Total cefazolin serum levels during elective valve replacement surgery on cardiopulmonary bypass

Daren Calleemalay

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of

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Declaration

I, Daren Calleemalay, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

.....

23rd day of May 2017.

Dedication

In memory of my grandfathers

Narainsamy Calleemalay & Ayasamy Goinden.

Abstract

Background:

Infections post cardiac surgery can have potentially devastating consequences. Adequate antimicrobial prophylaxis is therefore crucial to limit the occurrence of such complications. Cefazolin is a commonly prescribed prophylactic agent for major cardiac surgery requiring cardiopulmonary bypass (CPB). The effects of CPB on the pharmacokinetic profile of cefazolin are largely unknown. To date there is no published work determining the optimal bolus dosing of cefazolin required to achieve acceptable concentrations intra-operatively during and post CPB.

Aim:

The aim of this study was to describe the total serum cefazolin levels during elective valve replacement surgery on CPB at CMJAH.

Method:

A prospective, contextual, descriptive design was used in this study. Cefazolin plasma concentrations were analysed at specific pre-determined time intervals in adults patients scheduled for elective valve replacement surgery. Convenience sampling was used.

Results:

Sixteen patients were enrolled in the study with equal number of males and females, ranging from 18 years to 59 years of age and with a mean BMI of 28.2 kg/m² (range of 18.1 to 40.2 kg/m²). The mean trough for the unbound concentration of cefazolin was 5.02 μg/ml (range of 2.79 to 10.35 μg/ml). For 5 out of the 16 patients (31.25%) the targeted therapeutic goal of time above MIC (4 μg/ml) of 100% (T > MIC 100%) was not achieved. Seven corresponding pre and post CPB serum samples (A1-A7) were statistically analysed using the paired t-test. The results indicated no statistically significant differences between samples A1-A5 ($p = 0.11, 0.34, 0.46, 0.32$ and 0.98 respectively). There was a statistically significant difference between the samples A6 and A7 ($p = 0.024$ and 0.025), however, the clinical significance of these small differences is questionable.

Conclusion:

Surgical site infections not only result in significant morbidity and mortality but also lead to an increased financial burden to the country's economy. This study has shown that potentially 31.25% of the patients undergoing cardiac surgery may have an increased risk of acquiring infections due to sub-optimal levels of prophylactic antibiotic during the surgery. In addition, the findings point towards no sequestration of cefazolin in the CPB circuits.

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Abbreviations

AUC: Area under the curve

BTCCRC: Burns Trauma Critical Care Research Centre

CDC: Centre for Disease Control and Prevention

Cl: Clearance

Cmax: Maximal concentration

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

CPB: Cardiopulmonary bypass

CVC: Central venous catheter

EUCAST: European Committee on Antimicrobial Susceptibility Testing

HAI: Hospital acquired infection

IV: Intra-venous

t½: Half-life

MIC: Minimum inhibitory concentration

PBP: Penicillin binding protein

PD: Pharmacodynamics

PK: Pharmacokinetics

SIRS: Systemic inflammatory response syndrome

SSI: Surgical site infection

SWI: Sternal wound infection

U&E: Urea and electrolytes

Chapter 1: Overview of study

1.1 Introduction

Post-surgery the occurrence of surgical sites infections (SSIs) is always a concern, largely due to the morbidity and mortality associated with such infections. In the past SSI was thought to be primarily linked to the surgical team, however the impact of implementing other multi-disciplinary strategies is significant in minimising the incidence of SSI (1). A recent study demonstrated that among the nosocomial infections, SSIs are the most common having an incidence of 38% of all hospital acquired infections (HAIs) (2). Following cardiac surgery with cardiopulmonary bypass (CPB), SSIs, although rare, may occur in the lower limbs (venous graft site) or more worryingly involve the sternum, with devastating consequences.

The incidence of sternal wound infection (SWI) in the literature ranges from 0.5% to 10% (1, 3-10). Deep SWI (mediastinitis) has an incidence of 0.4% to 5% and is associated with a substantive morbidity and mortality, which may be up to 47% (3). Moreover, the associated treatment cost is a massive burden to the public health sector and as such antibiotic prophylaxis is imperative for CPB surgery (5). Cefazolin, a first generation cephalosporin, is generally considered to be an appropriate prophylactic antibiotic agent to reduce the impact of SSI post CPB surgery. However, there is a substantial variation in the precise regimen prescribed at various institutions globally (11).

1.2 Background

Current regimens for prophylaxis in CPB surgery were developed from empiric studies that observed post-operative infection reduction in both cardiac and non-cardiac surgeries. The current practice at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) consists of two grams of intra-venous (IV) cefazolin given at the induction of anaesthesia, with a repeat dose every four hours intra-operatively, taking into account the half-life (t½) of cefazolin. This is similar to The Society of Thoracic Surgeons recommendation of administering two grams (adult above 60 kg) of cefazolin within one hour of the skin incision and a repeat of one gram every three to four hours until the end of surgery (11). However, Bratzler et al (12) recommended a single pre-incision dose of two grams of cefazolin in adult patients for cardiac surgery. The recommendation from the Australian guidelines for antibiotics prophylaxis in cardiac surgery is one gram (or two grams, if weight above 80 kg) at the induction of anaesthesia and thereafter eight hourly for a further two doses (13). In South Africa, the South African Antibiotic Stewardship programme (14) recommends a single dose

of two grams of cefazolin given pre-operatively for cardiothoracic surgery and a repeat dose given should the surgery be prolonged or associated with severe blood loss. Additionally, there are some institutions that routinely administer a repeat dose upon completion of CPB, their rationale is to compensate for the losses that may occur in the CPB circuit (15). In light of the above discrepancy, it is unclear whether adequate cefazolin blood concentrations are produced with any of the regimens described. It is a concern that the presence of the CPB circuit may modify antibiotic levels in the patient, taking into consideration the vast physiological changes associated with CPB.

A literature review with regards to the serum cefazolin levels during CPB surgery yielded only three studies. Hutschala at al (3) investigated the extent of cefazolin penetration into soft tissue during cardiac surgery using in vivo microdialysis. The authors concluded that the interstitial tissue concentrations of cefazolin were within therapeutic range for the prevalent pathogens causing SWIs. However, this was achieved by using a higher dose of four grams of cefazolin before skin incision and a further two grams during skin closure. This practice is not in line with national or international recommendations and guidelines as the dose is higher than conventionally prescribed (11, 13). Theoretically this increases the risk of side effects.

Fellinger et al (16) did a pilot study to measure the serum levels of cefazolin during CPB surgery. The results showed serum levels of cefazolin remaining above MIC_{90} (Minimum inhibitory concentration at 90% inhibition) for only two pathogens. These were *Staphylococcus aureus and Staphylococcus epidermidis* and the serum levels of cefazolin were below MIC_{90} for all other prevalent pathogens.

Adembri et al (15) compared a bolus and continuous infusion of cefazolin in patients undergoing elective cardiac surgical procedures. The authors concluded that 90% of the patients in the continuous infusion group had a plasma level above MIC_{90} throughout surgery compared with the bolus group where this goal was only achieved for 30% of the group.

The influence of the CPB circuit amongst the bolus regimens that are currently in vogue needs to be ascertained first. Further research regarding continuous infusion regimens is also required as currently they are not recommended by any national or international guidelines. The lack of evidence based prophylaxis guidelines and the potential for prophylaxis failure in the bolus group results in a dilemma regarding what constitutes appropriate antibiotic prophylaxis in cardiac surgery.

In addition, Mets (17), in a review article detailed the effects of CPB on the pharmacokinetics of numerous commonly used anaesthetic drugs including induction agents, volatiles, opioids, muscle relaxants and antibiotics. He described the pharmacokinetic changes of cefazolin demonstrating a discontinuity from the normal decay

profile with a sudden decrease in plasma level coinciding with the initiation of CPB. The review failed to address the therapeutic implications of these changes (17).

1.3 Problem statement

There is paucity of literature pertaining to the pharmacokinetics of prophylactic antibiotics due to the physiological changes that occur during CPB. Little is known about the altered volume of distribution, from the circuit itself, the large volume of intravenous fluids (cardioplegia) used intra-operatively, actual antibiotic concentration during CPB, the aortic cross clamp time and the opposing effects of hypothermia and systemic inflammatory response syndrome (SIRS) on renal clearance.

Understanding the distribution and kinetics of cefazolin during CPB is necessary to help further inform the discussion on this uncertain matter. If there is indeed sequestration of cefazolin in the CPB circuit (18), resulting in the patient's serum concentration being below MIC_{90}. The dosing regimen will need to be reconsidered and evaluated.

Current practice at CMJAH is in alignment with the guidelines of the South African Antibiotic Stewardship Programme (14). It is not known if this practice does in fact provides adequate therapeutic levels of cefazolin prophylaxis to these patients.

1.4 Aim and objectives

1.4.1 Aim

The aim of this study was to describe the total serum cefazolin levels during elective valve replacement surgery on CPB at CMJAH.

1.4.2 Objectives

The primary objectives of this study were to:

- describe antibiotic levels in the patient's serum before, during and after CPB
- describe serial antibiotic levels in the circuits during the CPB period.

The secondary objective of this study was to describe the pre-operative and intra-operative creatinine clearance.

1.5 Research assumptions

The following definitions were used in this study.

Adult patient: a patient between 18 and 60 years.

CMJAH research group: researchers from the cardiothoracic ICU at CMJAH.

Burns Trauma Critical Care Research Centre (BTCCRC): research centre based at the University of Queensland, Australia.

1.6 Demarcation of study field

The study was conducted in the adult cardiothoracic theatre at CMJAH in Johannesburg, South Africa. It is a 950 bed central hospital affiliated to the University of the Witwatersrand. CMJAH is an academic referral centre for cardiothoracic patients in the Gauteng province and on average 400 cardiothoracic cases on CPB are done annually.

1.7 Research methodology

A prospective, contextual, descriptive research design was used in this study.

1.7.1 Study population

The study population comprised patients undergoing elective valve replacement on CPB from the cardiothoracic unit at CMJAH.

1.7.2 Study sample

A sample size of 16 patients was determined for this study in consultation with the BTCCRC, and convenience sampling was used. Inclusion and exclusion criteria for this study were defined.

1.7.3 Data collection

This research project was a collaborative project between the CMJAH research group and the BTCCRC.

Prior to data collection all the relevant approvals were obtained and the research was carried out adhering to the principles of the Declaration of Helsinki, 2013 (19) and the South African Good Clinical Practice Guidelines (20).

Data were collected by DC from the CMJAH research group, adhering strictly to the standard operating procedure for data collection.

The samples were couriered to the BTCCRC by a specialised courier service where they were analysed in an accredited laboratory.

1.7.4 Data analysis

Data were captured and analysed on a Microsoft® Excel 2013 spreadsheet. Descriptive and inferential statistics were used for analysis. Paired T tests were used to compare the pre-operative and intra-operative creatinine clearance and the pre and post CPB serum samples and a Kaplan Meier survival curve graphically represented the serial cefazolin levels.

1.8 Significance of the study

The findings of this study will shed light on the pharmacokinetics of prophylactic antibiotics during CPB. The results may lead to a review of the existing antibiotic prophylaxis regimen at CMJAH. Following analysis of the blood samples for cefazolin levels, it will be known if the intra-operative serum concentrations are within the therapeutic range and therefore adequate to prevent SSIs or if the current regimen must be adjusted.

1.9 Outline of research report

The following chapters are presented in the research report.

Chapter 1	Overview of study
Chapter 2	Literature review
Chapter 3	Research methodology
Chapter 4	Results and discussion
Chapter 5	Summary, limitation, recommendations and conclusion.

1.10 Summary

In this chapter an overview of the study was given. In the following chapter the literature review will be presented.

Chapter 2: Literature review

2.1 Introduction

Infections in cardiac surgery can have devastating outcomes in terms of morbidity and mortality. Antibiotic prophylaxis becomes crucial in order to limit these life threatening complications. But, with the additional effects of the aortic cross-clamp, hypothermia and the CPB circuit on the antibiotic, any deviation to the pharmacokinetic profile of the antibiotic is currently unknown. Little has been done linking the dosing of the antibiotic and its corresponding plasma level in this group of patients. In this chapter the need for antibiotic prophylaxis in cardiac surgery is highlighted, with particular focus on the pharmacokinetics of cefazolin. The literature review commences with a background of the problems, SSI, mediastinitis and the pathogenesis involved as well as elaborates on the resultant complications. The review then addresses antibiotic prophylaxis with reference to general principles and then specifically in cardiac surgery. Thereafter, the review examines the CPB machine followed by an overview of the physiological changes that occur. Finally, the discussion leads to the pharmacological properties of cefazolin, with emphasis on the pharmacokinetics.

2.2 Background

Joseph Lister at the Glasgow Royal Infirmary, originally introduced the principles of "antisepsis" in the late 1860's. This led the way to radical changes in surgical procedures. He acknowledged the hypothesis of Louis Pasteur regarding microbiology and developed the concept of sterile surgery by introducing carbolic acid. Prior to this, up to 80% of surgical patients developed sepsis. Unfortunately, most did not understand the pathological cause and process of the infection and believed that the resulting deaths of these patients were incidental (21). In this era, the common complications described post-surgery were pyrexia followed by purulent drainage from the incision site, profuse sepsis and often death. Even though Lister's principles had drastically improved morbidity and mortality outcomes, purulent drainage from the incision site, now referred to as SSI, remains a devastating complication following surgery (3).

2.3 Surgical site infection

Previous studies cited urinary tract infection as the most common HAI. Lewis et al (2) performed a study using HAI surveillance data from 15 community hospitals participating

in the Duke Infection Control Outreach Network. It included complete hospital-wide and surgical surveillance data from January 1, 2010 to June 30, 2012. They found that SSIs were the most common of the nosocomial infections, accounting for 38% of all HAIs (2).

In the past the main focus was on the surgical team to prevent SSIs. However, it has been shown that anaesthetists can also contribute in decreasing the incidence of SSIs both in the theatre and the critical care unit (1).

The United States Centre for Disease Control and Prevention (CDC) developed the National Nosocomial Infections Surveillance system that classified SSIs into three categories:

- superficial incisional SSI, limited to the skin and the underlying subcutaneous tissue
- deep incisional SSI, comprising the facial layer and muscle
- organ/space SSI, penetrating the deeper anatomical layers (22, 23).

The three classifications are illustrated in Figure 2.1.

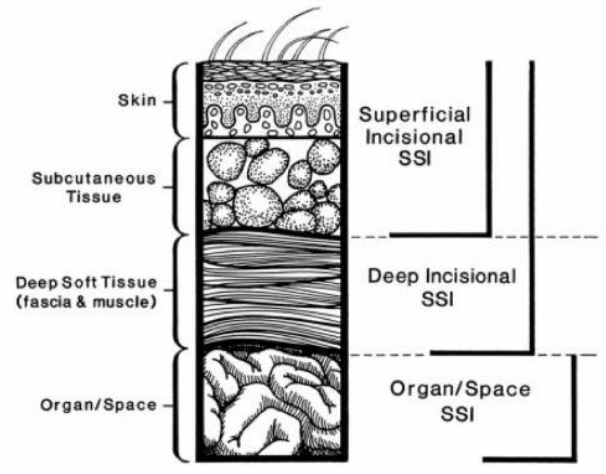


Figure 2.1 Cross-section of abdominal wall depicting CDC Classification of SSI (22)

The causative microbes leading to SSIs commonly originates from the patient's endogenous flora. Most frequently cultured pathogens are *Staphylococcus aureus,* coagulase-negative S*taphylococci, Enterococcus spp and Escherichia coli.* The incidence of these microbes is specific to the surgical procedure as detailed in Table 2.1. (24)

Table 2.1 Pathogens commonly associated with different surgical procedures (24)

Type of surgery	Common pathogens
Cardiac	*Staphylococcus aureus,* coagulase-negative *Staphylococcus*
Neurosurgery	*Staphylococcus aureus,* coagulase-negative *Staphylococcus*
Breast	*Staphylococcus aureus,* coagulase-*negative Staphylococcus*
Ophthalmic	*Staphylococcus aureus,* coagulase-negative *Staphylococcus, streptococci,* gram-negative bacilli
Vascular	*Staphylococcus aureus,* coagulase-negative *Staphylococcus*
Gastrointestinal	Gram-negative bacilli*,* anaerobes
Gynaecological	Gram-negative bacilli*,* anaerobes*, enterococci,* Group B *Streptococci*
Urological	Gram-negative bacilli

SSIs are not only associated with a significant increase in morbidity and mortality, prolonged hospital stay, median increase of 14 days (25), but also with increased cost (26). The financial burden of SSI on the health care system is quite substantial and estimated to be around US$1 billion to US$10 billion each year in direct or indirect medical costs in the United States of America (27). In a retrospective cohort study Perencevich et al (27), used patient questionnaires and administrative databases to assess the effect of SSIs, during the first eight weeks after being discharged. They found the incidence of SSI to be 1.9% (89 out of 4571 procedures) with a higher average medical expenditure for patients with SSI (US$5,155) than the patients without SSI (US$1,773). This translated to a resultant increase of 290% in cost. In addition patients with SSI used more healthcare resources, comprising mainly more homebased health aides, radiology, outpatient and emergency department visits. They also had a higher number of hospital readmissions (27).

In cardiac surgery the SSI can occur from the leg (venous graft site), but more worryingly from the sternum which can lead to devastating consequences.

2.4 Sternal wound infection

SWI is an uncommon but potentially life threatening complication that often develops into mediastinitis. In order to access the heart and surrounding structures, a median sternotomy incision is commonly used for open cardiac surgery. The less common incision is the transverse sternotomy with bilateral thoracotomy (clamshell) required for the surgical excision of large tumours, severe traumatic chest injuries or in bilateral lung

transplantation (28). The first median sternotomy was performed by Milton in 1897, to remove a tuberculous lymph node compressing the anterior mediastinum. However, his second case in 1901, demised from overwhelming sepsis after a foreign body was successfully removed from the patient's trachea (29). Although in 1953, Shumacker became the first surgeon to recommend a median sternotomy for elective heart surgery having successfully used it for a valvulotomy (29). It was only in 1957 that Julian and associates published a report of four patients requiring cardiac surgery where they avoided the bilateral anterior thoracotomy and used the median sternotomy (29). This surgical incision resulted in a marked reduction in operating time, an excellent global exposure of the heart and less pulmonary trauma making the median sternotomy what it is today (29-31).

With regard to sternal closure in low-risk patients, it is generally accomplished with simple closure. However, prophylactic sternal reinforcement techniques are used in most cases and these include: the common wiring technique (figure of 8, simple wires and the Robicsek weave) and rigid titanium plating with fixation of the sternum utilising screws (32). Although the wiring technique might be a more cost effective solution to sternal closure, in a prospective, randomized multicentre trial, Raman et al (33) have demonstrated that the mechanical benefits of the rigid plate fixation resulted in superior bone healing to that of common wiring, thereby improving morbidity. The subcutaneous tissues are typically closed with absorbable sutures and the skin closed in a subcuticular fashion (28).

The incidence of SWI in the literature varies with a range of 0.5% to 10% (1, 3-10). Sharma et al (4), reviewed patients that had coronary artery bypass graft surgery retrospectively between June 1997 to December 2000 and found 122 among the 3443 patients (3.5%) developed SWI.

SWI in the setting of cardiac surgery is not only a financial burden but increases morbidity and mortality. Various studies have highlighted multiple risk factors including diabetes, obesity, male gender, prolonged operative time, bilateral internal mammary artery grafts, post-operative transfusion, re-exploration for bleeding, renal insufficiency, chronic obstructive pulmonary disease, smoking, steroid use, peripheral vascular disease and prolonged mechanical ventilation. (5, 7, 9, 10, 34)

There have been various attempts in the literature to classify SWI, based on the anatomy, onset and management and these are described below.

El Oakley and Wright (5), classified mediastinitis in relation to the onset after surgery and the presence of risk factors. Whereas, Pairolero et al (35), describe their classification in chronological nature with regard to only the onset after surgery as displayed in Table 2.2. On the other hand, Jones et al (30) classified the wound in relation to the depth and tissue involvement, presented in Table 2.3, which was modified by Vlajcic et al (36), to include its resulting management.

Table 2.2 Pairolero's (35) classification of SWI based on onset time

Type	Onset of infection
I	Occurs within first few days
II	Occurs within first few weeks
III	Occurs months to years later

Table 2.3 Jones et al, (30) classification of SWI based on tissue involvement

Type	Depth	Tissue involvement
1a	Superficial	Skin and subcutaneous tissue dehiscence
1 b	Superficial	Exposure of sutured deep fascia
2a	Deep	Exposed bone, stable wired sternotomy
2b	Deep	Exposed bone, unstable wired sternotomy
3a	Deep	Exposed necrotic bone, unstable, heart exposed
3b	Deep	Types 2 or 3 with sepsis

However, to maintain consistency during data analysis, various reports have sub-divided SWI into two groups.

- superficial SWI: infection limited to the skin and subcutaneous tissue
- deep SWI: infection with sternal osteomyelitis with or without the involvement of the retrosternal space (mediastinitis) (5).

Mediastinitis

To understand the relevance of mediastinitis, a review of the anatomy of the mediastinum is primordial. The mediastinum is the mass of tissue between the two pulmonary spaces in the thoracic cavity. It is enclosed by the pleura and houses all the thoracic viscera with the exceptions of the lungs. It originates from the superior thoracic inlet to the diaphragm, anteriorly it is housed within the sternum and costal cartilages to the thoracic vertebrae posteriorly as illustrated Figure 2.2 (37). The critical and vital organs in the mediastinum explains why mediastinitis is regarded as a life threatening complication of cardiac surgery.

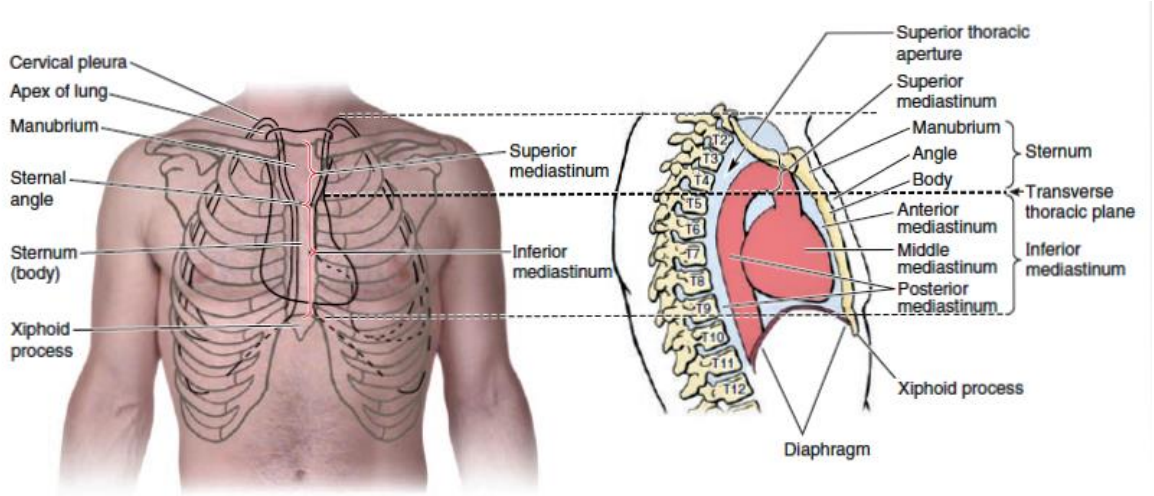


Figure 2.2 Anatomy of the mediastinum (37)

Mediastinitis is an inflammation of the cellular tissue in the mediastinum (36). In cardiac surgery it is a complication of a progressing superficial SWI. Sexton (38) stated that historically, prior to cardiac surgery, mediastinitis resulted mainly from the proliferation of odontogenic or retropharyngeal infections and oesophageal perforation. The author also mentioned that rare causes of primary infections of the mediastinum were either from penetrating traumatic injuries or metastatic proliferation of infections. Furthermore Sexton (38) concluded that in modern practice, mediastinitis is more commonly attributed to a complication of cardiothoracic surgical procedures.

Mediastinitis following cardiac surgery is an uncommon complication with an incidence rate between 0.4% to 5% reported in the literature (5). Tang et al (8) retrospectively reviewed 30102 cardiac surgical patients operated on between 1993 to 2003 and found an incidence of deep SWI of 0.77%. The authors also found a significantly higher mortality rate, three times more than in patients without mediastinitis. The mortality range documented in the literature varies from 14% to 47% following mediastinitis (5). Farinas et al (39) found

a mortality of 35% and two of the patients out of the 34 with mediastinitis (5.9%) develop chronic osteomyelitis of the sternum.

El Oakley and Wright (5) classified mediastinitis with respect to the timing of onset and the risk factors present and this is tabulated in Table 2.4.

Table 2.4 El Oakley and Wright's (5) classification of mediastinitis in patients undergoing CPB

Type	Presentation
Type 1	Mediastinitis developing within 2 weeks of surgery without any risk factors
Type 2	Mediastinitis presenting at 2 to 6 weeks after surgery without any risk factors
Type 3a	Mediastinitis type 1 including one or more risk factors
Type 3b	Mediastinitis type 2 including one or more risk factors
Type 4a	Mediastinitis type 1, 2 or 3 after one failed therapeutic trial
Type 4b	Mediastinitis type 1, 2 or 3 after more than one failed therapeutic trial
Type 5	Mediastinitis with initial presentation more than 6 weeks after a surgical procedure

Clinically, mediastinitis can follow a fulminant or subacute clinical course. Patients usually present with tachycardia, fever, chest pain, signs of SWI or purulent discharge from the mediastinal area with sternal instability (38). Farinas et al (39) found that sternal wound drainage and/or cellulitis were present in 29 of 34 patients (85%) with post-operative mediastinitis. Other clinical signs include oedema of the chest wall, crepitus and Hamman's sign (crunching sound on auscultation synchronous with the cardiac cycle) (38). Although signs of sternal wound infection can precede or follow the recognition of mediastinitis, fever and systemic symptoms are the main precursor (38).

2.5 Pathological aspects of sternal wound infection

Surgical contamination occurs in the peri-operative period. This is caused by the endogenous flora of the patient or the surgical team and the exogenous flora within the operating room.

The impact of endogenous flora is essential in understanding SWI. In the 1950s, it was hypothesised that endogenous *Staphylococcus aureus* was the primary cause of numerous SSIs. It was only in 1995 that Kluytmans et al (40) shed more light on the matter, following their study conducted in Netherlands. The study comprised of 1980 patients requiring cardiac surgery between 1988 and 1991. The authors concluded that nasal colonisation by *Staphylococcus aureus* was a risk factor for SWI. In their follow-up study, they concluded that peri-operative eradication of nasal flora using a mupirocin based nasal ointment significantly decreases the SSI rate in cardiothoracic surgery patients (41).

The exogenous causes of the SSIs are either handborne or airborne. Lepelletier et al (42) described the two components of airborne contamination, firstly, the presence of microorganisms (air bio-contamination) and secondly inert particles (air contamination). The author further explained that these microorganisms mainly results from the common air flora (rarely pathogenic) and the symbiotic human flora released by individuals. The inert particles are released by individuals (cutaneous squamous cells, skin appendages, respiratory droplets, and droplet nuclei), and textiles (surgical team's clothes and operative field drapes). These amounts correspond not only to the number of individuals in the operating theatre but to their movements as well as the textile material worn (42).

Nowadays, contamination by non-sterile material is becoming extremely rare due to stricter guidelines for the sterilisation and disinfection of materials, and also the increasing use of disposable sterile material (42). Other rare types of post-operative contamination are from the metastatic spread of infection occurring in the post-operative critical care unit (catheter bacteraemia or pneumonia) or by direct bacterial inoculation of the operative site during wound dressing (42).

2.6 Microbiology

Staphylococci species has been identified in multiple studies as the main pathogen responsible for post-operative SSIs. Their exact proportions do vary according to the reports (3, 4, 6, 30, 35, 42). Lepelletier et al (42) demonstrated that *Staphylococcus aureus* accounted for 40% to 60% of strains causing mediastinitis, closely followed by the coagulase-negative *staphylococcus*, involved in 20% to 30% of cases while the gram-negative bacilli (*Escherichia coli, Enterobacter, Klebsiella, Proteus, & Pseudomonas*) comprise up to 20% of cases as described in Table 2.5 .

Table 2.5 Microbiology of mediastinitis (42)

Microbiology of mediastinitis	Percentage
Gram-positive cocci	
Staphylococcus aureus	40%
Coagulase-negative *Staphylococcus*	30%
Gram-negative bacilli	
Escherichia coli	5%
Enterobacter spp.	10%
Klebsiella spp.	3%
Proteus spp.	2%
Pseudomonas spp.	2%
Other	
Candida	< 2%
Polymicrobial	10-40%

2.7. Antibiotics

The most common microorganisms causing SSIs from clean procedures are skin flora, including *Staphylococcus aureus, Streptococcal species and* coagulase-negative *Staphylococci* (43). It becomes essential to provide antibiotic prophylaxis against these pathogens, particularly in cardiac surgery, considering the devastating complications that may result from a SSI.

Antibiotic prophylaxis constitutes a practice that potentially leads to the prevention of SSIs. Bratzler et al (12) sub-divided antibiotic prophylaxis into three broad categories: primary prophylaxis, secondary prophylaxis and eradication.

According to Bratzler et al (12), primary prophylaxis points to the steps in the avoidance of acquiring a direct infection whereas secondary prophylaxis stands for the avoidance of recurrence or reactivation of a pre-existing infection. Eradication relates to the extermination of a colonized flora in order to avoid the proliferation of an infection (12).

The necessity for the routine use of prophylactic antibiotics in a simple, clean surgical intervention such as inguinal hernia repair or breast surgery has been debated in the literature. Mazaki et al (44), in a double blind randomised control trial of prophylactic antibiotic versus placebo involving 200 patients undergoing open-mesh plug hernia repair, found the incidence of SSI to be 2% for the antibiotic group versus 13% for the placebo group. Additionally, it has been argued that whilst the relative risk of infection in cardiac surgery is small (0.4%-10%), the resulting SWI leading to severe morbidity and mortality, justifies the use of prophylactic antibiotics (43).

The optimisation of antibiotic prophylaxis requires that multiple factors be considered. These issues are discussed below.

2.7.1 Preoperative-dose timing

Previously the recommended time for administration of a prophylactic antibiotic was during the induction of anaesthesia. The optimal time for the administration of preoperative doses is deemed to be within 60 minutes prior to incision. However, some agents, e.g. vancomycin, requires infusion over one to two hours and this needs to be individualised (12).

The latest recommendations by the Society of Thoracic Surgeons (45) encourage a continuation of prophylactic antibiotics for 48 hours or less.

2.7.2 Obesity

Multiple reports linked obesity with an increased risk for SSIs (5, 7, 10). The pharmacokinetics of antibiotics may be altered in this group of patients, therefore a dose adjustment based on body weight is often required (12).

2.7.3 Intra-operative re-dosing

There are instances for re-dosing to ensure therapeutic plasma and tissue concentrations of the antibiotic agent. These are the duration of the surgical procedure exceeding two half-lives of the drug and substantial blood loss occurring during the procedure (12). The half-lives of commonly used antibiotics in cardiac surgery are shown in Table 2.6.

Table 2.6 Antibiotics half-life in adults with normal renal function (12)

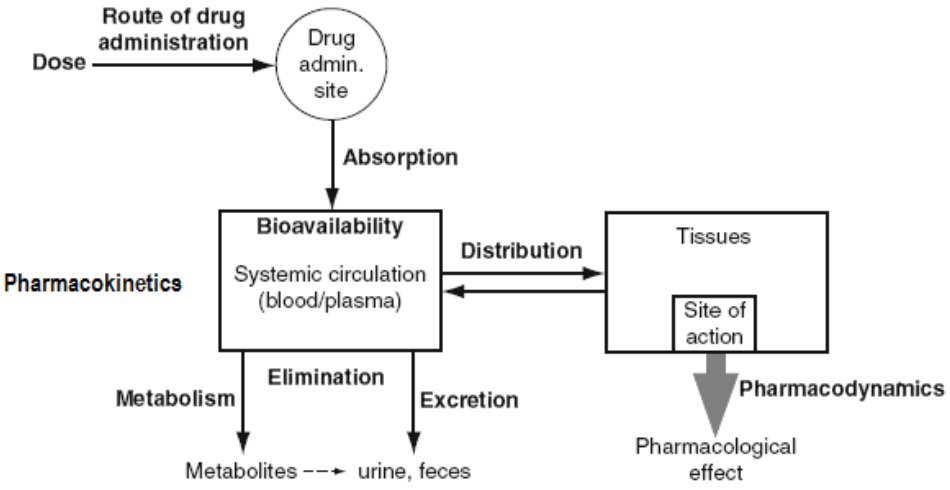
Antibiotic	Half-life in adults with normal renal function (hours)
Cefazolin	1.2-2.2
Cefuroxime	1-2
Erythromycin	3-5
Clindamycin	2-4
Gentamicin	2-3
Vancomycin	4-8
Ertapenem	3-5

2.7.4 Specific surgical procedure

Depending on the common pathogens involved, there might be a need to use specific antibiotics as prophylaxis. For instance, in cardiac surgery, as mentioned earlier these microbes commonly are the skin flora (42, 43). However, for surgery involving a viscus, the pathogens consist of the endogenous flora inhabiting the specific viscus or that of the nearby mucosal surface. These resulting infections are usually poly-microbial and require alternative agents to be used as prophylaxis (12, 26, 42, 43).

2.7.5 Pharmacokinetics

Pharmacokinetics (PK) is defined as "the effects of the body on the drug'' (46) or as stated by Roberts and Lipman (47), "Pharmacokinetics refers to the study of concentration changes of a drug over a given time period". On the other hand, pharmacodynamics (PD) refers to the drug concentration and its resulting pharmacological effect (48) or simply ''the effect of the drug on the body'' (46). The inter-relationship between PK and PD, illustrated in Figure 2.3, forms the basis of pharmacology. An understanding of these principles is essential to determine appropriate antibiotic dosing in order to achieve the desired clinical effect.



Simple schematic diagram illustrating common pharmacokinetic (PK) terms.

Figure 2.3 The Relationship between pharmacokinetics and pharmacodynamics (49)

PK can be sub-divided into four major components: absorption, distribution, metabolism and excretion.

Absorption

During cardiac surgery, considering the drugs are intravenously administered, the principle of absorption becomes irrelevant as opposed to the other routes of drug delivery, where the percentage of the drug absorbed is variable, since the bioavailability of intravascularly administered drug is 100% (48).

However, apart from route of administration there are numerous other factors altering drug absorption: “drug solubility, dissolution of the drug in a medium, nature of the vehicle dispersing the drug, concentration of drug, pH (for ionised drugs), circulation to the site of absorption and absorbing surface” (50).

Distribution

When giving a drug intravenously, its distribution throughout plasma and tissues can be likened to the process of dilution from the highly concentrated solution in the syringe to the less concentrated solution in plasma. This is due to the initial mixing of the drug into blood and being eventually transferred into tissues (51).

Byer and Sarver (49) explained the two compartment PK model as the molecular transfer of a drug from the central compartment to the surrounding tissues. They further elaborated on the bi-directional nature of this process including the molecular transfer of the drug from the central compartment to the tissues and subsequently from the tissues to the central compartment, until an equilibrium is reached. This process of distribution typically follows a three-stage sequence as illustrated in Figure 2.4 (49). Byer and Sarver (49) detailed the stage of distribution, which begins with the drug being absorbed into the systemic circulation. The net movement of the drug is initially from the plasma to the surrounding tissues. After the initial distribution equilibrium is achieved, the rate of transfer into the surrounding tissues is the same as the reverse transfer rate into the plasma, with no net molecular movement of drug. Following completion of the infusion, the plasma concentration of the drug begins to decrease more rapidly than that of the tissue concentrations. The tissues now serve as a drug reservoir, transporting the drug back into the plasma (49).

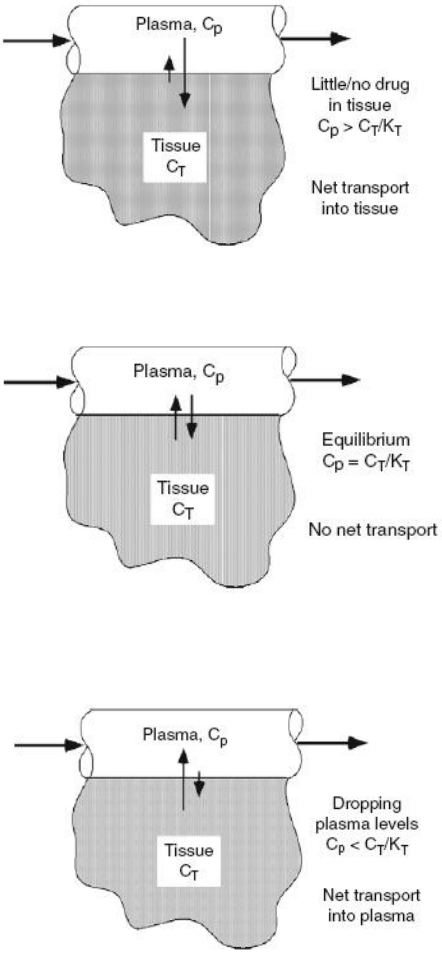


Figure 2.4 The stages of the distribution equilibrium (49)

C_P: plasma concentration; C_T: tissue concentration; K_T: rate constant.

However, most drugs displays a more complicated three compartment PK model, as illustrated in Figure 2.5. The central compartment which is the plasma equilibrates with the rapidly and the slow equilibrating compartment. And this can be explained graphically by three phases of redistribution, rapid, intermediate and slow as shown in Figure 2.6.

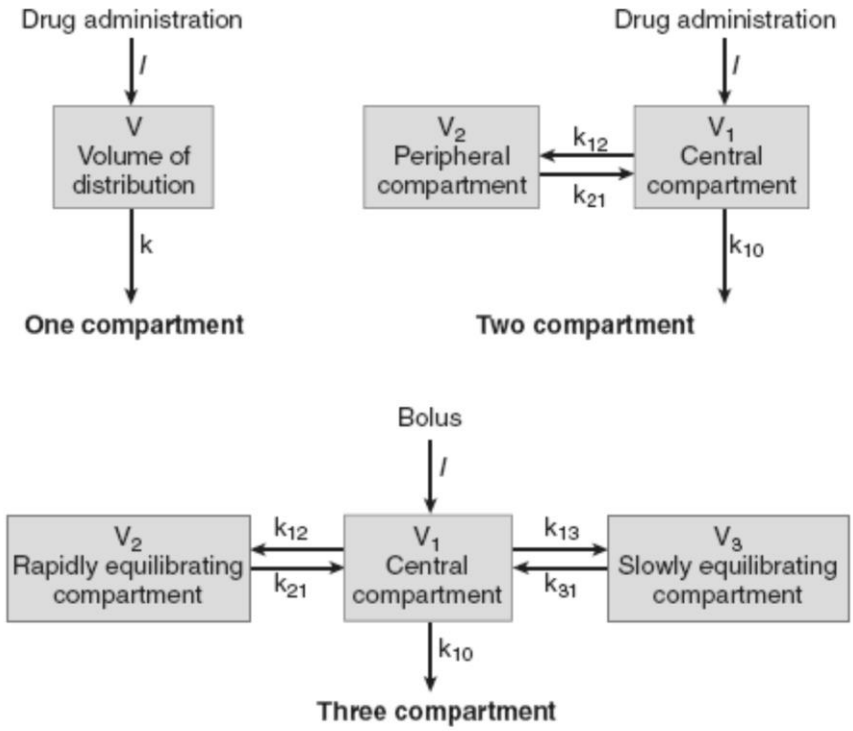


Figure 2.5 The different compartment model (51)

K_{10}: Clearance; K_{12}: rate constant for drug distribution from central compartment to rapidly equilibrating compartment; K_{13} rate constant for drug distribution from central compartment to slow equilibrating compartment; K_{21}: rate constant for drug distribution from rapid equilibrating compartment to central compartment; K_{31}: rate constant for drug distribution from slow equilibrating compartment to central compartment.

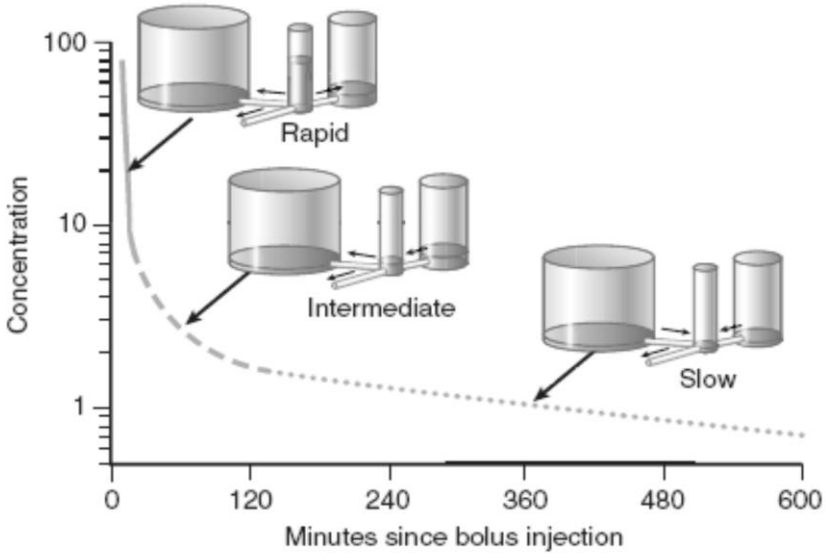


Figure 2.6 The three phases of redistribution (51)

Metabolism

Byers and Sarver (49) defined metabolism as the process whereby multiple enzymatic reactions alter the chemical structure of the drug molecules. The authors further identified cytochrome P450 enzymes as the most significant class of enzyme and their function lies in the Phase I biotransformation of a number of drugs, which include: oxidation, reduction, hydrolysis and de-alkylation. This takes place mainly in the liver, although the gastrointestinal tract, lungs and to some degree the skin also have substantial levels of cytochrome enzymes (49).

This transformation may be explained by the following three scenarios:

Active drug → inactive metabolite

Inactive drug → active metabolite

Active drug → active metabolite.

Phase 2 biotransformation: involves conjugation with glucuronic acid, acetate, amino acid or glutathione to facilitate the process of excretion in the urine or faeces (52).

To summarise this process, hepatic metabolism involves structural alterations of the drug molecule to a form that is either lipophilic for excretion in the bile and eventually in the faeces, or a hydrophilic structure for excretion by the kidneys (53).

Elimination

Clearance (Cl) defined as the volume of blood from which a drug is withdrawn per unit of time, with unit ml/min, forms the basic principle in the process of elimination that occurs mainly in the liver and kidney (47).

According to Shafer et al (51), hepatic Cl depends on the hepatic blood flow, enzyme activity and first pass effect for drugs administered orally. Hepatic Cl is equal to extraction ratio multiply by the liver blood flow. The resultant fat soluble drug and metabolite are excreted into the bile (51).

Cl of hydrophilic drugs usually occurs via excretion into the urine. Renal clearance depends on the glomerular filtration rate, active tubular secretion and renal disease (51).

2.7.5.1 Specific antibacterial pharmacokinetics

The pharmacokinetics properties of antibacterials are more complex with multiple additional factors influencing the concentrations of these drugs at their target sites (47, 54). These factors are discussed in the paragraph that follows.

Volume of distribution

Volume of distribution (Vd) displays the relationship between the total amount of drug in the body and the plasma drug concentration. It is a mathematical hypothesis, which does not necessarily reflect a physiological or real space such as plasma or extracellular volume. Therefore the Vd of many drugs, specially highly lipophilic drugs, can be much higher than the total body water (49). Examples of hydrophilic antibiotics are β-lactams, aminoglycosides and glycopeptides which have a much lower Vd (<0.2 L/Kg) compared to the lipophilic antibiotics, fluoroquinolones, macrolides and tigecycline, which tend to have a substantially higher Vd (> 1 L/Kg) (46, 54). In the critically ill, there are further changes to Vd, these changes mainly affect the hydrophilic antibiotics (increase in Vd), whereby an increase in the loading dose may be warranted (54).

Other factors influencing the Vd of a drug include: degree of ionisation, pKa (pH of a solution at which the ionised and unionised fraction of a drug is in equal amount), the size of the molecule and protein binding (52). Protein binding is predominantly with albumin (acidic and neutral drugs) and glycoprotein (basic drugs) (52, 55, 56).

Clearance

With regard to clearance of the drugs, it is either cleared by the kidney or liver.

Hydrophilic antibiotics are predominantly cleared via renal clearance compared to lipophilic antibiotics which mainly involve hepatic clearance (54).

Various factors affect Cl. An augmented renal clearance has been observed in critically ill patients without renal dysfunction (57-59). The patients with sepsis or increased inflammatory response often have higher than normal cardiac indices. Also in the absence of significant organ dysfunction there is often an increased renal preload and consequently increased drug clearance (47, 59). Declercq et al (60) found an augmented renal clearance in non-critically ill patients undergoing abdominal and trauma surgery, this phenomenon could easily be going on unnoticed during cardiac bypass surgery. With the compounding effect of the severe inflammatory response that occurs from the extracorporeal circuit there might be a significant increase in the renal clearance.

Half-life

t½ is defined as the time required for the plasma concentration of a drug to decrease by half (48). It is directly proportional to the Vd and inversely proportional to Cl, so the same factors that affect the changes in Vd and Cl will also significantly affect the t½ of the antibiotic (47).

Antimicrobial agents follow three modes of bacterial killing.

Concentration-dependent killing with post-antibiotic effect

The bacterial killing is proportional to maximal concentration (Cmax) attained compared to minimum inhibitory concentration (MIC) of targeted organism (Cmax/MIC). Therefore, only achieving most favourable killing once the maximal concentration passes a threshold peak to MIC ratio. Hence, the dosing goal is to enhance peak concentration of the antibacterial, e.g. aminoglycosides and fluroquinolones. (47, 53)

Time-dependent killing

In this pattern the bacterial killing is proportional to the extent of time the drug concentration is upheld above the MIC of the targeted organism (T > MIC). The goal of therapy is therefore to augment the duration of exposure by small dosing intervals, e.g. β-lactams. (47, 53)

Time-dependent killing with post-antibiotic effect

The total drug exposure, also referred to as area under the curve (AUC), relative to MIC of the targeted organism is proportional to bacterial killing (AUC/MIC). The goal of therapy is therefore to augment the quantity of drug available via both dose and interval, e.g. vancomycin, clindamycin and linezolid. (47, 53)

These three patterns are graphically represented in Figure 2.7(47).

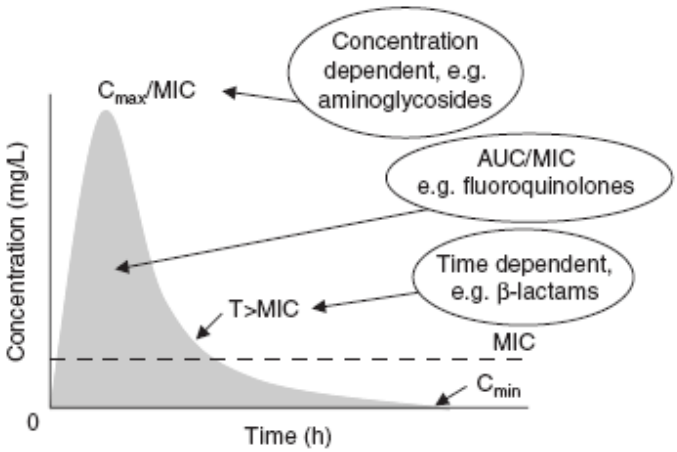


Figure 2.7 Pharmacokinetic and pharmacodynamics parameters of antibacterials (47)

With regard to post-antibiotic effect, it represents the continued suppression of bacterial proliferation for an extended period after the antibiotic level drop below the MIC of the specific bacteria. Most antibacterials exhibit a post-antibiotic effect. β-Lactams

demonstrate very little post-antibiotic effect against gram-positive organisms, but no post-antibiotic effect (with the exception of carbapenems) against gram-negative organisms, therefore the concentration of cefazolin has to be above MIC throughout the majority of the surgical procedure. However, aminoglycosides express a significant post-antibiotic effect of more than three hours (47).

2.7.6 Cardiopulmonary bypass

CPB was first used in 1953 by John Gibbon, Jr after 15 years of work in developing a machine with the capabilities of supporting respiration and providing an extracorporeal circulation. The goal of the extracorporeal circuit is to undertake four important vital functions: organ perfusion, systemic cooling and rewarming, respiratory functions, and optimisation of the surgical field by diverting blood from the heart during surgery. Deoxygenated venous blood is passively removed from the right atrium into a reservoir that additionally receives all blood suctioned during surgery, additional fluids, and drugs. Multiple factors determine the amount of venous blood drained from the heart and these are, the resistance in the venous circuit, the height from the patient to reservoir and central venous pressure. Leaving the reservoir, the blood is sieved of surgical debris and other contaminants via an arterial filter. It is then pumped to an oxygenator and heat exchanger unit before finally returning to the patient via the aortic cannula. The general components of the CPB circuitry is made up of a series of pumps and tubing for cardiotomy suction, bubble detectors, pressure monitors, venting and cardioplegia ports, and blood sampling ports including in-line blood gas analysers as described in Figure 2.8. (61, 62)

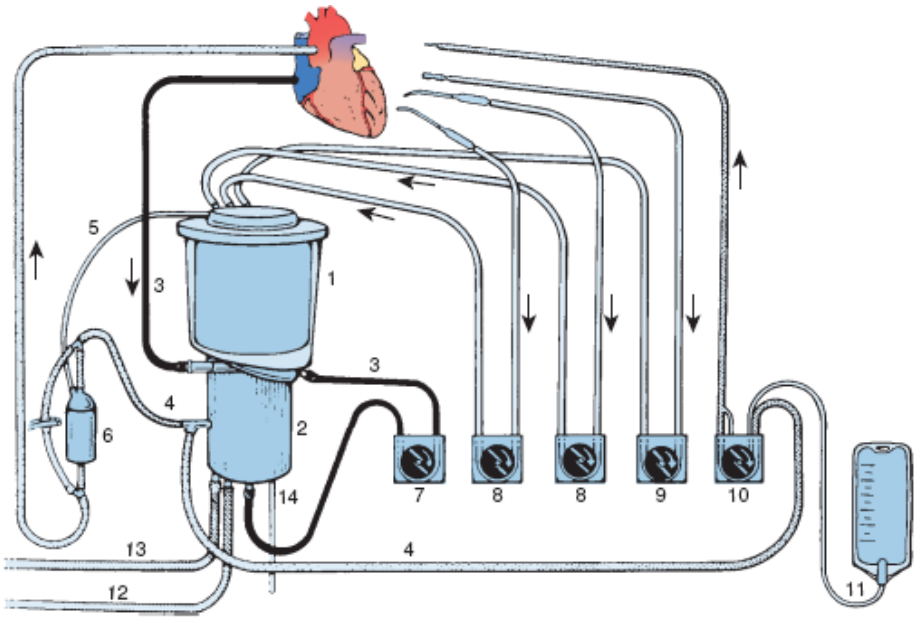


Figure 2.8 Schematic representation of the CPB circuit (62)

Labelling of the components from Figure 2.8 are as follows: [1] integral cardiotomy reservoir; [2] membrane oxygenator; [3] venous blood line; [4] arterial blood line; [5] arterial filter purge line; [6] arterial line filter; [7] venous blood pump (also called the arterial pump head); [8] cardiotomy suction pump; [9] ventricular vent pump; [10] cardioplegia pump; [11] crystalloid cardioplegia; [12] water inlet line; [13] water outlet line; and [14] gas inlet line. (62)

Depending on the surgical procedure, the type of CPB circuit used and cannulation sites may vary, however for the majority of elective cardiac procedures the full CPB circuitry is involved. There, blood is withdrawn from the right atrium and pumped back to the systemic and peripheral circulation via the aorta. Therefore, the CPB circuit takes over the complete function of the heart and lungs. (62)

During CPB, there is a vast alteration in the PK principles. There is limited data in the literature to guide therapy whilst the PK changes from CPB are in effect. Shekar et al (18) reviewed the pharmacokinetics properties during extracorporeal membrane oxygenation and found lipophilic drugs such as fentanyl and midazolam to be highly sequestered. PK studies in neonates, have demonstrated increased Vd and decreased drug CL during extra corporeal membrane oxygenation (18).

Miller et al (63) found a lower clearance of cefazolin during surgery (27.4 ml/min), compared to preoperative clearance (48.6 ml/min) and post-operative clearance (46.6 ml/min). Since, cefazolin is up to 90% eliminated by the kidney, the clearance was representative of the renal clearance. The authors also found the decrease in cefazolin clearance constant throughout the surgery, including the duration of CPB.

With regards to the pharmacokinetics of cefazolin during CPB surgery. From a pilot study in Vermont, USA in 2002, Fellinger et al (16) measured the serum levels of cefazolin during CPB surgery. Their observations were that therapeutic range was only achieved for two pathogens involved, namely *Staphylococcus aureus* and *Staphylococcus epidermidis.* However, the protocol at their institution was to give one gram of cefazolin at onset of anaesthesia and an additional gram at the onset of CPB. Therefore it is not possible to extrapolate their findings to our practice, given the difference in both the dose and dosing frequency.

Hutschala at al (3) in Austria, published in 2007, cefazolin penetration into soft tissue during cardiac surgery, using in vivo microdialysis. They found the median interstitial tissue concentration of cefazolin to be higher than the MIC_{90} for the prevalent pathogens involved in SWIs. The protocol used at their institution required four grams intravenous bolus before skin incision and a further two grams during skin closure. This is not a recommendation by any guidelines and therefore their findings cannot be extrapolated for any purposes.

In Firenze, Italy, Adembri et al (15), in 2010 compared a bolus and continuous infusion of cefazolin in patients undergoing elective cardiac surgical procedures. They prospectively and randomly divided their 20 patients into either a bolus or a continuous infusion group.

The former group received two grams of cefazolin as a prophylaxis dose 30 minutes prior to surgical incision with additional doses of one gram at the end of CPB, as well as at nine and fifteen hours after CPB. The second group received a continuous infusion of initially two grams of cefazolin, followed immediately by an infusion of three grams at a rate of one gram every six hours. They demonstrated that 90% of the patients in the continuous infusion group were within therapeutic range throughout surgery compared to the bolus group where this goal was only achieved for 30% of the group. The authors concluded that continuous infusion exhibits pharmacokinetic and pharmacodynamic advantages compared to the bolus administration. However there is currently no additional evidence supporting the use of continuous infusions in prophylactic regimens.

In a 2006 prospective study from Stanford University, California, Caffarelli et al (64) analysed the cefazolin serum levels of four groups of patients. Group A was the control group with 10 patients receiving cefazolin as antibiotic prophylaxis, undergoing vascular surgery with no CPB. Group B included 10 patients undergoing cardiac surgery with CPB time less than 120 minutes, Group C involved 10 patients where CPB time was more than 120 minutes and Group D had 10 patients for cardiac surgery involving CPB and hypothermic circulatory arrest. The results showed that in all three groups on CPB, the serum cefazolin level was below the MIC_{90} for *Staphylococcus aureus* at various stages of surgery in the majority of the patients. The authors suggested increasing the initial dose from one gram to two grams of cefazolin at induction and to repeat a second dose after 240 minutes.

Bertholee et al (65) investigated the concentration of cefuroxime whilst on CPB and found that the current antibiotic regimen used (one and a half gram at onset of anaesthesia followed by one and a half grams at onset of CPB) did not maintain cefuroxime concentrations above the MIC throughout the operation. This translates to ineffective antibiotic prophylaxis. They concluded that further research with different antibiotic dosing regimens was therefore necessary to obtain adequate prophylactic levels.

2.8 Cardiac surgery prophylactic antibiotics

Cephalosporins form the core of standard prophylactic antibiotics for cardiac surgery owing to their low toxicity and broad microbial coverage. Current regimens for prophylaxis in CPB surgery were developed from studies that reviewed post-operative infection in both cardiac and non-cardiac surgeries (16). Engelman et al (11), in their report on antibiotic prophylaxis in cardiac surgery, singled out β-lactams as the antibiotics of choice, with the exception of specific population with high incidence of methicillin-resistant *Staphylococcus aureus.*

Bolon et al (66) performed a multi-institutional, international meta-analysis on 5761 patients comparing the incidence of SSIs using a glycopeptide (vancomycin, teicoplanin) versus a β-lactams. The results showed that β-lactams were as effective as glycopeptides for the overall prevention of SSIs. Therefore, the authors recommended, based on availability and cost, the use cefazolin as standard cardiac surgery prophylaxis. Saginur et al (67), also concluded that cefazolin was a more effective prophylaxis and they did so following a multicentre double-blind randomized controlled trial comparing teicoplanin with cefazolin in elective cardiac surgery.

Regarding gram-positive bacteria, the peptidoglycan layer (hence Penicillin binding protein, PBP) is positioned on the outer surface of the cell wall whereas for gram-negative bacteria a complex lipopolysaccharide structure is located on the outermost layer, therefore cephalosporins have to diffuse across the lipopolysaccharide membrane to reach the PBPs. The PBPs within each and every bacterium vary by type and amount. These targets are numbered by convention on the basis of molecular weight, with letters differentiating proteins of similar molecular weight. The events following the covalent binding of cephalosporins to the PBP targets that lead to cell lysis and death are not entirely understood (51, 68).

Against gram-positive infections such as cellulitis, cefazolin remains a popular choice. This first generation cephalosporin is commonly used as part of home-based intravenous antibiotic programmes due to its relatively narrow spectrum, stability and side effects profile. In order to limit the rise of resistance and to decrease the growing antibiotic pressure, the use of a first generation cephalosporin is considered the sensible choice. (68)

2.9 Pharmacological properties of cefazolin

In order to maximise the efficiency of cefazolin as a prophylactic antibiotic and ensuring maximum time above MIC, knowledge of its specific pharmacology is of utmost importance.

Cefazolin comes from the family of cephalosporin and is a first generation cephalosporin.

Figure 2.9 Chemical structure of cefazolin (69)

Cephalosporins were first isolated from a filamentous fungus, known as cephalosporium. They were cultured from the sea near a Sardinian sewage outfall in 1945. Their molecular structure, illustrated in Figure 2.9, is closely related to that of penicillin. Since then, various semi-synthetic forms have been introduced. (70)

Cephalosporin is considered to be bactericidal (69). They are usually well tolerated, but unfortunately do come with side-effects, such as injection site pain, allergic reactions of the penicillin type, hypotension, drowsiness, headache, weakness, hives, skin rashes, confusion and gastrointestinal upset. The overall cross-allergy between penicillins and cephalosporins involves around 10% of patients. The overall rate of unwanted skin reactions (urticarial rashes and pruritis) is quite rare and ranges between 1% and 3% (53). Pain may be experienced at intravenous or intramuscular sites of injection. If continued use for more than two weeks, reversible thrombocytopenia, haemolytic anaemia, neutropenia, interstitial nephritis or abnormal liver function tests may occur (53).

Cefazolin's mechanism of action is similar to that of other β-lactam drugs. Like the β-lactams, cefazolin inhibits the bacterial cell wall synthesis. The peptidoglycan cross-linkage structure found within the bacterial cell wall is the primary target for these compounds. Peptidoglycans are made from cross-linked chains of polysaccharide consisting of alternating N-acetylglucosamine and N-acetylmuramic acid compounds resulting into a net-like structure. These structures via the action of several group of enzymes including transpeptidases, carboxypeptidases, and endopeptidases, are inserted into the bacterial cytoplasmic membrane. The lactam ring of the penicillins and cephalosporins has similar conformation to the terminal d-alanine-d-alanine of these pentapeptide enabling a covalent bond to be formed with these enzymes resulting in loss of enzyme activity. The enzyme drug targets are referred to as PBPs. (49)

2.9.1 Pharmaceutical properties

Cefazolin (as sodium) presents in a 500 mg vial and a one gram vial, it is in a powdered form when reconstituted and diluted can be given either intramuscularly or intravascularly. The dosage ranges between 25 to 100 mg/Kg over 24 hours. This can be divided into three to four doses. The adult dose for surgical prophylaxis is one- two grams or 30 mg/kg as an intravenous bolus 30 to 60 minutes prior to surgical incision. (71, 72)

Cefazolin is a polar, water-soluble compound, with a wide volume of distribution in the body (Vd of around 10L) which allows treatment of infection at most sites, including bone, soft tissue and muscle (56, 68, 70).

Up to 90% of cefazolin is eliminated unchanged via the kidney. The elimination is more by glomerular filtration than by tubular excretion. It is between 75% to 90% plasma protein bound and this slows the glomerular filtration of the drug, resulting in a t½ of 1.5 to 2 hours, which allows 8 and 12-hourly dosing (55, 56, 68, 70, 73). Cefazolin binds to albumin on either the warfarin site or the bilirubin site or both (55). The percentage binding is lower with lower concentrations of albumin occurring with renal and hepatic dysfunction and displacement by acidic drugs such as salicylic acid, valproic acid and furosemide or endogenous substrates such as bilirubin and free fatty acids (55). A lower percentage binding will increase the free fraction of cefazolin with a resultant increase in its Vd. The increased free fraction of the drug will be eliminated more rapidly by the kidney (increased clearance with a decreased t½), requiring adjustments to both drug dosing and frequency to maintain therapeutic levels (59).

2.10 Conclusion

There are numerous and vastly contrasting practices in antibiotic prophylaxis. Variation exist not only in adult doses ranging from one gram up to four grams, but also in duration extending from single dose, 24 hour duration and up to 48 hour duration (3, 11, 16, 45). Some of these practices have been investigated and concluded that sub-therapeutic doses were used intra-operatively in cardiac surgery (16). The fact that the pharmacokinetic changes in CPB surgery remains fairly unexplored leaves an obvious gap. The CPB circuit is still, after more than 50 years of being in use, a foreign concept where many important properties are being simply left for speculation. The principle of augmented renal clearance, which occurs from SIRS, together with the decrease renal clearance that is seen in hypothermia, leads to more uncertainty regarding the pharmacokinetic of prophylactic antibiotics during CPB (47, 63).

In view of all of the multiple confounding factors, it is difficult to predict the actual concentration of the drug that will reach the tissue site and if the actual concentration is

adequate for prophylaxis. Therefore, a study on the serum cefazolin levels during CPB surgery is vital to guide future acceptable prophylactic dosing strategies.

2.11 Summary

In this chapter a literature review was presented. In the following chapter the research methodology will be reviewed.

Chapter 3: Research methodology

3.1 Introduction

This chapter presents the problem statement, aim, objectives, ethical considerations, research methodology and the validity and reliability of this study.

3.2 Problem statement

Based on the South African Antibiotic Stewardship Programme guidelines (14), the current practice at CMJAH is to give a two grams bolus of cefazolin 30 minutes pre-operatively followed by a re-dose after four hours with prolonged surgery. The efficacy of this practice in achieving therapeutic levels of cefazolin intra-operatively cannot be confirmed due to paucity in the literature pertaining to the altered PK of antibiotics caused by the vast physiological changes that occur during cardiothoracic surgery and CPB.

An understanding of the distribution and kinetics of cefazolin during CPB would therefore further inform this discussion. If there is indeed sequestration of cefazolin in the circuit (18), leading to concentrations below MIC_{90}, then the dosing regimen will consequently require review.

3.3 Aim and objectives

3.3.1 Aim

The aim of this study was to describe the total serum cefazolin levels during elective valve replacement surgery on CPB at CMJAH.

3.3.2 Objectives

The primary objectives of this study were to:

- describe antibiotic levels in the patient's serum before, during and after CPB
- describe serial antibiotic levels in the circuits during the CPB period.

The secondary objective of this study was to describe the pre-operative and intra-operative creatinine clearance.

3.4 Ethical considerations

Approval to conduct this study was obtained from the Human Research Ethics Committee (Medical) (Appendix A) and the Postgraduate Studies Committee (Appendix B) of the University of the Witwatersrand. Written permission was obtained from the Chief Executive Officer of CMJAH (Appendix C). A change of title of the research report was approved from the Postgraduate Studies Committee (Appendix D).

Participants were approached the day before surgery and the researcher explained the study and invited them to take part. If they agreed, they received a study information letter and were asked to give written consent (Appendix E).

In order to maintain anonymity of the participants, their names or hospital numbers were delinked from the data capture sheets and the sample labels. Each participant was allocated a study number. Only the researcher had the information that links the participants name with the study number. This was kept in a separate file. Confidentiality was maintained as the researchers were the only people with access to the raw data.

Each participant had blood samples taken on a regular basis intra-operatively. To reduce the amount of blood sampled, the blood was collected in micro-tubes so that each sample did not exceed 2 ml. This amounted to a maximum of 50 ml being sampled per patient. Sampling would continue for up to five hours or until surgery ended, whichever came first and in the latter case sampling was reduced. Additionally the patient was under anaesthesia and the blood sampling did not involve venepuncture but the use of indwelling arterial or venous catheters (placed as per standard surgical requirements and not for study purposes). One sample was taken when the patient was awake and this sample was taken from the intravenous drip site when it was inserted by the anaesthetist.

The study records will be stored securely for six years following completion of the study.

The study was conducted in adherence to the principles of the Declaration of Helsinki, 2013 (19) and the South African Good Clinical Practice Guidelines (20).

3.5 Research methodology

3.5.1 Research design

A prospective, contextual, descriptive research design was used in this study.

Cefazolin plasma concentrations were analysed at specific pre-determined time intervals in adults patients scheduled for elective valve replacement surgery and there was no randomisation.

3.5.2 Study population

The study population comprised patients undergoing elective valve replacement, on CPB from the cardiothoracic unit at CMJAH.

3.5.3 Study sample

Sample size

Given that this was not an interventional study, a traditional sample size calculation was not possible. Based from the experience of the BTCCRC research group in conducting multiple similar studies, enrolling 12 patients with rich pharmacokinetic sampling will enable a reasonably accurate description of relevant pharmacokinetic parameters in the study population. This chosen sample size is supported by the published literature as well as the following peri-operative pharmacokinetic studies being conducted with antibiotics with similar characteristics to cefazolin, or studies being conducted with cefazolin. The sample size in these pharmacokinetic studies range between 6 and 20, Douglas et al (74) n=12, Naik et al (75) n=16, Elkomy et al (56) n=20 and Bertholee et al (65) n=17. This sample size is likely to have sufficient power to identify the most important covariates which are associated with pharmacokinetic variability. However, we realised that the surgeon in our cardiothoracic unit, had a practice of requesting the valve to be dipped in a specific antibiotic of choice prior to surgical implantation. When this came to our attention, after consulting with the BTCCRC research group and the ethics department of the university (Appendix F), we decided to increase the sample size to 16 patients. We then had 8 patients with valves dipped in cefazolin and the remainder dipped in gentamicin. Based on the results of this study, we would be able to derive a sample size calculation for any future interventional study.

Sampling Method

The process of selecting a group of individuals from a population, to accurately represent the population, forms the basis of a sampling method (76). In this study, the sampling method used was convenience sampling, which is detailed by Burns and Grove (76) as choosing available subjects for the study until an adequate sample size is reached.

Inclusion and exclusion criteria

Inclusion criteria for this study were:

- adult patients, aged between 18 and 60 years
- undergoing elective valve replacement surgery
- consenting to participate in the study.

Exclusion criteria for this study included:

- Jehovah's Witness
- pregnancy
- receiving dialysis
- septic shock
- emergency cases
- receipt of cefazolin in the last 72 hours
- deviation from the standard antibiotic protocol.

3.5.4 Data collection

This research project was a collaborative project between the CMJAH research group and the BTCCRC. This research report only addresses three objectives of the larger study therefore the data collection reflected more data than were necessary to answer these objectives.

Discussion of the data collection process include the data collector, data collection period and data collected with the process of collection.

Data was collected by one researcher (DC) from the CMJAH research group. All the members had an in depth knowledge of the data collection procedure to ensure standardised collection of data should the researcher not have been available.

Data was collected between 11 November 2014 and 26 March 2015.

The process of data collection is shown in Table 3.1.

Table 3.1 Data collected with the process of collection

Data Collection	Standard operating procedure
Invite patient	Participants were approached the day before surgery and the researcher explained the study and invited them to take part If they agreed, they received a study information letter and were asked to give written consent
Antibiotic prophylaxis • cefazolin 2 g IV within before 30 minutes surgical incision • repeat 2g IV dose intra-operatively if >4 hours	2 g of cefazolin was diluted in 20 ml saline and given intravenously over 5 minutes

Blood Samples **Pre-operative bloods** • routine albumin, Urea and electrolytes (U&E) **Intra-operative bloods** **Pharmacokinetic bloods** **Venous sampling** • pre antibiotic administration (from IV line insertion site) 30 minutes before incision • from the central venous catheter (CVC) at time 2, 5, 10, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes • CVC blood sampling at 5 minutes before and 5 minutes after CBP is initiated • CVC blood sample 5 minutes after CPB is completed **Arterial sampling from CPB circuit** • 5 mins after going on CPB • sample 2-6 to correspond with venous samplings	1. Pre-operative bloods and intra-operative U&E were tested at the National Health Laboratory Service 2. Pharmacokinetic blood samples were restricted for the duration of the surgery, e.g. meaning if the surgery was completed at 180 minutes then the blood sampling was be aborted 3. CVC and CPB samples were taken simultaneously each time a CVC sample is taken in point. This did not exceed 7 samples 4. CVC samples were taken in the following manner • 20 ml aspirated from the CVC line • a 2 ml volume was sampled in a yellow top microtube • 20 ml aspirate replaced • Microtube was labelled with a pre-printed self-adhesive label 5. CPB samples were taken in the following manner • were taken from the arterial port of the CPB circuit • a 2 ml sample was taken in a yellow top microtube • microtube was labelled with a pre-printed self-adhesive label 6. All blood sample were centrifuged • within minutes • centrifuged in theatre (one standard machine) • at 3000rpm for 10 minutes • the plasma was then transferred to a microtube with a disposable pipette • the microtube was labelled with a pre-printed self-adhesive label 7. Storage of blood sample: • In a -80°C freezer

	• In the Department of Medicine
	  ○ continuous temperature monitoring
	  ○ with back-up CO2
	  ○ back-up power from the hospital generator
	8. Courier of sample
	  • all blood sample were couriered at study completion
	  • by a courier, which specialises in transport of research sample
	9. Sample analysis
	  • sample were analysed at the BTCCRC
	  • accredited laboratory

Urine sample • collected at time of urine catheter insertion • repeated at post-surgery completion	• 10 ml was be collected • labelled with a pre-printed self-adhesive label • stored same as blood samples • couriered same as blood sample • analysed same as blood sample

No patients required snaring in this study, therefore blood was available from the CVC. Should any patients have required snaring, the surgeon would have been requested to intermittently release the snare to allow blood sample to be collected from the CVC.

Data collected

The following additional data were also collected on the data collection sheet (Appendix G):

- demographic data (gender, age, date of surgery, height, weight, APACHE II score, renal function and albumin)
- pre-operative antibiotics
- anaesthetic agents used
- intra-operative fluids, including blood products
- CPB time

- cross-clamp time
- vasopressor used
- Intra-operative complications
- duration in theatre.

3.5.5 Sample analysis

All blood samples were centrifuged after acquisition. Plasma was pipetted into appropriately labelled microtubes. The plasma and urine microtubes were stored at -80 ˚C. An export permit was obtained from the Department of Health, South Africa (Appendix H) and on completion of sample collection the samples were couriered on dry ice, in a temperature controlled container by a specialised medical specimen transport company to the BTCCRC for analysis. Ultra-high-performance liquid chromatography-tandem mass spectrometry was employed to analyse the study samples (77).

3.5.6 Data analysis

Data was captured and analysed on a Microsoft® Excel 2013 spreadsheet. Descriptive and inferential statistics was used. Categorical variables were described using frequencies and percentages, continuous variables were described using means and standard deviations. Time series analysis of the serum samples was done by the bio-statistician involved in the research group in Australia. This analysis was sent to the researcher for interpretation for this MMed project. A Kaplan-Meier curve was used to graphically display the serum samples. Paired T tests were used to compare both the pre-operative and intra-operative creatinine clearance and the arterial and corresponding venous samples on CPB. A p value of < 0.05 was considered statistically significant.

3.6 Reliability and validity

Bothma et al (78) defined validity of the study as " the degree to which measurement represents the true value" and reliability as "the consistency of the measure achieved".

The reliability and validity of this study was maintained by:

- using an appropriate study design and data capturing technique
- adhering strictly to the standard operating procedure for data collection
- collection of data by only one researcher (DC)
- storing specimen in a quality controlled -80 °C freezer
- using a courier service specialising in the transport of research samples

- analysing bloods and samples in an accredited facility
- analysing data using appropriate statistical methods in consultation with the biostatistician.

3.7 Summary

In this chapter the research methodology was described in details. In the following chapter the results and discussion will be presented.

Chapter 4: Results and discussion

4.1 Introduction

This chapter contains the results and discussion. The results are presented in accordance with the objectives of the study.

The primary objectives of this study were to:

- describe antibiotic levels in the patient's serum before, during and after CPB
- describe serial antibiotic levels in the circuits during the CPB period.

The secondary objective of this study was to describe the pre-operative and intra-operative creatinine clearance.

4.2 Sample realisation

A sample size of 16 patients was determined in consultation with the BTCCRC. Sixteen patients were enrolled from 11 November 2014 until 26 March 2015 and there were no exclusions.

4.3 Results

4.3.1 Patient demographics

The sixteen patients included comprised an equal number of males and females.

The patients' demographics are shown in Table 4.1.

Table 4.1 Patient demographics

Demographics	Mean (SD)	Minimum	Maximum
Age (years)	44 (11.98)	18	59
Height (cm)	163.88 (9.98)	144	180
Weight (kg)	75.06 (15.55)	51	97
BMI (kg/m^2)	28.22 (6.72)	18.1	40.2
APACHE II	16.63 (2.58)	12	21
Onset of CPB, from initiation of infusion of cefazolin (minutes)	71 (17.20)	44	102
Mean CPB time (minutes)	180 (55.41)	105	279

4.3.2 Objective: describe antibiotic levels in the patients' serum before, during and after CPB

Table 4.2 shows the total cefazolin serum levels of the 16 patients during cardiac valve surgery on CPB.

Table 4.2 Total cefazolin serum level (µg/ml) during CPB surgery

First dose of cefazolin ↓ Second dose of cefazolin ↓

Patient	Bld 1	Bld 2	Bld 3	Bld 4	Bld 5	Bld 6	Bld 7	Bld 8	Bld 9	Bld 10	Bld 11	Bld 12	Bld 13	Bld 14
1	0	398.33	272.82	197.43	156.44	126.46	67.02	58.30	54.51	52.12	45.04	39.14	170.59	
2	0	344.84	288.91	241.33	185.28	113.19	97.37	99.68	83.16	74.43	70.90			
3	0	349.34	331.18	271.92	224.39	95.95	84.05	82.101	73.71	59.81	49.77	43.22	163.38	130.01
4	0	301.43	248.37	214.88	156.63	86.94	73.80	61.15	55.43	50.93	43.49	40.22	192.49	
5	122.87	378.30	257.37	155.8	117.47	86.22	70.69	64.00	53.96	42.84	34.15	27.86	154.97	
6	0	274.72	248.47	213.98	159.71	93.59	75.73	64.03	65.16	60.46	55.97	48.36	163.36	138.60
7	0	256.54	235.82	202.36	151.59	120.25	69.3	63.42	54.1	31.88	34.18	35.13		
8	0	336.14	294.37	236.75	157.51	123.04	71.62	70.53	61.44	48.84	43.39	41.73		
9	0	186.26	152.42	123.88	80.23	61.15	51.27	48.43	40.41	34.92	33.78	31.56	120.36	86.67
10	0	334.60	313.16	266.26	209.37	162.79	105.35	92.77	86.62	71.24	62.03	57.57		
11	0	319.27	212.66	168.65	134.95	98.05	87.50	79.11	76.43	76.14	83.40	69.76	182.85	148.80
12	0	338.52	248.97	199.66	147.44	124.45	107.23	107.99	108.58	111.93	107.94	103.51	234.78	212.19
13	0	320.32	252.93	202.19	148.16	116.39	62.51	62.71	61.12	52.51	61.29	49.42	169.77	
14	0	324.61	282.98	235.53	170.08	127.54	80.73	74.74	77.00	82.76	79.71	66.3	145.55	125.55
15	0	270.34	219.54	199.26	147.93	113.92	87.45	50.32	45.01	42.5	32.54	32.12	142.52	96.42
16	0	335.87	295.45	226.68	195.10	140.81	86.36	73.56	65.26	87.42	67.82	66.80	191.57	164.38
Mean	7.68	316.84	259.71	209.79	158.89	111.92	79.87	72.05	66.37	61.30	56.59	50.18	169.35	137.83
SD	30.72	50.81	43.47	38.50	34.53	24.36	15.24	16.88	17.30	21.42	21.45	19.93	29.32	39.46

Bld = Blood sample.

	CPB
	Sub-therapeutic level

Blood 1 of all the patients, except patient 5, was taken prior to initiating the cefazolin infusion and had no time factor to it. The subsequent bloods were all sampled timeously as per protocol, i.e time 2, 5, 10, 30 minutes, etc. With regard to patient 5, the patient had difficult intravenous access resulting in a delay in setting up invasive lines (arterial line and CVP catheter), hence the antibiotic infusion was started without any further delay (ensuring that it was started 30 minutes prior to incision time) and blood 1 was obtained simultaneously. This lead to an erroneous value in blood 1 of patient 5, as the actual value should have been 0 µg/ml. The subsequent bloods of patient 5 were sampled timeously.

Therefore, the decision was made not to exclude patient 5 from the study as there was no actual deviation from the standard antibiotic protocol.

A centre specific practice by the surgeon in our cardiothoracic unit is to request the valve to be dipped in an antibiotic of choice prior to surgical implantation. When this came to our attention we decided to increase our sample size from 12 to 16 patients (with relevant ethics approval, Appendix F). Half of the patients had their valves dipped in cefazolin and the other 8 patients' valves were dipped in gentamicin prior to implantation. Of note, patient number 1, 2 and 11-16 had their valves dipped in cefazolin.

The measured total antibiotic concentration is the sum of the free antibiotic concentration (unbound) and the bound antibiotic concentration. The bound cefazolin concentration depends on plasma protein binding which has a range of 75% to 90% in the literature (55, 56, 68, 70, 73). In this study, based on the expertise of the BTCCRC group, the maximum protein binding value was used and hence a free level of 10% was calculated from the measured total cefazolin level. The unbound level has more clinical significance as it needs to correlate with the MIC of the relevant pathogens. The mean free cefazolin serum level is graphically represented in Figure 4.1.

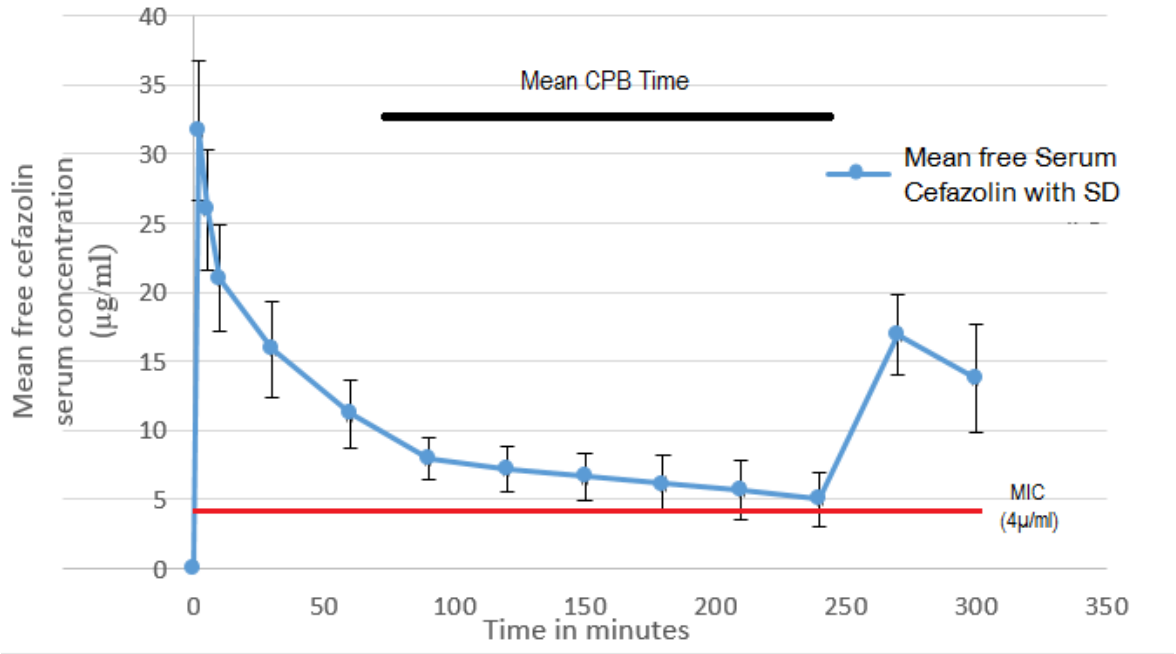


Figure 4.1 Mean free cefazolin serum concentration

The mean value of the trough from Figure 4.1 for the unbound concentration time curve was 5.02 µg/ml with a range of 2.79 to 10.35 µg/ml. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database from the European Society of Clinical Microbiology and Infectious Diseases, a value of 4 µg/ml and above of unbound cefazolin would be therapeutic against the common pathogens (73, 79). However, we found that this MIC level was not achieved in 5 out of the 16 patients (31.25 %) throughout surgery namely patient 1, 5, 7, 9 and 15. The Kaplan-Meier survival curve depicts this in Figure 4.2 below. Of note, patient 1 and 15 had their valves dipped in cefazolin and patients 5, 7 and 9 had their valves dipped in gentamicin.

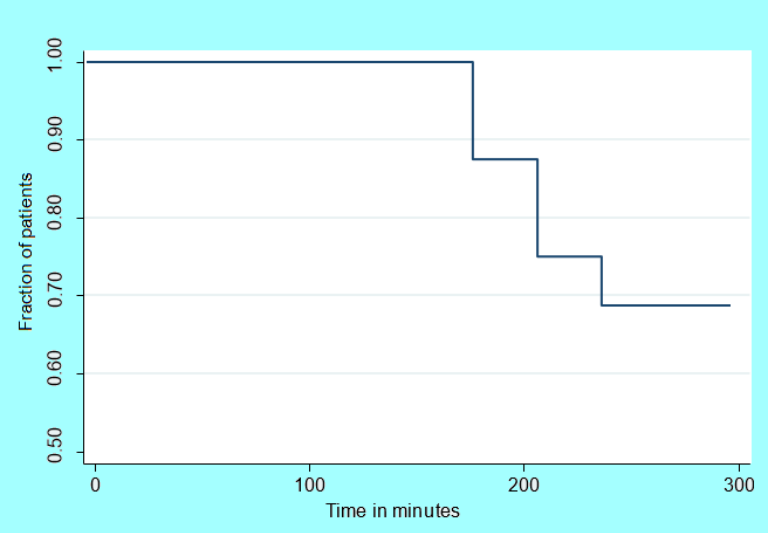


Figure 4.2 Kaplan-Meier survival curve for cefazolin concentration greater than 4µg/ml

4.3.3 Objective: describe serial antibiotic levels in the circuits during the CPB period

At the initiation of CPB a blood sample was taken from the arterial port of the CPB circuit and simultaneously a venous sample was withdrawn from the CVP. Following this, the arterial samples were taken simultaneously with the corresponding venous sample every 30 minutes as per the protocol.

The measured total cefazolin concentration is represented in Table 4.3 and graphically shown in Figure 4.3.

Table 4.3 Cefazolin serum level (µg/ml) of arterial and corresponding venous sample on CPB

Pt.	Art1	Ven	Art 2	Ven	Art 3	Ven	Art 4	Ven	Art 5	Ven	Art 6	Ven	Art 7	Ven
1	74.09	70.70	65.24	67.02	58.94	58.30	54.12	54.51	53.37	52.12				
2	119.90	122.17	117.45	113.19	97.30	97.37	94.61	99.68	78.05	83.16	73.483	74.43		
3	96.28	96.33	101.15	95.95	85.32	84.05	82.87	82.10	75.89	73.71	60.274	59.81		
4	91.58	129.52	82.84	86.94	72.76	73.80	58.33	61.15	53.79	54.43	49.824	50.93	41.65	43.49
5	66.09	70.69	65.38	64.00	60.50	53.96	52.85	42.84	40.49	34.15				
6	81.64	81.77	72.61	75.73	61.95	64.03	62.02	65.16	59.38	60.46	54.89	55.97	46.16	48.36
7	71.55	72.72	72.29	69.30	65.93	63.42	53.29	54.10	46.58	31.88	33.37	34.18		
8	69.95	68.07	71.40	71.62	67.36	70.53	59.60	61.44	34.77	48.84				
9	47.83	49.32	48.85	51.27	47.45	48.43	42.06	40.41	36.56	34.92	32.45	33.78	29.81	31.56
10	103.89	151.55	103.56	105.35	91.70	92.77	91.57	86.62	71.43	71.24				
11	96.16	93.49	84.07	87.50	76.35	79.11	74.18	76.42	75.42	76.14	82.91	83.39	58.15	69.76
12	130.27	130.42	103.20	107.99	102.87	108.58	106.98	111.90	109.77	107.94	97.85	103.51	229.45	234.77
13	61.77	62.07	66.22	62.71	61.69	61.12	60.76	52.51	58.14	61.29				
14	73.36	72.16	76.30	80.73	76.31	74.74	178.31	77.00	80.06	82.76	77.70	79.71	63.88	66.30
15	49.10	55.66	50.89	50.32	41.55	45.01	42.18	42.50	32.87	32.54				
16	89.18	95.40	80.91	86.36	72.16	73.56	65.40	65.26	85.97	87.42	66.83	67.82	65.02	67.82
Mean	82.66	88.88	78.90	79.75	71.26	71.80	73.69	67.10	62.03	62.06	62.96	64.35	76.30	80.29
SD	23.22	30.12	19.30	19.25	16.90	17.66	33.59	20.63	21.61	22.62	21.11	22.01	68.72	69.61

Pt = Patient, Art = Arterial blood (From Arterial port of CPB circuit), Ven = Corresponding blood from the CVP (From Blood 1-14) and Sub-therapeutic levels highlighted.

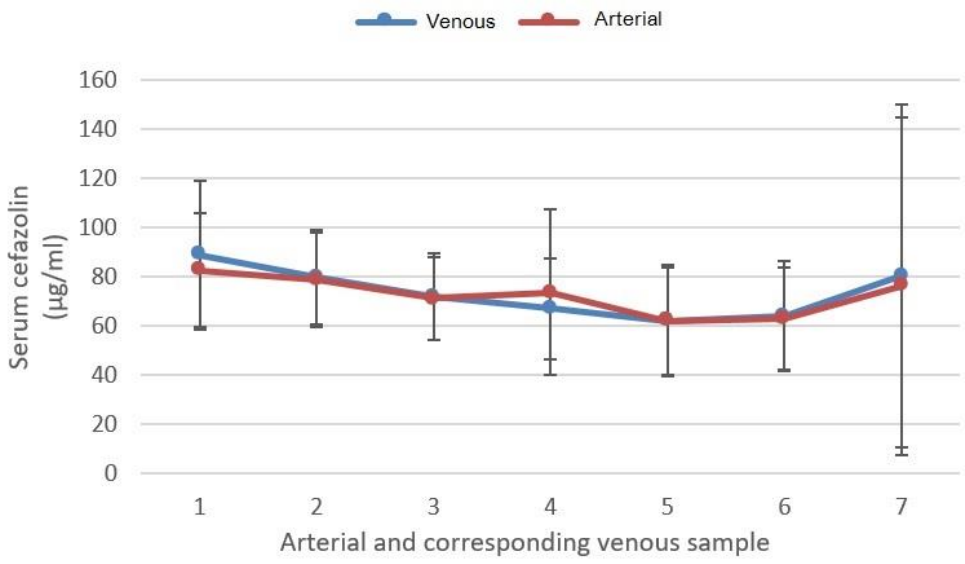


Figure 4.3 Mean total serum cefazolin concentration of arterial and venous samples

The arterial and corresponding venous samples were analysed using the paired t-test. There were no statistical differences between the arterial samples A1-A5 with their corresponding venous samples (p = 0.11, 0.34, 0.46, 0.32 and 0.98 respectively). However, there was a statistically significant difference between the arterial samples A6 and A7 and their corresponding venous samples (p = 0.024 and 0.025).

4.3.4 Secondary objective: Describe the pre-operative and intra-operative creatinine clearance

The patients' pre-operative creatinine clearance as calculated by the Cockcroft-Gault formula as well as their intra-operative measured creatinine clearance are shown in Table 4.4. As the pre-operative creatinine clearance was calculated, the values were rounded off to whole numbers.

Table 4.4 Patients' pre-operative and intra-operative creatinine clearance

Patient number	Pre-operative creatinine clearance (ml/min)	Intra-operative creatinine clearance (ml/min)
1	129	62.8
2	63	52.1
3	52	26.9
4	145	186
5	132	79.3
6	54	111.1
7	111	102.4
8	114	174.7
9	152	114.3
10	98	52.6
11	89	81.8
12	114	77.1
14	58	139
15	103	124.9
16	110	71.1
Mean (SD)	101.6 (32.6)	97.1 (45.3)

The urine sample of patient 13 was accidentally discarded by the laboratory technician, explaining why patient number 13 was removed from the urinalysis.

4.4 Discussion

The pharmacokinetics of cefazolin on CPB had not previously been investigated at CMJAH. A few studies have investigated the cefazolin level on CPB namely Fellinger et al (16), Hutschala et al (3) and Adembri et al (15). However their results cannot be extrapolated to our patients as their dosing regimens were different. Hutschala et al (3) used a substantially higher dose of cefazolin which is not based from any national or international guidelines. The consequences of providing a sub-therapeutic level of prophylactic antibiotics in this population group carries an increased risk of morbidity and mortality peri-operatively. There is a gap in the literature concerning the pharmacokinetics of prophylactic antibiotics within the physiology of CPB.

In our study, the total level of cefazolin was measured from all the blood samples that were collected. The total concentration is the sum of the bound concentration and the unbound or free concentration. The percentage binding of cefazolin (ranging from 75% to 90% in vivo studies) was estimated to be 90%, leaving a free fraction of 0.10 (10%) of the total concentration (55, 56, 73).

Miller et al (63) described a decrease in total body clearance of cefazolin during CPB compared to pre-operative and post-operative clearance. This was primarily accounted for by a decrease in renal clearance, since cefazolin is primarily excreted unchanged by the kidneys. There findings were in contrast to what we observed amongst our patients. The calculated mean pre-operative creatinine clearance was 101.6 ml/min (SD 32.6) with a range of 52 to 152 ml/min whereas the measured intra-operative mean creatinine clearance was 97.1 ml/min (SD 45.3) with a range of 26.9 to 186 ml/min and there was no statistically significant difference between the two sets of data ($p = 0.71$). This finding could be explained by the opposing effects that occur with renal clearance during surgery. The typical decrease in renal clearance on CPB as described by Miller et al (63) with the opposing effect of augmented renal clearance seen both in SIRS (57) and in patients undergoing abdominal and trauma surgery (60) resulted in an unaltered creatinine clearance.

The CPB time varied between patients with a mean of 180 minutes (SD 55.41), ranging from 105 to 279 minutes. The onset of CPB from the initial antibiotic infusion also had wide variation with a mean of 71 minutes (SD 17.20), ranging from 44 to 102 minutes. This may be due to CMJAH being an academic institution. Clinicians with varying degree of experience ranging from junior registrars to senior consultants are involved with the cases while teaching concurrently.

Based on the review article of Mets (17), we expected “a ‘break’ or ‘discontinuity’ in their plasma decay profile with the onset of bypass, with a sudden decrease in concentration” but the graphs of both the total and unbound level of cefazolin represented in Figure 4.1 follows a three compartment model as previously discussed in Chapter 2, Figure 2.6. Hence,

a three-compartment linear model best described the time-course of the 211 total serum concentrations collected in this study before, during and after CPB. This model included zero order input of drug into the central compartment as depicted in Figure 4.4.

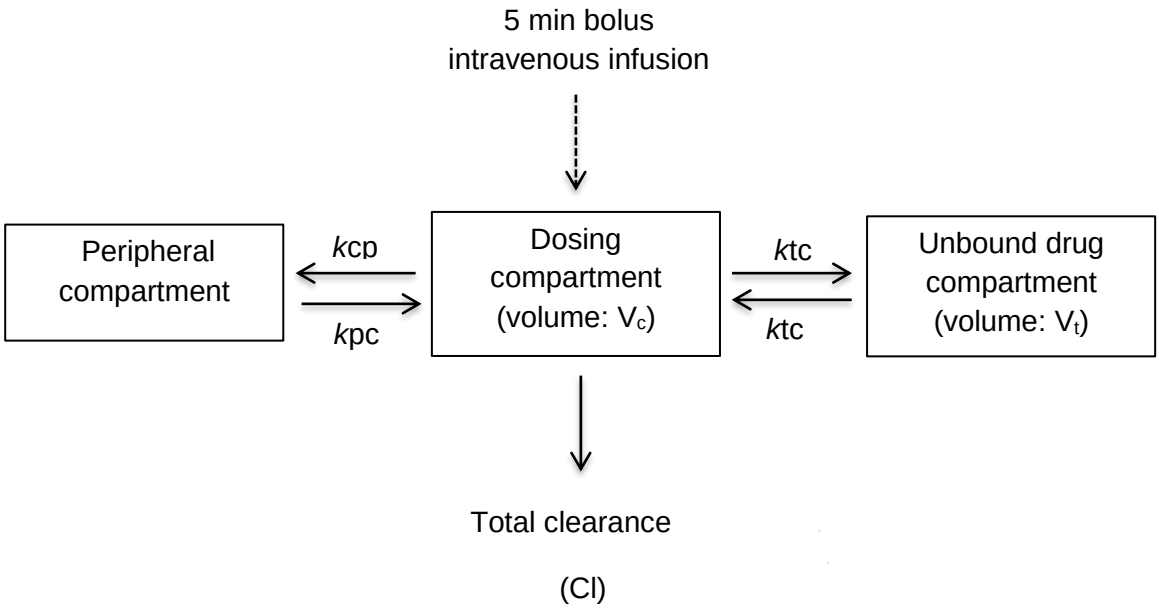


Figure 4.4 Structural pharmacokinetic model for cefazolin in CBP surgical patients (73)

Cl – Clearance; Vc –volume of the dosing compartment; Vt – volume of unbound drug compartment; kcp – rate constant for drug distribution from the central to peripheral compartment; kpc – rate constant for drug distribution from the peripheral to central compartment; kct – rate constant for drug distribution from the central to unbound drug compartment; ktc – rate constant for drug distribution from the unbound drug to central compartment.

Cefazolin being a β-lactam antibiotic exhibits time dependant killing with very little post-antibiotic effect. Thus to ensure adequate surgical prophylaxis the time above the MIC (T>MIC) should ideally be 100% of the dosing interval i.e. T>MIC should be achieved for the full surgical duration. With regards to the pathogens involved in SSIs, *Staphylococcus aureus,* coagulase-negative *Staphylococcus aureus (Staphylococcus epidermidis), Escherichia coli* and *Enterobacter* account for over 90% of the pathogens involved in SSIs (3, 4, 6, 30, 35, 42). Therefore an MIC of cefazolin suitable for these common pathogens should be attained throughout the surgical time. Based on the EUCAST database a value of 4 µg/ml would be effective prophylaxis against these pathogens (73, 79).

Our observation was that this MIC level was not attained in 5 out of the 16 patients (31.25 %) throughout surgery namely subject 1, 5, 7, 9 and 15. Furthermore subjects 7 and 9

demonstrated significant sub-therapeutic levels as early as 180 minutes from the initial infusion. Our current antibiotic regimen provided adequate antibiotic therapeutic levels to only 68.75% as depicted in the Figure 4.2. This is concerning for our population group since the occurrence of SSIs in the face of cardiac surgery can have drastic consequences on morbidity and mortality. These five subjects comprising of two males and three females had BMIs ranging from 21.2 to 29.8 with a mean of 25.08, which was less than the mean of the sample group 28.22. This indicates that the cause for their lower levels might be of an external nature. The remaining patients had antibiotic levels above MIC throughout the surgical time. In five patients the cefazolin levels of blood sample 12 taken four hours post initial infusion did approach MIC of 4 μg/ml. This only increased after a re-dose of two grams of cefazolin maintaining therapeutic levels in subsequent bloods sampled. This lends credibility to the theory that a re-dose is necessary after two t½ of the antibiotic.

With regards to the CPB circuit, in his review article titled "The pharmacokinetics of anaesthetic drugs and adjuvants during cardiopulmonary bypass" Mets (17) details the anaesthetics drugs that are sequestered in the oxygenator circuit. The author mainly found lipophilic drugs such as opioids to be highly sequestered. With the onset of CPB the plasma concentration of these opioids dropped significantly by up to 55%. He also explained that the degree of sequestration is proportional to the lipid solubility of the opioid. In contrast, cefazolin being a hydrophilic drug was not sequestered in the circuit but instead showed a discontinuity in the decay profile at the onset of CPB followed by a slight rise in plasma concentration then the normal decay curve.

While performing serial samples of both arterial (post CPB) and corresponding venous (pre CPB) blood on CPB we have been able to assess the degree of sequestration in the CPB circuit. The data is graphically represented in Figure 4.3. There was no statistically significant differences between the arterial samples A1 to A5 with their corresponding venous samples ($p > 0.05$). There was a statistically significant difference with the arterial samples A6 and A7 with their corresponding venous samples ($p < 0.05$). The clinical significance however of these differences is questionable. These findings point towards no sequestration in the CPB circuit, which is in line with the findings of Mets (17) in his review article.

4.5 Summary

In this chapter the results of the study as defined by the objectives were presented followed by a discussion of the results. In the following chapter a brief summary will be presented followed by limitations, recommendations and finally the conclusion.

Chapter 5: Summary, limitations, recommendations and conclusions

5.1 Introduction

In this chapter a summary of the study method and results will be briefly reviewed, the limitations of the study will be addressed, recommendations made and a conclusion presented.

5.2 Summary of the study

5.2.1 Aim

The aim of this study was to describe the total serum cefazolin levels during elective valve replacement surgery on CPB at CMJAH.

5.2.2 Objectives

The primary objectives of this study were to:

- describe antibiotic levels in the patient's serum before, during and after CPB
- describe serial antibiotic levels in the circuits during the CPB period.

The secondary objective of this study was to describe the pre-operative and intra-operative creatinine clearance.

5.2.3 Methodology

A prospective, contextual, descriptive design was used in this study. Cefazolin plasma concentrations were analysed at specific pre-determined time intervals in adults patients scheduled for elective valve replacement surgery at CMJAH. A sample size of 16 patients was determined based on the experience of the BTCCRC research group in conducting multiple similar studies. A convenience sampling method was used. The inclusion criteria for this study were consenting adults between 18 and 60 years, undergoing elective valve replacement surgery. The exclusion criteria for this study included Jehovah's Witness, pregnancy, receiving dialysis, septic shock, emergency cases, cefazolin use in the last 72 hours and deviation from the standard antibiotic protocol.

5.2.4 Results

Even with the addition of the CPB circuit the decay profile of cefazolin followed a three compartment linear model with no discontinuity or sudden decrease in plasma concentration at the onset of CPB, but instead had slow decline in plasma concentration over time on CPB. The decline was substantial and sub-optimal in 31.25% of the patients at some point during their surgery leaving them, at times, with inadequate antibiotic levels for surgical prophylaxis. No significant cefazolin sequestration was found on the CPB circuit since there was no statistically significant difference between the pre and post CPB samples A1-A5 ($p > 0.05$). There was a statistically significant difference with the samples A6 and A7 ($p < 0.05$), however the clinical significance of this remains questionable. Similar findings were shown for hydrophilic drugs by Mets (17). There was no statistically significant difference between the pre-operative creatinine clearance and the intra-operative creatinine clearance, contrasting to the findings of Miller et al (63).

5.3 Limitations

The assumption that the percentage protein binding of cefazolin remains constant throughout CPB surgery is a limitation. With the acute haemodilution experienced at the onset of bypass, due to the priming solution used on the CPB circuit, we would expect a change in percentage protein binding. For a drug that has a high plasma protein binding, haemodilution from the blood/prime mixture might result in a relatively larger increase in free fraction (17). Additionally, with the effect of heparin which results in the release of lipoprotein lipase hydrolysing plasma triglycerides into fatty acids that eventually competitively binds to plasma protein leading to a potentially higher free level of cefazolin. A change or a lower protein binding of cefazolin during CPB could be expected to be seen (17).

5.4 Recommendations for clinical practice and future research

The observation that potentially 31.25% of patients had sub-optimal protection in this study is concerning. We thus recommend that we follow with a larger, adequately powered study in order to ascertain if this observation is significant or not. And secondly, the assumption that the protein binding capacity of cefazolin remains constant throughout a surgical procedure where vast pharmacokinetic and physiological changes are expected to occur is a substantial limitation. Further analysis of the samples for the free plasma level would shed some light as to the exact free level concentration of the prophylactic antibiotics.

5.4 Conclusion

SSIs have become the most common cause of nosocomial infections, not only significantly affecting morbidity and mortality rates but also placing further strain on the country's economy. In cardiac surgery specifically, the risks associated with developing a SSI can quickly lead to the direst outcomes. This study has revealed that potentially 31.25% of the patients undergoing cardiac surgery may be at a higher risk of acquiring SSIs due to sub-therapeutic dosing of prophylactic cefazolin being administered during surgery. In addition, the findings point towards no sequestration of cefazolin in the CPB circuits.

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Appendix A:

R14/49 Dr Daren Calleemalay and Prof Fathima Paruk et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140662

NAME: (Principal Investigator)	Dr Daren Calleemalay and Prof Fathima Paruk et al
DEPARTMENT:	Anaesthesiology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Cefazolin Kinetics during Cardiopulmonary Bypass Surgery
DATE CONSIDERED:	27/06/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor A Woodiwiss, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/09/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date 25/09/2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B:

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

17 May 2016
Person No: 0200954N
PAG

Dr D Calleemalay
Unit 12
Glen Devon II
284 Wilson Street
Fairlands
2170
South Africa

Dear Dr Calleemalay

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Pharmacokinetics of cefazolin during elective valve replacement surgery on cardiopulmonary bypass* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix C:

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. L. Mngomezulu
Office of the Director: Clinical Services
Tell: (011): 488-3365
Fax: (011): 488-3753
02nd September 2014

Dr. D. Calleemalay
Registrar
Anaesthesia - CMJAH

Dear Dr. Calleemaly

RE: "Cefazolin pharmacokinetic during cardiopulmonary bypass surgery at CMJAH"

Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic hospital will not in anyway incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the Head of Department and Unit Manager or Sister in Charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Director: Clinical Services
DATE:

Approved / not approved

Ms. G. Bogoshi
Chief Executive Officer
DATE: 4/9/2014

Appendix D:

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

29 March 2017
Person No: 0200954N
TAA

Dr D Calleemalay
Unit 12
Glen Devon II
284 Wilson Street
Fairlands
2170
South Africa

Dear Dr Calleemalay

Master of Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Medicine** has been approved:

From:	**Pharmacokinetics of cefazolin during elective valve replacement surgery on cardiopulmonary bypass**
To:	**Total cefazolin serum levels during elective valve replacement surgery on cardiopulmonary bypass**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix E:

Information sheet and consent form for Study on cefazolin pharmacokinetics during CPB surgery.

Hello, my name is Dr Daren Calleemalay. I am a medical doctor and I work in this hospital as an anaesthesiologist in training. I understand that you are going to have an operation soon to replace a valve. I would like to chat with you and invite you to take part in a study that is looking at what happens to the antibiotic that is given to you for the operation.

For major operations like the one you are having, it is our practice to administer an antibiotic just before the surgeon starts to operate and then to continue for 24 hours thereafter. This is to prevent the occurrence of wound infections. The risk for a wound infection is 1-10%. In our hospital the risk is just under 5%. We would like to avoid such infections as they are associated with longer stays in hospital and a higher chance of patients not doing well.

As your surgeon may have explained to you, during the procedure the blood that is returning to your heart and lungs will be diverted to a machine called the cardiopulmonary bypass (CPB) machine. This diversion will permit the surgeon to work on your heart. The machine also enriches your blood with oxygen as it is sent back to your body.

What we do not know at the moment is, whether the CPB collects and retains some of the antibiotics, when your blood passes through the machine. If this is the case then it may mean that in future we may need to increase the amount of antibiotics we give when patients are attached to a CPB machine.

We would thus like to ask you if you would be happy to participate in our study, which looks at the level of the antibiotic (that was given to you) that is retained in the CPB machine, your blood and your urine. This will help us determine if we need to change how we administer antibiotics in the future. Whilst the study may assist future patients it will not influence your management. I will also be using the results of this study to obtain an additional degree from the University of Witwatersrand (it is called a master in Medicine degree).

If you participate we will take blood samples from you at different times whilst you are in theatre. The blood will be taken once when your drip is inserted for the operation and then when you are asleep. The total amount will not exceed 50 ml (5 tablespoons). Two samples of urine (2 tablespoons and part of the amount that is normally collected during the operation) will also be taken. The other tests are routine tests that you have before surgery. Your charts and theatre records will also be used. However no one will be able to identify you as your name will be removed. The samples will be stored in a fridge in this hospital (in a secure site with other samples for research) and then sent to Australia where it will be stored until it is analysed (the researchers from Australia are known to us). Once the testing is done the samples will be destroyed.

It is your choice entirely-whether you wish to take part in this study. If you decide not to take part, that is perfectly fine and you will still get all the necessary treatment that you require.

If you have any queries or concerns about this study you are most welcome to contact Professor Cleaton-Jones at 011-7171234. He is the Head of a committee called the Human

Research Ethics Committee that approved this study to happen. You may also contact Professor Fathima Paruk (one of the investigators) if you have queries about the study. Her number is 011-4884344.

If you do choose to participate, I will ask you to indicate below that you have understood what I have told you and that you are willing to take part in this study. I will give you a copy of the information provided to you. Thank-you for listening to me.

Consent form for study on cefazolin pharmacokinetics during cardiopulmonary bypass

The doctor has explained the contents of the information leaflet (pertaining to the above study) and I have understood what he has discussed with me.

I …… give consent (provide permission) for myself to participate in the above study.

	Name	Signature	Date
Patient			
Researcher			

Appendix F:

8 December 2014

The Chair
HREC
University of Witwatersrand

Protocol no: M140662
Project Title: Cefazolin Kinetics during Cardiopulmonary Bypass Surgery

RE: Request to increase participant number from 12 to 16 (4 additional patients)

Dear Professor Cleaton-Jones

The above study explores what happens to antibiotic concentrations when a patient is subjected to cardiopulmonary bypass. We are taking blood samples to ascertain these levels pre and post bypass. The study is being conducted in patients undergoing valve replacement.

During our recruitment we have come to realise that the perfusionist is dipping the new valves in either Cefazolin or an aminoglycoside. We were unaware of this practice. Our concern is that these antibiotics may influence the results (although it a small dose and a momentary dip).

Following expert consultation we have been advised as a precautionary measure to increase our study population by 4 more participants (such that we will have 50% each with Cefazolin and 50% with aminoglycoside exposure). So far we have recruited 8/12 participants. With your permission we would like to include 4 additional participants (Total of 16 as opposed to original plan of 12 participants)

Apologies for this inconvenience.

Yours sincerely

Fathima Paruk

approved
13/3/15

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
10 MAR 2015
RESEARCH OFFICE
JOHANNESBURG

Appendix G:

Data sheet.

Patient Initials	
Research Number	
Gender	
Age	
Date of Surgery	
Height	
Weight	
APACHE II	
Renal Function	**Urea** **Creat**
Albumin	

PRE- OPERATIVE ANTIBIOTICS

Antibiotic Name	Dose Given	Time Started	Route

ANAESTHETIC AGENTS USED

Drug Name	Total amount given

ALL FLUIDS GIVEN IN THEATRE

Fluid Name	Volume Given	Time Given

Total Volume of Fluids given Intra-operatively: _____

CEPHAZOLIN: 2 g diluted to 20ml of 0.9% saline and give over 5 min.

	Blood (CVP)	
Enter OT		
IV access	Tpreop (±30min before incision)	Blood sample no. 1 Time taken____
Induction	Insert IDC and volume	Urine Sample no. 1 Time taken____
Start of Surgery	Antibiotic given 30	

Venous (CVP) SPECIFIC TIMING TO CPB	
Venous sample 5minutes before going ON CPB	Venous sample no.1 Time taken____
Venous sample 5 minutes after going ON CPB	Venous sample no.2 Time taken____
Venous sample 5 **minutes** 5minutes after coming OFF CPB	Venous sample no.3

	minutes pre-incision	
	T=2min	Blood sample no. 2 Time taken____
	T=5min	Blood sample no.3 Time taken____
	T=10min	Blood sample no. 4 Time taken____
	T=30 min	Blood sample no: 5 Time taken____
	T=60min	Blood sample no.6 Time taken____
	T= 90min	Blood sample no. 7 Time taken____
	T= 120 min	Blood sample no. 8 Time taken____
	T= 150 min	Blood sample no. 9 Time taken____

	Time taken____

Arterial sample (from Circuit): WHEN ON CPB	
sample 5 minutes after going ON CPB	Arterial sample no. 1 Time taken____
When on CPB, take a specimen when blood is being sampled	Arterial sample no. 2 Time taken____
When on CPB, take a specimen when blood is being sampled	Arterial sample no. 3 Time taken____
When on CPB, take a specimen when blood is being sampled	Arterial sample no. 4 Time taken____
When on CPB, take a specimen when blood is being sampled	Arterial sample no. 5 Time taken____
When on CPB, take a specimen when blood is being sampled	Arterial sample no.6 Time taken____

	T= 180min	Blood sample no. 10 Time taken____
	T= 210min	Blood sample no. 11 Time taken____
REDOSE ANTIBIOTIC	T= 240min	Blood sample no. 12 Time taken____
	T= 270min	Blood sample no. 13 Time taken____
	T= 300min	Blood sample no. 14 Time taken____
End of Surgery	Empty IDC and volume	Urine Sample no. 2 Time taken____

ON CPB TIME _____

OFF CPP TIME _____

CROSS CLAMP ON TIME: _____

CROSS CLAMP OFF TIME: _____

Surgical time interval

	Details	Time
Induction		
Post Intubation		
CVL Insertion		
Incision		
X Clamp ON		
X Clamp OFF		
CPB ON		
CPB OFF		
Vaso-active		
Vaso-active		
Vaso-active		
Vaso-active		
ICU		

URINE COLLECTION

Empty bag on arrival to ICU or when going to ward: Leave bottle at bedside in ICU and place pink sticker on urine bag.

Start: _____ Stop: _____ U&E time (end of 4 hour collection): _____

Total Length of Time in OT: Start _____ Finish _____

Appendix H:

health

Department:
Health
REPUBLIC OF SOUTH AFRICA

Private Bag X828, PRETORIA, 0001. 27th Floor, Room 2710, Civitas, Cnr Thabo Sehume & Struben Street, PRETORIA, 0001
Tel: +27 (0) 12 395 8000, Fax: +27 (0) 12 395 8422

Reference : J1/2/4/2 No 1/15
Enquiry : Ms L Motopi
Tel : (012) 395 8366/8965
email : importexportpermit@health.gov.za

EXPORT PERMIT

In terms of Section 68 of the National Health Act 2003 (Act No. 61 of 2003) –

Professor Fathima Panik
Head,Cardiothoracle ICU,Johannesburg Hospital
University of Witwattersrand & CM Johannesburg Academic Hospital
York Road
Park town
Tel. No.: (011) 4888 4399 E-Mail:Fathima.Paruk@wits.ac.za

is hereby authorised to export from the Republic of South Africa –

384 tubes Plasma

to – Professor Jeffrey Lipman
University of Queensland School pf Med
Royal Brisbane & women's hospital
Herston QLD 4029, Australis
Tel: No: +617 36468897 Fax: +61736463542

For – Clinical study.

This export permit is subject to the following conditions:

1. The substance shall be imported into the country specified above, within the legal requirements of that country.
2. The substance shall be exported from South Africa and handled in accordance with the provisions of the National Health Act 2003 (Act No. 61 of 2003), and the regulations made in terms of the Act.
3. The export permit shall not be used for any trade or advertising purposes.
4. **This export permit shall expire on 30 April 2016.**

DIRECTOR-GENERAL: HEALTH
Date: 14/4/2015
Ms P Netshidzivhani