase. [1, 2, 15]

An important finding of this study was that bi-ventricular systolic function was significantly reduced in the sildenafil group compared to placebo. This contractile change was measured using tissue Doppler peak systolic velocities, which are limited by their

load-dependant; right ventricular velocities could be reduced by acute elevations in afterload. [14, 20, 21] However, this mechanism is not supported by the study findings of similar post-operative PA pressures between groups. Furthermore the velocities were reduced in all myocardial areas measured. Post-operatively, this was associated with a significant reduction in cardiac index in the sildenafil group which also impacted on oxygen delivery. To our knowledge such a negative inotropic effect has not been described in any clinical studies although transient falls in systemic blood pressure are widely reported. One possible explanation for the impaired contractility demonstrated in the sildenafil group could have been an increase in intra-cardiac shunting and subsequent volume loading through reduced PVR. However the Qp:Qs derived by spectral Doppler, on-table immediately prior to surgery, did not indicate an increased shunt volume in sildenafil group compared with placebo.

Experimental models of isolated cardiomyocytes, perfused rodent hearts and human studies of normal and heart failure patients at the time of cardiac catheterisation have shown that elevated levels of cGMP (produced through iNO or oral sildenafil) can suppress contractility by decreasing myocardial calcium sensitivity, cAMP inhibition and blunting the contractile response to adrenergic stimulation. [17, 22-24] We propose that this is relevant in the context of paediatric cardiac surgery where myocardial contraction is modulated by endogenous and exogenous catecholamine stimuli and may explain why pre-operative sildenafil inhibits peri-operative contractility in this patient group. Ultimately, the reduced contractility seen in the sildenafil group was not clinically apparent in terms of increased inotrope usage, ventilation time or intensive care stay.

In this study the pre-operative administration of oral sildenafil resulted in a significant deterioration in post-operative tissue oxygenation. This was demonstrated through

increasing post-operative oxygenation index and significant reduction in oxygen delivery from admission to PICU. In contrast to iNO which is selectively delivered to ventilating areas of the lung, the systemic administration of sildenafil acts throughout the pulmonary vasculature with resulting dose-dependent increase in ventilation-perfusion mismatch seen in both human and animal studies. [1, 2, 15, 25] Stocker et al showed that intravenous sildenafil (0.35mg/kg) resulted in a significant decrease in $PaO_2$ post-operatively associated with increased oxygenation index and alveolar-arterial gradient. [15] Crucially these deleterious effects on oxygenation were not reversed by iNO and the trial was terminated early. Similarly Schulze-Neick et al demonstrated significant dose-dependent increases in intra-pulmonary shunt ratio during sildenafil infusion in children following corrective cardiac surgery. [2] Kleinsasser et al investigated the dose dependent effects of oral sildenafil (25-100mg) on pulmonary function in anaesthetised and ventilated pigs. All doses of sildenafil caused significant increases in intrapulmonary shunt flow measured by inert gases and reflected through marked decreases in $PaO_2$. [25] This is further exacerbated by the acute lung injury, related to the systemic inflammatory response, that is associated with CPB. [5] The effects on post-operative oxygenation were well tolerated in our stable group of study patients and were not associated with prolonged ventilation or PICU stay. However, in a group of children with higher ventilatory requirements or borderline pulmonary function a similar degree of impairment could have adverse effects.

Certain limitations of this study should be mentioned. Intra-pulmonary shunt was not measured as part of the study and as such we can only infer this probable hypoxic mechanism. Pre-operative PVRI was also not measured and as such the compounding effects of CPB could not be excluded or evaluated. Peri-operative plasma sildenafil concentrations were not measured making it impossible to comment on the intra-individual variations in drug levels post-operatively and the effects if any of CPB.

## 4.7 Conclusion

Oral sildenafil (0.5mg/kg) administered before elective cardiac surgery in children with VSD physiology resulted in modest elevations in plasma cGMP levels and post-operative NO production but no effect on pulmonary haemodynamics or clinical outcome. Significant impairments to global peri-operative systolic function and post-operative oxygenation would caution the use of pre-operative sildenafil.

## 4.8 Acknowledgements

This study was supported through a successful research grant application to 'The Yorkhill Children's Foundation'.

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# CHAPTER 5: FINAL DISCUSSION

The aim of this thesis was primarily to investigate end organ effects of modern day cardiopulmonary bypass in children with common congenital heart defects undergoing elective repair utilising cardiopulmonary bypass. End organ function was investigated through two prospective clinical trials which in turn examined the relationships between tissue Doppler imaging and myocardial injury, cystatin C and renal dysfunction and sildenafil and pulmonary endothelial dysfunction.

It is clear from Chapter 2 that myocardial dysfunction is commonplace following paediatric cardiac surgery with some recovery of at least left ventricular function evident within twenty four hours after surgery. TDI appears to offer a sensitive, non-invasive, load-independent, real time assessment of peri-operative myocardial function which until the completion of this study was not routinely used within the Department of Paediatric Cardiac Surgery, Yorkhill Hospital. Indeed there are very few studies published in the literature which characterise the typical pattern of change of these parameters within this patient population.

Right ventricular dysfunction is often clinically difficult to treat and the unique ability of TDI to examine the longitudinal fibres predominating in this ventricle makes it a very attractive tool in this regard. This study has demonstrated that TDI parameters can be easily and reliably obtained within the paediatric population allowing the possibility for pre-operative risk stratification and earlier post-operative optimisation, further preventing additional end organ effects of low cardiac output. As mentioned previously TDI parameters were not analysed beyond the first post-operative day and it would be interesting to see when and if bi-ventricular systolic function returned to normal pre-operative values. With the advent of 3D echocardiography absolute and derived TDI

parameters are likely to play an important role in the assessment of myocardial function in children in the future.

Despite extensive and ongoing research in the prediction and treatment of acute renal injury in the post-operative paediatric cardiac surgery population there has been limited success in altering patient outcomes. Wide ranging definitions of injury and the limited availability of reliable serum biomarkers further complicates this problem. Cystatin C is a novel biomarker which, as demonstrated in chapter 3 of this thesis, is a sensitive measure of early post-operative renal injury that may be in a position to play a pivotal role in the identification, classification and customisation of treatment therapies. Moreover, this research has shown that cystatin C is also predictive of other clinical outcomes including ventilation time and intensive care stay further enhancing its profile.

This is one of the very few studies in the literature which has investigated the effectiveness of cystatin C in such a patient group and the findings are therefore clinically important. The independence of cystatin C of age, sex and mass is a key prerequisite of the paediatric population and immediately separates it from the most commonly used serum marker, creatinine. However, the assay used to measure cystatin C is relatively expensive and only available in specialised centres in the UK making it difficult for clinicians to routinely access at present. As a result, despite growing evidence the likelihood of this biomarker predominating within the clinical environment remains to be seen.

When compared to medicine as a whole the specialty of paediatric cardiac surgery is still in its youth. However, 50 years have passed since the advent of cardiopulmonary bypass and still there are no defining guidelines regarding optimal perfusion. In chapter 3 of this thesis in addition to exploring the use of cystatin C as a marker of renal injury its

association with intra-operative perfusion-related factors of renal injury was investigated. The relative ease with which renal injury can be monitored makes it an attractive organ to assess perfusion. In keeping with the published literature this research demonstrated that an ischaemic insult was the most likely intra-operative mechanism responsible for post-operative renal dysfunction. A critical dysoxic threshold was apparent below which significant injury occurred. Interestingly, oxygen delivery during cardiopulmonary bypass is not currently calculated, even though the individual variables of the equation are all noted by the perfusionist, and furthermore does not represent a parameter by which perfusion is altered. This is an important clinical consideration which can be drawn from this work and is currently being taken forward within the Department of Paediatric Cardiac Surgery at Yorkhill Hospital. Further work in this area would be merited in the form of a trial specifically designed to evaluate whether targeting a specific oxygen delivery during bypass improves outcome overall.

In chapter 4 of this thesis the vicious circle of pulmonary hypertension, right ventricular failure and decreased oxygenation often seen post-operatively in children with high flow pulmonary physiology was investigated. The administration of pre-operative oral sildenafil to this patient group, particularly as a randomised clinical trial, has not been previously undertaken. The minimal effect of pre-operative sildenafil on pulmonary vascular resistance seen in this study is not surprising when one considers that none of the children enrolled in the trial had or indeed developed post-operative pulmonary hypertension. However, some of the findings were both surprising and concerning with regards to peri-operative ventricular function. Considering the relatively routine use of this drug within the paediatric intensive care environment the consistent reduction in peri-operative ventricular function demonstrated in our trial raises significant clinical concerns and future work in this area is needed to further clarify or refute these findings. One of the main limitations of

this trial was that intra-cardiac shunt was not formally assessed and although data regarding Doppler-derived pulmonary and systemic flows was available a sildenafil related increase in shunt as the cause of ventricular impairment was not conclusively exclude.

In addition to the concerns raised regarding contractility pre-operative sildenafil was also associated with reduced oxygen delivery and a trend for increasing ventilatory requirements post-operatively. Although not formally analysed in this trial several other studies have demonstrated the association between systemic sildenafil therapy and ventilation perfusion uncoupling. Furthermore the impact of the systemic inflammatory response, to the process of cardiopulmonary bypass, on pulmonary function was not addressed in this chapter or as part of this thesis. This is an important limitation which should be factored into any further studies specifically addressing this issue.

In conclusion this thesis has, through a prospective observational study and a randomised controlled trial, successfully considered some key issues and developments in the management and assessment of end organ dysfunction commonly seen after paediatric heart surgery. Several important contributions to clinical knowledge have been made with as many questions raised for future deliberation.

# CHAPTER 6: APPENDICIES

## 6.1 Prospective Observational Study

### 6.1.1 Appendix 1: Local Research Ethics Committee Approval

SL14 Favourable opinion following consideration of further information
Version 2, October 2004

**Yorkhill Local Research Ethics Committee**
Yorkhill
Dalnair Street
Glasgow
G3 8SJ

02 March 2005

Mr Tony Vassalos
Research Fellow, Department of Cardiac Surgery,
Yorkhill
NHS
Yorkhill Department of Cardiac Surgery
Royal Hospital for Sick Children
Glasgow
G3 8SJ

Dear Mr Vassalos

***Full title of study:*** ***End organ effects of pediatric cardiopulmonary bypass.***
***REC reference number:*** ***04/S0708/75***
***Protocol number:***

Thank you for your letter of 02 March 2005, responding to the Committee's request for further information on the above research.

The further information has been considered on behalf of the Committee by the Chair the Vice-Chair.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation.

**Conditions of approval**

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

| Document Type: | Version: | Dated: | Date Received: |
|---|---|---|---|
| Application | | 21/12/2004 | 21/12/2004 |
| Investigator CV | | 21/12/2004 | 21/12/2004 |
| Protocol | | 21/12/2004 | 21/12/2004 |

SL14 Favourable opinion following consideration of further information
Version 2, October 2004

| | | | |
|---|---|---|---|
| Covering Letter | | 21/12/2004 | 21/12/2004 |
| Participant Information Sheet | | 21/12/2004 | 21/12/2004 |
| Participant Consent Form | | 21/12/2004 | 21/12/2004 |
| Response to Request for Further Information | | 02/03/2005 | 02/03/2005 |
| Other | | 21/12/2004 | 21/12/2004 |

**Management approval**

The study should not commence at any NHS site until the local Principal Investigator has obtained final management approval from the R&D Department for the relevant NHS care organisation.

**Membership of the Committee**

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

| | |
|---|---|
| **04/S0708/75** | **Please quote this number on all correspondence** |

With the Committee's best wishes for the success of this project,

Yours sincerely,

**Chair**

E-mail: liz.meenagh@yorkhill.scot.nhs.uk

*Enclosures* *List of names and professions of members who were present at the meeting and those who submitted written comments*

*Standard approval conditions*

*Site approval form (SF1)*

### 6.1.2 Appendix 2: Data Entry Proforma

| Patient ID: |
|---|
| |

| PATIENT FACTORS | | | | | |
|---|---|---|---|---|---|
| 1. | Age (Months) | | 5. | BSA ($m^2$) | |
| 2. | Weight (Kg) | | 6. | WCC (Preop) ……/…. | |
| 3. | Height (cm) | | 7. | Admitted | / / |
| 4. | Sex (M/F) | | 8. | Discharged | / / |

| PREOPERATIVE FACTORS | | |
|---|---|---|
| 1. | Cardiac Diagnosis | |
| | | |
| 2. | Non-Cardiac Diagnosis | |
| | | |
| 3. | Cardiac Catheter (PAP/AO/PVR PCWP/$Q_p$:$Q_s$) | |
| | | |
| 4. | Signs / Symptoms (L > R Shunt) | |
| | | |
| 5. | Medication | |
| | | |
| 6. | Aspirin / Warfarin / Sildenafil | |

| OPERATIVE FACTORS | | | | | |
|---|---|---|---|---|---|
| 1. | DATE | ….. / ….. / ….. | 7. | CPB Temp (minimum rectal) | |
| 2. | XC time (mins) | | 8. | UF volume (mls) | |
| 3. | BP time (mins) | | | | |
| 4. | Surgeon & Anaesthetist | / | | | |
| 5. | Inotropes (coming off) | | | | |
| | | | | | |
| 6. | Aprotinin Regimen | L = | P = | M = | |

| RENAL OUTCOMES | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test \ Timing | | PreOp Day… …/… | Th 1st 12hrs | Th 2nd 12hrs | Postop Day 1 .../... | 2 …/.. | 3 …/.. | 4 …/.. | 5 …/.. | 6 …/.. | 7 …/.. | |
| 1. | SCr (µmol/l) (18-40) | | | | | | | | | | | |
| 2. | CrCl (54-86) (ml/min/1.73m$^2$) | | | | | | | | | | | |
| *(Normal values in parenthesis)* | | 1hr | 2hrs | 3hrs | 4hrs | 5hrs | | Total Urine 1st 24hr | | Diuretics (Inf / Bolus) | | |
| 3. | Urine (mls) | | | | | | | | | | | |
| 4. | Dopamine (µg/kg/min) | | | | | | | | | | | |

<table>
<tr><td colspan="7">RESPIRATORY OUTCOMES</td></tr>
<tr><td colspan="2">Timing / Test</td><td>Post Intubation</td><td>Post CPB</td><td>2 hrs Postop</td><td>4hrs Postop</td><td>8am Day 1</td></tr>
<tr><td>1.</td><td>Oxygenation Index (OI)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>2.</td><td>Dynamic Compliance ($C_L$) (ml/cm$H_2O$/kg)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>3.</td><td>Mech. Ventilation</td><td colspan="2">(Hrs)<br>(Days)</td><td>Sildenafil (dose/days)</td><td colspan="2" rowspan="2"></td></tr>
<tr><td>4.</td><td>Period in PICU</td><td colspan="2">(Hrs)<br>(Days)</td><td>Hospital Stay (days)</td><td></td></tr>
</table>

Oxygenation Index: Mean airway pressure ($cmH_2O$ x 100 x $FiO_2$ / $PaO_2$ (mmHg))

| ECHOCARDIOGRAPHIC OUTCOMES | | | | |
|---|---|---|---|---|
| Test / Timing | | Pre-Op | <1hr post return to PICU | Day 1 Post-Op |
| 1. | $LV_{EDD}$ (mm) [23 +/- 3] | | | |
| M-mode [mean +/- SD] | $LV_{ESD}$ (mm) [14 +/- 2] | | | |
| | $RV_{EDD}$ (mm) [9-13] | | | |
| | LV % FC [38.9 +/- 4.1] | | | |
| | LV EF % [52-65] (1-18yr olds) | | | |
| | $V_P$ (cm/sec) [54.6 +/- 14] (2mn – 6yrs) | | | |
| 2. | Peak AO (LVOT) (cm/sec) median 107.0* [73-141] | | | |
| SPECTRAL | Peak PA (RVOT) (cm/sec) median 84.4* [63.8-70.5] | | | |
| | AO annular diameter [11-13mm] | | | |
| | PA annular diameter [8.4 – 11.6mm] (up to 10kg) | | | |
| | $VTI_{PA}$ (stroke distance cm) [heart rate] | | | |
| | $VTI_{AO}$ (stroke distance cm) [heart rate] | | | |
| | MV Peak E wave (cm/sec) [79.7 +/- 18.8] | | | |
| | MV Peak A wave (cm/sec) [65.3 +/- 13.3] | | | |
| 3. | MV E/A Ratio [1.24 +/- 0.3] | | | |
| 4. | Regurgitation (L + R AV) (Y/N) | | | |
| 5. | $S_a$: Lateral Mitral (5.7 +/- 1.6) [5.3 – 6.1] | | | |
| TDI cm/sec (mean +/- SD) [95% CI] | $S_a$: Lateral Tricuspid (10.2 +/- 5.5) [8.8 – 11.7] | | | |
| | $S_a$: Septum (5.4 +/- 1.2) [5.1 – 5.7] | | | |
| | IVA ($cm/sec^2$) [248 +/- 35] | | | |
| | $E_a$: Lateral Mitral (9.7 +/- 3.3) [8.8 – 10.5] | | | |
| | $E_a$: Lateral Tricuspid (13.8 +/- 8.2) [11.7 – 15.9] | | | |
| | $E_a$: Septum (8.1 +/- 2.5) [7.5-8.7] | | | |
| | $A_a$: Lateral Mitral (5.7 +/- 1.8) [5.3 – 6.2] | | | |
| | $A_a$: Lateral Tricuspid (9.8 +/- 2.4) [9.1 - 10.5] | | | |
| | $A_a$: Septum (6.1 +/- 1.5) [5.7 – 6.4] | | | |
| | $E/E_a$ Ratio (L AV valve) (8.8 +/- 2.7) [8.1 – 9.5] | | | |

*(Normal values in parenthesis)*

| Timing / Test | | Pre-op | 1st 24Hrs Post-Op | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | <1 Hr | 4hr | 8hr | 12 Hr | 16 Hr | 20 Hr | 24 Hr |
| 1. | TEMP ($^{O}$C) Periph / Core | | | | | | | | |
| | | | | | | | | | |
| 2. | INOTROPE Score | | | | | | | | |
| 3. | LA pressure | | | | | | | | |

| HAEMATOLOGY OUTCOMES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1hr Post-op | 2hr Post-op | 3hr Post-op | 4hr Post-op | 5hr Post-op | Total 1st 24 hrs | | |
| 1. | Chest Tube Drainage (ml) | | | | | | | | |
| | | Intra-operatively | | | Post-operatively | | | | |
| 2. | PRC (units/ml) | | | | | | | | |
| 3. | FFP (units/ml) | | | | | | | | |
| 4. | Platelets (units/ml) | | | | | | | | |
| 5. | Other: | | | | | | | | |

| COMMENTS / COMPLICATIONS: |
|---|
| - |
| - |
| - |

### 6.1.3 Appendix 3: Lab Specimen Data Proforma

| CPB SAMPLES | ALIQUOT Nos. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. 5mins into CPB | | | | | | | | | | |
| 2. 30mins into CPB | | | | | | | | | | |
| 3. 2mins off X-clamp | | | | | | | | | | |
| 4. Coming off | | | | | | | | | | |

| RETAINED PLASMA | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pre-OP Samples Aliquot Nos. | | | | | | | | | | |
| Volume P (µL) | | | | | | | | | | |
| Day 1 Samples Aliquot Nos. | | | | | | | | | | |
| Volume D1 (µL) | | | | | | | | | | |
| Day 2 Sample Aliquot Nos. | | | | | | | | | | |
| Volume D2 (µL) | | | | | | | | | | |
| Post-Op Samples | Th | D1 | D2 | D3 | D4 | D5 | D6 | D7 | …… | …… |
| Tick if present | | | | | | | | | | |

### 6.1.4 Appendix 4: Patient Protocol

PRE-ASSESSMENT

- Study introduced to parents by consultant cardiac surgeon at the clinic
- Routine cardiology investigations (echocardiography)

ADMISSION/PRE-OPERATIVELY

- Study re-introduced to parents by consultant cardiac surgeon
- If parents agreeable, study discussed in greater detail by principal investigator
- Parental consent for study obtained
- Plasma retained from routine bloods (day 2 pre-op)

POST-OPERATIVELY (PICU)

- Plasma retained from routine bloods on admission to PICU
- Transthoracic echocardiography and Doppler tissue imaging <2hr post admission to PICU
- Two 12hr urine collections into provided containers (over 1$^{st}$ 24hrs)
- Transthoracic echocardiography and Doppler tissue imaging (day 1 post op)
- Plasma retained from routine bloods (day 1 post-op)
- Plasma retained from routine bloods (day 2 post-op – if still in PICU)

POST-OPERATIVELY (WARD)

- Plasma retained from routine bloods (prior to discharge)

DISCHARGE

- Patient's involvement in the study ends on hospital discharge

### 6.1.5 Appendix 5: Echocardiography Protocol

TIMING: pre-operatively vs. <2hr admission to PICU and day-1 post-operatively

1. M-MODE & SPECTRAL DOPPLER

| SYSTOLIC FUNCTION (M-MODE) | SYSTOLIC FUNCTION (SPECTRAL DOPPLER) |
|---|---|
| - $LV_{EDD}$ | - Peak AO (aortic, LVOT) velocity |
| - $LV_{ESD}$ | - Peak PA (pulmonary, RVOT) velocity |
| - $RV_{EDD}$ | - AO annular diameter |
| - Fractional Shortening (% FS) | - PA annular diameter |
| - Ejection Fraction (EF %) | - $VTI_{PA}$ |
| | - $VTI_{AO}$ |

| DIASTOLIC FUNCTION (SPECTRAL DOPPLER) |
|---|
| - E & A wave velocity across mitral valve |
| - Note any regurgitation |

2. TISSUE DOPPLER IMAGING

| SYSTOLIC FUNCTION |
|---|
| - $S_a$ wave velocity at septum, lateral mitral and tricuspid annuli |
| - IVA isovolumic acceleration at lateral tricuspid and mitral annuli |

| DIASTOLIC FUNCTION |
|---|
| - $E_a$ and $A_a$ wave velocity at septum, lateral mitral and tricuspid annuli |

### 6.1.6 Appendix 6: Parent Information Sheet

Version 2 (01 February 2005)

1. **Study Title:** End organ effects of paediatric cardiopulmonary bypass

2. **Lay Title**: Effects of heart surgery, requiring the use of the heart-lung bypass machine, on the function of vital body organs in children

3. **Invitation paragraph:** You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

   Thank you for reading this.

**Q1. What is the purpose of the study?**

**Ans:** The purpose of this initial (pilot) study is to investigate the effects of heart surgery using the heart-lung bypass machine on the function of the heart, lungs and kidneys.

**Q2. Why have I been chosen?**

**Ans:** Participation in this study is being offered to all parents with children aged over 3 months with inborn acyanotic heart disease who require heart surgery using the heart-lung bypass machine.

**Q3. Do I have to take part?**

**Ans: NO.** It is up to you to decide whether or not you wish your child to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

**Q4. What will happen to my child, if my child takes part?**

**Ans:** If you decide that you would like your child to take part:

- Your child's surgery and treatment will follow the normal practice of your consultant heart surgeon.
- Your child's progress before and after surgery will be closely monitored.

  (a) Data from ward charts, intensive care charts and routine blood results will be recorded.
  (b) Images from routine echocardiography used before and after surgery to assess the heart's function will be studied on the computer.
  (c) Urine samples normally discarded in intensive care will be sent to the laboratory to measure markers of kidney function.
  (d) Blood samples taken for routine tests will be stored and used to test for markers of heart, lung and kidney function.

- Your child will remain part of the study until the day of discharge (usually 5 days).
- You and your child will not need to visit your GP or attend any additional clinics.
- Your child will not need to take any specific medications.

**Q5. What do I have to do?**

**Ans:** There are no associated restrictions with taking part in this study. Your child's stay in hospital should follow the normal course.

**Q6. What is the drug or procedure that is being tested?**

**Ans:** There are no drugs or procedures being tested in this initial (pilot) study.

**Q8. What are the side effects of any treatment received when taking part?**

**Ans:** Your child will not be receiving any treatment specifically related to this study.

**Q9. What are the possible disadvantages and risks of taking part?**

**Ans:** This initial study is mainly an observational study aimed at following your child's progress through hospital more closely. It is not associated with any specific therapy and we do not foresee any potential risks to them.

**Q10. What are the possible benefits of taking part?**

**Ans:** By taking part in the study your child's progress through hospital will be closely monitored. As a result we would expect that any change to your child's condition would be detected at an early stage and the clinical team could make the relevant decisions earlier.

**Q11. What if new information becomes available?**

**Ans:** All relevant additional information on the function of the heart, lungs or kidneys found during the study will be made available to the clinical team caring for your child to allow them to make the appropriate clinical decisions early.

**Q12. What happens when the research study stops?**

**Ans:** The research study stops when your child is discharged from hospital. There is no further participation or responsibility.

**Q13. What if something goes wrong?**

**Ans:** If taking part in this research project harms your child, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

**Q14. Will my taking part in this study be kept confidential?**

**Ans:** All information that is collected about your child during the course of the research will be kept strictly confidential. Any information about your child which leaves the hospital/surgery will have his/her name and address removed so that he/she cannot be recognised from it.

**Q15. What will happen to the results of the research study?**

**Ans:** The results of this initial study will hopefully serve as a basis for further more specific research into this area. Any publications that arise should be available to everyone on-line. The information may also be used in presentations to local charities and parent groups. You will not be specifically identified in any report or publication.

**Q16. Who is organising and funding the research?**

**Ans:** Laboratory tests associated with this study are being funded locally.

**Q17. Who has reviewed the study?**

**Ans:** The Research Ethics Committee based in Yorkhill Hospital has reviewed the study. The study has also been reviewed by the doctors and other clinical staff involved in your child's care.

**Q18. Who can I contact for further information?**

**Ans:** If there is anything you do not understand or feel you would like further information on please do not hesitate to contact either of the persons named below through the Cardiac Surgery secretaries at Yorkhill Hospital (0141 201 0251).
- Mr Tony Vassalos, Research Fellow, Department of Cardiac Surgery, Yorkhill
- Mr Mark Danton, Consultant Paediatric Cardiac Surgeon, Yorkhill

**Q19. With whom can I register any complaints?**

**Ans:** Any complaints should be directed to the complaints officer or research fellow detailed below. All complaints will follow the standard NHS complaints mechanism.
- Mrs Kate Colquhoun, Complaints Officer, Yorkhill NHS Trust, Dalnair Street, Glasgow G3 8SJ. (0141 201 9278)
- Mr Tony Vassalos, Research Fellow, Department of Cardiac Surgery, Level 5 Children's Hospital, Yorkhill NHS Trust, Dalnair Street, Glasgow G3 8SJ.

All patients precipitating in this study will be given a copy of the information sheet and a signed consent form to keep.

Thank you for your co-operation and patience.

### 6.1.7 Appendix 7: Parent Consent Form

Centre Number::
Study Number: 05/CA/01
Patient Identification Number for this trial:

# CONSENT FORM

**Title of Project: End organ effects of paediatric cardiopulmonary bypass**

Name of Researcher: Mr Tony Vassalos

**Please initial box**

1. I confirm that I have read and understand the information sheet dated 1 February 2005 February 2005 (version 2) for the above study and have had the opportunity to ask opportunity to ask questions. ☐
2. I understand that my child's participation is voluntary and that I am free to withdraw at withdraw at any time, without giving any reason, without my child's medical care or legal medical care or legal rights being affected. ☐
3. I understand that sections of my child's medical notes may be looked at by responsible by responsible individuals from the Yorkhill Division or from regulatory authorities where authorities where it is relevant to my child taking part in research. I give permission for permission for these individuals to have access to my child's records. ☐
4. I give permission for my child to take part in the above study. ☐

| _____ | _____ | _____ |
|---|---|---|
| Name of Parent or Guardian | Date | Signature |
| _____ | _____ | _____ |
| Researcher | Date | Signature |

1 for parent/guardian; 1 for researcher; 1 to be kept with hospital notes

## 6.2 Randomised Controlled Trial

### 6.2.1 Appendix 1: Study Protocol

**A phase IV, randomised, double-blind, placebo-controlled, single-centre study of the pre-operative effect of Sildenafil Citrate on pulmonary related complications following cardiopulmonary bypass in children undergoing cardiac surgical repair**

| | |
|---|---|
| **Sponsor:** | NHS Greater Glasgow<br>Yorkhill Division, Royal Hospital for Sick Children<br>Dalnair Street, Glasgow G3 8SJ |
| **Chief Investigator:** | Mr Tony Vassalos (CI), Research Fellow Cardiac Surgery<br>Yorkhill Division, Royal Hospital for Sick Children<br>Dalnair Street, Glasgow G3 8SJ |
| **Supervisors:** | Mr Mark Danton (PI), (Consultant Cardiac Surgeon)<br>Professor Fiona Lyall (Professor of Maternal and Fetal Health) |
| **Collaborators:** | Dr Peter Galloway, Mr Kenneth MacArthur, Mr Jim Pollock, Mr Stuart Lilley, Dr Brodie Knight, Dr Alastair Gracie, Dr Jennifer Scarth, Dr Crispin Best, Sr Gael Tinney, Mrs Ida Torrance, Dr James Paton, Dr Brenda Gibson, Dr David Young, Ms Sarah Casey |
| **Main Funder:** | Yorkhill Children's Foundation<br>Royal Hospital for Sick Children<br>Glasgow, G3 8SJ<br>(Tele: 0141 201 0723) |
| **Study Product:** | Sildenafil Citrate (Viagra, Pfizer) |
| **Protocol Code No:** | 05/CH/01 |
| **EudraCT Number:** | 2005-001034-32 |
| **Protocol Version:** | Version 1 (10/11/2005) |

**CONFIDENTIAL**

**List of Abbreviations** (*In order of appearance*)

- CI : chief investigator
- PI : principal investigator
- cGMP : cyclic guanosine monophosphate
- PVRI : pulmonary vascular resistance index
- PED : pulmonary endothelial dysfunction
- CPB : cardiopulmonary bypass
- PICU : pediatric intensive care unit
- NO : nitric oxide
- PDE5 : phosphodiesterase-5
- iNO : inhaled nitric oxide
- $T_{max}$ : time from administration to peak plasma concentration
- $C_{max}$ : peak plasma concentration
- $V_{ss}$ : volume of distribution
- $CL_{cr}$ : creatinine clearance
- AUC : area under the plasma concentration-time curve
- $IC_{50}$ : half-maximal inhibition
- RCT : randomised controlled trial
- CHD : congenital heart disease
- PVR : pulmonary vascular resistance
- NYHA : New York Heart Association
- $WUm^2$ : woods units per metre squared body surface area
- PA : pulmonary artery
- LA : left atrium
- $VTI_{PA}$ : velocity time integral of the pulmonary artery
- TDI : tissue Doppler imaging
- RV : right ventricle
- IVA : peak myocardial acceleration during isovolumetric contraction
- IVC : isovolumetric contraction
- OI : oxygenation index
- ERS : European Respiratory Society
- ELISA : enzyme linked immunosorbent assay
- VCAM-1 : vascular cell adhesion molecule
- REC : Research Ethics Committee
- SAE : serious adverse event
- ASD : atrial septal defect
- VSD : ventricular septal defect
- AV Canal : atrioventricular defect
- ECMO : extra-corporeal membrane oxygenation

## STUDY SUMMARY

**Title:** Does pre-operative Sildenafil protect against pulmonary related complications following cardiopulmonary bypass? A randomised trial in children undergoing cardiac surgical repair

**Short Title:** Randomised trial of pre-operative Sildenafil in children

**Lay Title**: Will giving the drug Sildenafil to children before open heart surgery improve the function of the lungs after surgery?

**Design:** Phase IV, randomised, double-blind, placebo-controlled

**Duration:** 12 months

**Centre:** Single-centre

**Research Hypothesis**
Oral Sildenafil (Viagra, Pfizer) given pre-operatively to children with left to right intracardiac shunts, undergoing corrective surgery utilizing cardiopulmonary bypass, will amplify cGMP levels in the pulmonary endothelium and reduce post-operative pulmonary dysfunction.

**Number of subjects**
A sample size of 12 in each group will have 80% power to detect a minimum difference in PVRI of 4.14 $WUm^2$ between the control and intervention groups. This computation is based on data published by Schulze-Neick et al. (Circulation 2003:108[suppl II];II-167-II-173) where a mean of 8.32 $WUm^2$ and standard deviation of 3.464 was observed in the post-operative group. The sample size calculation was done using nQuery, version 5.0, under the supervision of Dr David Young (Senior Statistician, Yorkhill Hospital).

**Inclusion Criteria**
- pediatric patients undergoing open heart surgery utilising cardiopulmonary bypass to correct acyanotic congenital heart disease
- age >3 months
  parents that show a good understanding of their child's condition and are happy for their child to participate in the study

**Exclusion Criteria**
- patients with known organ dysfunction prior to surgery (pulmonary, renal or hepatic)
- communication barriers resulting in poor basic comprehension of the proposed study (e.g. language barrier)
- patients with cyanotic heart disease
- patients undergoing heart surgery without the use of cardiopulmonary bypass
- patients who do not tolerate oral Sildenafil (e.g. Vomiting) or whose surgery is subsequently cancelled

**Study Product**

- Name : Sildenafil Citrate (Viagra, Pfizer)
- Dose : 0.5mg/kg (Max 100mg/day)
- Route : Oral (suspension)
- Regimen : 6hrly (day before surgery only), 4 doses total

**Reference Therapy**

Alternatively, an identical and inactive Placebo (equal volume) will be given 6hrly the day before surgery.

**Data Analysis**

Data will be tested for normality and two-sample t-tests or Mann-Whitney tests done accordingly. Analysis will be done on an intention-to-treat basis with any missing values being replaced by previous recorded measures, or based on group averages as appropriate. All analyses will be done using Minitab (version 14) with a significance level of 5% and results displayed as between-group effect sizes with 95% confidence intervals. Analysis will be carried out under the supervision of Dr David Young (Senior Statistician, Yorkhill Hospital).

**Introduction**

This document is a clinical research protocol. The research trial detailed within will be conducted in compliance with the principles of the Declaration of Helsinki (1989), the principles of ICH-GCP and all applicable regulatory requirements.

**Background**

Specific congenital heart defects such as ventricular septal defects that result in left to right intracardiac shunting lead to excessive blood flow through the pulmonary circulation. This has an adverse effect on the pulmonary endothelium clinically manifest as episodes of pulmonary hypertension and hemodynamic instability. This phenomenon of 'pulmonary endothelial dysfunction' (PED) is exacerbated by cardiopulmonary bypass (CPB) utilised during surgical repair. [1] Post-operatively such patients are prone to developing pulmonary infiltrations, decreased oxygenation and pulmonary hypertension with associated right-sided heart failure and hemodynamic instability. This often results in a prolonged stay in intensive care (PICU) and is associated with significant morbidity and mortality. [1, 2]

In the normal lung, nitric oxide (NO) is produced by the endothelium to regulate blood flow and optimise ventilation. PED is defined as a failure of the pulmonary endothelium to produce adequate amounts of NO. [2] This failure is amplified by cardiac surgery and is thought to be a specific consequence of a more generalised inflammatory response to CPB. NO acts through a secondary messenger, intracellular cyclic guanosine monophosphate (cGMP), to dilate pulmonary blood vessels. cGMP is metabolized by phosphodiesterase-5 (PDE5) which is the predominant phosphodiesterase in normal lung and may be up regulated in patients with PED. [2, 3, 4]

Administration of inhaled NO (iNO) is currently the standard treatment for post-operative pulmonary hypertension. Although this has proved to be clinically effective, significant disadvantages do exist with some patients failing to respond and others developing life-threatening pulmonary hypertension upon withdrawal. [5, 6] Recently Sildenafil (Viagra, Pfizer), a potent PDE-5 inhibitor that prevents the breakdown of cGMP, has been introduced as a potential new therapy and has been shown to be as effective as iNO in treating pulmonary hypertension. [7, 8] Sildenafil, however, does not require a special delivery system and does not cause life-threatening rebound pulmonary hypertension. Oral

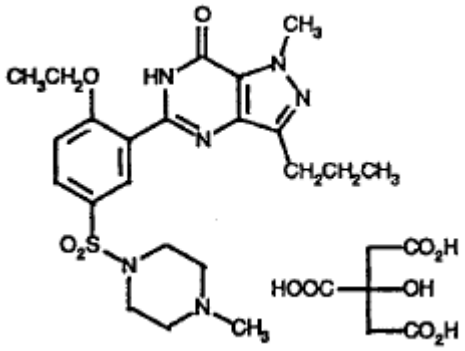
Sildenafil is now an established treatment for chronic pulmonary hypertension in children and is safe and effective when given at therapeutic doses (0.5-2.0mg/kg; 4hrly). [9] Currently, Sildenafil is used on an 'ad hoc' basis in our PICU to treat post-operative pulmonary hypertension with or without iNO.

Our hypothesis is that oral Sildenafil liquid (0.5mg/kg) administered 6hrly the day before surgery will maintain intracellular cGMP levels during the insult of cardiopulmonary bypass, protecting the pulmonary endothelium and avoiding the consequences of PED in the post-operative period. To date, the limited number of trials reporting the use of Sildenafil have been small in number and not specifically related to surgery. [10] Furthermore, the pre-operative role of Sildenafil in protecting the pulmonary endothelium has not been previously addressed.

**Investigational Medicinal Product** *(direct from Pfizer)*

| | |
|---|---|
| **Generic Name:** | Sildenafil Citrate (Discovered in 1989) |
| **Trade Name:** | Viagra |
| **Chemical Name:** | 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl) phenylsulfonyl |
| **Molecular Formula:** | $C_{22}H_{30}N_6O_4S \cdot C_6H_8O_7$ |
| **Molecular Weight:** | 666.7 (citrate salt) |
| **Pharmacological Class:** | cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) inhibitor |
| **Formulation and Dose:** | Oral Suspension; 0.5mg/kg; 6hrly (Max 100mg/day) |
| **Route of Administration:** | Oral |

**Structure:**




**Rationale**

Normally, stimulation of the pulmonary vascular endothelium causes local release of NO which in turn regulates pulmonary blood flow to optimize ventilation. NO activates the enzyme guanylate cyclase, which in turn results in increased levels of cyclic guanosine monophosphate (cGMP), producing smooth muscle relaxation in the pulmonary vasculature and reducing pulmonary vascular resistance. Sildenafil has no direct relaxant effect on isolated pulmonary blood vessels, but enhances the effect of NO by inhibiting phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP.

Studies *in vitro* have shown that Sildenafil is selective for PDE5. Its effect is more potent on PDE5 than on other known phosphodiesterases (10-fold for PDE6, >80-fold for PDE1,

>700-fold for PDE2, PDE3, PDE4, PDE7, PDE8, PDE9, PDE10, and PDE11). The approximately 4,000-fold selectivity for PDE5 versus PDE3 is important because PDE3 is involved in control of cardiac contractility. Sildenafil is only about 10-fold as potent for PDE5 compared to PDE6, an enzyme found in the retina which is involved in the phototransduction pathway of the retina. This lower selectivity is thought to be the basis for abnormalities related to color vision observed with higher doses or plasma levels. PDE5 is found in high concentrations in human corpus cavernosum and pulmonary vascular smooth muscle. PDE5 is also found in lower concentrations in other tissues including platelets, vascular and visceral smooth muscle, and skeletal muscle. The inhibition of PDE5 in these tissues by sildenafil may be the basis for the enhanced platelet antiaggregatory activity of nitric oxide observed *in vitro*, an inhibition of platelet thrombus formation *in vivo* and peripheral arterial-venous dilatation *in vivo*.

**Pharmacokinetics and Metabolism**
Sildenafil is rapidly absorbed after oral administration, with absolute bioavailability of about 40%. Its pharmacokinetics are dose-proportional over the recommended dose range. It is eliminated predominantly by hepatic metabolism (mainly cytochrome P450 3A4) and is converted to an active N-demethylated metabolite with properties similar to the parent, sildenafil. The concomitant use of potent cytochrome P450 3A4 inhibitors (e.g., erythromycin, ketoconazole, itraconazole) as well as the nonspecific CYP inhibitor, cimetidine, is associated with increased plasma levels of sildenafil. Both sildenafil and the metabolite have terminal half lives of about 4 hours.

**Absorption and Distribution**
Sildenafil is rapidly absorbed. Maximum observed plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of oral dosing in the fasted state. When Sildenafil is taken with a high fat meal, the rate of absorption is reduced, with a mean delay in Tmax of 60 minutes and a mean reduction in Cmax of 29%. The mean steady state volume of distribution (Vss) for sildenafil is 105 L, indicating distribution into the tissues. Sildenafil and its major circulating N-desmethyl metabolite are both approximately 96% bound to plasma proteins. Protein binding is independent of total drug concentrations.

**Metabolism and Excretion**
Sildenafil is cleared predominantly by the CYP3A4 (major route) and CYP2C9 (minor route) hepatic microsomal isoenzymes. The major circulating metabolite results from N-desmethylation of sildenafil, and is itself further metabolized. This metabolite has a PDE selectivity profile similar to sildenafil and an *in vitro* potency for PDE5 approximately 50% of the parent drug. Plasma concentrations of this metabolite are approximately 40% of those seen for sildenafil, so that the metabolite accounts for about 20% of sildenafil's pharmacologic effects. After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the feces (approximately 80% of administered oral dose) and to a lesser extent in the urine (approximately 13% of the administered oral dose). Similar values for pharmacokinetic parameters were seen in normal volunteers and in the patient population, using a population pharmacokinetic approach.

**Pharmacokinetics in Special Populations**
- **Renal Insufficiency:** In volunteers with mild (CLcr=50-80 mL/min) and moderate (CLcr=30-49 mL/min) renal impairment, the pharmacokinetics of a single oral dose of Sildenafil (50 mg) were not altered. In volunteers with severe (CLcr=<30 mL/min) renal impairment, sildenafil clearance was reduced, resulting in approximately doubling of AUC and Cmax compared to age-matched volunteers with no renal impairment.

- **Hepatic Insufficiency:** In volunteers with hepatic cirrhosis (Child-Pugh A and B), sildenafil clearance was reduced, resulting in increases in AUC (84%) and Cmax (47%) compared to age-matched volunteers with no hepatic impairment.

Therefore, hepatic impairment and severe renal impairment are associated with increased plasma levels of sildenafil. A starting oral dose of 25 mg should be considered in those patients.

**Pre-clinical Data**

Sildenafil is a potent and selective inhibitor of 3'5'-cGMP-specific PDE-5 with a half-maximal inhibition ($IC_{50}$) of PDE-5 activity at a concentration of 3.5nmol/L. Sildenafil has a substantially lower affinity for the other PDE isoenzymes (at least 11 isoforms of PDE have been discovered) manifested by the much higher concentrations needed to inhibit 50% of their activities ($IC_{50}$). The tissue distribution of PDE-5 is well defined and the effects of Sildenafil can be expected to be limited to these tissues (corpus cavernosum, platelets, skeletal muscle, vascular and visceral smooth muscle).[11-13] The estimated degree of PDE-5 inhibition in humans after oral dosing would be as follows: 25mg (~11nmol/L free drug) would give approximately 76% inhibition; 50 mg (~22nmol/L free drug) approximately 86% inhibitions and 100mg (~47nmol/L free drug) approximately 93% inhibition. These estimations are based on a number of assumptions, one of which is that the inhibition of the enzyme is sigmoid with respect to inhibitor concentration.[14]

**Clinical Data to Date**

In the levels of clinical evidence, the randomised controlled trial (RCT) is generally considered the best approach to ascertain the value of a particular therapy. To date only one RCT has investigated the effects of Sildenafil in children undergoing corrective cardiac surgery for congenital heart disease (CHD). Stocker et al [15] investigated the acute effects of intravenous Sildenafil in 16 ventilated infants at risk of pulmonary hypertension after cardiac surgery on haemodynamics, oxygenation and its interaction with iNO. The study was completed in 15 patients. Seven infants received iNO (20ppm) first with the addition of intravenous Sildenafil (0.35mg/kg over 20mins) after 20mins. Eight infants received Sildenafil first, iNO was added after 20mins. In infants receiving iNO first, iNO lowered the PVRI from 3.45 to 2.95 units ($p<0.01$); Sildenafil further reduced PVRI to 2.45 units ($p<0.05$). In those receiving Sildenafil first, PVRI was reduced from 2.84 to 2.35 units ($p<0.05$) with Sildenafil and fell to 2.15 units ($p<0.01$) with the addition of iNO. In both groups, these potentially beneficial effects were associated with a significant reduction in systemic blood pressure and deterioration in oxygenation that was not improved by iNO ($p<0.05$). The latter effects were well tolerated by all study patients and were not associated with any clinical decline.

In a prospective non-randomised study, Schulze-Neick et al [2] compared the effects of iNO before and after specific PDE-5 inhibition by intravenous Sildenafil in pre- and post-operative children with increased pulmonary vascular resistance (PVR) due to CHD. 12 children (0.2-15.7yrs) with CHD and increased mean pulmonary artery pressure and 12 children (0.11-0.65yrs) with increased PVR were studied during cardiac catheterisation or within 2hrs after return from cardiac surgery, respectively. All were sedated, tracheally intubated and paralysed. In the pre-operative group 0.33-0.66mg/kg of intravenous Sildenafil more effectively reduced PVR than iNO (11.5% versus 4.3%) ($p<0.05$). This result was repeated in the post-operative group where intravenous Sildenafil 0.025-0.25mg/kg more effectively reduced PVR than iNO (25.8% versus 14.6%) ($p=0.09$). This was however associated with a clinically insignificant increase in intrapulmonary shunting post-operatively ($p=0.04$).

Kothari et al [16] reported the outcome of long-term oral Sildenafil therapy in 14 patients with severe pulmonary artery hypertension, 5 of whom had surgery for CHD. Sildenafil was started at low dose and empirically increased such that the upper dosage limit of Sildenafil was not strictly defined but left to the functional improvement in individual cases (75-300mg/day). On a mean follow up 7.3 +/- 2.4 months oral Sildenafil was well tolerated and produced a significant and sustained improvement in exercise performance (Six-minute walk test), New York Heart Association (NYHA) functional class, right ventricular and pulmonary artery pressures ($p<0.002$). Two patients died during follow up despite clinical improvement.

Humpl et al [17] performed a 12 month open-label, single-drug, pilot study into the effects of oral Sildenafil in 14 children (5.3-18yrs) with pulmonary artery hypertension, 7 of whom had had surgery for CHD. The median time from cardiac surgical repair to start of Sildenafil therapy was 7 years. Oral Sildenafil (0.25mg/kg for 2 doses and then increased to 1mg/kg; 4 times daily) was commenced after baseline assessment of haemodynamics by cardiac catheterisation and exercise performance (Six-minute walk test). Exercise performance was repeated in all children at 6 weeks and at 3, 6 and 12 months. Cardiac catheterisation was repeated in 9 children after a median follow up of 10.8 months (6-15.3). Oral Sildenafil improved exercise performance at 6 months (278+/-114 to 443+/-107m) ($p=0.02$) and at 12 months (432+/-156m) ($p=0.005$). A plateau was reached between 6 and 12 months ($p=0.48$). Mean pulmonary artery pressure decreased from a median of 60mmHg to 50mmHg ($p=0.014$) and mean PVR decreased from 15WUm$^2$ to 12WUm$^2$ ($p=0.024$). Sildenafil was well tolerated and no patient withdrew. There was no change in urea, creatinine, liver function tests or platelet count.

**Dose Rationale and Risk/Benefits**

Clinical evidence relating to the efficacy of Sildenafil in reducing pulmonary hypertension due to congenital heart disease is limited and described in detail above. The current best available evidence suggests that a dosing range of 0.025-0.66mg/kg of intravenous Sildenafil and 0.25-1mg/kg (6hrly) of oral Sildenafil would safely and significantly reduce pulmonary hypertension in this patient group. Our personal clinical experience to date also supports the opinion that oral Sildenafil (commenced at 0.5mg/kg and increased up to 2mg/kg; 6hrly) is a safe and effective treatment for post-operative pulmonary hypertension in this patient group with or without iNO.

In general, Sildenafil has been very well tolerated in all clinical trials with the most commonly reported adverse events being headache, flushing, dyspepsia and nasal congestion. Adverse events were generally transient and mild to moderate. Furthermore, no serious adverse events were judged to be related to Sildenafil therapy. [18, 19] Oral Sildenafil is now an established treatment for chronic pulmonary hypertension in children and is safe and effective when given at therapeutic doses (0.5-2.0mg/kg; 4hrly). [9]

It is on this basis that the dosing regimen for oral Sildenafil in the proposed trial was chosen: *0.5mg/kg; 6hrly*. Due to the sigmoidal dose-related response of Sildenafil and in an attempt to reduce the risk of potential adverse effects we elected a dosing regimen representative of the lower end of our normal post-operative prescribing practice.

**Study Objectives**

- **Primary Objective:** Does pre-operative administration of Sildenafil (Viagra, Pfizer) reduce the lung injury associated with cardiopulmonary bypass in children undergoing corrective surgical repair of congenital heart disease?

- **Secondary Objectives:** To further delineate the mechanism(s) of action of Sildenafil in this setting. Specifically, does pre-operative Sildenafil:
  − reduce post-operative pulmonary vascular resistance index?
  − improve post-operative oxygenation?
  − affect cardiac contractility?
  − increase peri-operative cGMP levels?

**Study Design**

**General Design**

- Phase IV, randomised, double-blind, placebo-controlled, single-centre study

**Primary Study Endpoints**

The efficacy of pre-operative Sildenafil given to children undergoing corrective heart surgery for congenital heart disease in reducing PVRI, the systemic inflammatory response to CPB and improving oxygenation post-operatively.

**Primary Safety Endpoints**

- Anaphylaxis
- Clinically significant hemodynamic instability pre-operatively

**End of Study Definition**

The study will end when 24 patients with completed data sets have been enrolled or in the event that criteria for stopping the study early are met.

**Subject Selection and Withdrawal**

**Inclusion criteria**

- pediatric patients undergoing open heart surgery utilising cardiopulmonary bypass to correct acyanotic congenital heart disease
- age >3 months
- parents that show a good understanding of their child's condition and are happy for their child to participate in the study

**Exclusion criteria**

- patients with known organ dysfunction prior to surgery (pulmonary, renal or hepatic)
- communication barriers resulting in poor basic comprehension of the proposed study (e.g. language barrier)
- patients with cyanotic heart disease
- patients undergoing heart surgery without the use of cardiopulmonary bypass
- patients who do not tolerate oral Sildenafil (e.g. Vomiting) or whose surgery is subsequently cancelled

**Subject Enrolment**

- *Identification:* patients who fulfill the inclusion criteria on paper will be invited to take part in this randomized clinical trial.
- *Introduction:* the randomized trial will be introduced to the identified patient's parents by their consultant cardiac surgeon at the pre-assessment clinic. A patient information sheet will also be available for those who want one. Patients will be electively admitted to the cardiology ward 48hrs prior to surgery as is the current practice. The chief investigator will introduce himself at that time to potential participants and their families to further discuss the randomized clinical trial and potential recruitment.
- *Recruitment:* during the pre-operative period a comprehensive explanation specifically discussing potential risks and benefits will be given to parents regarding their child. Thereafter, they will be offered the opportunity to participate and complete the informed consent. There will be no obligation to take part in the trial. Everyone involved appreciates and understands the emotional turmoil associated with this type of surgery.

**Early Withdrawal of Subjects**

**When and How to Withdraw Subjects**

Subjects will be withdrawn from the trial prior to the expected completion where:

- their subsequent surgery is cancelled
- there is a failure of the subject to adhere to protocol requirements
- the study drug is not tolerated (e.g. Vomiting)
- subject consent is withdrawn

We do not anticipate abrupt withdrawal from the trial to impact subject safety or clinical management thereafter.

**Data Collection and Follow-up for Withdrawn Subjects**

There will be no specific follow up associated with participation in this randomised trial. In the event that a subject is withdrawn from the trial due to their surgery being cancelled or intolerability to the study drug their data will not be included in the final analysis and they will be replaced. Where a subject's consent is withdrawn and in the event that the study drug has been successfully administered an attempt will be made to obtain permission to record survival data up to the protocol-described end of subject participation. Serious adverse events will be recorded in all cases irrespectively.

**Study Drug**

**Description**

- Sildenafil (Viagra, Pfizer), is a potent and selective inhibitor of cGMP-specific PDE5. It is rapidly absorbed orally with dose-proportional peak plasma concentrations within 1 hour of administration. It is eliminated predominantly by hepatic metabolism (mainly cytochrome P450 3A4) and is converted to an active metabolite with properties similar to parent. The elimination half-life for both Sildenafil and its active metabolite is 3 to 5 hours. Sildenafil is excreted as metabolites predominantly in the faeces (~80%) and to a lesser extent in the urine (~13%). Hepatic and severe renal impairment are associated with increased plasma levels.
- Drug product will appear as: 'Sildenafil / Placebo Suspension of 2.5 mg/ml'. Total volume, expiry date and date dispensed will be stated on the drug product bottle.

**Treatment Regimen**
Sildenafil regimen prescribed: 0.5mg/kg, orally, 6hrly (4 doses; Max 100mg/day), day before surgery

**Method for Assigning Subjects to Treatment Groups**
Randomisation of the patients to receive oral Sildenafil or equivalent volume of Placebo liquid will be undertaken by the clinical trials technician using a minimisation programme (MINIM). Only the clinical trials technician will know which subjects have received the active drug. This will be disclosed at the interim analysis, in the event of an emergency and at the end of the trial to the chief investigator once all data has been recorded and analysed. Each subject will be given a specific number upon trial entry, the identity of which will only be known by the chief investigator and the clinical trials technician.

**Preparation and Administration of Study Drug**

**Placebo**
The placebo suspension will be prepared by the Pharmacy Production Unit based at the Western Infirmary Glasgow (IMP Licence Number 18324). The ingredients of this suspension include Ora Plus Suspending Vehicle and Ora Sweet Syrup Sugar Free.

**Sildenafil**
The Sildenafil suspension (2.5mg/ml) will be prepared by Pharmacy Production Unit based at the Western Infirmary Glasgow (IMP Licence Number 18324). The ingredients of this suspension include Sildenafil tablets (25-100mg), Ora Plus Suspending Vehicle and Ora Sweet Syrup Sugar Free.

**Administration**
Upon successful informed consent, a study specific prescription sheet will be completed for each participant. Following randomisation an appropriately labelled bottle (Sildenafil / Placebo) will be dispensed by the Pharmacy Department in Yorkhill and stored in a locked locker at the participant's bed space on the Cardiology Ward (5A). The appropriate dose detailed on the label and prescribed on the drug Kardex will then be administered by the nursing staff. The suspension will be placed in the participant's mouth either directly (oral syringe) or using a spoon as per normal practice.

**Subject Compliance Monitoring**
Compliance to the study treatment regimen will be overlooked by the clinical trials technician, ward pharmacist and the chief investigator. As the study regimen only involves 4 doses we expect subject compliance to be easily monitored. Any subject that is non-compliant with the study treatment regimen will be withdrawn from the trial.

**Prior and Concomitant Therapy**
The concomitant use of potent cytochrome P450 3A4 inhibitors (e.g., erythromycin, ketoconazole, itraconazole) as well as the nonspecific CYP inhibitor, cimetidine, is associated with increased plasma levels of Sildenafil and will be avoided the day before surgery. Otherwise all patients will be managed according to the normal practice of the Cardiology/Cardiac Surgery Departments at Yorkhill Hospital.

**Packaging**

**Placebo**
The placebo suspension supplied by the Pharmacy Production Unit, Glasgow in *3 x 50ml, amber bottles*. The bottles will be clearly labelled and transported individually as required in a small cardboard box wrapped in bubble-wrap.

**Sildenafil**
Sildenafil suspension supplied by the Pharmacy Production Unit, Glasgow in *3 x 50ml, amber bottles* and received in bulk-pack by courier. Each batch will have a corresponding batch number and expiry date. Bottles will be wrapped in bubble-wrap and packed into a cardboard box for transportation.

**Blinding of Study Drug**
Randomisation for this study will be undertaken by the clinical trial technician once informed consent has been obtained using the minimisation programme MINIM. The participants will therefore be placed into either the active (Sildenafil) arm or inactive (Placebo) arm of the study. This will be recorded by the clinical trials technician on individual patient study files held in the clinical trials department. The code will be broken for interim analysis, in the event of an emergency and then at the end of the trial.

**Receiving, Storage, Dispensing and Return**

**Receipt of Drug Supplies**
The Study medication bottles will be delivered to pharmacy stores by courier. Packed in cardboard box, bottles will be wrapped in bubble wrap. The pharmacy store staff will check the delivery receipt and match with corresponding order. After the paper work has been checked and completed the clinical trials department will accept delivery and complete the drug accountability record sheet before supplies are stored in a locked cupboard in the Clinical Trials Department.

**Storage**
Sildenafil and corresponding placebo suspension will be stored (below 25° C) in a locked cupboard in the Clinical Trials Department at Yorkhill Hospital. The expiry date for both is 28 days. Temperatures will be checked and recorded Monday-Friday.

**Dispensing of Study Drug**
The Study medication will be dispensed into 50ml, amber glass bottles with 22mm click lock caps. The label will contain all the information required to meet the applicable regulatory requirements. A register of pharmacy personnel dispensing and checking trial medication will be completed and filed in the Study filing cabinet. A dispensing record and prescription sheet will be completed and checked for each patient.

**Return or Destruction of Study Drug**
Study medication that has been returned or expired will be checked and recorded before destruction. Each trial subject will have a returns record sheet and drug container disposal procedure. Certification of destruction is retained with the Directory of Pharmacy, Mr James Wallace.

**Study Procedures**

The trial will be introduced to the parents by their consultant cardiac surgeon at the pre-assessment clinic. Upon admission to the ward for elective surgery the parents will meet the chief investigator if willing to discuss the trial further. If consent is given, the pharmacy will randomise patients to receive Sildenafil or Placebo using a minimisation programme (MINIM). The corresponding suspension (0.5mg/kg Sildenafil or equivalent volume placebo) will be dispensed to be taken 6hrly (orally) the day before surgery. Complete surgical repair and associated medical care thereafter will be undertaken according to the normal practice of Cardiology/Cardiac Surgery Departments at Yorkhill Hospital. Participation in the trial will end upon hospital discharge.

- Data collection under standardised conditions ($FiO_2$ 0.65) will occur at ***2hrs*** and ***24hrs*** post-operatively in the paediatric intensive care unit to assess:

**Pulmonary Haemodynamics:**

• *Measured indices:*

- Pressure catheter measurements: pulmonary artery (PA) and left atrial (LA) pressures recorded from routine invasive monitoring lines placed in theatre.

• *Echocardiography*:

- *Velocity time integral ($VTI_{PA}$):* spectral Doppler of the right ventricular outflow tract will be used together with the pulmonary annular measurement to estimate pulmonary blood flow.
- *Tissue Doppler Imaging (TDI):* load independent assessment of right ventricular (RV) function. Pulsed wave TDI in the apical 4-chamber view will be used to obtain the $S_a$, $E_a$ and $A_a$ wave velocities and the peak myocardial IVC acceleration at the lateral tricuspid annulus. Each parameter will be measured over 3 consecutive cardiac cycles and the average value used in the analysis (100mm/s sweep speed). On and off-line analysis will be used to assess RV systolic and diastolic function.

  Peak myocardial IVC acceleration (IVA): represents global myocardial contractility and is calculated by dividing peak myocardial velocity during isovolumetric contraction (IVC) by the time interval from the onset of this wave to the time at peak velocity. The period of IVC is taken as the onset of the QRS complex, on simultaneous ECG, to the beginning of the $S_a$ wave. This index has been shown to be a sensitive indicator of RV systolic function.

• *Derived indices:*

- *Transpulmonary gradient:* calculated by subtracting the LA pressure from the PA pressure measurement

- *Pulmonary vascular resistance index (PVRI) ($WUm^2$):* is derived from the transpulmonary gradient and estimated pulmonary blood flow on spectral Doppler. The value is standardised against body surface area.

**Ventilation:**

• *Oxygenation Index (OI) Calculation*: *[mean airway pressure (cm H20) x 100 x FiO2 / PaO2 (mmHg)]* where the mean airway pressure is recorded off the ventilator, Fi02 refers to the inspired oxygen concentration (standardised at 65%) and PaO2 refers to the arterial oxygen concentration. A number of different indices have been used to quantify the

severity of respiratory illness in ventilated patients. The OI is generally preferred because it is incorporates a measure of ventilator pressures.

- *Oxygen delivery ($DO_2$) Calculation:* Oxygen delivery calculated post-operatively using Doppler derived CO ($Q_S$) and arterial oxygen content according to the equation: $DO_2 = Q_s$ *x* [Haemoglobin x 1.34 x Haemoglobin saturation + 0.003 x arterial oxygen tension (mmHg)].

- *Period of ventilation & length of stay in PICU*: data extracted directly from PICU charts

**cGMP:**

- *cGMP levels:* measured using commercially available radioimmunoassay (Du Pont Speciality Diagnostics).

**Statistical Plan**

**Statistical Methods and Sample Size Determination**

A sample size of 12 in each group will have 80% power to detect a minimum difference in PVRI of 4.14 $WUm^2$ between the control and intervention groups. This computation is based on data published by Schulze-Neick et al. (Circulation 2003:108[suppl II];II-167-II-173) where a mean of 8.32 $WUm^2$ and standard deviation of 3.464 was observed in the post-operative group. The sample size calculation was done using nQuery, version 5.0, under the supervision of Dr David Young (Senior Statistician, Yorkhill Hospital).

A mid-point analysis of the data will be undertaken to evaluate trends and any unforeseen adverse effects. The interim and complete data set will be tested for normality and two-sample t-tests or Mann-Whitney tests done accordingly. Analysis will be done on an intention-to-treat basis with any missing values being replaced by previous recorded measures, or based on group averages as appropriate. All analyses will be done using Minitab (version 14) with a significance level of 5% and results displayed as between-group effect sizes with 95% confidence intervals. Analysis will be carried out under the supervision of Dr David Young.

**Safety and Adverse Events**

**Definitions**

Research participant safety will be closely monitored during the study as is the case in all pediatric cardiac surgical cases. This will involve routine case conference, physical examination, echocardiography +/- cardiac catheterisation, monitoring of vital signs and blood tests pre-operatively. All participants will undergo routine intra-operative echocardiography to assess the cardiac surgical repair. Thereafter all participants will be admitted to PICU where they will be invasively monitored, undergo routine post-operative echocardiography and overlooked by the cardiac surgery, cardiology and intensive care teams. Normally children with intracardiac shunts undergoing cardiac surgical correction are expected to remain in PICU for 48hrs post-operatively before being transferred to the Cardiology Ward prior to discharge around day 7. Adverse event recording will be undertaken throughout the admission.

**Potential Serious Adverse Events Associated with Study Population**

**Pre-operative**

- Cardiac arrhythmias
- Episodic pulmonary hypertension
- Eisenmenger Syndrome
- Embolism
- Left / Right sided heart failure
- Endocarditis
- Anaphylaxic reaction to anaesthetic agents

**Intra-operative**

- Cardiopulmonary bypass circuit failure +/- air embolus, circulatory arrest, multi-organ dysfunction
- Myocardial infarction
- Mortality (1% for ASD/VSD; <5% for AV Canal)

**Post-operative**

- Cardiac Tamponade +/- Re-sternotomy
- Cardiac arrhythmias
- Episodic severe pulmonary hypertension
- Myocardial infarction
- Right / Left sided heart failure +/- multi-organ dysfunction, prolonged inotropic support or extra-corporeal membrane oxygenation (ECMO)
- Cerebral injury (hypoxic, embolic, hemorrhagic)
- Acute renal failure +/- renal replacement therapy
- Hepatic failure
- Ischaemic gastrointestinal tract
- Sternal wound infection +/- Mediastinitis
- Systemic sepsis +/- multi-organ dysfunction
- Coagulopathies
- Tension Pneumothorax / Simple Pneumothorax / Chylothorax

**Potential Adverse Events Associated with Sildenafil Toxicity** *(directly from Pfizer)*

ADVERSE EVENTS REPORTED BY ≥2% OF PATIENTS TREATED WITH VIAGRA AND MORE FREQUENT ON DRUG THAN PLACEBO IN PRN FLEXIBLE-DOSE PHASE II/III STUDIES

| Adverse Event | Percentage of Patients Reporting Event | |
|---|---|---|
| | VIAGRA [N=734] | PLACEBO [N=725] |
| Headache | 16% | 4% |
| Flushing | 10% | 1% |
| Dyspepsia | 7% | 2% |
| Nasal Congestion | 4% | 2% |
| Urinary Tract Infection | 3% | 2% |
| Abnormal Vision† | 3% | 0% |
| Diarrhea | 3% | 1% |
| Dizziness | 2% | 1% |
| Rash | 2% | 1% |

†Abnormal Vision: Mild and transient, predominantly color tinge to vision, but also increased sensitivity to light or blurred vision. In these studies, only one patient discontinued due to abnormal vision.

**Potential Risks or Hazards for Research Participants**
The mainstay of surgical and medical management of all research participants before, during and after surgery will follow the normal clinical practice of their consultant cardiac surgeon, cardiologist and intensive care team. Possible disadvantages and risks would therefore be related to taking 4 oral doses of Sildenafil liquid (0.5mg/kg) the day before surgery. As we are not currently aware of any significant clinical side effects associated with oral Sildenafil at this dosage we do foresee any clinical disadvantages or side effects. We do not anticipate any potential for pain, discomfort, distress, inconvenience or changes to lifestyle directly related to taking part in this study.

**Potential Benefit to Research Participants**
We anticipate that pre-operative Sildenafil will protect the lungs during cardiopulmonary bypass to improve lung function and reduce related complications post-operatively. Clinically this may manifest as reduced episodic pulmonary hypertension, improved hemodynamics, reduced ventilation period, reduced requirements of Sildenafil or iNO and reduced overall intensive care and hospital stay. All children undergoing corrective heart surgery are closely monitored throughout their admission. Although this will also be the case for all research participants they will undergo a more detailed echocardiographic assessment both before and after surgery, providing additional clinical information that may be beneficial to the caring medical team.

**Recording of Adverse Events**
Adverse event reporting will be undertaken throughout the admission period. All events will be evaluated for seriousness, followed until resolution, recorded and stored in the master file. Each individual adverse event will be discussed in detail by the CI, project supervisors and the Sponsor. Any serious adverse event that occurs after the study period and is considered to be related to the study treatment or study participation will be recorded and reported immediately.

**Reporting of Serious Adverse Events**
All serious adverse events will be reported to the Sponsor by telephone (Dr Alison Wood, ext 86935) within *24 hrs* of the event. A serious adverse event (SAE) form will also be completed by the PI and given to the Sponsor, a copy of which will be stored in the master file.

**Study Sponsor Notification by Investigator**
All safety and adverse events (as defined in the Protocol) will be reported by the CI to the Sponsor *via* the arrangements set up by the Yorkhill R&D Office within 24hrs.

**MHRA and REC Notification by Sponsor**
The Sponsor has delegated the task of reporting safety and adverse events to the MHRA and the REC to the Yorkhill R&D Department, who undertake to meet appropriate time-lines for reporting to the relevant authorities.

**Annual Safety Reports**
The Sponsor has delegated the task of providing the MHRA and the REC with an annual safety report on the investigational medicinal product used in the study to the Yorkhill R&D Department who will undertake to meet appropriate time-lines for reporting to the relevant authorities.

**Unblinding Procedures**
Any concerns with study participants should be highlighted to the CI or consultant cardiac surgeon responsible for their care in the first instance. In the event that the code needs to be broken to ensure subject's safety the clinical trials technician will be available during normal working hours (9am-5pm) to provide the relevant information (Ms Sarah Casey, ext 89367). Between the hours of 5pm and 9am and during the weekend the on-call pharmacist at Yorkhill will undertake this responsibility.

**Stopping Rules**
The mainstay of clinical care will be unchanged by the participation in this study. Criteria for electively stopping the trial prematurely would be dependent on new contraindications to giving pre-operative Sildenafil arising in the literature or directly from the manufacturer. Adverse advents will be monitored throughout the trial by the CI and the pharmacy department at Yorkhill. In addition a midpoint review of the trial data will be undertaken to highlight any unforeseen issues.

**Medical Safety Monitoring**
The CI will undertake the responsibility of overseeing the safety of the study. In this respect a monitoring form will be constructed in conjunction with the clinical trials pharmacist and the R&D Department at Yorkhill Hospital prior to commencement of the study and attached to the protocol. This will include a regular assessment of the number and type of serious adverse events.

**Data Handling and Record Keeping**

**Confidentiality**
Information about study subjects will be kept confidential and managed according to the requirements of the Data Protection Act (1998). Informed consent will be obtained from the parents of research participants in accordance with regulations outlined in the stated act. In the event that authorisation to collect or use confidential information is revoked by a subject's parents, the investigator, by regulation, will retain the ability to use all information collected prior to the revocation or subject authorisation. Additional attempts will be made in this situation to obtain permission to collect information on vital status at the end of the study.

Individual patient's samples will be labelled with an allocated patient number to maintain anonymity during analysis. All samples will be stored for the duration of the trial within the laboratory at Yorkhill. The CI and lab supervisor will have access to the stored samples. Results will be stored on a Yorkhill desktop computer based in Cardiology.

**Source Documentation**
All source documentation will be held by the CI in a trial master file.

**Case Report Forms**
An appropriate datasheet, detailing all mandatory data fields, will be constructed prior to commencement of the trial and attached to the study protocol. All missing data will be explained.

**Archiving/Record Retention**
Data will be archived for 10 years from the end of the study, in accordance with MRC guidelines on Good Research Practice.

## Study Monitoring, Auditing and Inspecting

### Study Monitoring Plan

The study will be largely self-monitored by the research team, following a monitoring plan agreed with the Sponsor of the study, NHS Greater Glasgow, Yorkhill Division. In addition, members of the Yorkhill R&D Department may attend study team meetings to discuss monitoring reports.

### Auditing and Inspecting

The PI will permit study-related monitoring, auditing and inspections by ethics committees, the Sponsor, regulatory bodies and the R&D Department of all study-related documents.

### Ethical Considerations

This protocol and any amendments will be submitted to a properly constituted Research Ethics Committee (REC) in agreement with local legal prescriptions, for formal approval of study conduct. A copy of the documented decision of the REC concerning the conduct of the study will be provided to the Sponsor before commencement of the study.

Parent's whose children meet the entry criteria and who are willing to discuss the study further will be provided with a consent form describing this study and providing sufficient information to enable an informed decision about their child's participation in this study. (Appendix 2) This consent form will be submitted with the protocol for review and approval by the REC for the study. The formal consent of a subject, using the REC-approved consent form will be obtained before that subject is submitted to any study procedure. The consent form will be signed by the subject's parents and the PI.

### Study Finances

The study is being funding from a successful research grant application to the Yorkhill Children's Foundation. As the study will not commence until R&D Management approval has been obtained, the study will be covered by the NHS CNORIS arrangements for acts of negligence.

### Publication policy

The primary responsibility for publication of the results of this study is held by the PI. Approval must be obtained directly from the PI before any information can be used or passed onto a third party.

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**Signatures**

- Principal Investigator: Mr Mark Danton
  ……..

- Sponsor (Representative): Dr Alison Wood
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### 6.2.2 Appendix 2: Investigational Medicinal Product Dossier (IMPD)

# INVESTIGATIONAL MEDICINAL PRODUCT DOSSIER (IMPD)

**Sponsor:** NHS Greater Glasgow
Yorkhill Division, Royal Hospital for Sick Children
Dalnair Street, Glasgow G3 8SJ

**Drug Substance:** VIAGRA (Sildenafil Citrate - discovered in 1989)

**Edition No:** 1

**Date:** 6 December 2005

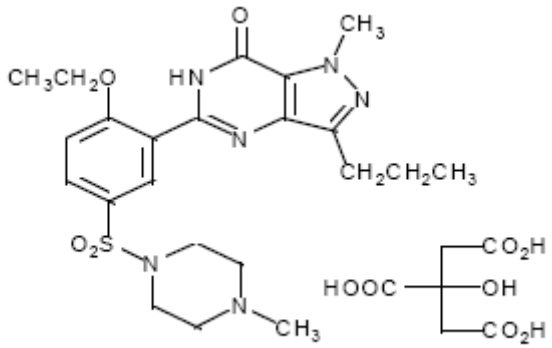
## LIST OF ABBREVIATIONS

- cGMP : cyclic guanosine monophosphate
- PDE5 : phosphodiesterase type 5
- NO : nitric oxide
- $T_{max}$ : time from administration to peak plasma concentration
- $C_{max}$ : peak plasma concentration
- $V_{ss}$ : volume of distribution
- $CL_{cr}$ : creatinine clearance
- AUC : area under the plasma concentration-time curve
- ECG : electrocardiography
- ED : erectile dysfunction
- IIEF : the international index of erectile function
- CABG : coronary artery bypass grafting
- TURP : transurethral resection of the prostate
- NAION : non-arteritic anterior ischaemic optic neuropathy
- HIV : human immunodeficiency virus
- MRHD : maximum recommended human daily dose
- MTD : maximum tolerated dose

VIAGRA® (sildenafil citrate)
*Tablets*

## 1. DESCRIPTION

VIAGRA®, an oral therapy for erectile dysfunction, is the citrate salt of sildenafil, a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Sildenafil citrate is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate and has the following structural formula:



Sildenafil citrate is a white to off-white crystalline powder with a solubility of 3.5 mg/mL in water and a molecular weight of 666.7. VIAGRA (sildenafil citrate) is formulated as blue, film-coated rounded-diamond-shaped tablets equivalent to 25 mg, 50 mg and 100 mg of sildenafil for oral administration. In addition to the active ingredient, sildenafil citrate, each tablet contains the following inactive ingredients: microcrystalline cellulose, anhydrous dibasic calcium phosphate, croscarmellose sodium, magnesium stearate, hypromellose, titanium dioxide, lactose, triacetin, and FD & C Blue #2 aluminum lake.

## 2. CLINICAL PHARMACOLOGY

### 2.1 Mechanism of Action

The physiologic mechanism of erection of the penis involves release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. NO then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. Sildenafil has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of nitric oxide (NO) by inhibiting phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP in the corpus cavernosum. When sexual stimulation causes local release of NO, inhibition of PDE5 by sildenafil causes increased levels of cGMP in the corpus cavernosum, resulting in smooth muscle relaxation and inflow of blood to the corpus cavernosum. Sildenafil at recommended doses has no effect in the absence of sexual stimulation.

Studies *in vitro* have shown that sildenafil is selective for PDE5. Its effect is more potent on PDE5 than on other known phosphodiesterases (10-fold for PDE6, >80-

fold for PDE1, >700-fold for PDE2, PDE3, PDE4, PDE7, PDE8, PDE9, PDE10, and PDE11). The approximately 4,000-fold selectivity for PDE5 versus PDE3 is important because PDE3 is involved in control of cardiac contractility. Sildenafil is only about 10-fold as potent for PDE5 compared to PDE6, an enzyme found in the retina which is involved in the phototransduction pathway of the retina. This lower selectivity is thought to be the basis for abnormalities related to color vision observed with higher doses or plasma levels (see Pharmacodynamics). In addition to human corpus cavernosum smooth muscle, PDE5 is also found in lower concentrations in other tissues including platelets, vascular and visceral smooth muscle, and skeletal muscle. The inhibition of PDE5 in these tissues by sildenafil may be the basis for the enhanced platelet antiaggregatory activity of nitric oxide observed *in vitro*, an inhibition of platelet thrombus formation *in vivo* and peripheral arterial-venous dilatation *in vivo*.

## 2.2 Pharmacokinetics and Metabolism

VIAGRA is rapidly absorbed after oral administration, with absolute bioavailability of about 40%. Its pharmacokinetics are dose-proportional over the recommended dose range. It is eliminated predominantly by hepatic metabolism (mainly cytochrome P450 3A4) and is converted to an active metabolite with properties similar to the parent, sildenafil. The concomitant use of potent cytochrome P450 3A4 inhibitors (e.g., erythromycin, ketoconazole, itraconazole) as well as the nonspecific CYP inhibitor, cimetidine, is associated with increased plasma levels of sildenafil (see DOSAGE AND ADMINISTRATION). Both sildenafil and the metabolite have terminal half lives of about 4 hours.

Mean sildenafil plasma concentrations measured after the administration of a single oral dose of 100 mg to healthy male volunteers is depicted below:

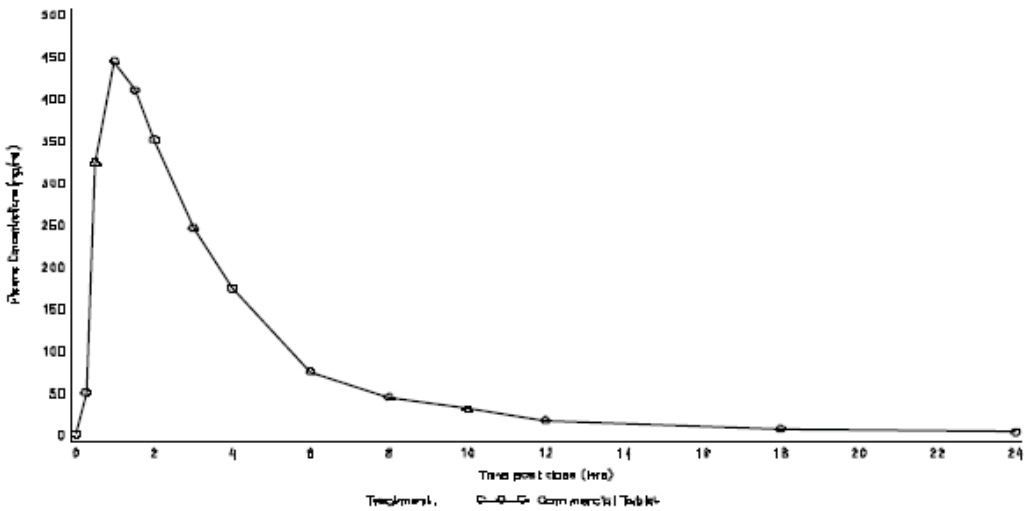



Figure 1: Mean Sildenafil Plasma Concentrations n Healthy Male Volunteers.

## 2.3 Absorption and Distribution

VIAGRA is rapidly absorbed. Maximum observed plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of oral dosing in the fasted state. When VIAGRA is taken with a high fat meal, the rate of absorption is reduced, with a mean delay in Tmax of 60 minutes and a mean reduction in Cmax of 29%. The mean steady state volume of distribution (Vss) for sildenafil is 105 L, indicating distribution into the tissues. Sildenafil and its major circulating N-desmethyl metabolite are both approximately 96% bound to plasma proteins. Protein binding is independent of total drug concentrations. Based upon measurements of sildenafil in semen of healthy volunteers 90 minutes after dosing, less than 0.001% of the administered dose may appear in the semen of patients.

## 2.4 Metabolism and Excretion

Sildenafil is cleared predominantly by the CYP3A4 (major route) and CYP2C9 (minor route) hepatic microsomal isoenzymes. The major circulating metabolite results from N-desmethylation of sildenafil, and is itself further metabolized. This metabolite has a PDE selectivity profile similar to sildenafil and an *in vitro* potency for PDE5 approximately 50% of the parent drug. Plasma concentrations of this metabolite are approximately 40% of those seen for sildenafil, so that the metabolite accounts for about 20% of sildenafil's pharmacologic effects. After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the feces (approximately 80% of administered oral dose) and to a lesser extent in the urine (approximately 13% of the administered oral dose). Similar values for pharmacokinetic parameters were seen in normal volunteers and in the patient population, using a population pharmacokinetic approach.

## 2.5 Pharmacokinetics in Special Populations

Geriatrics: Healthy elderly volunteers (65 years or over) had a reduced clearance of sildenafil, with free plasma concentrations approximately 40% greater than those seen in healthy younger volunteers (18-45 years).

Renal Insufficiency: In volunteers with mild (CLcr=50-80 mL/min) and moderate (CLcr=30-49 mL/min) renal impairment, the pharmacokinetics of a single oral dose of VIAGRA (50 mg) were not altered. In volunteers with severe (CLcr=<30 mL/min) renal impairment, sildenafil clearance was reduced, resulting in approximately doubling of AUC and Cmax compared to age-matched volunteers with no renal impairment.

Hepatic Insufficiency: In volunteers with hepatic cirrhosis (Child-Pugh A and B), sildenafil clearance was reduced, resulting in increases in AUC (84%) and Cmax (47%) compared to age-matched volunteers with no hepatic impairment.

Therefore, age >65, hepatic impairment and severe renal impairment are associated with increased plasma levels of sildenafil. A starting oral dose of 25 mg should be considered in those patients (see DOSAGE AND ADMINISTRATION).

## 3. PHARMACODYNAMICS

### 3.1 Effects of VIAGRA on Erectile Response

In eight double-blind, placebo-controlled crossover studies of patients with either organic or psychogenic erectile dysfunction, sexual stimulation resulted in improved erections, as assessed by an objective measurement of hardness and duration of erections (RigiScan®), after VIAGRA administration compared with placebo. Most studies assessed the efficacy of VIAGRA approximately 60 minutes post dose. The erectile response, as assessed by RigiScan®, generally increased with increasing sildenafil dose and plasma concentration. The time course of effect was examined in one study, showing an effect for up to 4 hours but the response was diminished compared to 2 hours.

### 3.2 Effects of VIAGRA on Blood Pressure

Single oral doses of sildenafil (100 mg) administered to healthy volunteers produced decreases in supine blood pressure (mean maximum decrease in systolic/diastolic blood pressure of 8.4/5.5 mmHg). The decrease in blood pressure was most notable approximately 1-2 hours after dosing, and was not different than placebo at 8 hours. Similar effects on blood pressure were noted with 25 mg, 50 mg and 100 mg of VIAGRA, therefore the effects are not related to dose or plasma levels within this dosage range. Larger effects were recorded among patients receiving concomitant nitrates (see CONTRAINDICATIONS).

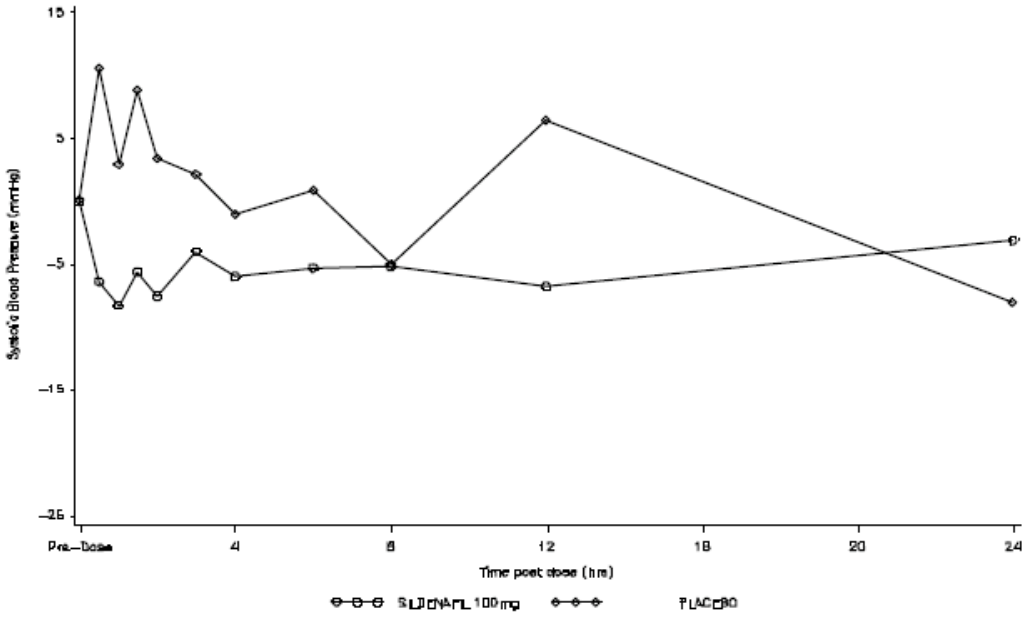



Figure 2: Mean Change from Baseline in Sitting Systolic Blood Pressure, Healthy Volunteers.

### 3.3 Effects of VIAGRA on Cardiac Parameters

Single oral doses of sildenafil up to 100 mg produced no clinically relevant changes in the ECGs of normal male volunteers. Studies have produced relevant data on the effects of VIAGRA on cardiac output. In one small, open-label, uncontrolled, pilot study, eight patients with stable ischemic heart disease underwent Swan-Ganz catheterization. A total dose of 40 mg sildenafil was administered by four intravenous infusions.

The results from this pilot study are shown in Table 1; the mean resting systolic and diastolic blood pressures decreased by 7% and 10% compared to baseline in these patients. Mean resting values for right atrial pressure, pulmonary artery pressure, pulmonary artery occluded pressure and cardiac output decreased by 28%, 28%, 20% and 7% respectively. Even though this total dosage produced plasma sildenafil concentrations which were approximately 2 to 5 times higher than the mean maximum plasma concentrations following a single oral dose of 100 mg in healthy male volunteers, the hemodynamic response to exercise was preserved in these patients.

TABLE 1. HEMODYNAMIC DATA IN PATIENTS WITH STABLE ISCHEMIC HEART DISEASE AFTER IV ADMINISTRATION OF 40 MG SILDENAFIL

| Means ± SD | At rest | | | | After 4 minutes of exercise | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Baseline (B2) | n | Sildenafil (D1) | n | Baseline | n | Sildenafil |
| PAOP (mmHg) | 8 | 8.1 ± 5.1 | 8 | 6.5 ± 4.3 | 8 | 36.0 ± 13.7 | 8 | 27.8 ± 15.3 |
| Mean PAP (mmHg) | 8 | 16.7 ± 4 | 8 | 12.1 ± 3.9 | 8 | 39.4 ± 12.9 | 8 | 31.7 ± 13.2 |
| Mean RAP (mmHg) | 7 | 5.7 ± 3.7 | 8 | 4.1 ± 3.7 | - | - | - | - |
| Systolic SAP (mmHg) | 8 | 150.4 ± 12.4 | 8 | 140.6 ± 16.5 | 8 | 199.5 ± 37.4 | 8 | 187.8 ± 30.0 |
| Diastolic SAP (mmHg) | 8 | 73.6 ± 7.8 | 8 | 65.9 ± 10 | 8 | 84.6 ± 9.7 | 8 | 79.5 ± 9.4 |
| Cardiac output (L/min) | 8 | 5.6 ± 0.9 | 8 | 5.2 ± 1.1 | 8 | 11.5 ± 2.4 | 8 | 10.2 ± 3.5 |
| Heart rate (bpm) | 8 | 67 ± 11.1 | 8 | 66.9 ± 12 | 8 | 101.9 ± 11.6 | 8 | 99.0 ± 20.4 |

In a double-blind study, 144 patients with erectile dysfunction and chronic stable angina limited by exercise, not receiving chronic oral nitrates, were randomized to a single dose of placebo or VIAGRA 100 mg 1 hour prior to exercise testing. The primary endpoint was time to limiting angina in the evaluable cohort. The mean times (adjusted for baseline) to onset of limiting angina were 423.6 and 403.7 seconds for sildenafil (N=70) and placebo, respectively. These results demonstrated that the effect of VIAGRA on the primary endpoint was statistically non-inferior to placebo.

### 3.4 Effects of VIAGRA on Vision

At single oral doses of 100 mg and 200 mg, transient dose-related impairment of color discrimination (blue/green) was detected using the Farnsworth-Munsell 100-hue test, with peak effects near the time of peak plasma levels. This finding is consistent with the inhibition of PDE6, which is involved in phototransduction in the retina. An evaluation of visual function at doses up to twice the maximum recommended dose revealed no effects of VIAGRA on visual acuity, intraocular pressure, or pupillometry.

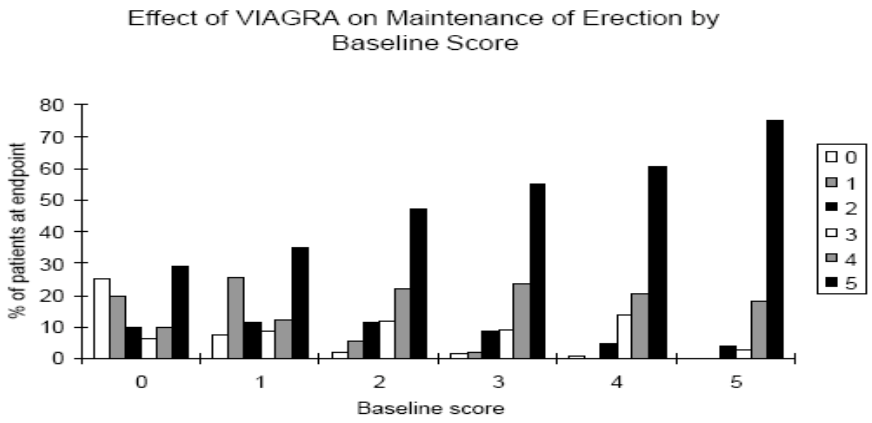
## 4. CLINICAL STUDIES

In clinical studies, VIAGRA was assessed for its effect on the ability of men with erectile dysfunction (ED) to engage in sexual activity and in many cases specifically on the ability to achieve and maintain an erection sufficient for satisfactory sexual activity. VIAGRA was evaluated primarily at doses of 25 mg, 50 mg and 100 mg in 21 randomized, double-blind, placebo-controlled trials of up to 6 months in duration, using a variety of study designs (fixed dose, titration, parallel, crossover). VIAGRA was administered to more than 3,000 patients aged 19 to 87 years, with ED of various etiologies (organic, psychogenic, and mixed) with a mean duration of 5 years. VIAGRA demonstrated statistically significant

improvement compared to placebo in all 21 studies. The studies that established benefit demonstrated improvements in success rates for sexual intercourse compared with placebo.

The effectiveness of VIAGRA was evaluated in most studies using several assessment instruments. The primary measure in the principal studies was a sexual function questionnaire (the International Index of Erectile Function - IIEF) administered during a 4-week treatment-free run-in period, at baseline, at follow-up visits, and at the end of double-blind, placebo-controlled, at-home treatment. Two of the questions from the IIEF served as primary study endpoints; categorical responses were elicited to questions about (1) the ability to achieve erections sufficient for sexual intercourse and (2) the maintenance of erections after penetration. The patient addressed both questions at the final visit for the last 4 weeks of the study. The possible categorical responses to these questions were (0) no attempted intercourse, (1) never or almost never, (2) a few times, (3) sometimes, (4) most times, and (5) almost always or always. Also collected as part of the IIEF was information about other aspects of sexual function, including information on erectile function, orgasm, desire, satisfaction with intercourse, and overall sexual satisfaction. Sexual function data were also recorded by patients in a daily diary. In addition, patients were asked a global efficacy question and an optional partner questionnaire was administered.

The effect on one of the major end points, maintenance of erections after penetration, is shown in Figure 3, for the pooled results of 5 fixed-dose, dose-response studies of greater than one month duration, showing response according to baseline function. Results with all doses have been pooled, but scores showed greater improvement at the 50 and 100 mg doses than at 25 mg. The pattern of responses was similar for the other principal question, the ability to achieve an erection sufficient for intercourse. The titration studies, in which most patients received 100 mg, showed similar results. Figure 3 shows that regardless of the baseline levels of function, subsequent function in patients treated with VIAGRA was better than that seen in patients treated with placebo. At the same time, on-treatment function was better in treated patients who were less impaired at baseline.



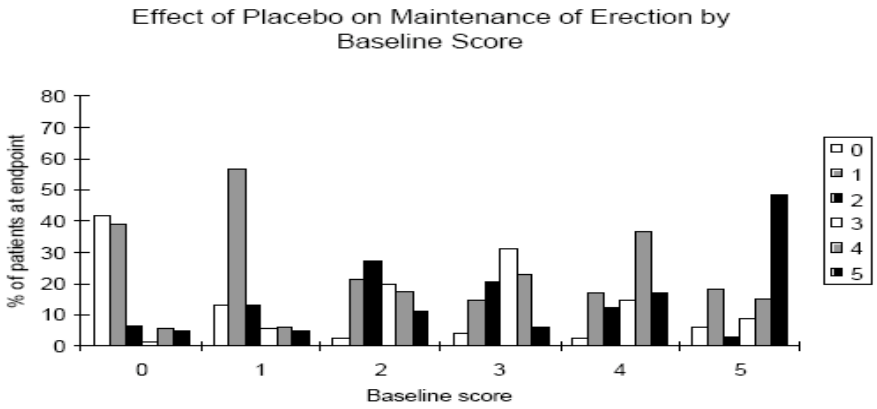



Figure 3. Effect of VIAGRA and Placebo on Maintenance of Erection by Baseline Score

The frequency of patients reporting improvement of erections in response to a global question in four of the randomized, double-blind, parallel, placebo-controlled fixed dose studies (1797 patients) of 12 to 24 weeks duration is shown in Figure 4. These patients had erectile dysfunction at baseline that was characterized by median categorical scores of 2 (a few times) on principal IIEF questions. Erectile dysfunction was attributed to organic (58%; generally not characterized, but including diabetes and excluding spinal cord injury), psychogenic (17%), or mixed (24%) etiologies. Sixty-three percent, 74%, and 82% of the patients on 25 mg, 50 mg and 100 mg of VIAGRA, respectively, reported an improvement in their erections, compared to 24% on placebo. In the titration studies (n=644) (with most patients eventually receiving 100 mg), results were similar.

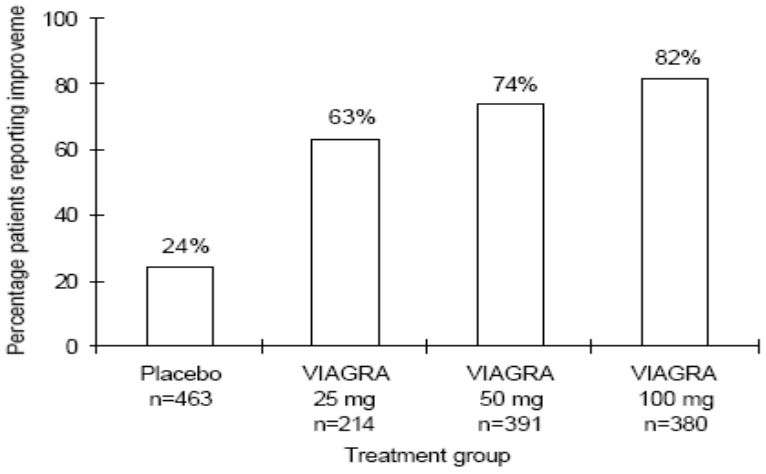



Figure 4. Percentage of Patients Reporting an Improvement in Erections.

The patients in studies had varying degrees of ED. One-third to one-half of the subjects in these studies reported successful intercourse at least once during a 4-week, treatment-free run-in period.

In many of the studies, of both fixed dose and titration designs, daily diaries were kept by patients. In these studies, involving about 1600 patients, analyses of patient diaries showed no effect of VIAGRA on rates of attempted intercourse (about 2 per week), but there was clear treatment-related improvement in sexual function: per patient weekly success rates averaged 1.3 on 50-100 mg of VIAGRA vs. 0.4 on placebo; similarly, group mean success rates (total successes divided by total attempts) were about 66% on VIAGRA vs about 20% on placebo.

During 3 to 6 months of double-blind treatment or longer-term (1 year), open-label studies, few patients withdrew from active treatment for any reason, including lack of effectiveness. At the end of the long-term study, 88% of patients reported that VIAGRA improved their erections.

Men with untreated ED had relatively low baseline scores for all aspects of sexual function measured (again using a 5-point scale) in the IIEF. VIAGRA improved these aspects of sexual function: frequency, firmness and maintenance of erections; frequency of orgasm; frequency and level of desire; frequency, satisfaction and enjoyment of intercourse; and overall relationship satisfaction.

One randomized, double-blind, flexible-dose, placebo-controlled study included only patients with erectile dysfunction attributed to complications of diabetes mellitus (n=268). As in the other titration studies, patients were started on 50 mg and allowed to adjust the dose up to 100 mg or down to 25 mg of VIAGRA; all patients, however, were receiving 50 mg or 100 mg at the end of the study. There were highly statistically significant improvements on the two principal IIEF questions (frequency of successful penetration during sexual activity and maintenance of erections after penetration) on VIAGRA compared to placebo. On a global improvement question, 57% of VIAGRA patients reported improved erections versus 10% on placebo. Diary data indicated that on VIAGRA, 48% of intercourse attempts were successful versus 12% on placebo.

One randomized, double-blind, placebo-controlled, crossover, flexible-dose (up to 100 mg) study of patients with erectile dysfunction resulting from spinal cord injury (n=178) was conducted. The changes from baseline in scoring on the two end point questions (frequency of successful penetration during sexual activity and maintenance of erections after penetration) were highly statistically significantly in favor of VIAGRA. On a global improvement question, 83% of patients reported improved erections on VIAGRA versus 12% on placebo. Diary data indicated that on VIAGRA, 59% of attempts at sexual intercourse were successful compared to 13% on placebo.

Across all trials, VIAGRA improved the erections of 43% of radical prostatectomy patients compared to 15% on placebo.

Subgroup analyses of responses to a global improvement question in patients with psychogenic etiology in two fixed-dose studies (total n=179) and two titration studies (total n=149) showed 84% of VIAGRA patients reported improvement in erections compared with 26% of placebo. The changes from baseline in scoring on the two end point questions (frequency of successful penetration during sexual activity and maintenance of erections after penetration) were highly statistically significantly in favor of VIAGRA. Diary data in two of the studies (n=178) showed rates of successful intercourse per attempt of 70% for VIAGRA and 29% for placebo.

A review of population subgroups demonstrated efficacy regardless of baseline severity, etiology, race and age. VIAGRA was effective in a broad range of ED patients, including those with a history of coronary artery disease, hypertension, other cardiac disease, peripheral vascular disease, diabetes mellitus, depression, coronary artery bypass graft (CABG), radical prostatectomy, transurethral

resection of the prostate (TURP) and spinal cord injury, and in patients taking antidepressants / antipsychotic and antihypertensive / diuretics.

Analysis of the safety database showed no apparent difference in the side effect profile in patients taking VIAGRA with and without antihypertensive medication. This analysis was performed retrospectively, and was not powered to detect any pre-specified difference in adverse reactions.

## 5. INDICATION AND USAGE

VIAGRA is indicated for the treatment of erectile dysfunction.

## 6. CONTRAINDICATIONS

Consistent with its known effects on the nitric oxide/cGMP pathway (see CLINICAL PHARMACOLOGY), VIAGRA was shown to potentiate the hypotensive effects of nitrates, and its administration to patients who are using organic nitrates, either regularly and/or intermittently, in any form is therefore contraindicated.

After patients have taken VIAGRA, it is unknown when nitrates, if necessary, can be safely administered. Based on the pharmacokinetic profile of a single 100 mg oral dose given to healthy normal volunteers, the plasma levels of sildenafil at 24 hours post dose are approximately 2 ng/ml (compared to peak plasma levels of approximately 440 ng/mL) (see CLINICAL PHARMACOLOGY: Pharmacokinetics and Metabolism). In the following patients: age >65, hepatic impairment (e.g., cirrhosis), severe renal impairment (e.g., creatinine clearance <30 mL/min), and concomitant use of potent cytochrome P450 3A4 inhibitors (erythromycin), plasma levels of sildenafil at 24 hours post dose have been found to be 3 to 8 times higher than those seen in healthy volunteers. Although plasma levels of sildenafil at 24 hours post dose are much lower than at peak concentration, it is unknown whether nitrates can be safely co-administered at this time point. VIAGRA is contraindicated in patients with a known hypersensitivity to any component of the tablet.

## 7. WARNINGS

There is a potential for cardiac risk of sexual activity in patients with preexisting cardiovascular disease. Therefore, treatments for erectile dysfunction, including VIAGRA, should not be generally used in men for whom sexual activity is inadvisable because of their underlying cardiovascular status.
VIAGRA has systemic vasodilatory properties that resulted in transient decreases in supine blood pressure in healthy volunteers (mean maximum decrease of 8.4/5.5 mmHg), (see CLINICAL PHARMACOLOGY: Pharmacodynamics). While this normally would be expected to be of little consequence in most patients, prior to prescribing VIAGRA, physicians should carefully consider whether their patients with underlying cardiovascular disease could be affected adversely by such vasodilatory effects, especially in combination with sexual activity.

Patients with the following underlying conditions can be particularly sensitive to the actions of
vasodilators including VIAGRA – those with left ventricular outflow obstruction (e.g. aortic stenosis, idiopathic hypertrophic subaortic stenosis) and those with severely impaired autonomic control of blood pressure. There is no controlled clinical data on the safety or efficacy of VIAGRA in the following groups; if prescribed, this should be done with caution.

- Patients who have suffered a myocardial infarction, stroke, or life-threatening arrhythmia within the last 6 months;
- Patients with resting hypotension (BP <90/50) or hypertension (BP >170/110);
- Patients with cardiac failure or coronary artery disease causing unstable angina;
- Patients with retinitis pigmentosa (a minority of these patients have genetic disorders of retinal phosphodiesterases).

Prolonged erection greater than 4 hours and priapism (painful erections greater than 6 hours in duration) have been reported infrequently since market approval of VIAGRA. In the event of an erection that persists longer than 4 hours, the patient should seek immediate medical assistance. If priapism is not treated immediately, penile tissue damage and permanent loss of potency could result.

The concomitant administration of the protease inhibitor ritonavir substantially increases serum concentrations of sildenafil (11-fold increase in AUC). If VIAGRA is prescribed to patients taking ritonavir, caution should be used. Data from subjects exposed to high systemic levels of sildenafil are limited. Visual disturbances occurred more commonly at higher levels of sildenafil exposure. Decreased blood pressure, syncope, and prolonged erection were reported in some healthy volunteers exposed to high doses of sildenafil (200-800 mg). To decrease the chance of adverse events in patients taking ritonavir, a decrease in sildenafil dosage is recommended (see Drug Interactions, ADVERSE REACTIONS and DOSAGE AND ADMINISTRATION).

## 8. PRECAUTIONS

### 8.1 General

The evaluation of erectile dysfunction should include a determination of potential underlying causes and the identification of appropriate treatment following a complete medical assessment.

Before prescribing VIAGRA, it is important to note the following:

Patients on multiple antihypertensive medications were included in the pivotal clinical trials for VIAGRA. In a separate drug interaction study, when amlodipine, 5 mg or 10 mg, and VIAGRA, 100 mg were orally administered concomitantly to hypertensive patients mean additional blood pressure reduction of 8 mmHg systolic and 7 mmHg diastolic were noted (see Drug Interactions).

When the alpha blocker doxazosin (4 mg) and VIAGRA (25 mg) were administered simultaneously to patients with benign prostatic hyperplasia (BPH), mean additional reductions of supine blood pressure of 7 mmHg systolic and 7 mmHg diastolic were observed. When higher doses of VIAGRA and doxazosin (4 mg) were administered simultaneously, there were infrequent reports of patients who experienced symptomatic postural hypotension within 1 to 4 hours of dosing. Simultaneous administration of VIAGRA to patients taking alpha-blocker therapy may lead to symptomatic hypotension in some patients. Therefore, VIAGRA doses above 25 mg should not be taken within 4 hours of taking an alpha-blocker.

The safety of VIAGRA is unknown in patients with bleeding disorders and patients with active peptic ulceration.

VIAGRA should be used with caution in patients with anatomical deformation of the penis (such as angulation, cavernosal fibrosis or Peyronie's disease), or in patients who have conditions which may predispose them to priapism (such as sickle cell anemia, multiple myeloma, or leukemia).

The safety and efficacy of combinations of VIAGRA with other treatments for erectile dysfunction have not been studied. Therefore, the use of such combinations is not recommended.

In humans, VIAGRA has no effect on bleeding time when taken alone or with aspirin. *In vitro* studies with human platelets indicate that sildenafil potentiates the antiaggregatory effect of sodium nitroprusside (a nitric oxide donor). The combination of heparin and VIAGRA had an additive effect on bleeding time in the anesthetized rabbit, but this interaction has not been studied in humans.

**8.2 Information for Patients**

Physicians should discuss with patients the contraindication of VIAGRA with regular and/or
intermittent use of organic nitrates. Physicians should discuss with patients the potential cardiac risk of sexual activity in patients with preexisting cardiovascular risk factors. Patients who experience symptoms (e.g., angina pectoris, dizziness, nausea) upon initiation of sexual activity should be advised to refrain from further activity and should discuss the episode with their physician.

Physicians should advise patients to stop use of all PDE5 inhibitors, including VIAGRA, and seek medical attention in the event of a sudden loss of vision in one or both eyes. Such an event may be a sign of non-arteritic anterior ischemic optic neuropathy (NAION), a cause of decreased vision including permanent loss of vision, that has been reported rarely post-marketing in temporal association with the use of all PDE5 inhibitors. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or to other factors. Physicians should also discuss with patients the increased risk of NAION in individuals who have already experienced NAION in one eye, including whether such individuals could be adversely affected by use of vasodilators, such as PDE5 inhibitors (see POST-MARKETING EXPERIENCE/Special Senses). Physicians should warn patients that prolonged erections greater than 4 hours and priapism (painful erections greater than 6 hours in duration) have been reported infrequently since market approval of VIAGRA. In the event of an erection that persists longer than 4 hours, the patient should seek immediate medical assistance. If priapism is not treated immediately, penile tissue damage and permanent loss of potency may result.

Physicians should advise patients that simultaneous administration of VIAGRA doses above 25 mg and an alpha-blocker may lead to symptomatic hypotension in some patients. Therefore, VIAGRA doses above 25 mg should not be taken within four hours of taking an alpha-blocker.

The use of VIAGRA offers no protection against sexually transmitted diseases. Counseling of patients about the protective measures necessary to guard against sexually transmitted diseases, including the Human Immunodeficiency Virus (HIV), may be considered.

## 9. Drug Interactions

### 9.1 Effects of Other Drugs on VIAGRA

*In vitro* studies: Sildenafil metabolism is principally mediated by the cytochrome P450 (CYP)
isoforms 3A4 (major route) and 2C9 (minor route). Therefore, inhibitors of these isoenzymes may reduce sildenafil clearance.

*In vivo* studies: Cimetidine (800 mg), a nonspecific CYP inhibitor, caused a 56% increase in plasma sildenafil concentrations when coadministered with VIAGRA (50 mg) to healthy volunteers.

When a single 100 mg dose of VIAGRA was administered with erythromycin, a specific CYP3A4 inhibitor, at steady state (500 mg bid for 5 days), there was a 182% increase in sildenafil systemic exposure (AUC). In addition, in a study performed in healthy male volunteers, co-administration of the HIV protease inhibitor saquinavir, also a CYP3A4 inhibitor, at steady state (1200 mg tid) with VIAGRA (100 mg single dose) resulted in a 140% increase in sildenafil Cmax and a 210% increase in sildenafil AUC. VIAGRA had no effect on saquinavir pharmacokinetics. Stronger CYP3A4 inhibitors such as ketoconazole or itraconazole would be expected to have still greater effects, and population data from patients in clinical trials did indicate a reduction in sildenafil clearance when it was co-administered with CYP3A4 inhibitors (such as ketoconazole, erythromycin, or cimetidine) (see DOSAGE AND ADMINISTRATION).

In another study in healthy male volunteers, co-administration with the HIV protease inhibitor ritonavir, which is a highly potent P450 inhibitor, at steady state (500 mg bid) with VIAGRA (100 mg single dose) resulted in a 300% (4-fold) increase in sildenafil Cmax and a 1000% (11-fold) increase in sildenafil plasma AUC. At 24 hours the plasma levels of sildenafil were still approximately 200 ng/mL, compared to approximately 5 ng/mL when sildenafil was dosed alone. This is consistent with ritonavir's marked effects on a broad range of P450 substrates. VIAGRA had no effect on ritonavir pharmacokinetics (see DOSAGE AND ADMINISTRATION).

Although the interaction between other protease inhibitors and sildenafil has not been studied, their concomitant use is expected to increase sildenafil levels.

It can be expected that concomitant administration of CYP3A4 inducers, such as rifampin, will decrease plasma levels of sildenafil.

Single doses of antacid (magnesium hydroxide/aluminum hydroxide) did not affect the bioavailability of VIAGRA.

Pharmacokinetic data from patients in clinical trials showed no effect on sildenafil pharmacokinetics of CYP2C9 inhibitors (such as tolbutamide, warfarin), CYP2D6 inhibitors (such as selective serotonin reuptake inhibitors, tricyclic antidepressants), thiazide and related diuretics, ACE inhibitors, and calcium channel blockers. The AUC of the active metabolite, N-desmethyl sildenafil, was increased 62% by loop and potassium-sparing diuretics and 102% by nonspecific beta-blockers. These effects on the metabolite are not expected to be of clinical consequence.

## 9.2 Effects of VIAGRA on Other Drugs

*In vitro* studies: Sildenafil is a weak inhibitor of the cytochrome P450 isoforms 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 (IC50 >150 μM). Given sildenafil peak plasma concentrations of approximately 1 μM after recommended doses, it is unlikely that VIAGRA will alter the clearance of substrates of these isoenzymes.

*In vivo* studies: When VIAGRA 100 mg oral was coadministered with amlodipine, 5 mg or 10 mg oral, to hypertensive patients, the mean additional reduction on supine blood pressure was 8 mmHg systolic and 7 mmHg diastolic. No significant interactions were shown with tolbutamide (250 mg) or warfarin (40 mg), both of which are metabolized by CYP2C9.

VIAGRA (50 mg) did not potentiate the increase in bleeding time caused by aspirin (150 mg).

VIAGRA (50 mg) did not potentiate the hypotensive effect of alcohol in healthy volunteers with mean maximum blood alcohol levels of 0.08%.

In a study of healthy male volunteers, sildenafil (100 mg) did not affect the steady state pharmacokinetics of the HIV protease inhibitors, saquinavir and ritonavir, both of which are CYP3A4 substrates.

## 9.3 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sildenafil was not carcinogenic when administered to rats for 24 months at a dose resulting in total systemic drug exposure (AUCs) for unbound sildenafil and its major metabolite of 29- and 42-times, for male and female rats, respectively, the exposures observed in human males given the Maximum Recommended Human Dose (MRHD) of 100 mg. Sildenafil was not carcinogenic when administered to mice for 18-21 months at dosages up to the Maximum Tolerated Dose (MTD) of 10 mg/kg/day, approximately 0.6 times the MRHD on a mg/m2 basis.

Sildenafil was negative in *in vitro* bacterial and Chinese hamster ovary cell assays to detect mutagenicity, and *in vitro* human lymphocytes and *in vivo* mouse micronucleus assays to detect clastogenicity.

There was no impairment of fertility in rats given sildenafil up to 60 mg/kg/day for 36 days to females and 102 days to males, a dose producing an AUC value of more than 25 times the human male AUC. There was no effect on sperm motility or morphology after single 100 mg oral doses of VIAGRA in healthy volunteers.

## 9.4 Pregnancy, Nursing Mothers and Pediatric Use

VIAGRA is not indicated for use in newborns, children, or women.

## 9.5 Pregnancy Category B.

No evidence of teratogenicity, embryotoxicity or fetotoxicity was observed in rats and rabbits which received up to 200 mg/kg/day during organogenesis. These doses represent, respectively, about 20 and 40 times the MRHD on a mg/m2 basis in a 50 kg subject. In the rat pre- and postnatal development study, the no observed adverse effect dose was 30 mg/kg/day given for 36 days. In the nonpregnant rat the AUC at this dose was about 20 times human AUC. There are no adequate and well-controlled studies of sildenafil in pregnant women.

### 9.6 Geriatric Use

Healthy elderly volunteers (65 years or over) had a reduced clearance of sildenafil (see CLINICAL PHARMACOLOGY: Pharmacokinetics in Special Populations). Since higher plasma levels may increase both the efficacy and incidence of adverse events, a starting dose of 25 mg should be considered (see DOSAGE AND ADMINISTRATION).

## 10 ADVERSE REACTIONS

### 10.1 Pre-marketing experience

VIAGRA was administered to over 3700 patients (aged 19-87 years) during clinical trials worldwide. Over 550 patients were treated for longer than one year.

In placebo-controlled clinical studies, the discontinuation rate due to adverse events for VIAGRA (2.5%) was not significantly different from placebo (2.3%). The adverse events were generally transient and mild to moderate in nature.

In trials of all designs, adverse events reported by patients receiving VIAGRA were generally similar. In fixed-dose studies, the incidence of some adverse events increased with dose. The nature of the adverse events in flexible-dose studies, which more closely reflect the recommended dosage regimen, was similar to that for fixed-dose studies.

When VIAGRA was taken as recommended (on an as-needed basis) in flexible-dose, placebo-controlled clinical trials, the following adverse events were reported:

TABLE 2. ADVERSE EVENTS REPORTED BY ≥2% OF PATIENTS TREATED WITH VIAGRA AND MORE FREQUENT ON DRUG THAN PLACEBO IN PRN FLEXIBLE-DOSE PHASE II/III STUDIES

| Adverse Event | Percentage of Patients Reporting Event | |
|---|---|---|
| | VIAGRA | PLACEBO |
| | N=734 | N=725 |
| Headache | 16% | 4% |
| Flushing | 10% | 1% |
| Dyspepsia | 7% | 2% |
| Nasal Congestion | 4% | 2% |
| Urinary Tract Infection | 3% | 2% |
| Abnormal Vision† | 3% | 0% |
| Diarrhea | 3% | 1% |
| Dizziness | 2% | 1% |
| Rash | 2% | 1% |

†Abnormal Vision: Mild and transient, predominantly color tinge to vision, but also increased sensitivity to light or blurred vision. In these studies, only one patient discontinued due to abnormal vision.

Other adverse reactions occurred at a rate of >2%, but equally common on placebo: respiratory tract infection, back pain, flu syndrome, and arthralgia.

In fixed-dose studies, dyspepsia (17%) and abnormal vision (11%) were more common at 100 mg than at lower doses. At doses above the recommended dose range, adverse events were similar to those detailed above but generally were reported more frequently.

The following events occurred in <2% of patients in controlled clinical trials; a causal relationship to VIAGRA is uncertain. Reported events include those with a plausible relation to drug use; omitted are minor events and reports too imprecise to be meaningful:

Body as a whole: face edema, photosensitivity reaction, shock, asthenia, pain, chills, accidental fall, abdominal pain, allergic reaction, chest pain, accidental injury.

Cardiovascular: angina pectoris, AV block, migraine, syncope, tachycardia, palpitation, hypotension, postural hypotension, myocardial ischemia, cerebral thrombosis, cardiac arrest, heart failure, abnormal electrocardiogram, cardiomyopathy.

Digestive: vomiting, glossitis, colitis, dysphagia, gastritis, gastroenteritis, esophagitis, stomatitis, dry mouth, liver function tests abnormal, rectal hemorrhage, gingivitis.
Hemic and Lymphatic: anemia and leukopenia.

Metabolic and Nutritional: thirst, edema, gout, unstable diabetes, hyperglycemia, peripheral edema, hyperuricemia, hypoglycemic reaction, hypernatremia.
Musculoskeletal: arthritis, arthrosis, myalgia, tendon rupture, tenosynovitis, bone pain, myasthenia, synovitis.

Nervous: ataxia, hypertonia, neuralgia, neuropathy, paresthesia, tremor, vertigo, depression, insomnia, somnolence, abnormal dreams, reflexes decreased, hypesthesia.

Respiratory: asthma, dyspnea, laryngitis, pharyngitis, sinusitis, bronchitis, sputum increased, cough increased.

Skin and Appendages: urticaria, herpes simplex, pruritus, sweating, skin ulcer, contact dermatitis, exfoliative dermatitis.

Special Senses: mydriasis, conjunctivitis, photophobia, tinnitus, eye pain, deafness, ear pain, eye hemorrhage, cataract, dry eyes.

Urogenital: cystitis, nocturia, urinary frequency, breast enlargement, urinary incontinence, abnormal ejaculation, genital edema and anorgasmia.

### 10.3 Post-marketing experience

Cardiovascular and cerebrovascular: serious cardiovascular, cerebrovascular, and vascular events, including myocardial infarction, sudden cardiac death, ventricular arrhythmia, cerebrovascular hemorrhage, transient ischemic attack, hypertension, subarachnoid and intracerebral hemorrhages, and pulmonary hemorrhage have been reported post-marketing in temporal association with the use of VIAGRA. Most, but not all, of these patients had preexisting cardiovascular risk factors. Many of these events were reported to occur during or shortly after sexual activity, and a few were reported to occur shortly after the use of VIAGRA without sexual activity. Others were reported to have occurred hours to days after the use of VIAGRA and sexual activity. It is not possible to determine whether these events are related directly to VIAGRA, to sexual activity, to the patient's underlying cardiovascular disease, to a combination of these factors, or to other factors (see WARNINGS for further important cardiovascular information).

Other events reported post-marketing to have been observed in temporal association with VIAGRA and not listed in the pre-marketing adverse reactions section above include:

Nervous: seizure and anxiety.

Urogenital: prolonged erection, priapism (see WARNINGS) and hematuria.

Special Senses: diplopia, temporary vision loss/decreased vision, ocular redness or bloodshot appearance, ocular burning, ocular swelling/pressure, increased intraocular pressure, retinal vascular disease or bleeding, vitreous detachment/traction, paramacular edema and epistaxis.

Non-arteritic anterior ischemic optic neuropathy (NAION), a cause of decreased vision including permanent loss of vision, has been reported rarely post-marketing in temporal association with the use of phosphodiesterase type 5 (PDE5) inhibitors, including VIAGRA. Most, but not all, of these patients had underlying anatomic or vascular risk factors for developing NAION, including but not necessarily limited to: low cup to disc ratio ("crowded disc"), age over 50, diabetes, hypertension, coronary artery disease, hyperlipidemia and smoking. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors, to the patient's underlying vascular risk factors or anatomical defects, to a combination of these factors, or to other factors (see PRECAUTIONS/Information for Patients).

## 11. OVERDOSAGE

In studies with healthy volunteers of single doses up to 800 mg, adverse events were similar to those seen at lower doses but incidence rates were increased.

In cases of overdose, standard supportive measures should be adopted as required. Renal dialysis is not expected to accelerate clearance as sildenafil is highly bound to plasma proteins and it is not eliminated in the urine.

## 12. DOSAGE AND ADMINISTRATION

For most patients, the recommended dose is 50 mg taken, as needed, approximately 1 hour before sexual activity. However, VIAGRA may be taken anywhere from 4 hours to 0.5 hour before sexual activity. Based on effectiveness and toleration, the dose may be increased to a maximum recommended dose of 100 mg or decreased to 25 mg. The maximum recommended dosing frequency is once per day.

The following factors are associated with increased plasma levels of sildenafil: age >65 (40% increase in AUC), hepatic impairment (e.g., cirrhosis, 80%), severe renal impairment (creatinine clearance <30 mL/min, 100%), and concomitant use of potent cytochrome P450 3A4 inhibitors [ketoconazole, itraconazole, erythromycin (182%), saquinavir (210%)]. Since higher plasma levels may increase both the efficacy and incidence of adverse events, a starting dose of 25 mg should be considered in these patients.

Ritonavir greatly increased the systemic level of sildenafil in a study of healthy, non-HIV infected volunteers (11-fold increase in AUC, see Drug Interactions.) Based on these pharmacokinetic data, it is recommended not to exceed a maximum single dose of 25 mg of VIAGRA in a 48 hour period. VIAGRA was shown to potentiate the hypotensive effects of nitrates and its administration in patients who use nitric oxide donors or nitrates in any form is therefore contraindicated.

Simultaneous administration of VIAGRA doses above 25 mg and an alpha-blocker may lead to symptomatic hypotension in some patients. Doses of 50 mg or 100 mg of VIAGRA should not be taken within 4 hours of alpha-blocker administration. A 25 mg dose of VIAGRA may be taken at any time.

## 13. HOW SUPPLIED

VIAGRA® (sildenafil citrate) is supplied as blue, film-coated, rounded-diamond-shaped tablets containing sildenafil citrate equivalent to the nominally indicated amount of sildenafil as follows:

| | 25 mg | 50 mg | 100 mg |
|---|---|---|---|
| Obverse | VGR25 | VGR50 | VGR100 |
| Reverse | PFIZER | PFIZER | PFIZER |
| Bottle of 30 | NDC-0069-4200-30 | NDC-0069-4210-30 | NDC-0069-4220-30 |
| Bottle of 100 | N/A | NDC-0069-4210-66 | NDC-0069-4220-66 |

Recommended Storage: Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

## 14. PATIENT SUMMARY OF INFORMATION ABOUT
*(Sildenafil citrate) tablets*

This summary contains important information about VIAGRA®. It is not meant to take the place of your doctor's instructions. Read this information carefully before you start taking VIAGRA. Ask your doctor or pharmacist if you do not understand any of this information or if you want to know more about VIAGRA.

This medicine can help many men when it is used as prescribed by their doctors. However, VIAGRA is not for everyone. It is intended for use only by men who have a condition called erectile dysfunction. VIAGRA must never be used by men who are taking medicines that contain nitrates of any kind, at any time. This includes nitroglycerin. If you take VIAGRA with any nitrate medicine your blood pressure could suddenly drop to an unsafe or life threatening level.

WHAT IS VIAGRA?
VIAGRA is a pill used to treat erectile dysfunction (impotence) in men. It can help many men who have erectile dysfunction get and keep an erection when they become sexually excited (stimulated). You will not get an erection just by taking this medicine. VIAGRA helps a man with erectile dysfunction get an erection only when he is sexually excited.

HOW SEX AFFECTS THE BODY
When a man is sexually excited, the penis rapidly fills with more blood than usual. The penis then expands and hardens. This is called an erection. After the man is done having sex, this extra blood flows out of the penis back into the body. The erection goes away. If an erection lasts for a long time (more than 6 hours), it can permanently damage your penis. You should call a doctor immediately if you ever have a prolonged erection that lasts more than 4 hours. Some conditions and medicines interfere with this natural erection process. The penis cannot fill with enough blood. The man cannot have an erection. This is called erectile dysfunction if it becomes a frequent problem. During sex, your heart works harder. Therefore sexual activity may not be advisable for people who have heart problems. Before you start any treatment for erectile dysfunction, ask your doctor if your heart is healthy enough to handle the extra strain of having sex. If you have chest pains, dizziness or nausea during sex, stop having sex and immediately tell your doctor you have had this problem.

HOW VIAGRA WORKS
VIAGRA enables many men with erectile dysfunction to respond to sexual stimulation. When a man is sexually excited, VIAGRA helps the penis fill with enough blood to cause an erection. After sex is over, the erection goes away.

VIAGRA IS NOT FOR EVERYONE
As noted above (*How Sex Affects the Body*), ask your doctor if your heart is healthy enough for sexual activity. If you take any medicines that contain nitrates – either regularly or as needed – you should never take VIAGRA. If you take VIAGRA with any nitrate medicine or recreational drug containing nitrates, your blood pressure could suddenly drop to an unsafe level. You could get dizzy, faint, or even have a heart attack or stroke. Nitrates are found in many prescription medicines that are used to treat angina (chest pain due to heart disease) such as:

- nitroglycerin (sprays, ointments, skin patches or pastes, and tablets that are swallowed
  or dissolved in the mouth)

- isosorbide mononitrate and isosorbide dinitrate (tablets that are swallowed, chewed, or
  dissolved in the mouth)

Nitrates are also found in recreational drugs such as amyl nitrate or nitrite ("poppers"). If you are not sure if any of your medicines contain nitrates, or if you do not understand what nitrates are, ask your doctor or pharmacist. VIAGRA is only for patients with erectile dysfunction. VIAGRA is not for newborns, children, or women. Do not let anyone else take your VIAGRA. VIAGRA must be used only under a doctor's supervision.

WHAT VIAGRA DOES NOT DO

- VIAGRA does not cure erectile dysfunction. It is a treatment for erectile dysfunction.
- VIAGRA does not protect you or your partner from getting sexually transmitted diseases, including HIV—the virus that causes AIDS.
- VIAGRA is not a hormone or an aphrodisiac.

WHAT TO TELL YOUR DOCTOR BEFORE YOU BEGIN VIAGRA

Only your doctor can decide if VIAGRA is right for you. VIAGRA can cause mild, temporary lowering of your blood pressure. You will need to have a thorough medical exam to diagnose your erectile dysfunction and to find out if you can safely take VIAGRA alone or with your other medicines. Your doctor should determine if your heart is healthy enough to handle the extra strain of having sex.

Be sure to tell your doctor if you:

- have ever had any heart problems (e.g., angina, chest pain, heart failure, irregular heart
  beats, heart attack or narrowing of the aortic valve)
- have ever had a stroke
- have low or high blood pressure
- have ever had severe vision loss
- have a rare inherited eye disease called retinitis pigmentosa
- have ever had any kidney problems
- have ever had any liver problems
- have ever had any blood problems, including sickle cell anemia or leukemia
- are allergic to sildenafil or any of the other ingredients of VIAGRA tablets
- have a deformed penis, Peyronie's disease, or ever had an erection that lasted more than
  4 hours
- have stomach ulcers or any types of bleeding problems
- are taking any other medicines

VIAGRA AND OTHER MEDICINES

Some medicines can change the way VIAGRA works. Tell your doctor about any medicines you are taking. Do not start or stop taking any medicines before

checking with your doctor or pharmacist. This includes prescription and nonprescription medicines or remedies:

- Remember, VIAGRA should never be used with medicines that contain nitrates (see *VIAGRA Is Not for Everyone*).
- If you are taking alpha-blocker therapy for the treatment of high blood pressure or prostate problems, you should not take a dose of greater than 25 mg of VIAGRA at the same time (within 4 hours) as you take your dose of alpha-blocker.
- If you are taking a protease inhibitor, your dose may be adjusted (please see *Finding the Right Dose for You*).
- VIAGRA should not be used with any other medical treatments that cause erections. These treatments include pills, medicines that are injected or inserted into the penis, implants or vacuum pumps.

FINDING THE RIGHT DOSE FOR YOU
VIAGRA comes in different doses (25 mg, 50 mg and 100 mg). If you do not get the results you expect, talk with your doctor. You and your doctor can determine the dose that works best for you.

- Do not take more VIAGRA than your doctor prescribes.
- If you think you need a larger dose of VIAGRA, check with your doctor.
- VIAGRA should not be taken more than once a day.

If you are older than age 65, or have serious liver or kidney problems, your doctor may start you at the lowest dose (25 mg) of VIAGRA. If you are taking protease inhibitors, such as for the treatment of HIV, your doctor may recommend a 25 mg dose and may limit you to a maximum single dose of 25 mg of VIAGRA in a 48 hour period. If you are taking alpha-blocker therapy, you should not take a dose of greater than 25 mg of VIAGRA at the same time (within 4 hours) as your dose of alphablocker.

HOW TO TAKE VIAGRA
Take VIAGRA about one hour before you plan to have sex. Beginning in about 30 minutes and for up to 4 hours, VIAGRA can help you get an erection if you are sexually excited. If you take VIAGRA after a high-fat meal (such as a cheeseburger and french fries), the medicine may take a little longer to start working. VIAGRA can help you get an erection when you are sexually excited. You will not get an erection just by taking the pill.

POSSIBLE SIDE EFFECTS
Like all medicines, VIAGRA can cause some side effects. These effects are usually mild to moderate and usually don't last longer than a few hours. Some of these side effects are more likely to occur with higher doses. The most common side effects of VIAGRA are headache, flushing of the face, and upset stomach. Less common side effects that may occur are temporary changes in color vision (such as trouble telling the difference between blue and green objects or having a blue color tinge to them), eyes being more sensitive to light, or blurred vision.

In rare instances, men taking PDE5 inhibitors (oral erectile dysfunction medicines, including VIAGRA) reported a sudden decrease or loss of vision in one or both eyes. It is not possible to determine whether these events are related directly to these medicines, to other factors such as high blood pressure or diabetes, or to a

combination of these. If you experience sudden decrease or loss of vision, stop taking PDE5 inhibitors, including VIAGRA, and call a doctor right away. In rare instances, men have reported an erection that lasts many hours. You should call a doctor immediately if you ever have an erection that lasts more than 4 hours. If not treated right away, permanent damage to your penis could occur (see *How Sex Affects the Body*).

Heart attack, stroke, irregular heart beats, and death have been reported rarely in men taking VIAGRA. Most, but not all, of these men had heart problems before taking this medicine. It is not possible to determine whether these events were directly related to VIAGRA. VIAGRA may cause other side effects besides those listed on this sheet. If you want more information or develop any side effects or symptoms you are concerned about, call your doctor.

ACCIDENTAL OVERDOSE
In case of accidental overdose, call your doctor right away.

STORING VIAGRA
Keep VIAGRA out of the reach of children. Keep VIAGRA in its original container. Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

FOR MORE INFORMATION ON VIAGRA
VIAGRA is a prescription medicine used to treat erectile dysfunction. Only your doctor can decide if it is right for you. This sheet is only a summary. If you have any questions or want more information about VIAGRA, talk with your doctor or pharmacist, visit www.viagra.com, or call 1-888-4VIAGRA.


*Distributed by*
LAB-0221-2 Revised June 2005
Pfizer Labs
Division of Pfizer Inc, NY, NY 10017

### 6.2.3 Appendix 3: Medicines and Healthcare products Regulatory Agency Approval

Safeguarding public health

MHRA

Direct Line: 0207 084-2456
Facsimile: 0207 084-2443
Room 12- 242

**Our reference:** 24712/0003/001-0001
**Eudract Number:** 2005-001034-32

Dr A Wood
NHS GREATER GLASGOW
YORKHILL DIVISION
DALNAIR STREET
GLASGOW
G3 8SJ
UNITED KINGDOM

RESEAR

- 6 FEB 2006

02/02/2006

Dear Dr A Wood

**THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 1031**
**Product Type:** General Medicinal Product
**Product:** Sildenafil Citrate
**Protocol number:** 05/CH/01

**NOTICE OF ACCEPTANCE**

I am writing to confirm that the Licensing Authority, acting under regulation 18(2)(b) or (c), or 19(2)(a) or (8), or 20(2)(a) or (5), of the Regulations and according to the type of medicinal product involved[1], accepts your request to carry out a clinical trial in accordance with your application received on 12/12/2005 which we acknowledged in our letter dated 31/01/2006 subject to you receiving a favourable opinion from the relevant ethics committee in accordance with regulation 15(1). You may therefore carry out the trial as notified, but I must remind you of the Authority's powers under regulation 31 to suspend or terminate a clinical trial if the conditions set out in regulation 31(1)(a) and (b) are satisfied.

Remarks:
* Further information on the composition, specification, release testing and stability of the active product is required before the study commences.
A copy of the manufacturer's authorisation for the manufacture of investigational medicinal products [MA(IMP)]for the active and placebo products should be provided before the study commences.
* A sample of the labelling to be used should be provided.

[1] The Licensing Authority's authorisation powers for clinical trials are regulation 18 for those involving general medicinal products, regulation 19 for those involving medicinal products for gene therapy etc., and regulation 20 for those involving medicinal products with special characteristics.

Medicines and Healthcare products Regulatory Agency
Market Towers 1 Nine Elms Lane London SW8 5NQ
T 020 7084 2000 F 020 7084 2353 www.mhra.gov.uk

An executive agency of the Department of Health

The authorisation is effective from the date of this letter and may continue under this authorisation. In accordance with regulation 27, you must notify the Licensing Authority within 90 days of the conclusion of the trial, that it has ended.

Yours sincerely

pp

**Dr Martyn Ward**
**Head of Clinical Trial Unit**

## 6.2.4 Appendix 4: Local and Regional Ethics Approval

**West Glasgow Ethics Committee 1**

**LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION**

*For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.*

| | | | | | |
|---|---|---|---|---|---|
| **REC reference number:** | 06/S0703/8 | **Issue number:** | 2 | **Date of issue:** | 16 January 2006 |
| **Chief Investigator:** | Mr Tony Vassalos | | | | |
| **Full title of study:** | Does preoperative Sildenafil protect against pulmonary related complications following cardiopulmonary bypass? A randomised trial in children undergoing cardiac surgical repair. | | | | |

*This study was given a favourable ethical opinion by West Glasgow Ethics Committee 1 on 10 January 2006. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.*

| *Principal Investigator* | *Post* | *Research site* | *Site assessor* | *Date of favourable opinion for this site* | *Notes [1]* |
|---|---|---|---|---|---|
| Mr Mark Danton | Consultant Cardiac Surgeon | Yorkhill Hospital | Yorkhill Research Ethics Committee | 13/01/2006 | |

Approved by the Chair on behalf of the REC:

.....(Signature of Chair/Administrator)

(delete as applicable)

.....(Name)

### 6.2.5 Appendix 5: Parent Information Sheet

VERSION 2 (18/01/2006)

**Study Title:** Does pre-operative Sildenafil protect against pulmonary related complications following cardiopulmonary bypass? A randomised trial in children undergoing cardiac surgical repair.

**Lay Title**: Will giving the drug Sildenafil to children before open-heart surgery improve the function of the lungs after surgery?

**Invitation paragraph:** You and your child are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with family, medical or nursing staff if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

**Q1. What is the purpose of the study?**

**Ans:** The purpose of this study is to investigate whether giving the drug Sildenafil (Viagra, Pfizer) to children before open-heart surgery improves the function of the lungs after surgery.

**Q2. Why have I been chosen?**

**Ans:** Participation in this study is being offered to all parents with children aged over 3 months undergoing heart surgery to correct inborn heart defects that allow increased blood flow within the lungs and may be associated with lung related complications (pulmonary hypertension) after surgery.

**Q3. Do I have to take part?**

**Ans: NO.** It is up to you to decide whether or not you wish your child to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives.

**Q4. What will happen to my child, if my child takes part?**

**Ans:** If you decide that you would like your child to take part:

- The day immediately before surgery your child will be randomly (like the toss of a coin) put into a group that is either given the drug *Sildenafil (1/2 teaspoon liquid)* or *Placebo (1/2 teaspoon inactive liquid)*, *6hrly* (4 doses) by the ward nursing staff. This will be given in addition to their normal medications.
- Otherwise, your child's surgical and medical care both before, during and after surgery will follow the normal practice of their consultant heart surgeon.

- Your child's progress before, during and after surgery will be closely monitored.

(e) Data from ward charts, intensive care charts and routine blood results will be recorded.
(f) Images from routine echocardiography used before, during and after surgery to assess the heart's function will be studied on the computer.
(g) Any blood that remains after routine investigations have been carried out by the laboratory at Yorkhill will be stored and used to measure markers of inflammation / infection. No extra blood will be taken from your child specifically for the trial.

- Your child will remain part of the trial until the day of discharge (usually 5 days).
- You and your child will not need to visit your GP or attend any additional clinics. Your child's GP will however be informed of your child's participation in this trial.

**Q5. What do I have to do?**

**Ans:** There are no associated restrictions with taking part in this study. Your child's stay in hospital should follow the normal course.

**Q6. What is the drug or procedure that is being tested?**

**Ans:** The drug being tested is called *Sildenafil (Viagra, Pfizer)*. Some children undergoing heart surgery to correct inborn heart defects that allow increase blood flow within the lungs are prone to lung related complications (pulmonary hypertension) after surgery. Oral *Sildenafil liquid (0.5mg/kg)* is routinely used in our pediatric intensive care unit after heart surgery to treat these complications. We hope that giving *4 doses* of *oral Sildenafil (0.5mg/kg, equivalent to ½ teaspoon liquid per dose)* the day before surgery will prevent or reduce lung related complications after surgery.

**Q7. What are the side effects of any treatment received when taking part?**

**Ans:** Sildenafil is not licensed in the UK for the treatment of pulmonary hypertension. Sildenafil is however effectively and regularly used after heart surgery in our pediatric intensive care unit to treat post-operative pulmonary hypertension. Clinical trials assessing the use of Sildenafil in children undergoing heart surgery are limited but to date there are no clinically significant side effects reported in giving oral Sildenafil (0.5mg-2mg/kg; 6hrly) to children. This opinion is also supported by our own clinical experience. Although some children in the trial will receive 4 doses of Sildenafil the day before surgery, after surgery Sildenafil could be given to any child if that is deemed necessary by the consultant cardiac surgeon and the medical team responsible for their care. All clinically significant side effects thought to be related to Sildenafil administration in this study will be recorded and reported to the relevant authorities.

**Q8. What are the possible disadvantages and risks of taking part?**

**Ans:** The mainstay of your child's surgical and medical care before, during and after surgery will follow the normal clinical practice of their consultant cardiac surgeon. Possible disadvantages and risks would therefore be related to taking 4 oral doses of Sildenafil liquid (0.5mg/kg) the day before surgery. Side effects including headache, flushing, diarrhoea, rash and heartburn have been reported in the literature but we do not foresee any clinical disadvantages or side effects associated with the dosage being

used in this study. Oral Sildenafil (0.5-2mg/kg/4-6hrs) is routinely used after heart surgery in our pediatric intensive care unit without clinically significant side effects.

**Q9. What are the possible benefits of taking part?**

**Ans:** We hope that giving 4 doses of the drug Sildenafil before surgery will protect the lungs during surgery to improve lung function and reduce related complications after surgery. If this is the case it would reduce the overall recovery time and intensive care stay after surgery. All children undergoing corrective heart surgery are closely monitored throughout their admission. Although this will also be the case for all research participants they will undergo a more detailed echocardiographic assessment both before and after surgery, providing additional clinical information that may be beneficial to the caring medical team.

**Q10. What if new information becomes available?**

**Ans:** All relevant additional information on the function of the heart and lungs found during the trial will be made available to the clinical team caring for your child to allow them to make the appropriate clinical decisions early.

**Q11. What happens when the research study stops?**

**Ans:** The research study stops when your child is discharged from hospital. There is no further participation or responsibility.

**Q12. What if something goes wrong?**

**Ans:** If taking part in this research project harms your child, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

**Q13. Will my taking part in this study be kept confidential?**

**Ans:** All information that is collected about your child during the course of the research will be kept strictly confidential. Any information about your child, which leaves the hospital/surgery, will have his/her name and address removed so that he/she cannot be recognised from it.

**Q14. What will happen to the results of the research study?**

**Ans:** The principal aim of this study is to improve the management and care of children with inborn heart defects that make them prone to developing severe pulmonary hypertension after corrective heart surgery. We also plan to publish our results in peer reviewed medical journals. Any publications that arise should be available to everyone on-line. The information may also be used in presentations to local and international congenital heart disease forums as well as local charities and parent groups. You will not be specifically identified in any report or publication.

**Q15. Who is organising and funding the research?**

**Ans:** This study is being funded by successful research grant application to the Yorkhill Children's Foundation. It has been organised by Mr Tony Vassalos (Research Fellow, Cardiac Surgery) under the supervision of Mr Mark Danton (Consultant Cardiac Surgeon), Professor Fiona Lyall (Professor of Maternal and Fetal Health) and key Cardiac Surgery, Cardiology, Intensive Care and Pharmacy collaborators.

**Q16. Who has reviewed the study?**

**Ans:** As mentioned above the study is being funded by a successful research grant application to the Yorkhill Children's Foundation. This involved a stringent, 2 phase, internal and external review process of the proposed study. The study has also been reviewed and approved by the Research Ethics Committee based in the Western Infirmary, Glasgow.

**Q17. Who can I contact for further information?**

**Ans:** If there is anything you do not understand or feel you would like further information on please do not hesitate to contact either of the persons named below through the Cardiac Surgery secretaries at Yorkhill Hospital (0141 201 0251) or the Cardiac Liaison Sisters who will introduce themselves during your child's admission:

- Mr Mark Danton, Consultant Paediatric Cardiac Surgeon, Yorkhill
- Mr Tony Vassalos, Research Fellow, Department of Cardiac Surgery, Yorkhill
- Cardiac Liaison Sisters (0141 201 9291)

**Q18. With whom can I register any complaints?**

- **Ans:** The standard NHS complaints procedure will be open to you.

Parents of children participating in this trial will be given a copy of the information sheet and a signed consent form to keep.

Thank you for your co-operation and patience.

### 6.2.6 Appendix 6: Parent Consent Form

Centre Number:
Study Number:
Patient Identification Number for this trial:

# CONSENT FORM

**Title of Project:** Does pre-operative Sildenafil protect against pulmonary related complications following cardiopulmonary bypass? A randomised trial in children undergoing cardiac surgical repair.

Name of Researcher: Mr Tony Vassalos

1. I confirm that I have read and understand the information sheet dated 18 January 2006 (version 2) for the above trial and have had the opportunity to ask questions. ☐
2. I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected. ☐
3. I understand that sections of my child's medical notes may be looked at by responsible individuals from the Yorkhill Division or from regulatory authorities where it is relevant to my child taking part in research. I give permission for these individuals to have access to my child's records. ☐
4. I give permission for my child to take part in the above trial. ☐

| _____ | _____ | _____ |
|---|---|---|
| Name of Parent or Guardian | Date | Signature |
| _____ | _____ | _____ |
| Researcher | Date | Signature |

1 for parent/guardian; 1 for researcher; 1 to be kept with hospital notes

### 6.2.7 Appendix 7: Data Entry Proforma

| Patient ID: |
|---|
| |

| PATIENT FACTORS | | | | | |
|---|---|---|---|---|---|
| 1. | Age (Months) | | 5. | BSA ($m^2$) | |
| 2. | Weight (Kg) | | 6. | WCC (Preop) …./…. | |
| 3. | Height (cm) | | 7. | Admitted | / / |
| 4. | Sex (M/F) | | 8. | Discharged | / / |

| PREOPERATIVE FACTORS | | |
|---|---|---|
| 1. | Cardiac Diagnosis | |
| | | |
| 2. | Non-Cardiac Diagnosis | |
| | | |
| 3. | Cardiac Catheter (PAP/AO/PVR PCWP/$Q_p$:$Q_s$) | |
| | | |
| 4. | Signs / Symptoms (L > R Shunt) | |
| | | |
| 5. | Medication | |
| | | |
| 6. | Aspirin / Warfarin / Sildenafil | |

| OPERATIVE FACTORS | | | | | |
|---|---|---|---|---|---|
| 1. | DATE | ….. / ….. / ….. | 7. | CPB Temp (minimum rectal) | |
| 2. | XC time (mins) | | 8. | UF volume (mls) | |
| 3. | BP time (mins) | | | | |
| 4. | Surgeon & Anaesthetist | / | | | |
| 5. | Inotropes (coming off) | | | | |
| | | | | | |
| 6. | Aprotinin Regimen | L = | P = | M = | |

| RENAL OUTCOMES | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test \ Timing | | PreOp Day… …/… | Th 1st 12hrs | Th 2nd 12hrs | Postop Day 1 .../... | 2 …/.. | 3 …/.. | 4 …/.. | 5 …/.. | 6 …/.. | 7 …/.. | |
| 1. | SCr (μmol/l) (18-40) | | | | | | | | | | | |
| 2. | CrCl (54-86) (ml/min/1.73m$^2$) | | | | | | | | | | | |
| *(Normal values in parenthesis)* | | 1hr | 2hrs | 3hrs | 4hrs | 5hrs | | Total Urine 1st 24hr | | Diuretics (Inf / Bolus) | | |
| 3. | Urine (mls) | | | | | | | | | | | |
| 4. | Dopamine (μg/kg/min) | | | | | | | | | | | |

| RESPIRATORY OUTCOMES | | | | | | |
|---|---|---|---|---|---|---|
| Test \ Timing | | Post Intubation | Post CPB | 2 hrs Postop | 4hrs Postop | 8am Day 1 |
| 1. | Oxygenation Index (OI) | | | | | |
| 2. | Dynamic Compliance ($C_L$) (ml/cm$H_2O$/kg) | | | | | |
| 3. | Mech. Ventilation | (Hrs) / (Days) | | Sildenafil (dose/days) | | |
| 4. | Period in PICU | (Hrs) / (Days) | Hospital Stay (days) | | | |

Oxygenation Index: Mean airway pressure (cm$H_2O$ x 100 x $FiO_2$ / $PaO_2$ (mmHg)

| Test \ Timing | | 1st 24Hrs Post-Op | | | | | | | Days Post-Op | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2hr | 4hr | 8hr | 12 Hr | 16 Hr | 20 Hr | 24 Hr | 2 | ../.. | ../.. | ../.. |
| 1. | PVRI ($WU/m^2$) | | | | | | | | | | | |
| 5. | Lactate / Base Excess | | | | | | | | | | | |
| 6. | Inotrope Score | | | | | | | | | | | |
| 7. | LA pressure | | | | | | | | | | | |
| 8. | PA pressure | | | | | | | | | | | |
| 9. | Transpulmonary Gradient | | | | | | | | | | | |
| 10. | PaO2 | | | | | | | | | | | |
| 11. | PCO2 | | | | | | | | | | | |

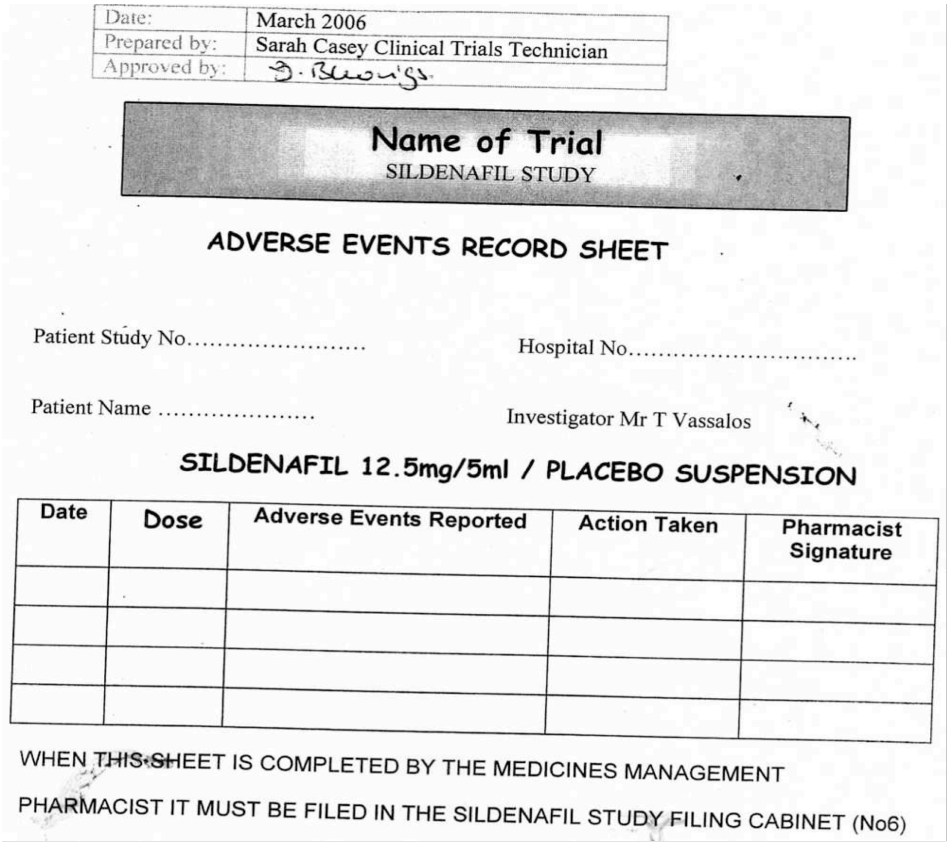
| ECHOCARDIOGRAPHIC OUTCOMES | | | | |
|---|---|---|---|---|
| Test / Timing | | Pre-Op | <1hr post return to PICU | Day 1 Post-Op |
| 1. | $LV_{EDD}$ (mm) [23 +/- 3] | | | |
| M-mode [mean +/- SD] | $LV_{ESD}$ (mm) [14 +/- 2] | | | |
| | $RV_{EDD}$ (mm) [9-13] | | | |
| | LV % FC [38.9 +/- 4.1] | | | |
| | LV EF % [52-65] (1-18yr olds) | | | |
| | $V_P$ (cm/sec) [54.6 +/- 14] (2mn – 6yrs) | | | |
| 2. | Peak AO (LVOT) (cm/sec) median 107.0* [73-141] | | | |
| SPECTRAL | Peak PA (RVOT) (cm/sec) median 84.4* [63.8-70.5] | | | |
| | AO annular diameter [11-13mm] | | | |
| | PA annular diameter [8.4 – 11.6mm] (up to 10kg) | | | |
| | $VTI_{PA}$ (stroke distance cm) [heart rate] | | | |
| | $VTI_{AO}$ (stroke distance cm) [heart rate] | | | |
| | MV Peak E wave (cm/sec) [79.7 +/- 18.8] | | | |
| | MV Peak A wave (cm/sec) [65.3 +/- 13.3] | | | |
| 3. | MV E/A Ratio [1.24 +/- 0.3] | | | |
| 4. | Regurgitation (L + R AV) (Y/N) | | | |
| 5. | $S_a$: Lateral Mitral (5.7 +/- 1.6) [5.3 – 6.1] | | | |
| TDI cm/sec (mean +/- SD) [95% CI] | $S_a$: Lateral Tricuspid (10.2 +/- 5.5) [8.8 – 11.7] | | | |
| | $S_a$: Septum (5.4 +/- 1.2) [5.1 – 5.7] | | | |
| | IVA ($cm/sec^2$) [248 +/- 35] | | | |
| | $E_a$: Lateral Mitral (9.7 +/- 3.3) [8.8 – 10.5] | | | |
| | $E_a$: Lateral Tricuspid (13.8 +/- 8.2) [11.7 – 15.9] | | | |
| | $E_a$: Septum (8.1 +/- 2.5) [7.5-8.7] | | | |
| | $A_a$: Lateral Mitral (5.7 +/- 1.8) [5.3 – 6.2] | | | |
| | $A_a$: Lateral Tricuspid (9.8 +/- 2.4) [9.1 - 10.5] | | | |
| | $A_a$: Septum (6.1 +/- 1.5) [5.7 – 6.4] | | | |
| | $E/E_a$ Ratio (L AV valve) (8.8 +/- 2.7) [8.1 – 9.5] | | | |

*(Normal values in parenthesis)*

| HAEMATOLOGY OUTCOMES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1hr Post-op | 2hr Post-op | 3hr Post-op | 4hr Post-op | 5hr Post-op | Total 1st 24 hrs | | |
| 1. | Chest Tube Drainage (ml) | | | | | | | | |
| | | Intra-operatively | | | Post-operatively | | | | |
| 2. | PRC (units/ml) | | | | | | | | |
| 3. | FFP (units/ml) | | | | | | | | |
| 4. | Platelets (units/ml) | | | | | | | | |
| 5. | Other: | | | | | | | | |

| COMMENTS / COMPLICATIONS: |
|---|
| - |
| - |
| - |

## 6.2.8 Appendix 8: Adverse Events Record Sheet



| | |
|---|---|
| Date: | March 2006 |
| Prepared by: | Sarah Casey Clinical Trials Technician |
| Approved by: | |

**Name of Trial**
SILDENAFIL STUDY

**ADVERSE EVENTS RECORD SHEET**

Patient Study No…… Hospital No……

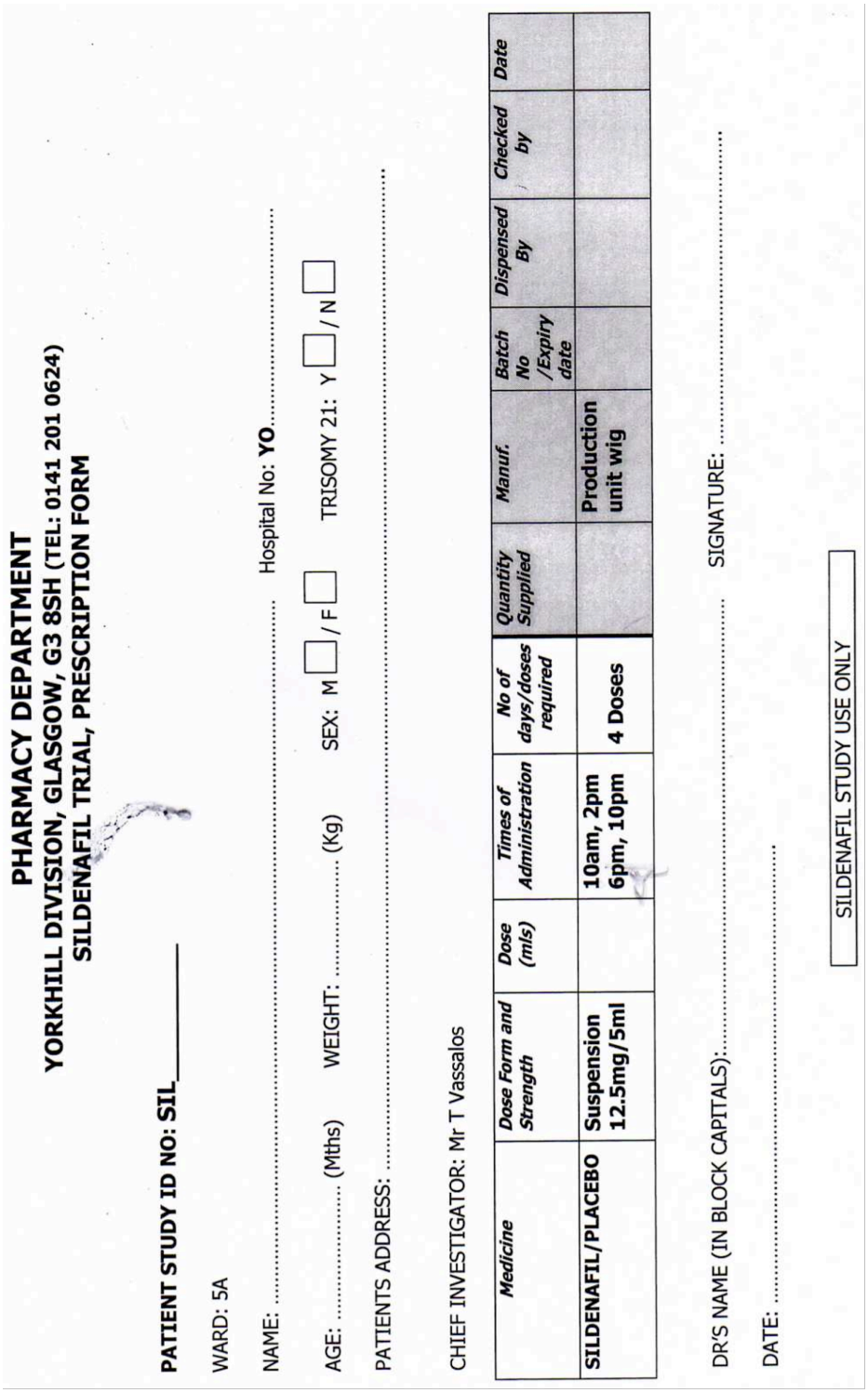
Patient Name …… Investigator Mr T Vassalos

**SILDENAFIL 12.5mg/5ml / PLACEBO SUSPENSION**

| Date | Dose | Adverse Events Reported | Action Taken | Pharmacist Signature |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

WHEN THIS SHEET IS COMPLETED BY THE MEDICINES MANAGEMENT PHARMACIST IT MUST BE FILED IN THE SILDENAFIL STUDY FILING CABINET (No6)

## 6.2.9 Appendix 9: Sildenafil Trial Prescription Form



**PHARMACY DEPARTMENT**
**YORKHILL DIVISION, GLASGOW, G3 8SH (TEL: 0141 201 0624)**
**SILDENAFIL TRIAL, PRESCRIPTION FORM**

**PATIENT STUDY ID NO: SIL_____**

WARD: 5A

NAME: ..... Hospital No: **YO**.....

AGE: ..... (Mths) WEIGHT: ..... (Kg) SEX: M ☐ / F ☐ TRISOMY 21: Y ☐ / N ☐

PATIENTS ADDRESS: .....

CHIEF INVESTIGATOR: Mr T Vassalos

| *Medicine* | *Dose Form and Strength* | *Dose (mls)* | *Times of Administration* | *No of days/doses required* | *Quantity Supplied* | *Manuf.* | *Batch No /Expiry date* | *Dispensed By* | *Checked by* | *Date* |
|---|---|---|---|---|---|---|---|---|---|---|
| **SILDENAFIL/PLACEBO** | **Suspension 12.5mg/5ml** | | **10am, 2pm 6pm, 10pm** | **4 Doses** | | **Production unit wig** | | | | |

DR'S NAME (IN BLOCK CAPITALS):..... SIGNATURE: .....

DATE: .....

SILDENAFIL STUDY USE ONLY

## 6.2.10 Appendix 10: Protocol Registration Receipt

*ClinicalTrials.gov*
*Protocol Registration System*

**Protocol Registration Receipt**
2006-07-07

### Does Sildenafil Protect Against Pulmonary Related Complications Following Cardiopulmonary Bypass?

This study is currently recruiting patients.
Verified by NHS Greater Glasgow Yorkhill Division 2006-07

| | |
|---|---|
| **Sponsored by:** | NHS Greater Glasgow Yorkhill Division |
| **Information provided by:** | NHS Greater Glasgow Yorkhill Division |
| **ClinicalTrials.gov Identifier:** | |

**Purpose**

Does pre-operative administration of Sildenafil (Viagra, Pfizer) reduce the lung injury associated with cardiopulmonary bypass in children undergoing corrective surgical repair of congenital heart disease.

| Condition | Treatment or Intervention | Phase |
|---|---|---|
| Cardiopulmonary Bypass | Drug: Sildenafil Citrate | Phase 4 |

Study Type: Interventional
Study Design: Treatment, Randomized, Double Blind, Placebo Control, Single Group Assignment

Official Title: A Randomised, Double-Blind, Placebo-Controlled, Single-Centre Study of the Pre-Operative Effect of Sildenafil Citrate on Pulmonary Related Complications Following Cardiopulmonary Bypass in Children Undergoing Cardiac Surgical Repair.

Further Study Details:

Expected Total Enrollment: 12

Study Start: 2006-06

**Eligibility**

Ages Eligible for Study: 3 Months - 5 Years, Genders Eligible for Study: Both

- Page 1 of 2 -

Criteria

Inclusion Criteria:

Paediatric patients undergoing open heart surgery utilising cardiopulmonary bypass to correct acyanotic congenital heart disease.

Age > 3 months Parents that show a good understanding of their child's condition and are happy for their child to participate in the study.

Exclusion Criteria:

Patients with known organ dysfunction prior to surgery (pulmonary, renal or hepatic) Communication barrier resulting in poor basic comprehension of the proposed study (e.g. language barrier) Patients with cyanotic heart disease Patients undergoing heart surgery without the use of cardiopulmonary bypass Patients who do not tolerate oral Sildenafil (e.g. Vomiting) or whose surgery is subsequently cancelled.

## Location and Contact Information

Tony Vassalos, MB Ch B 0141 201 1000 tonyv@doctors.org.uk

**United Kingdom, Strathclyde**
Royal Hospital for Sick Children, Glasgow, Strathclyde, G3 8SJ, United Kingdom; Recruiting
Tony Vassalos 0141 201 1000 tonyv@doctors.org.uk
Mark Danton 0141 201 1000 mark.danton@yorkhill.scot.nhs.uk
Tony Vassalos, Principal Investigator

Study chairs or principal investigators

Tony Vassalos, MB Ch B, Principal Investigator
Royal Hospital for Sick Children

## More Information

Study ID Numbers 05/CA/01
Health Authority: UK: Medicines for Healthcare Regulatory Agency

- Page 2 of 2 -

## 6.2.11 Appendix 11: R&D Management Approval

NHS
Greater
Glasgow

Yorkhill Division
Research & Development Office
Dalnair Street
Glasgow
G3 8SJ

Tel: (0141) 201 0005
E-mail:alison.wood@yorkhill.scot.nhs.uk

11th January 2006

Dr Tony Vassalos
Department of Cardiology
RHSC
NHS Greater Glasgow – Yorkhill Division
Dalnair Street
G3 8SJ

**Yorkhill R&D Management Approval**

Dear Dr Vassalos,

**Re: Does preoperative Sidenafil protects against pulmonary complications**
**R&D Project Number: 05/CA/01 Ethics Ref: 06/S0703/8**

Thank you for submitting a copy of your ethics submission for the above project to the R&D Office. I am pleased to inform you that your project has been approved by the Yorkhill Division R&D department. This letter ensures that you and the researchers working with you, who hold substantive or honorary contracts, are indemnified by the NHS under the CNORIS scheme. This means you can now proceed with your project at Yorkhill once you have written confirmation of ethics approval for the study.

Amendments – The R&D office needs to be kept informed of any changes to the project for example regarding patient recruitment, funding, personnel changes or your project status. If changes are made to the protocol they will need to be considered by the ethics committee.

Should you have any queries please contact the R&D office quoting the Project ID number. Please let me know if the R&D office help in any way with the study. May I wish you every success with your research.

With very best wishes,
Yours sincerely,

Alis ~ Wood .

**Dr. Alison Wood**
**Research & Development Manager**

cc. Mr Mark Danton

---

We would be grateful if you could complete & return this slip once the study has commenced.

**Name:** Dr T Vassalos **Department:** Cardiology
**Project No:** 05/CA/01
**Project Name:** Does preoperative Sidenafil protects against pulmonary complications

The above study commenced on ..... Signed.....

Please retun slip to the Research & Development Office, 1st Floor Corridor,
Royal Hospital for Sick Children, Glasgow G3 8SJ
On commencment of study.