

A Pharmacokinetic/Pharmacodynamic Evaluation of
Cefazolin Antimicrobial Prophylaxis in Cardiac Surgery with Cardiopulmonary Bypass

by
Divna Calic

A Thesis Submitted to the Faculty of Graduate Studies of
The University of Manitoba
In Partial Fulfilment of the Requirement for the Degree of

Doctor of Philosophy

College of Pharmacy
Rady Faculty of Health Sciences
University of Manitoba
Winnipeg, MB, Canada

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ABSTRACT

Surgical site infections (SSI) following cardiac surgery result in significant patient morbidity and mortality. Antimicrobial prophylaxis (AP) with cefazolin is a core strategy for preventing SSI. This was the first pharmacokinetic-pharmacodynamic study of guideline-recommended cefazolin AP in cardiac surgery with cardiopulmonary bypass (CPB).

In this non-interventional study, subjects ($n = 55$, 65 ± 10 years, 80 ± 19 mL/min/72kg) undergoing cardiac surgery received cefazolin AP and had blood samples collected at relevant times intraoperatively. Total and free cefazolin concentrations were measured by LC-MS/MS. Subjects were monitored for SSI during and after hospitalization. Total cefazolin concentrations at wound closure were compared to target concentrations ≥ 40 mg/L and risk factors for below-target concentrations identified. Free cefazolin concentrations were used to characterize cefazolin protein binding (PB). A population-pharmacokinetic model was constructed to characterize the pharmacokinetics of cefazolin during cardiac surgery. Finally, a pharmacodynamic analysis was conducted to investigate the relationship between cefazolin concentrations and SSI development.

Almost 10% of cefazolin concentrations at wound closure were < 40 mg/L. Shorter surgery duration, lower body weight and lower total cefazolin dose per hour of surgery were independently associated with below-target concentrations. A significant dose threshold of 7.6 mg/kg_{DW}/h was identified and used to optimize the AP regimen, which demonstrated the inadequacy of 1-gram doses. The PB of cefazolin during surgery was $72 \pm 8\%$ compared to the usual 80%. PB saturation

was observed during/post-CPB. The elimination rate constant was similar ($0.35 \pm 0.07 \text{ h}^{-1}$), while the volume of distribution was higher ($0.14 \pm 0.05 \text{ L/kg}$) during cardiac surgery with CPB versus healthy volunteers. In the PD analysis, longer duration of surgery and lower total cefazolin closure concentrations were independently associated with SSI. Significant increases in risk of SSI were identified at thresholds of surgery duration > 5.8 hours and total closure concentrations $< 104 \text{ mg/L}$.

Most importantly, the findings of this study were translated to clinical practice. An AP regimen designed to achieve target closure concentrations during cardiac surgery with CPB has been adopted where cefazolin AP consists of 2 grams (3 grams if $\geq 120 \text{ kg}$) preoperatively and re-dosed every 3 hours intraoperatively, with adjustments for renal function.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Sheryl Zelenitsky, for her guidance, encouragement and understanding during my research and Ph.D. studies. Thank you for believing in me and providing me with both the assistance and the independence I needed to develop as a researcher and academic. It has been a pleasure to work with and learn from you over these years. I would also like to thank the members of my Advisory Committee, Dr. Robert Ariano, Dr. Ted Lakowski and Dr. Hilary Grocott for your invaluable support throughout my studies.

To my loving husband, Geoff Namaka, thank you for always believing in me and helping me to pursue my dreams. I thank my parents, Dr. Neda Bankovic-Calic and Mile Calic for their endless love and encouragement. The three of you share in this achievement with me as I couldn't have done it without you.

I would also like to acknowledge the generous financial support of my studies by the Leslie F. Bugey Graduate Scholarship in Pharmacy and the University of Manitoba Graduate Fellowship.

DEDICATION

I dedicate this work to my little sunshine, Luka. I hope that one day when you're grown up, this will encourage you to work hard and pursue your dreams, whatever they may be. I love you to the planets and back.

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LIST OF ABBREVIATIONS

ACCF	American College of Cardiology Foundation
AHA	American Heart Association
AIC	Akaike Information Criterion
AP	Antimicrobial prophylaxis
ASA	American Society of Anesthesiologists
ASHP	American Society of Health-System Pharmacists
ASP	Antimicrobial stewardship program
AUC	Area under the concentration-time curve
AUROC	Area under receiver operating curves
BIC	Bayesian Information Criterion
BMI	Body mass index
CABG	Coronary artery bypass grafting
CANWARD	Canadian Ward Surveillance Study
CARA	Canadian Antimicrobial Resistance Alliance
CART	Classification and regression tree analysis
CDC	Centers for Disease Control and Prevention
CDI	<i>Clostridioides difficile</i> or <i>Clostridium difficile</i> infection
CI	Confidence interval
CL	Clearance
Clcr	Creatinine clearance
CLSI	Clinical and Laboratory Standards Institute
COPD	Chronic obstructive pulmonary disease
CPB	Cardiopulmonary bypass
CSHP	Canadian Society of Hospital Pharmacists
CTSN	Cardiothoracic Surgical Trials Network
CV	Coefficient of variation
DPIN	Drug Program Information Network
DW	Dosing weight

EACTS	European Association for Cardio-Thoracic Surgery
ESBL	Extended-spectrum β -lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
GAIS	Global Alliance for Infections in Surgery
HPLC	High-performance liquid chromatography
HREB	Health Research Ethics Board
IBW	Ideal body weight
ICU	Intensive care unit
ID	Infectious disease
IDSA	Infectious Diseases Society of America
IMA	Internal mammary artery
IQR	Interquartile range
IS	Internal standard
IV	Intravenous
JAC	Journal of Antimicrobial Chemotherapy
Ke	Elimination rate constant
kg _{DW}	Kilograms dosing-weight
LAA	Left atrial appendage
LC-MS/MS	Liquid chromatography and tandem mass spectrometry
LLOQ	Lower limit of quantification
LVAD	Left ventricular assist device
m/z	Mass-to-charge ratio
MDR	Multi-drug resistant
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MRM	Multiple reactions monitoring
MS	Mass spectrometer
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NHSN	National Healthcare Safety Network
NICE	National Institute for Health and Care Excellence
NIH	National Institutes of Health

NPAG	Nonparametric adaptive grid
OR	Operating ratio; operating room
ORL	Otorhinolaryngology
PAR	Peak area ratio
PB	Protein binding
PD	Pharmacodynamic, pharmacodynamics
PFO	Patent foramen ovale
PICS	Prevention of Infections in Cardiac Surgery Study
PIDS	Pediatric Infectious Diseases Society
PK	Pharmacokinetic, pharmacokinetics
PO	Oral
Pop-PK	Population-pharmacokinetic
r	Pearson correlation coefficient
R ²	Coefficient of determination
RCPI	Royal College of Physicians of Ireland
RCSI	Royal College of Surgeons in Ireland
RCT	Randomized controlled trial
RED	Rapid Equilibrium Dialysis
RR	Relative risk
RRC	Research Review Committee
SD	Standard deviation
SHEA	Society for Healthcare Epidemiology of America
SIGN	Scottish Intercollegiate Guidelines Network
SIRS	Steroids in Cardiac Surgery trial
SIS	Surgical Infection Society
SSI	Surgical site infection
STS	Society of Thoracic Surgeons
SVG	Saphenous vein graft
t _{1/2}	Half-life
TBW	Total body weight

TCPS 2: CORE	Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics
Vd	Volume of distribution
VRE	Vancomycin-resistant <i>Enterococci</i>
WHO	World Health Organization
WSES	World Society of Emergency Surgery

1. INTRODUCTION

1.1. Antimicrobial Stewardship

Antimicrobial resistance is a global challenge resulting in increased patient morbidity and mortality, as well as increased healthcare expenditures.¹ If left unaddressed, antimicrobial resistance may threaten some of the major medical achievements of the past century, including surgery, anesthesia and chemotherapy. The Canadian Antimicrobial Resistance Surveillance System update for 2018 reported that community rates of methicillin-resistant *Staphylococcus aureus* (MRSA) have nearly doubled from 2012 to 2017, colonization with carbapenemase-producing organisms has increased in both hospitals and in the community and that infections caused by vancomycin-resistant *Enterococci* (VRE) are increasingly reported in hospitalized patients.² The rising resistance of microorganisms coupled with the scarcity of new antimicrobials emphasizes the need for prudent use of available agents through antimicrobial stewardship.

The Infectious Diseases Society of America (IDSA), the Pediatric Infectious Diseases Society (PIDS) and the Society for Healthcare Epidemiology of America (SHEA) define antimicrobial stewardship as “coordinated interventions designed to improve and measure the appropriate use of [antimicrobial] agents by promoting the selection of the optimal drug regimen including dosing, duration of therapy, and route of administration.”³ Antimicrobial stewardship has also been described as “the optimal selection, dosage, and duration of antimicrobial treatment that results in the best clinical outcome for the treatment or prevention of infection, with minimal toxicity to the patient and minimal impact on subsequent resistance.”⁴ The three main goals of antimicrobial

stewardship include the selection and use of the most appropriate antimicrobial for the patient, the prevention of overuse of antimicrobials and minimizing antimicrobial resistance.⁵ Optimal antimicrobial use is often defined by the “4 D’s”; the right drug administered at the correct dose, de-escalation of therapy and optimal duration.⁶ The right antimicrobial is the one which is effective for the particular pathogen and infection and is not contraindicated in the affected patient due to allergy or other comorbidities. The right dose is the one which will most effectively cure the infection, while accounting for patient variables such as renal function as well as the antimicrobial’s pharmacokinetics (PK) and pharmacodynamics (PD). De-escalation of therapy refers to both de-escalation from broad-spectrum to pathogen-specific narrow-spectrum antimicrobials and the de-escalation from intravenous (IV) to oral (PO) antimicrobial use as soon as it is appropriate.^{3,5} The right duration of antimicrobial is the shortest duration which is effective for the treatment of infection.³ The second aim of stewardship is to preclude overuse of antimicrobials (e.g., in viral infections), as well as their misuse (e.g., not adjusting therapy based on culture results) and abuse (e.g., preferential use of one antimicrobial over another in absence of objective reasoning).⁵ The third goal of minimizing resistance to antimicrobials is applicable at both the individual and the institutional level as susceptibility patterns are affected by antimicrobial use. It has been estimated that 30 – 50% of antimicrobial use in hospitals is for surgical antimicrobial prophylaxis (AP).⁷ As such, employing antimicrobials rationally in AP has far-reaching consequences.

Successful antimicrobial stewardship requires a multifaceted approach, commonly employing a combination of front-end and back-end techniques.⁵ Front-end, or pre-prescription approaches are aimed at preventing inappropriate antimicrobial use from occurring, through formulary and

prescribing authority restrictions. Selective formularies promote the use of certain antimicrobials, while limiting that of others through requirements for prior approval from antimicrobial stewards or by setting criteria that must be met prior to use.⁵ Utilizing pre-made order sets for specific indications is another front-end approach that can aid in ensuring appropriate and evidence-based empiric therapy. Computer-based decision support systems can also aid in antimicrobial stewardship as “these programs can identify allergies, inappropriate dosages, and, with the appropriate software and information technology systems, mismatches between drug and susceptibility.”⁵ Back-end, or post-prescription approaches to antimicrobial stewardship are interventions that occur after therapy has started. These include prescription review and feedback such that the stewardship team reviews antimicrobial orders and provides advice on (dis)continuation or adjustments to therapy based on clinical features and microbiology culture results.⁵ Pharmacy plays a key part in back-end stewardship interventions, particularly in IV to PO conversion programs and in pharmacy-led dosing programs in which pharmacists are tasked with the dosing and appropriate monitoring of antimicrobials such as aminoglycosides and vancomycin. Regardless of the type of approach chosen, education is paramount to any successful antimicrobial stewardship program. Education can take the form of formal lectures or occur more informally, for example through telephone conversations.

Implementing antimicrobial stewardship programs (ASP) can result in several important benefits, including “improved patient outcomes, reduced adverse events including *Clostridium difficile* infection (CDI), improvement in rates of antibiotic susceptibilities to targeted antibiotics, and optimization of resource utilization across the continuum of care.”³ In a single-center study by Malani *et al.*, the prospective auditing of new orders for and weekly use of target antimicrobials

by the ASP team resulted in an almost 50% reduction in a patient's odds of developing CDI (OR = 0.46; 95% confidence interval (CI) 0.25 – 0.82).⁸ This intervention also reduced the use of the targeted antimicrobials, with a 25.4% reduction in defined daily doses, along with a 15.2% reduction in the antimicrobial budget.⁸ Yong *et al.* examined the effects of a computerized antimicrobial decision support system on the susceptibility of bacteria.⁹ The computer system studied provided recommendations on antimicrobial use based on patient information, such as site of infection, allergy and antimicrobial susceptibility. This ASP approach resulted in reduction in broad-spectrum antimicrobial use as well as significant improvement in *Pseudomonas aeruginosa* susceptibility to imipenem and gentamicin compared to the pre-intervention time period.⁹ In the study by Dortch *et al.*, the effects of implementation of combined infection control and antimicrobial stewardship protocols in the intensive care units (ICU) of Vanderbilt University Hospital were studied.¹⁰ The program included protocols for the treatment of healthcare-associated infections and surgical AP, as well as rotation or restriction of antimicrobial classes. These interventions resulted in a decrease in healthcare-associated infections caused by multi-drug resistant (MDR) Gram-negative organisms from 37.4% to 8.5%, along with an associated increase in pan-sensitive organisms from 34.1% to 53.2%.¹⁰ As previously mentioned, education is the cornerstone of ASPs and the effectiveness of education in ASP outcomes was assessed by Chang *et al.*¹¹ This study demonstrated that monthly targeted educational detailing of antimicrobial prescribers by infectious disease (ID) physicians resulted in a significant reduction in overall inpatient antimicrobial consumption, as well as significant reduction in targeted antimicrobial use, including carbapenems and glycopeptides.¹¹

Often, more than one benefit is realized by the implementation of an ASP. Wu *et al.* reported that an ASP which focused on culture-guided antimicrobial therapy resulted in a significant reduction in length of hospital stay, reduction in antimicrobial costs and doses administered, as well as a decline in antimicrobial resistance.¹² Pasquale *et al.* specifically examined the impact of ASP on patients with bacterial skin infections.¹³ ASP interventions, including changes in antimicrobial dosage or regimen, antimicrobial de-escalation and consultation with an ID physician resulted in a significant reduction in mean length of hospital stay (4.4 days versus 6.2 days, $P < 0.001$) as well as reduced 30-day readmission due to any cause (6.5% versus 16.7%, $P = 0.05$) compared with historic data.¹³ Although the primary goal of ASP programs is not economy, the implementation of ASP programs does often result in cost savings. It has been demonstrated that even relatively simple interventions, including IV to PO antimicrobial conversions, batch production of IV maintenance doses to reduce waste, formulary restrictions and therapeutic substitutions result in considerable cost reductions.¹⁴

ASP has been successfully implemented in surgical programs as well. A recent study by Donà *et al.* examine the effects of ASP on AP in pediatric surgery.¹⁵ The ASP intervention included the implementation of a clinical pathway for improving adherence to AP guidelines. The clinical pathway outlined the selection, preoperative dosage, intraoperative re-dosing and duration of appropriate AP for different types of surgeries.¹⁵ It was applied to all pediatric patients undergoing surgical procedures, with the exception of those with “concomitant infections, ongoing antibiotic therapy, complicated abdominal infection, immunodeficiency, immunosuppressive therapy, patients who underwent neurosurgical, vascular, ORL [otorhinolaryngology], and ocular procedures”.¹⁵ The correct administration of AP, defined as both correct type and correct duration

of antimicrobial therapy increased from 48.9% in the 6 months before intervention to 60.1% in the 6 months after the intervention ($P = 0.03$).¹⁵ In the same time period, the choice of appropriate antimicrobial improved from 81.0% to 91.9% ($P = 0.02$) for monotherapy and from 65.9% to 100% ($P = 0.004$) for combination AP. The duration of AP was also reduced in the post-intervention group, such that 66.7% of AP was discontinued within 24 hours, compared to 45.1% prior to the intervention ($P < 0.001$).¹⁵ Moreover, no significant difference in SSI rate were reported between the pre- and post-intervention periods at 2.5% and 1.9% ($P = 0.54$).

1.2. Surgical Site Infections in Cardiac Surgery

Surgical site infection (SSI) is a potential complication after cardiac surgery. The Centers for Disease Control and Prevention (CDC) and the National Healthcare Safety Network (NHSN) have developed the most commonly applied SSI definitions. SSIs are classified as superficial when involving the skin or subcutaneous tissues; deep when affecting soft tissue such as fascia or muscle; or organ/space when other manipulated areas such as bone are involved.¹⁶ Superficial infections of the incision site are attributable to the operative procedure if infection occurs within 30 days of surgery, whereas surveillance for deep or organ/space SSIs extends to 90 days for certain procedures, including cardiac surgery.

1.2.1. Incidence

The incidence of SSI following cardiac surgery was determined retrospectively until a landmark study in 2014 by Gelijns *et al.* at the Cardiothoracic Surgical Trials Network (CTSN) prospectively examined the occurrence of SSIs following a variety of cardiac surgeries at all 10 CTSN sites in

the United States.¹⁷ A total of 5,158 patients were followed for 65 days post-surgery for the development of major infections, including “deep incisional surgical site infection occurring at the primary chest incision, deep incisional surgical site infection (SSI) occurring at a secondary incision site (e.g., saphenous harvest and groin cannulation sites), mediastinitis, infectious myocarditis or pericarditis, endocarditis, cardiac device infection, pneumonia, empyema, *Clostridium difficile* colitis, and bloodstream infection.”¹⁷ Minor infections, including superficial SSIs, urinary tract infections and bacteriuria were secondary endpoints. Major infections occurred in 237 patients (4.6%) with the most common infection being pneumonia (2.4%), followed by bloodstream infection (1.1%) and *C. difficile* colitis (1.0%). The most common major SSI was deep incisional chest infection (0.6%), mediastinitis (0.2%), and groin deep incisional infection (0.2%). Minor SSIs were much more common with 2.9% of patients experiencing a superficial incisional SSI of the chest and 1.6% of the groin.

The 2015 study by Lemaigen *et al.* focused on the incidence of sternal wound infections in patients undergoing open heart surgery in a large hospital in Paris, France.¹⁸ A total of 7170 adult patients were studied and 4.1% developed sternal SSI, defined in the study as reoperation due to local or systemic infection of the sternotomy scar. In this study, SSIs were further classified as CDC-positive if the patient had a minimum of one positive blood culture which grew the same microorganism as the wound sample; otherwise the SSI was classified as CDC-negative. Overall, 1.4% of patients had deep, CDC-positive SSIs, while 0.7% had superficial CDC-positive SSIs. Another novel finding of this study was that once the study data was stratified by type of surgery, the risk of sternal wound infection were highest with coronary artery bypass grafting (CABG) procedures (7.3%), followed by combined procedures (5.8%) and lowest for valve surgery (1.4%).

This finding may be due to CABG surgery potentially having two surgical sites that could become infected as in the case of saphenous vein grafting (SVG).

In 2017, Gulack *et al.* analyzed data previously collected in the CTSN study to characterize the occurrence of secondary SSIs in following CABG with SVG. Of the 2174 patients from the CTSN study who met this criteria, 3.0% had a secondary leg or groin SSI within 65 days of operation, with the majority of these (88%) defined as superficial infections.¹⁹

1.2.2. Microbiology

Gram-positive organisms, including *Staphylococcus aureus* and coagulase-negative *Staphylococcus epidermidis* are the most common pathogens associated with SSI in cardiac surgery.²⁰ The CDC/NHSN regularly perform surveillance on a number of healthcare associated infections, including SSIs. In 2016, the report covering NHSN's surveillance efforts from 2011 to 2014 was released, representing data from 4515 hospitals in the United States.²⁰ Of the 11,381 cardiac SSIs reported during the study period, 30.4% were caused by *S. aureus* and 14.5% by coagulase-negative staphylococci.²⁰ The most common Gram-negative pathogens reported were *P. aeruginosa* (5.8%), *Klebsiella pneumoniae/oxytoca* (5.8%) and *Enterobacter* spp. (5.8%). Other notable pathogens reported included *Escherichia coli*, *Proteus* spp. and *Enterococcus faecalis* at 5.7%, 4.6% and 2.9%, respectively.²⁰ The aforementioned proportion of pathogens appear to be holding steady from the previous iteration of this surveillance report, covering the period from 2009 to 2010, where *S. aureus* accounted for 30.7% and coagulase-negative staphylococci for 16.7% of the cardiac SSI isolates.²¹ The incidence of *P. aeruginosa* and *Klebsiella* spp. were also

similar at 7.9%, 5.9%, respectively. In the 2013 report, *E. coli* made up a larger percentage of the isolates than in the report from 2016 at 6.4%, while *Enterobacter* spp. accounted for a smaller percentage at 5.1%.

The distribution of cardiac SSI pathogens at the St. Boniface Hospital (Winnipeg, Manitoba, Canada) where our study took place is somewhat different than that reported by the CDC/NHSN. Between January 2014 and December 2016, the majority (52.0%) of cardiac surgery SSIs at the St. Boniface Hospital were caused by coagulase-negative staphylococci, which is a greater percentage than in CDC/NHSN report. (Unpublished data, S. Zelenitsky, University of Manitoba). Similar to the CDC/NHSN report, other common pathogens included *S. aureus* (15.4%), *Enterobacteriaceae* spp. (8.9%), *Enterococcus* spp. (7.3%) and *P. aeruginosa* (4.9%).

The CDC/NHSN 2016 surveillance report included aggregate data on trends of antimicrobial resistance in select pathogens in SSIs.²⁰ The rates of methicillin-resistance in *S. aureus* were stable between 43% and 45% during the 2011 to 2014 study period, while vancomycin resistance in *E. faecalis* was reported in 4 – 5% of tested pathogens during the same period. Seven to eight percent (7 – 8%) of *P. aeruginosa* isolates were resistant to aminoglycosides, 10 – 12% to cefepime or ceftazidime, 12 – 14% to fluoroquinolones, 8 – 10% to carbapenems, and 7 – 8% to piperacillin/tazobactam.²⁰ The number of isolates of *K. pneumoniae/oxytoca* resistant to extended-spectrum cephalosporins (i.e., cefepime, ceftriaxone, ceftazidime or cefotaxime) and carbapenem antimicrobials was 10 – 11% and 3 – 5%. In isolates of *Enterobacter* spp., 26 – 28% were resistant to extended-spectrum cephalosporins and 3 – 4% to carbapenems.²⁰

For comparison, the Canadian Antimicrobial Resistance Alliance (CARA) conducts surveillance of pathogens, infections and antimicrobial resistance through the Canada-wide Canadian Ward Surveillance Study (CANWARD). The results of the 2017 national susceptibility report reveal that 100% of methicillin-sensitive *S. aureus* (MSSA), 65.8% of *E. coli*, 80.7% of *K. pneumoniae*, 15.1% of *K. oxytoca*, 2.3% of *Proteus mirabilis* and 1.7% of *Enterobacter cloacae* were susceptible to cefazolin.²² Susceptibility data was not reported for *S. epidermidis*, *P. aeruginosa*, or *E. faecalis*. Locally, the St. Boniface Hospital publishes an annual antibiogram outlining the *in vitro* activity of selected antimicrobial agents against common pathogens. The 2019 antibiogram reports that 49% of systemic *E. coli* isolates were susceptible to cefazolin, as were 82% of *K. pneumoniae* and 11% of other *Klebsiella* spp.²³ The susceptibility to cefazolin of common Gram-positive pathogens, as determined by oxacillin testing were 63% for *S. aureus* and 36% for *S. epidermidis*.

1.2.3. Risk Factors

In 2005, Fowler *et al.* utilized the Society of Thoracic Surgeons (STS) National Cardiac Database to analyze 331,429 cases of CABG from January 1, 2002 to December 31, 2003 for clinical predictors of major infection post-surgery.²⁴ A total of 11,636 patients (3.5%) experienced major infection, most commonly septicemia (35.0%), vein harvest site infection (32.6%) and mediastinitis (25.1%). The affected patients were more likely to have hospital stays in excess of 14 days than those unaffected (47.0% versus 5.9%, $P < 0.0001$). In addition to significant resources and treatment costs, the affected patients also had a higher mortality of 17.3% versus 3.0% in those unaffected, $P < 0.0001$. The researchers utilized the database information to build a risk scoring system which estimated probability of major infection following surgery. The results of the

analysis combining both preoperative and intraoperative variables indicated greatest risk in procedures involving morbidly obese patients (body mass index (BMI) > 40 kg/m²), perfusion times exceeding 200 minutes and use of intra-aortic balloon pumps. Other notable variables included renal failure, BMI of 30 to 40 kg/m², diabetes, congestive heart failure, chronic lung disease and perfusion for 100 to 200 minutes.

A similar, more recent multi-center study by Gelijns *et al.* of 5158 patients undergoing cardiac surgery reported patient characteristics that were associated with increased risk of major infection.¹⁷ Increased risk for major infection was seen in patients with “chronic lung disease, heart failure, elevated creatinine, use of corticosteroids, LVAD [left ventricular assist device] and transplant surgery, leaving an open sternum for secondary closure and longer surgery time.”¹⁷ Conversely, higher hemoglobin values were associated with a reduced risk of infection.¹⁷ This study also identified that AP duration exceeding 48 hours, mechanical ventilation lasting longer than 48 hours, hyperglycemia and transfusion of packed red blood cells were also associated with increased risk of major infection. These results are consistent with findings from other studies and summarized in the 2013 clinical practice guidelines, which reported “diabetes, hyperglycemia, peripheral vascular disease, chronic obstructive pulmonary disease [COPD], obesity (BMI > 30 kg/m²), heart failure, advanced age, involvement of internal mammary artery, reoperation, increased number of grafts, long duration of surgery, and *S. aureus* nasal colonization” as risk factors for SSI following cardiac surgery.²⁵

Lemaignen *et al.* examined risk factors associated with sternal wound infections specifically.¹⁸ Similar to the findings by Fowler *et al.*, this study also identified obesity, COPD and diabetes as independent risk factors for sternal wound infections in multivariate analysis. Similar to the results of Gelijns *et al.*, the study by Lemaignen also reported that higher serum creatinine, perioperative blood transfusion and prolonged mechanical ventilation were independent risk factors for sternal wound infections. Additional identified risk factors included older age, critical status preoperatively, CABG using one or more internal thoracic artery and the need for vasopressor support.¹⁸

In 2016, studies by Meszaros *et al.* and Figuerola-Tejerina *et al.* examined whether different types of cardiac surgeries were associated with different risk factors for infection. The surveillance study conducted in Switzerland by Meszaros *et al.* studied 3249 patients undergoing one of: CABG only, valve operation only, or CABG plus valve procedures.²⁶ Patients were followed for the development of sternal wound infections during hospital stay as well as through follow-up phone calls at 30 days and 1 year post-procedure. The overall incidence of SSIs was 3.8% and deep sternal wound infections occurred in 2.3% of patients. For CABG surgery alone ($n = 1857$), independent risk factors for development of sternal infections were the use of bilateral internal thoracic arteries for bypass grafting, surgery length exceeding 300 minutes, COPD, diabetes, female sex and obesity. In single-valve surgery ($n = 799$), wound revision due to bleeding was the only independent risk factor identified. In combination procedures ($n = 593$), operative length in excess of 300 minutes and wound revision were independent risk factors for sternal wound infections.

The prospective cohort study by Figuerola-Tejerina *et al.* assessed risk factors for SSI following cardiac surgery in 1557 patients.²⁷ SSIs occurred in 4% of patients, including 1.3% superficial SSIs, 1.6% deep SSIs and 1.4% mediastinitis. When all surgeries were assessed together, multivariate analysis identified diabetes, obesity, operative time and reoperation due to bleeding as independent risk factors²⁷, similar to the findings of the studies by Fowler, Gelijns and Lemaigen.^{17,18,24} However, when were analyzed separately, diabetes and obesity were independent risk factors in valve surgery ($n = 1119$), whereas diabetes and reoperation were independent risk factors in CABG procedures ($n = 281$).²⁷

In 2018, researchers at McMaster University utilized data from the large international, multi-center Steroids in Cardiac Surgery (SIRS) trial to conduct a cohort study to identify risk factors for SSI 30 days following various types of cardiac surgery with cardiopulmonary bypass (CPB).²⁸ Data from 7406 patients from 18 countries was analyzed and SSIs were reported in 4.8% of cases. Independent risk factors for SSI included diabetes, female sex, renal failure, CPB time greater than 96 minutes, BMI $> 30 \text{ kg/m}^2$ or $< 22 \text{ kg/m}^2$, higher peak 24-hour ICU glucose, CABG surgery and use of inotropes.

1.2.4. Clinical Impact

SSIs following cardiac surgery are a source of significant patient morbidity and mortality and carry a large economic burden as well. A landmark study published in 1999 by Kirkland *et al.* was the first to establish the increased mortality, excess length of hospitalization and the associated costs directly attributable to SSI.²⁹ In the study, patients with SSIs were matched to uninfected controls

by surgical procedure, age, National Nosocomial Infection Surveillance System risk index, surgeon and date of surgery. A total of 255 matched pairs were assessed and it was determined that patients with SSI had a higher mortality compared to their uninfected matches (7.8% versus 3.5%, relative risk (RR) 2.2, 95% CI 1.1 – 4.5).²⁹ Infected patients were also more likely to be admitted to the ICU (29% versus 18%, RR 1.6, 95% CI 1.3 – 2.0) and had longer median length of hospital stays (11 days versus 6 days). As such, the excess hospital stay due to the SSI was 6.5 days (95% CI 5 – 8 days) which resulted in median excess direct medical costs of \$3,080 USD at the time of the study. Two-hundred and twenty-nine (229) patients and matched controls survived the initial hospitalization and were followed for 30 days post-discharge. Patients with infection had higher 30-day readmission rates than those uninfected (41% versus 7%, RR 5.5, 95% CI 4.0 – 7.7).²⁹

One of the most serious complications of cardiac surgery is mediastinitis.³⁰ Mediastinitis affects both short and long-term survival as demonstrated in a prospective cohort study of 36,078 consecutive CABG surgery patients by Braxton *et al.*³⁰ Survival analysis was conducted using propensity scores which accounted for baseline differences in patient and disease characteristics, including age, surgery priority (emergency, urgent, or elective), obesity, diabetes mellitus, heart failure, ejection fraction, peripheral vascular disease, COPD, renal failure and the number of affected coronary arteries. Adjusted hazard ratios comparing patients with mediastinitis and those without mediastinitis demonstrated the both acute (<6 month post-op) and long-term (> 6 month post-op) mortality rates were higher in affected patients at 2.12 and 1.70, respectively.³⁰ The 1-year survival for patients with mediastinitis was 81.3% compared with 94.7% in unaffected

patients. By year 10 this gap had widened to 39.4% survival in affected patients, compared to 70.0% in unaffected patients.

1.3. Antimicrobial Prophylaxis in Cardiac Surgery

AP is the practice of administering antimicrobials preoperatively in order to prevent SSI postoperatively. The goal of AP is not to sterilize the tissue, but to reduce the bacterial burden at the surgical site to an inoculum that can be managed by host defenses.³¹ To achieve this, the agent used for AP is ideally “active against the pathogens most likely to contaminate the surgical site, given in an appropriate dosage and at a time that ensures adequate serum and tissue concentrations during the period of potential contaminations, safe, and administered for the shortest effective period to minimize adverse effects, the development of resistance, and costs.”²⁵ Cefazolin and cefuroxime are the American Society of Health-System Pharmacists (ASHP) guideline-recommended first-line agents for AP in cardiac surgery, with clindamycin and vancomycin listed as options for a β -lactam allergic patients.²⁵

The first prospective study of prophylactic antimicrobials in cardiac surgery was published in 1968, comparing the efficacy of penicillin G with streptomycin, oxacillin and placebo in the prevention of postoperative infections.³² At the time, the authors concluded that the use of antimicrobials did not affect the occurrence of postoperative infections, however this conclusion was based on the composite endpoint of major infections, including endocarditis, bacteremia, wound, urinary tract and pulmonary infections. When the study data was examined separately for wound infections, the frequency of infections was substantially lower in the groups treated with

antimicrobials (6.6% and 3.7%) compared with the placebo group (20%). Although the use of AP in cardiac surgery continued, it was not supported by definitive evidence until almost a decade later. A landmark prospective, double-blind study in 1979 by Fong *et al.* compared methicillin AP to placebo for the prevention of infection after surgery.³³ The total infection rates were significantly lower in the methicillin-treated group compared to the placebo (8.6% versus 51.1%, $P < 0.001$).³³ Moreover, sternal wound infections were also significantly lower in the antimicrobial-treated group (0% versus 21.3%, $P < 0.01$).³³

Cefazolin was patented in 1967 by the Japanese pharmaceutical company Fujisawa (now a part of Astellas Pharma Inc.) and was introduced to the United States in 1974 by SmithKline.^{34–36} It has been utilized for AP in cardiac surgery since 1978, and along with cefuroxime, it is the current ASHP guideline-recommended agent for cardiac surgery AP.^{25,37} Cefazolin is a first-generation cephalosporin with activity against both Gram-positive (MSSA, methicillin-susceptible coagulase-negative *Staphylococci*, penicillin-sensitive *Streptococcus pneumoniae*, β -hemolytic *Streptococci*) and Gram-negative bacteria (*E. coli*, *P. mirabilis*, susceptible *Klebsiella* spp).^{38,39} In terms of cardiac SSI pathogens, notable exceptions to cefazolin's spectrum of activity include MRSA, *E. faecalis*, *P. aeruginosa* and *Enterobacter* spp.

1.3.1. Role of Vancomycin

The gaps in cefazolin's spectrum of activity, particularly against MRSA, have raised concerns at a time of increasing antimicrobial resistance. In the St. Boniface Hospital antibiogram (2019), 37% of *S. aureus* isolates were resistant to oxacillin.²³ Oxacillin, like methicillin, is a penicillinase-

stable penicillin and it is used for detection of resistance in *S. aureus*.⁴⁰ The current clinical practice guidelines for AP in cardiac surgery recommend vancomycin, along with an agent active against Gram-negative pathogens if a patient is colonized with MRSA.²⁵ In patients colonized with MRSA and without a β -lactam allergy, vancomycin plus cefazolin should be used since vancomycin is less active against MSSA and lacks Gram-negative activity.²⁵

In 2002, researchers compared the cefazolin and vancomycin for SSI prevention after cardiac surgery in an Israeli medical center with high MRSA infection or colonization.⁴¹ Patients undergoing cardiac surgery were randomly assigned to receive AP with either vancomycin (1 gram IV during anesthesia induction and 1 gram 12 hours later) or cefazolin (1 gram at anesthesia induction and every 8 hours for two additional doses). A total of 886 patients were followed for a minimum 30 days postoperatively for SSI development. No significant differences were observed between the groups in overall SSI rate or in rates of superficial SSI, deep-incisional SSI or organ/space SSI.⁴¹ Pathogens causing SSIs were similar, with the exception of MSSA which were significantly higher in the vancomycin group. Furthermore, no differences were observed in the length of hospital stay or patient mortality between groups.

A meta-analysis conducted by Bolon *et al.* combined the results of seven randomized trials to compare SSI rates in patients receiving glycopeptide AP to AP with any penicillin or cephalosporin.⁴² No difference was observed between the glycopeptides (vancomycin or teicoplanin) and β -lactams with respect to the primary outcome of SSI at 30 days. Further analysis revealed that β -lactam AP was superior for preventing chest SSIs, while glycopeptide AP was

superior for preventing leg SSIs and MRSA infections. AP with a β -lactam also demonstrated a trend towards superiority with respect to preventing deep-chest SSIs and Gram-positive SSIs, although these findings were not statistically significant.

A pre- and post-intervention analysis by Walsh *et al.* assessed the addition of vancomycin to the cardiac surgery AP in a New York state hospital with a high rate of MRSA (54% of all SSIs caused by MRSA).⁴³ In the pre-intervention period, cefazolin AP was used, whereas during the intervention vancomycin was added to the cefazolin AP in patients with MRSA nasal carriage. During the intervention period, patients and staff were screened for MRSA and decolonized if it was identified. Additionally, mupirocin nasal ointment was used for five days in all patients regardless of MRSA status, chest and mediastinal tube exit sites were covered with a mupirocin-coated gauze and MRSA screening was repeated on hospital discharge.⁴³ The bundled approach described resulted in a significant reduction in SSI rate from 2.1% in the 3-year pre-intervention period to 0.8% in the 3-year post-intervention period ($P < 0.001$). The authors also reported a 93% reduction in MRSA SSI during the post-intervention period. Although the benefits of this intervention are clear, as a bundled approach was utilized it is unclear if the same results would have been achieved with only the addition of vancomycin to the AP regimen.

A recent prospective study utilized data from the Swiss national SSI surveillance system to compare AP regimens.⁴⁴ When compared to cefuroxime AP, cefazolin AP had a significantly lower risk of SSI (adjusted OR 0.64, 95% CI 0.49 – 0.84, $P = 0.001$), while combined AP with vancomycin and cefuroxime had a higher risk of SSI, although this difference did not reach

statistical significance (adjusted OR 1.05, 95% CI 0.82 – 1.34, $P = 0.689$).⁴⁴ The authors suggested that the observed differences may have been due to confounding as complete information on patient, surgery and prophylaxis-related variables was not available. Furthermore, the median administration time of the dual antimicrobial regimen was 50 minutes prior to incision, compared to 45 minutes for the single agent regimens.⁴⁴ To minimize adverse effects, vancomycin is usually administered over a minimum of one hour and ASHP guidelines recommend that the administration of vancomycin is initiated 120 minutes pre-incision.²⁵ As such, it may be postulated that vancomycin AP was not optimized and that the true effects of the combination regimen on SSI have not been elucidated in this study. Furthermore, when deep and organ/space SSIs were examined separately, no difference between was observed between the different AP regimens.⁴⁴

An ongoing multi-national Prevention of Infections in Cardiac Surgery Study (PICS) is attempting to answer some of the questions surrounding optimal AP in cardiac surgery. This factorial cluster randomized crossover trial will compare the efficacy of cefazolin alone to AP consisting of cefazolin plus vancomycin for the prevention of deep and organ/space SSIs.⁴⁵ Additionally it will test the non-inferiority of using AP only during surgery compared to continuing AP postoperatively. The pilot study, which will test the feasibility of the full-scale trial is currently taking place in three hospitals in Canada and has an estimated completion date of October 2020.⁴⁶

1.3.2. *Gram-negative Coverage*

Cefazolin and the second-generation cephalosporin, cefuroxime, are the two first-line agents recommended for AP in cardiac surgery with CPB.²⁵ Compared to cefazolin, cefuroxime has

broader Gram-negative coverage, including enhanced *Enterobacteriaceae* spp. coverage as well as activity against *Haemophilus influenza*, *Neisseria meningitis* and *Neisseria gonorrhea*.⁴⁷ The superiority of one agent over the other in cardiac surgery AP has been a source of debate. A systematic review by Lador *et al.* analyzed 36 randomized controlled trials (RCTs) comparing AP with enhanced Gram-negative activity (second or third generation cephalosporin) to AP with a penicillinase-stable penicillin or a first generation cephalosporin.⁴⁸ No difference in SSI was observed, although a reduced risk of pneumonia (RR 0.66, 95% CI 0.51 – 0.86) and death due to any cause (RR 0.69, 95% CI 0.50 – 0.93) was associated with the use of second or third generation cephalosporins.⁴⁸ It should be noted however, that the results of this meta-analysis spanned a long period and that during this time “surgical techniques and postoperative management have changed, as have the antibiotics used and bacterial resistance patterns.”⁴⁸ Moreover, it is not the goal of AP to prevent infections beyond the surgical site.

Recently, the results of a multi-center cohort study by Sommerstein *et al.* of over 21,000 Swiss patients undergoing cardiac surgery and participating in the Swiss national SSI surveillance program were published.⁴⁴ The overall SSI rate was lower for cefazolin AP compared to cefuroxime AP (4.5% versus 5.6%, $P < 0.001$) or the combination of cefuroxime and vancomycin (4.5% versus 6.1%, $P < 0.001$).⁴¹ For deep or organ/space SSI, cefazolin AP also had lower SSI rates at 1.8% compared to 2.9% with cefuroxime and 1.9% with cefuroxime/vancomycin combination ($P < 0.001$).⁴⁴ However, due to the observational nature of the study, confounding cannot be excluded as the reason for the observed differences in efficacy and a large RCT is needed to confirm the conclusions.

Both cefazolin and cefuroxime lack activity against a few clinically important pathogens, including *P. aeruginosa* and *Enterobacter* spp.^{38,47} In the latest NHSN report, the aforementioned pathogens were responsible for 8.1% and 5.8%, respectively of the 11,281 cardiac SSI pathogens reported.²⁰ At the St. Boniface Hospital, *P. aeruginosa* caused 4.9% of cardiac SSIs between 2014 and 2016. (Unpublished data, S. Zelenitsky, University of Manitoba) Of the alternative agents recommended for AP in cardiac surgery, aztreonam, gentamicin and fluoroquinolones have activity against *P. aeruginosa*. In 1987, Kaiser *et al.* compared the efficacy of cefazolin with or without gentamicin to cefamandole with or without gentamicin in preventing SSIs in cardiac surgery.⁴⁹ In this study, an analysis comparing AP regimens with gentamicin to those without gentamicin demonstrated no difference in infections between the two, suggesting that regular addition of gentamicin to a cephalosporin-based regimen was unnecessary. However, shifts in pathogen type and sensitivity have occurred in the 32 years since this study was published and if repeated today, the study may have different results.

Treatment of infections caused by *Enterobacter* spp. is complicated by the presence of multiple resistance mechanisms and as such treatment is guided by susceptibility.⁵⁰ Although aminoglycosides and fluoroquinolones can sometimes be utilized to treat *Enterobacter* infections, susceptibility to these agents is not uniform and as such using them in AP would not provide reliable coverage against this pathogen.

1.4. Clinical Practice Guidelines

The aim of clinical practice guidelines is to aid practitioners in applying a standardized, evidence-based approach to clinical situations. Guidelines for AP in surgery are published by various national and international stakeholders. This section will review the contents of the current North American, European and global guidelines regarding AP in cardiac surgery. A summary of the recommendations is provided in Table 1.

In North America, groups including the Society of Thoracic Surgeons (STS), the American College of Cardiology Foundation (ACCF), the American Heart Association (AHA), ASHP, IDSA, the Surgical Infection Society (SIS), SHEA, the National Institutes of Health (NIH) and the CDC publish guidelines for prevention of SSI and AP in surgery. Although there is much overlap in the recommendations, there are differences in the level of details provided to the recommendations.

The most current version of the STS guidelines for AP in surgery, published in 2006 and 2007 recommends the use of β -lactams, specifically cefazolin, for AP in cardiac surgery in the absence of high MRSA incidence.^{51,52} The administration of 2 grams of cefazolin within 60 minutes prior to surgical incision and 1-gram doses administered every 3 to 4 hours during surgery is recommended.^{51,52} The addition of vancomycin to cefazolin AP is advised if the patient is colonized with *S. aureus* or is at risk for MRSA colonization, if the hospital has high incidence of MRSA, or if a prosthetic valve or graft are inserted. Risk factors for MRSA colonization include hospitalization for more than 3 days, transfer from another healthcare facility or current

antimicrobial treatment. The recommended dose of vancomycin is either 1 to 1.5 grams or 15 mg/kg infused over one hour and ending 60 minutes prior to incision.^{51,52} In a patient with an IgE-mediated β -lactam allergy, vancomycin combined with an aminoglycoside, usually a single dose of 4 mg/kg of gentamicin, is recommended for AP. It is recommended that the duration of AP not exceed 48 hours.^{51,52} If outbreaks of deep wound infections due to a specific pathogen occur, the abovementioned regular AP should be supplemented with agents active against the causative pathogen. In addition, it is recommended that all patients undergoing cardiac surgery receive intranasal mupirocin for staphylococcal eradication prior to surgery, unless absence of staphylococcal colonization is documented.

The ACCF and AHA have been publishing guidelines on cardiovascular care since 1980. In 2011, they published the Guideline for Coronary Artery Bypass Graft Surgery.⁵³ These guidelines recommend a first or a second-generation cephalosporin, i.e., cefazolin or cefuroxime, for AP in patients who are not colonized with MRSA.⁵³ If MRSA colonization is suspected or known, vancomycin or a combination of vancomycin and other agents should be used. With the exception of vancomycin which is administered two hours prior to surgery, AP is administered 30 to 60 minutes preoperatively. The recommended AP consists of cefazolin 1 gram or cefuroxime 1.5 grams preoperatively and re-dosed if the operation lasts longer than 3 hours.⁵³ No specific dosages for vancomycin AP are provided and the duration of AP is not specified.

In 2013, ASHP, IDSA, SIS and SHEA published joint guidelines for AP in surgery.²⁵ For cardiac procedures, cefazolin or cefuroxime administered within one hour before surgical incision and re-

dosed every 4 hours during surgery or for extensive blood loss (> 1500 mL) are recommended.²⁵ This volume was identified in a study of adults undergoing surgery with spinal instrumentation ($n = 11$) that demonstrated diminished ability of preoperative cefazolin AP to maintain adequate serum and tissue concentrations when blood loss exceeded 1500 mL.⁵⁴ Weight-based dosing for cefazolin (2 grams, 3 grams if patient > 120kg) or 1.5 grams of cefuroxime are recommended. In the presence of a β -lactam allergy, clindamycin (900 mg preoperatively and every 6 hours intraoperatively) or vancomycin (15 mg/kg preoperatively) are the suggested alternatives. Vancomycin AP is recommended in patients colonized with MRSA.²⁵ If vancomycin or clindamycin AP is used and hospital surveillance identifies Gram-negative pathogens in cardiac SSI, an additional agent with Gram-negative activity is recommended. Choices of additional agent include cefazolin, aztreonam, an aminoglycoside or a fluoroquinolone, as determined by surveillance data and the patient's allergy profile. Similar to the aforementioned ACCF/AHA guidelines, no recommendations are made on the duration of AP.²⁵ It is also advised that patients colonized with *S. aureus* receive intranasal mupirocin ointment preoperatively for staphylococcal eradication.

In 2014, the National Institutes of Health (NIH) published Strategies to Prevent Surgical Site Infections in Acute Care Hospitals.⁵⁵ These recommendations are very similar to the ASHP guidelines and include the administration of AP within one hour before incision (two hours for vancomycin and fluoroquinolones). Although no specific antimicrobials are recommended, it is advised that the choice of agent be based on the surgery type and most common SSI pathogens. Weight-based antimicrobial dosing is recommended, such as cefazolin 2 grams (3 grams if > 120 kg), 15 mg/kg of vancomycin or 5 mg/kg of gentamicin.⁵⁵ It is advised that vancomycin be reserved

for MRSA SSI outbreaks, high endemic MRSA rates or for patients at risk of MRSA SSI.⁵⁵ Re-dosing of antimicrobials is recommended every two half-lives or for significant blood loss. An AP duration of 24 hours is recommended as is *S. aureus* screening and decolonization, although specific decolonization regimens are not specified.

In 2017, CDC published guidelines for the Prevention of Surgical Site Infection.⁵⁶ Although not specific for cardiac surgery, these guidelines provide general recommendations that apply to all surgical procedures. It is recommended that preoperative antimicrobials be administered according to published clinical practice guidelines and at a time which allows bactericidal concentrations to be achieved in serum and tissue at the time of incision.⁵⁶ Additionally, in clean and clean-contaminated surgeries, AP should not continue postoperatively. Cardiac surgery is considered a clean surgical procedure and this is the first North American guideline which recommends no postoperative antimicrobials in cardiac surgery.

European guidelines on AP in surgery are largely in agreement with those published in North America. In 2012, the Royal College of Surgeons in Ireland (RCSI) and the Royal College of Physicians of Ireland (RCPI) published key recommendations for preventing SSIs in various surgical procedures.⁵⁷ These include administering AP within 60 minutes before surgical incision and re-dosing if blood loss exceeds 1500 mL or if surgeries last longer than 4 hours and a short acting antimicrobial, such as cefazolin, is used for AP. No specific agents are recommended for AP in cardiac surgery other than to recommend that the choice of agent be based on local susceptibility. For cardiac surgery, it is recommended that the duration of AP is between 24 and 48 hours.

The Scottish Intercollegiate Guidelines Network (SIGN) published national guidelines on Antimicrobial Prophylaxis in Surgery in 2014.⁵⁸ Specific antimicrobial agents are not recommended, but it is recommended that the choice of agent be based on common SSI pathogens and local resistance patterns. This is the only guideline that explicitly considers the cost of antimicrobials in addition to their spectrum, such that inexpensive and narrow spectrum prophylactic agents are preferred to broad-spectrum and more costly agents. It is recommended that AP occur within 60 minutes prior to incision, but ideally as close as possible to the incision time. It is advised that the doses used for AP be equivalent to those used in the treatment of infection. If cefazolin or an antibiotic with a similar half-life ($t_{1/2}$) is used, re-dosing is advised if surgery lasts longer than 4 hours or if blood loss of more than 1500 mL occurs.⁵⁸ The maximum recommended duration of AP in cardiac surgery is 48 hours. MRSA eradication therapy in the form of intranasal mupirocin is recommended in cardiac surgery patients who are known *S. aureus* or MRSA carriers. It is also recommended that MRSA-positive patients undergoing cardiac surgery receive AP with an agent with proven activity against local MRSA strains, typically a glycopeptide such as vancomycin.⁵⁸

In 2017, the European Association for Cardio-Thoracic Surgery (EACTS) published a guideline on medication use during cardiac surgery, including the use of antimicrobials for AP.⁵⁹ Cefazolin or cefuroxime are recommended for AP, with vancomycin and clindamycin as alternatives in β -lactam allergic patients. It is advised that AP is administered within one hours of incision (two hours for vancomycin) and that re-dosing occur if two $t_{1/2}$ of the agent are exceeded or if there is excessive blood loss.⁵⁹ Standardized, rather than weight-based doses for AP agents are recommended in order to avoid dosing errors. The recommended AP duration is between 24 and

48 hours. Intranasal mupirocin applied twice per day starting four days preoperatively is recommended for *S. aureus* eradication.⁵⁹

National Institute for Health and Care Excellence (NICE) guidelines on SSI treatment and prevention were published in April 2019.⁶⁰ Intranasal mupirocin along with a chlorhexidine body wash is recommended prior to cardiac surgery. A single dose of AP is recommended on anesthesia induction with re-dosing if the surgical procedure exceeds one $t_{1/2}$ of the antimicrobial used, although no specific agents are recommended. Gentamicin-impregnated collagen implants are recommended for consideration in cardiac surgery.⁶⁰

Finally, two global documents addressing AP in cardiac surgery have been published. The World Health Organization (WHO) published Global Guidelines for the Prevention of Surgical Site Infection in 2016.⁶¹ As these guidelines are meant to be used globally and for all types of surgeries, these recommendations are more general than in many of the previous documents. It is advised that AP administration occur 120 minutes before incision, unless agents with shorter $t_{1/2}$ are used, in which case administration closer to time of incision is recommended.⁶¹ No recommendations were made on antimicrobial doses or intraoperative re-dosing schedules. The WHO recommends that AP be stopped after completion of cardiac surgery, citing low to very low quality of studies demonstrating benefit of prolonged AP.⁶¹ Intranasal mupirocin applied twice daily for 5 days preoperatively is recommended in cardiac surgery patients with *S. aureus* nasal carriage.

In 2017, a global declaration for appropriate antimicrobial use in surgery was published by the Global Alliance for Infections in Surgery (GAIS) and World Society of Emergency Surgery (WSES) with endorsements by numerous North American, European, African and global stakeholders.⁶² Experts from 83 countries agreed on general recommendations for optimal antimicrobial use across the surgical pathway. The recommendations include AP for all procedures with high SSI rates or if foreign material is implanted. It is advised that agents active against the most likely SSI pathogens be administered 30 to 60 minutes before incision.⁶² Specific dosing recommendations are not provided other than to advise that the dose chosen provide adequate serum and tissue concentrations. Repeat doses are recommended if procedures exceed two $t_{1/2}$ of the antimicrobial agent or if blood loss is greater than 1500 mL.⁶² Postoperative AP is not recommended, and each hospital is advised to develop their own guidelines for surgical AP.

1.5. Antimicrobial Pharmacodynamics in Surgical Prophylaxis

Antimicrobial pharmacodynamics describe the relationship between antimicrobial concentrations and clinical effects, i.e., bacterial eradication and clearance of infection. Antimicrobials are broadly divided into two pharmacodynamic groups, time-dependent and concentration-dependent.⁶³ The activity of time-dependents agents, including β -lactams, is enhanced by maximizing the portion of the dosing interval that antimicrobial concentrations remain above MIC. The activity of concentration-dependent agents, such as aminoglycosides or fluoroquinolones, is enhanced by increasing the concentration of antimicrobial relative to the MIC.

Precise PD targets for AP in surgery have not been defined to date, although the importance of PD in cardiac surgery have been suggested in previous studies. Goldmann *et al.* reported that following valve replacement surgery, SSIs were more common in longer surgeries or if patients had lower cephalothin concentrations at the end of surgery.⁶⁴ More recently, Kosaka *et al.* reported that in patients undergoing elective cardiac surgery and receiving cefazolin AP, SSIs were more common in patients with better renal function.⁶⁵ For renally cleared antimicrobials such as cefazolin, renal function may potentially be a surrogate for lower antimicrobial concentrations during surgery, further suggesting the importance of PD in successful AP.

The 2013 ASHP clinical practice guidelines state that the main goals of AP are to prevent SSI and the associated morbidity and mortality.²⁵ As previously stated, in order to meet this goal, it is recommended that antimicrobials for surgical AP be “given in an appropriate dosage and at a time that ensures adequate serum and tissue concentrations during the period of potential contamination”.²⁵ Without further details, the interpretation of this statement has been left to researchers and clinicians. In previous studies of cefazolin in the adult cardiac surgery population, “adequate serum and tissue concentrations” to prevent SSI has been interpreted as cefazolin concentrations exceeding a target concentration, usually the MICs of common SSI pathogens.^{65–72} Depending on the pathogen targeted and the MIC of that pathogen at the time of the study, the target cefazolin concentrations have ranged from 4 mg/L to 16 mg/L. Considering the abovementioned goals of AP, a target total concentration of ≥ 40 mg/L, representing free concentration of 8 mg/L, assuming 80% protein binding is expected to provide reasonable coverage against the most likely SSI pathogens.⁷³ A cefazolin concentration of 8 mg/L was also the Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoint of cefazolin for

staphylococci prior to the introduction of ceftazidime testing for methicillin resistance and the susceptibility breakpoint for *Enterobacteriaceae* spp. prior to it being lowered for ESBL identification.⁷⁴ The second part of the clinical practice guidelines recommendations, i.e., that antimicrobial concentrations exceed targets “during the period of potential contamination” can be understood as the time from surgical incision to wound closure.

1.6. PREVIOUS STUDIES

1.6.1. Cefazolin Pharmacokinetics in Healthy Volunteers

The Canadian Pharmaceutical Association’s monograph of cefazolin reports that cefazolin is 74-86% bound to plasma proteins and has a $t_{1/2}$ of 1.2 to 2.2 hours in patients with normal renal function.³⁸ Although a precise volume of distribution (Vd) is not defined, it is noted that cefazolin is extensively distributed throughout the body. Much of the data in the product monograph originates from studies of healthy volunteers. Kirby *et al.* administered cefazolin to healthy adult male volunteers and collected blood samples to determine PK parameters.⁷⁵ The serum $t_{1/2}$, clearance (CL), Vd and protein binding were 1.8 ± 0.2 h, 62 ± 6 mL/min/1.73m², 10 ± 0.4 L/1.73m² and 86%, respectively.⁷⁶ Rattie *et al.* also administered cefazolin to healthy adult males and reported a $t_{1/2}$ of 1.4 ± 0.2 h after IV administration and 2.0 ± 0.3 h after IM administration.⁷⁷ The Vd of cefazolin was 6.94 L. Lavillaureix *et al.* measured cefazolin serum concentrations in patients with normal and impaired renal function or receiving hemodialysis.⁷⁸ The mean $t_{1/2}$ of cefazolin was 2.2 h in subjects with normal renal function after IM dosing and 1.8 h after IV dosing, while it was prolonged in patients with renal dysfunction (median $t_{1/2}$ = 9.1 h (IQR 5.5, 12.6)). The Vd in healthy subjects was 8.6 ± 1.0 L and the CL was 52 ± 3.6 mL/min.⁷⁸

1.6.2. Cefazolin Pharmacokinetics during Cardiac Surgery with CPB

During cardiac surgery, CPB is used to take over the functions of the heart and lungs to enable blood and oxygen circulation to the body while the heart is stopped to allow the surgeon to operate.⁷⁹ The use of CPB is associated with a number of factors which can potentially impact the PK of drugs. These factors include hemodilution, lung isolation, protein denaturation, hypotension and hypothermia.^{80,81} The 1.5 to 2 liters of priming fluid typically administered at the start of CPB result in hemodilution with an associated reduction in plasma proteins by up to 50% in adults.⁸⁰ The lungs are temporarily isolated from circulation during CPB and can act as drug reservoirs, such that upon CPB completion, the accumulated drug can recirculate and result in elevated systemic drug concentrations.^{79,81} Protein denaturation can occur through contact with the tubing or the oxygenator of the CPB apparatus.⁸⁰ Hypotension during CPB reduces the blood flow to various tissues, including the skin, adipose tissues, muscles, bones and kidneys which may alter drug distribution and elimination. Enzymatic processes in the liver and kidneys are temperature-sensitive and hypothermia during CPB can slow these processes, thus affecting drug metabolism. With multiple factors occurring simultaneously during CPB, it is difficult to predict the ultimate effect of CPB on drug PK.

At the start of our study, the PK of cefazolin during cardiac surgery with CPB were not well described. In 1980, Miller *et al.* reported the preoperative, intraoperative and postoperative PK of cefazolin in 8 patients undergoing cardiac surgery with CPB.⁸² The $t_{1/2}$ was similar in the pre- and postoperative period at 2.8 ± 1.6 h and 3.6 ± 2.0 h, respectively and prolonged during surgery at 11.0 ± 8.1 h. Similarly, V_d expanded during surgery to 0.26 ± 0.07 L/kg, compared to 0.14 ± 0.06 L/kg preoperatively and 0.16 ± 0.10 L/kg postoperatively.⁸² In the study by Lehot *et al.*, the authors

utilized serum cefazolin concentration over time data collected from 10 patients undergoing elective cardiac surgery with CPB to calculate the $t_{1/2}$, Vd and CL.⁸³ During surgery, the values for $t_{1/2}$, Vd and CL were 3.85 ± 2.1 h, 244 ± 50 mL/kg and 1.05 ± 0.49 mL/kg/min, respectively.⁸³ These results demonstrate differences in $t_{1/2}$ and Vd between patients undergoing cardiac surgery and those previously reported in healthy volunteers. However, the traditional approach to PK study (intensive sampling of a small number of patients) used in the studies by Miller and Lehot precludes the identification and quantification of any sources of variability in drug concentrations, which is important for individualization of therapy in the target population. Although Kosaka *et al.* also reported Vd, k_e , $t_{1/2}$ and CL of cefazolin during cardiac surgery, the study results were not analysed separately for patients undergoing surgery with and without CPB.⁶⁵ As such, the effects of CPB on cefazolin PK cannot be clearly delineated from these results.

At the beginning of our study, only two studies characterized the protein binding of cefazolin during cardiac surgery with CPB. In seven patients undergoing valve replacement, Hutschala *et al.* reported that maximum cefazolin concentrations in the interstitial fluid of the subcutaneous adipose layer and muscle tissue were 22.6% and 19.4%, respectively.⁶⁸ As only non-protein bound free drug is able to diffuse into interstitial fluids and tissues, these findings approximate protein binding of 77.4% and 80.6%, respectively. Conversely, Andreas *et al.* reported that mean protein binding of cefazolin was $40 \pm 40\%$ in eight patients undergoing elective CABG.⁷⁰ The results of these two studies demonstrated significant variation in cefazolin protein binding during cardiac surgery with CPB. What was unknown was whether the variability was due to the small number of patients studied or due to inherent changes in protein binding during cardiac surgery with CPB.

1.6.3. Cefazolin Concentrations during Cardiac Surgery with CPB

At the outset of our study in 2014, there were eight studies of cefazolin AP in adult cardiac surgery with CPB. Although each study provided valuable foundational knowledge regarding cefazolin in this patient population, none of the studies examined guideline-recommended cefazolin AP regimens. The first available study, published in 1990 by Lehot *et al.* reported the serum concentrations of cefazolin in 10 patients undergoing elective cardiac surgery with CPB.⁸³ AP consisted of cefazolin 25 mg/kg preoperatively and every 8 hours along with netilmicin 2mg/kg preoperatively and 1 mg/kg every 8 hours for a total of 48 hours.⁸³ Blood samples were collected from each patient, including before and after antimicrobial infusion, before and after CPB start, during CPB, at the end of CPB and at wound closure. Serum cefazolin concentrations were determined using a microbiological assay and the time-course of cefazolin during cardiac surgery described. Upon CPB start, cefazolin concentrations were reduced by $28 \pm 7\%$ and the concentrations at the end of surgery were 41 ± 12 mg/L.⁸³ Since the publication of this study, more accurate and precise measurements of antimicrobial concentrations have replaced microbiological assays for determination of antimicrobial concentrations in biological samples, including notably liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Fellinger *et al.* measured serum cefazolin concentrations in ten CABG surgery patients receiving cefazolin AP consisting of 1 gram preoperatively and at the start of CPB.⁶⁶ Blood samples were collected before and after CPB start, after the second cefazolin dose, before and after CPB end and at sternal closure. Total cefazolin concentrations were measured by high-performance liquid chromatography (HPLC) and free concentrations estimated based on 80% protein binding. Significant variability was observed in cefazolin concentrations at each time point and the

estimated mean free cefazolin concentrations at wound closure were 10.22 ± 3.76 mg/L⁶⁶, i.e., not every patient exceeded the most current CLSI breakpoint of 8 mg/L.

Waltrip *et al.* measured cefazolin concentrations in 34 patients receiving cefazolin AP by intermittent dosing or by continuous infusion in CABG procedures.⁸⁴ Cefazolin AP consisted of one of three regimens; 1 gram preoperatively and at end of CPB (Regimen 1), 2 grams preoperatively, followed by a continuous infusion of 20 mg/min (1.2 g/h) during surgery (Regimen 2) or 3 grams preoperatively and 15 mg/min (0.9 g/h) during surgery (Regimen 3). Total cefazolin serum concentrations and cefazolin concentrations in subcutaneous fat tissue from the sternal wound were determined by HPLC. Blood and adipose tissue samples were collected “at incision, 0.25 h, 0.5 h, and 1 h; at end of cardiopulmonary bypass; and at wound closure.”⁶⁶ Regimen 3 resulted in the highest cefazolin concentrations, followed by Regimen 2 and then Regimen 1. Although the authors concluded that these results demonstrated superiority of continuous infusions over intermittent dosing, the comparison lacked validity as higher cefazolin doses were administered by continuous infusion. Moreover, no statistically significant difference was observed in chest wound infections between the groups.⁸⁴

Caffarelli *et al.* measured cefazolin concentrations in 30 patients undergoing cardiac surgery with CPB following AP with 1 gram of cefazolin preoperatively and at wound closure.⁶⁷ Patients were divided into three groups; CPB shorter than 120 minutes (Group 1), CPB longer than 120 minutes and CPB combined with profound hypothermic circulatory arrest during which the patient was cooled to achieve a 20°C tympanic membrane temperature. Blood samples were collected before

cefazolin doses, at incision, before and after CPB and hourly during surgery and CPB. Total cefazolin serum concentrations were determined by a radial diffusion assay. In Group 1, cefazolin concentrations were below 16 mg/L in 30% of patients, but remained above 8 mg/L in all throughout surgery.⁶⁷ In Groups 2 and 3, 60% and 66% of patients, respectively had cefazolin plasma concentrations below 16 mg/L and 50% and 10% of patients, respectively were below 8 mg/L. The authors concluded that the aforementioned AP regimen was only adequate in surgeries with shorter CPB duration. However, as the assessment of antimicrobial activity was based on total, rather than free, pharmacologically active concentrations, this assessment may have overestimated the efficacy of the studied AP regimen.

Hutschala *et al.* measured cefazolin plasma, subcutaneous and muscle concentrations in seven patients undergoing aortic valve replacement receiving cefazolin AP of 4 grams preoperatively and 2 grams at wound closure.⁶⁸ Cefazolin concentrations in subcutaneous adipose layer and muscle layer of the thigh were measured by microdialysis. Blood and microdialysis samples were collected every 20 minutes for the first two hours after the preoperative dose and hourly thereafter for 10 hours.⁶⁸ Cefazolin concentrations were determined by capillary zone electrophoresis. The penetration of cefazolin into muscle and the subcutaneous adipose layer was similar, and both were significantly lower than serum cefazolin concentrations. With the studied AP regimen, plasma, subcutaneous and muscle tissue concentrations remained above 4 mg/L for the entire study duration.⁶⁸

Similar to the study by Waltrip *et al.*, Adembri *et al.* compared cefazolin AP by continuous infusion to that administered by standard intermittent dosing. Unlike the study by Waltrip, Adembri *et al.* compared equivalent doses of cefazolin administered by either continuous infusion or as intermittent bolus doses. The intermittent dosing group received 2 grams of cefazolin preoperatively and 1 gram at the end of CPB and at 9 and 15 hours thereafter while the continuous infusion group received 2 grams of cefazolin preoperatively and a continuous infusion providing 1 gram of cefazolin every 6 hours for 18 hours.⁶⁹ Ten patients in each group were studied and blood samples were collected before and after the preoperative dose, at each peak and trough in the intermittent group and at the matching times in the continuous infusion group. Total cefazolin concentrations in serum samples were determined by agar diffusion technique and free concentrations estimated based on 80% protein binding.⁶⁹ Compared to continuous infusion, cefazolin concentrations in the intermittent dosing group showed greater interpatient variability. Furthermore, continuous infusion maintained free concentrations greater than 8 mg/L for the entire study duration, whereas all free trough concentrations were below 8 mg/L in the intermittent group.⁶⁹ Although this suggests the superiority of continuous infusions to maintaining target concentrations, whether that translates to improved efficacy in terms of SSI prevention was not addressed.

Kosaka *et al.* investigated the effect of renal function on cefazolin AP in cardiac surgery.⁶⁵ A total of 62 patients underwent cardiac surgery, 44% of which required the use of CPB. Patients were grouped by renal function into one of three groups; creatinine clearance (Clcr) ≥ 50 mL/min, Clcr between 10 and 49 mL/min and patients receiving hemodialysis.⁶⁵ Cefazolin AP was administered as 2 grams preoperatively, 1 gram in CPB priming solution and 1 gram every 6 hours for up to 24

hours postoperatively, with the exception of patients on hemodialysis who did not receive any postoperative cefazolin doses. Blood samples were collected at incision, 6 hours after the preoperative dose, at the end of the procedure and at 22 and 24 hours after the preoperative dose.⁶⁵ Total and free cefazolin concentrations were measured in serum and ultrafiltrate by HPLC. Patients with better renal function had lower cefazolin concentrations at each time period, demonstrating that renal function is an important determinant of cefazolin concentrations in the studied population.

Finally, Andreas *et al.* assessed the effects of left internal mammary artery (IMA) harvesting on the penetration of cefazolin into pre-sternal subcutaneous tissue in eight patients undergoing elective CABG.⁷⁰ Cefazolin AP consisted of 4 grams administered preoperatively and 2 grams at skin closure. Blood and microdialysis samples were collected every 20 minutes for the first two hours and then hourly for the next 8 hours. The cefazolin concentrations in the collected samples were quantified by HPLC.⁷⁰ Cefazolin penetration into subcutaneous tissue was significantly reduced on the left side compared to the surgically unaffected right side. Moreover, in surgically unaffected tissues cefazolin concentrations exceeded 4 mg/L for the entire study duration, while in three patients the cefazolin concentration on the left side failed to do the same. These findings demonstrated local differences in cefazolin penetration during cardiac surgery with IMA harvesting.

After reviewing the available evidence, several important gaps in knowledge were identified. It was unclear whether adequate free cefazolin concentrations were being achieved during cardiac

surgery with CPB. This was due to few studies, various cefazolin AP regimens, different targets and significant interpatient variability in cefazolin concentrations. Furthermore, there was a notable lack of study of guideline-recommended AP regimens commonly used in clinical practice (see Section 1.4).

2. SCIENTIFIC RATIONALE

Cardiac surgery with CPB is a complex and highly invasive procedure. Faced with an aging population and increasing heart disease, the demand for cardiac surgery procedures is not expected to diminish. One of the potential complications of cardiac surgery is the development of SSIs, which are associated with significant patient morbidity and mortality, in addition to increased healthcare resource use.²⁹ Considering the deleterious effects of SSIs after cardiac surgery, prevention through a multi-faceted approach, including AP, is of paramount importance.

Prior to our study, the ASHP guideline-recommended regimen for cefazolin AP in cardiac surgery, i.e., cefazolin administered preoperatively and every 4 hours during surgery for up to 48 hours postoperatively, had not been studied. It was thus unknown whether this regimen resulted in adequate antimicrobial concentrations in all patients. By measuring the antimicrobial concentrations achieved during cardiac surgery, characterizing cefazolin protein binding, constructing a population-pharmacokinetic model and following patients for the development of SSI for up to 90 days postoperatively, this study makes important contributions to the assessment and optimization of cefazolin AP in cardiac surgery with CPB.

3. THESIS OBJECTIVES

The overall goal of my research was to enhance the scientific knowledge regarding cefazolin AP in cardiac surgery with CPB and thus optimize AP in the targeted population. My objectives were:

Objective 1. Cefazolin Concentrations: To characterize total cefazolin peak, intraoperative trough and closure concentrations, evaluate closure concentrations against the target of ≥ 40 mg/L, and investigate subject-, surgery-, and AP-related variables thereby identifying risk factors for below-target skin/chest closure concentrations when administered in accordance with guidelines.

Objective 2. Cefazolin Protein Binding: To measure free cefazolin peak, intraoperative trough and closure concentrations, determine the protein binding of cefazolin during cardiac surgery with CPB and investigate variables associated with protein binding.

Objective 3. Cefazolin Population Pharmacokinetics: To construct a population-pharmacokinetic model of cefazolin during cardiac surgery with CPB thereby providing estimates of significant PK variables, including the V_d , elimination rate constant (k_e) and CL of cefazolin in this special patient population.

Objective 4. Cefazolin Pharmacodynamics: To characterize SSI events, investigate subject-, surgery- and AP-related variables in relation to the development of SSI, and to identify potential cefazolin PD targets for preventing SSI in cardiac surgery with CPB.

4. HYPOTHESES

My hypotheses were that the:

1. ASHP guideline-recommended cefazolin AP regimen would not maintain cefazolin concentrations above the target of 40 mg/L in all patients during cardiac surgery with CPB.
2. Cefazolin pharmacokinetics, including protein binding would be affected by cardiac surgery with CPB, and would therefore be different than in other surgical populations.
3. Lower cefazolin concentrations during cardiac surgery with CPB, particularly at wound closure, would be associated with a higher risk of SSIs.

5. PREVIOUS PUBLICATION OF STUDY DATA

Some of the study data presented in this thesis has been previously published.

The results of Objective 1., i.e., the analysis of total cefazolin concentrations with respect to target closure concentrations of ≥ 40 mg/L has previously been published in the Journal of Antimicrobial Chemotherapy. (Calic D, Ariano RE, Arora RC, et al. *Evaluation of cefazolin antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass. J Antimicrob Chemother.* December 2017. doi:10.1093/jac/dkx439). The original article (version of record) is available at <https://academic-oup-com.uml.idm.oclc.org/jac/article/73/3/768/4717791> .

The results of Objective 4, i.e., pharmacodynamic analysis of postoperative SSI events has been published in Antimicrobial Agents and Chemotherapy. (Zelenitsky S, Calic D, Arora RC, et al. *Antimicrobial prophylaxis for patients undergoing cardiac surgery: Intraoperative cefazolin concentrations and sternal wound infections. Antimicrob Agents Chemother* 2018). The original article is available at <https://aac-asm-org.uml.idm.oclc.org/content/62/11/e01360-18.long> Copyright © American Society for Microbiology, Antimicrobial Agents and Chemotherapy, 62, 2018, e01360-18. DOI 10.1128/AAC.01360-18.

6. METHODS

6.1. Study Design

The study was designed as a prospective, non-interventional PK study of cefazolin AP in adult patients undergoing elective cardiac surgery with CPB. The prospective and non-interventional design was chosen as a pragmatic way to enable researchers to collect information on all variables of interest and thus most accurately assess the state of cefazolin AP in a real-world setting as compared to idealized study conditions.

The study was conducted within the Cardiac Sciences Program at the St. Boniface Hospital in Winnipeg, Manitoba. The Cardiac Sciences Program at the St. Boniface Hospital is the sole provider of tertiary-level cardiac care for Manitoba, a province with a 2016 population of almost 1.3 million people.^{85,86}

The study was conducted in collaboration with Dr. Robert Ariano, PharmD, Clinical Pharmacist at the St. Boniface Hospital, Dr. Hilary Grocott, MD, Professor of Anesthesia at the University of Manitoba and Dr. Rakesh Arora, MD, Associate Professor of Surgery at the University of Manitoba. The study was financially supported by the Canadian Society of Hospital Pharmacists (CHSP) Foundation Grant (grant no. 317380). The CSHP Foundation is an organization supporting “research and educational programs that advance patient-centred pharmacy practice in hospitals and other collaborative healthcare settings for the betterment of public health.”⁸⁷

Prior to commencement, the study protocol, consent forms, data collection forms and study budget were reviewed and approved by the University of Manitoba Health Research Ethics Board (HREB). HREB approved the study in May 2014 (HREB #H2014:142) and the St. Boniface Hospital Research Review Committee (RRC) granted approval in July 2014 (#RRC/2014/1408). Due to the non-interventional nature of the study, registration of the study protocol was not required by the HREB or RRC. Copies of the HREB and RRC approval letters are included in Appendices A and B, respectively. Additionally, study personnel completed the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans Course on Research Ethics (TCPS 2:CORE). This online tutorial presented by the Canadian Panel of Research Ethics is a requirement for all researchers whose work engages human subjects.

Prior to patient recruitment, the study proposal was communicated to the cardiac surgeons and anesthesiologists at St. Boniface Hospital to familiarize them with the study prior to commencement. Patient recruitment started in August 2014 and was completed in May 2015.

6.2. Sample Size Determination

Several methods were used to determine an appropriate sample size to describe the PK of cefazolin during cardiac surgery with CPB. As this was the first study of the guideline-recommended cefazolin regimen, the incidence rate of cases with sub-therapeutic closure concentrations was speculative. Based on significant patient and surgery-related variability, a sample size of 50 with a predicted event (i.e., below-target closure concentration) rate of 10 – 20% was needed to provide 5 – 10 cases in which risk factor analysis could be conducted.

Next, the sample size required for descriptive studies to estimate a mean value was calculated according to Equation 1:

$$N = \frac{4\sigma^2(Z_{crit})^2}{D^2} \quad \text{Equation 1}$$

Where N is the sample size, σ is the standard deviation (SD), Z_{crit} is the standard normal deviate and D is the total width of the confidence interval.⁸⁸

This equation was applied to the sample size needed to describe the PK parameter of cefazolin CL. In a previous study of cefazolin in pediatric patients undergoing cardiac surgery, the parameter estimate for CL was 1.56 L/h with an SD of 0.583, which was applied in this calculation.⁸⁹ A CI width of 0.312 was selected to represent $\pm 10\%$ of the CL estimate from the aforementioned study. Based on a Z_{crit} of 1.96 (i.e., 95% CI), a sample size of 54 patients was required to describe the mean cefazolin CL in this study. The number was also consistent with an analysis by Tam *et al.* that determined that a minimum sample size of 50 subjects was necessary to obtain robust PK predictions from nonparametric PK modeling.⁹⁰

At study commencement, a target sample size of 55 subjects was selected as it was expected to provide a sufficient number of cases of below-target closure concentrations for risk factor analysis as well as robust estimates of PK parameters in the target population. Furthermore, it also represented the largest PK study of cefazolin AP in adult patients undergoing cardiac surgery with CPB.

6.3. Patient Recruitment

Patients were screened for study eligibility in the preoperative assessment clinic at the St. Boniface Hospital. Patients were considered eligible for study inclusion if they were scheduled to undergo elective cardiac surgery with CPB, including, but not limited to, CABG and heart valve repairs or replacements. Patients had to be 18 years or older at the time of surgery and have no known contraindications to receiving cefazolin AP (e.g., known or suspected allergy). Exclusion criteria included documented or suspected infection, such as infective endocarditis, at the time of surgery. Furthermore, patients treated with systemic antimicrobials within 3 days of the operation or those that received more than one cefazolin dose preoperatively were ineligible for participation. Additional exclusion criteria included documented chronic liver disease or preoperative Clcr < 50 mL/min/72 kg.^{91,92} If ambiguity existed about eligibility for the study, the author conferred with the Principal Investigator, Dr. Sheryl Zelenitsky, for decisions on patient inclusion.

The patient's chart was reviewed for study eligibility and if the criteria were met, a member of the preoperative assessment clinic staff inquired if the patient was interested in participating in a research study. If the patient agreed, the author met with the patient following their preoperative assessment appointment. The author explained the goals and potential risks and benefits of participation to the patient before inquiring about their willingness to participate. If the patient confirmed interest in the study, the informed consent form was reviewed in detail. Written informed consent was obtained from all patients prior to study enrollment. Copies of the signed consent form were provided to the patient and placed in their medical chart, while the author kept the original copy. Study participation and informed consent were documented directly in the patient's medical chart as well to ensure that all health care providers were aware of the patient's

study enrollment. A copy of the Research Participant Information and Consent Form is included in Appendix C.

Following study enrollment, the study participant was assigned a study number that was used for all subsequent data collection and analysis. The cardiac surgery scheduling office was also notified to ensure that the study name would appear next to the patient name on the operating room (OR) schedule. This flag informed all health care providers of the patient's study participation, and helped the author recognize study participants on the OR schedule and thus plan sample collection. Due to the dynamic nature of the OR schedule, the flag was useful to ensure that sample collection was not missed due to surgery delays or rescheduling.

6.4. Clinical and Surgery Data Collection

Study subject data, including demographics, comorbidities, surgery information and SSI surveillance was collected from St. Boniface Hospital patient records or following discharge, as appropriate. All data was recorded on standardized data collection sheets (Appendix D).

General demographics, including age, gender, height and weight were collected and BMI was calculated.⁹³ Social history (i.e., smoking status, alcohol consumption) was recorded including quantities of cigarettes or alcoholic drinks consumed per week. Previous surgical procedures and hospitalizations were also noted. All prescription and non-prescription medications, as well as vitamins, minerals and herbal supplements that the study subject was taking prior to surgery were documented. For each, the name of the product, dosage and directions were documented. To ensure accuracy and completeness, two sources of drug information were used, including the

medication reconciliation conducted by the nurse practitioner at the preoperative assessment clinic and the list of medications in the patient's chart obtained from the Drug Program Information Network (DPIN). The DPIN is a prescription drug database that provides Manitoba Health real-time drug profiles of prescription use by Manitoba residents in outpatient pharmacies.⁹⁴ Information available from DPIN includes name of the drug dispensed, the dose, the quantity dispensed, the day supply, prescribing physician and dispensing pharmacy. Drug, food and environmental allergies, including type of reaction, were noted as well.

All comorbidities (e.g., hypertension, diabetes mellitus, heart failure, chronic lung disease, ischemic heart disease, cerebrovascular disease, malignancy) were documented and a Charlson comorbidity index was calculated.⁹⁵ The Charlson comorbidity index is used to predict 10-year survival based on patient comorbidities, with higher scores indicating lower survival.⁹⁵ It was developed in a cohort of 559 medical inpatients and validated in 685 breast cancer patients. The first "test" population was followed for 1 year and used to construct the index which assigned different scores to different co-morbidities such that, for example, myocardial infarctions and metastatic tumors were assigned scores of 1 and 6, respectively. Each comorbidity was assigned a score and the risk of mortality was calculated based on the total score. The second population was used to validate the ability of the aforementioned index to predict risk of death from comorbidities during a 10-year follow-up period. The 10-year mortality risk was 8% for patients with an overall comorbidity index of 0, 25% for those with a total score of 1, 48% for scores of 2 and 59% for scores equal or greater than 3.

The surgery data recorded included the type of surgery, number and type of grafts or stents placed, if applicable, and the American Society of Anesthesiologists (ASA) Physical Status score. The ASA score, as assigned by the attending anesthesiologist, is a global measure of physical health prior to surgery, with higher scores indicative of poorer health.⁹⁶ The ASA score ranges from I to VI, with each representing “a normal healthy patient, a patient with mild systemic disease, a patient with severe systemic disease, a patient with severe systemic disease that is a constant threat to life, a moribund patient who is not expected to survive without the operation, and a declared brain-dead patient whose organs are being removed for donor purposes”⁹⁶, respectively.

Relevant surgical times were recorded, including surgical incision, the start and end of CPB, as well as wound closure. All IV fluids administered during surgery, including blood products and CPB priming fluid, were recorded. Loss of fluids, including urine and blood, was documented and a net fluid balance was calculated based on the difference between fluid administration and fluid loss during surgery. Laboratory data, including serum creatinine, albumin, blood glucose and the most recent hemoglobin A1C were collected as well. Details of intraoperative complications were noted, as were cases requiring surgical re-exploration following primary wound closure. The pre- and postoperative durations of hospital stay were recorded.

6.5. Cefazolin Prophylaxis Regimen

As this was a non-interventional study, study subjects received cefazolin AP as per the standard hospital protocol in place at the time of the study and in line with clinical practice guidelines.^{25,53} Cefazolin was administered in a weight-based manner, such that individuals weighing less than 80 kg received 1-gram doses, while those 80 kg or greater received 2-gram doses. This dosing

represented a hybrid between the ACCF/AHA guidelines which recommended 1 gram cefazolin doses and the ASHP guidelines which recommended 2 grams (or 3 grams if $\geq 120\text{kg}$).^{25,53} Cefazolin was administered by the attending anesthesiologist as an IV bolus dose within one hour prior to surgical incision and at intervals of every 4 hours during surgery as per the ASHP guidelines. AP was continued for 48 hours postoperatively with the same cefazolin dose as above administered at intervals of every 8 hours. Information about perioperative cefazolin doses, including date and time of administration were documented for each study subject.

6.6. Sample Collection

Up to three 6mL blood samples were collected from each study subject. This included a peak sample collected approximately 30 minutes following the preoperative cefazolin dose, an intraoperative trough sample collected prior to the administration of an intraoperative cefazolin dose and a closure sample collected within 15 minutes of surgical wound closure. (Figure 1)

Blood samples were drawn by the anesthesiologist from an arterial cannula routinely placed for perioperative blood pressure monitoring,⁹⁷ or by the perfusionist directly from the CPB circuit, depending on the collection time. Blood samples were collected in plain Vacutainer® tubes (BD Canada, Mississauga, Canada) without anticoagulant and transferred immediately to the laboratory for analysis.

The whole blood samples were centrifuged at 1300 g at 4°C for 10 minutes in an Eppendorf® 5804R centrifuge (Eppendorf AG, Hamburg, Germany) to separate plasma from other blood components. The resultant plasma was used to measure total cefazolin concentrations. One mL of

the plasma was transferred into a Centrifree® device (EMD Millipore Corporation, Billerica, MA) and centrifuged at 2000 x g for 45 minutes at ambient temperature to separate ultrafiltrate which was used to measure free cefazolin concentrations.⁹⁸ The plasma and ultrafiltrate samples were transferred into labelled cryogenic vials and frozen at -80°C until analysis.

Equilibrium dialysis and ultrafiltration, the two most common methods for measuring protein binding *in vitro*, were considered for the determination of free cefazolin concentrations.⁹⁹ Equilibrium dialysis utilizes “a dialysis cell which contains two reservoirs separated by a semipermeable dialysis membrane.”¹⁰⁰ The dialysis membrane separating the plasma and buffer reservoirs is selected based on the molecular weights of the protein and the compound being studied. The dialysis system is allowed to reach equilibrium at 37°C, after which drug concentrations are measured in each reservoir to determine the degree of protein binding.¹⁰⁰ The drug concentration in the buffer reservoir represents free drug concentrations, while that in the plasma reservoir represents total drug concentrations.

The advantage of equilibrium dialysis is its precision due to which it is considered the “reference method” for determining drug protein binding.⁹⁹ Despite this, equilibrium dialysis had several important limitations for the current application. Sample analysis in equilibrium dialysis must be conducted in batches as automated equilibrium dialysis devices, such as the Rapid Equilibrium Dialysis (RED) device, are currently only available as 96-well plates. This necessitates the freezing and thawing of samples prior to analysis. As protein binding is affected by temperature, freezing and thawing is undesirable as it may affect the final measurement, potentially overestimating free drug concentrations. Additionally, non-specific drug adsorption to the dialysis membrane can

affect the results by potentially leading to erroneously low free drug concentrations.¹⁰⁰ Another disadvantage is the relatively long time to reach equilibrium and the changes in drug concentrations that occur during that process. This is especially important for drugs with concentration-dependent protein binding because protein binding may be different from beginning to end of the process.¹⁰⁰ Finally, the use of a non-physiological buffer presents challenges as the ions in the buffer can affect the protein binding of drugs.

Ultrafiltration performed with the use of an ultrafiltration device is another method for measuring protein binding.¹⁰⁰ Similar to equilibrium dialysis, the ultrafiltration device consists of two reservoirs which are separated by a filter. Unlike with equilibrium dialysis, ultrafiltration does not require a buffer solution and instead utilizes centrifugal force to push the sample containing free drug from the upper reservoir, through the semi-permeable membrane and into the lower reservoir while retaining the protein binding target, albumin, in the upper reservoir. Drug concentrations in the lower ultrafiltrate compartment represent free drug concentration, allowing for determination of protein binding by comparison to unfiltered samples. Unlike with equilibrium dialysis, ultrafiltration is conducted for each sample individually, thus avoiding the freezing and thawing required for batch analysis. A disadvantage to ultrafiltration is that only small volumes of ultrafiltrate (10 – 15% of initial plasma volume) are collected, which may pose a challenge for subsequent drug concentration measurement.¹⁰⁰ Similar to equilibrium dialysis, drug adsorption to the ultrafiltration membrane is a potential disadvantage to ultrafiltration as well, which if present may result in the underestimation of free drug concentrations.

After considering the advantages and disadvantages of both methods, ultrafiltration was selected for this study. Ultrafiltration was chosen as it did not require freezing and thawing of samples prior to analysis, a non-physiological buffer solution was not required, and the shorter analysis time (45 minutes versus 4 hours for RED plates) avoided potential measurement errors resulting from differences in protein binding from beginning to end of analysis. Following the choice of method, the selection of ultrafiltration device was considered. Although there are a number of devices on the market, the Centrifree® device was chosen based on its favorable characteristics and validated performance.

The Centrifree® ultrafiltration device (EMD Millipore Corporation, Billerica, USA) consists of sample and ultrafiltrate containers separated by a hydrophilic membrane with a 30 kDa molecular mass cut-off.^{65,98} For comparison, the molecular mass of albumin is 66 kDa.¹⁰¹ The regenerated cellulose membrane retains 99.9% of serum proteins, allowing for effective separation of free drug. As previously stated, membrane adsorption is a potential drawback to ultrafiltration and the low-adsorption membrane utilized in the Centrifree® was a distinct advantage. In a study by Kosaka *et al.*, the adsorption of cefazolin to the Centrifree® ultrafiltration device was assessed by comparing the concentration of cefazolin in cefazolin aqueous solutions directly and after ultrafiltration.⁶⁵ Similar cefazolin concentrations were reported in the nonfiltered aqueous cefazolin solutions and those that were filtered using the Centrifree® device. Furthermore, by studying cefazolin serum samples, the authors concluded that cefazolin did not adhere to the Centrifree® membrane in the presence of serum either.⁶⁵ After reviewing this evidence, the decision to use Centrifree® in the current study was clear as it enabled the accurate determination of protein binding while mitigating the risk of membrane adsorption.

6.7. Sample Analysis

6.7.1 *Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS)*

Total and free cefazolin concentrations in plasma and ultrafiltrate, respectively, were measured by LC-MS/MS using a Shimadzu Nexera ultra-high-performance liquid chromatography apparatus and an LCMS 8040 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). Liquid chromatography separates analytes based on the difference in partition between stationary and mobile phases, i.e., column retention time. Following chromatographic separation, mass spectrometry enables the identification and quantification of the analyte of interest based on its precursor and product ion mass-to-charge ratio (m/z).¹⁰²

The sample is initially fractionated via chromatography with a column. Charged analyte elutes from the column and is vaporized through electrospray ionization forming gas phase ion analytes (cefazolin) that are analyzed by three tandem quadrupole mass spectrometers (MS).¹⁰² In the first MS, the analyte of interest is selected based on its precursor ion m/z (typically (molecular weight + H)/+1 charge). In the second MS, the analyte is bombarded with high-energy argon gas fracturing the compound into multiple fragments (product ions), the m/z of which are uniquely dependent on the structure of the analyte.¹⁰² The third MS selects one of the analyte product ions via its diagnostic m/z for detection, with the intensity of the signal being linearly dependant on the initial concentration of the analyte in the sample prior to analysis. The triple MS configuration affords three complementary means of identification of the correct analyte in a complex matrix such as plasma: 1) the unique chromatographic retention time, 2) the analyte precursor m/z , and 3) the diagnostic product ion m/z .

The mass spectrometer was operated in the positive ion mode with multiple reactions monitoring (MRM). An Acquity UHPLC BEH C₁₈ 1.7 µm 2.1 x 100 mm column (Waters Corporation, Milford, USA) was used for chromatographic separation with a mobile phase that consisted of 85% acetonitrile in water with 0.1% formic acid. The mobile phase flow rate was 0.4 mL/min and column oven temperature was maintained at 40°C. Cefazolin sodium salt (Toronto Research Chemicals, Toronto, Canada) was used for standardization and quantification by a peak area ratio (PAR) of the stable isotope internal standard (IS), ¹³C₂¹⁵N cefazolin (Toronto Research Chemicals, Toronto, Canada). With the addition of two Carbons (¹³C) and one Nitrogen (¹⁵N), the stable isotope IS has three mass units more than cefazolin itself. The advantage of using the stable isotope IS was that other than the extra mass, it is identical to the compound of interest.¹⁰³ As a result, it behaves in the same way as cefazolin during sample preparation and analysis, but can also be uniquely identified compared to natural cefazolin owing to its higher mass. This reduces variation and improves accuracy and precision of analysis. The retention time for cefazolin and the IS was 2.8 minutes. The precursor to product ion transitions were *m/z* 454.6 to 322.9 for cefazolin and *m/z* 458.0 to 326.0 for the IS. Data acquisition and analysis was carried out using the LabSolutions system (Shimadzu Corporation, Kyoto, Japan).

The assay was based on a previously developed method to measure cefazolin in serum and adipose tissue.¹⁰⁴ However, for this study the method was validated over a wider concentration range following guidelines outlined in the Food and Drug Administration (FDA) Guidance for Industry: Bioanalytical Method Validation document.¹⁰⁵ These guidelines include acceptable measures of accuracy and precision and acceptable methods for standard curve construction and validation. Accuracy, as determined by repeated analyses of samples containing known quantities of the

analyte, is determined based on a minimum of five measurements per known concentration and a minimum of three different quality control concentrations covering the expected concentration range.¹⁰⁵ Accuracy of the assay is considered acceptable if the mean value of the measured concentrations is within 15% of the actual concentration, with the exception of the lower limit of quantification (LLOQ) at which the deviation could not exceed 20%. Precision describes how close individual measures of concentration are following repeated analysis. Similar to the determination of assay accuracy, a minimum of five measurements per known concentration with a minimum of three different quality control concentrations are utilized for precision analysis.¹⁰⁵ An acceptable coefficient of variation (CV) is 15% or less for the method to be considered adequately precise for analysis. The FDA guideline also advises that the same biological matrix be used during validation as was present in the study samples in order to minimize matrix effects.¹⁰⁵ Standard curve concentrations that cover the expected range of concentrations in the study and include a blank, a zero sample and six to eight other concentrations are advised. The blank sample consists of the biological matrix on its own, while the zero sample consists of the biological matrix with IS.¹⁰⁵ Per FDA guidelines, an adequate standard curve is one in which a minimum of four out of six non-zero samples have deviations of less than 15%. The exception to this is at the LLOQ, at which the deviation from known concentration could not exceed 20%. The LLOQ is identified as the lowest concentration on the standard curve that still has an analyte response of at least five times greater than that of the blank sample and a minimum precision and accuracy of 20% and 80 to 120%, respectively.¹⁰⁵ The FDA guidance document also advises that the simplest model be used to describe the relationship between concentration and response, which for our purpose was linear.

Calibration curves and quality controls were prepared using cefazolin sodium salt and IS in pooled normal human plasma (Innovative Research Inc., Novi, USA). Standard curves were validated for linearity and quantitative limits using PAR between cefazolin and its stable isotope IS. The curves were linear and quantitative from 4 to 100 mg/L for total cefazolin concentrations (93% accuracy, 97% precision) and 1 to 100 mg/L for free cefazolin concentrations (98% accuracy, 96% precision). Fresh standard curve and four quality control samples (5, 25, 50 and 75 mg/L) were prepared and analyzed on each day of analysis. All study subject samples were prepared and analyzed in duplicate. Samples outside the linear range of the assay or replicates with CV exceeding 10% were re-prepared, with or without dilution and re-analyzed.

For the measurement of total cefazolin concentrations, plasma samples were thawed at room temperature and the plasma sample and IS were combined. The solution was vortexed prior to the addition of 1 mL of acetonitrile at -20°C. The solution was vortexed then centrifuged at $15000 \times g$ for 10 minutes to separate plasma proteins. The supernatant was transferred to a new tube and dried *in vacuo* for 40 minutes at 45°C in a Savant SPD1010 vacuum centrifuge (Thermo Fisher Scientific, Waltham, USA). The samples were reconstituted in 200 µL of mobile phase and transferred to a VWR Centrifugal Filter 82031-358 (VWR International LLC, Radnor, USA) and re-centrifuged at $15000 \times g$ for 15 minutes to remove any particulate matter. The filtrate was transferred to HPLC vials and injected into the LC-MS/MS for analysis. For the measurement of free cefazolin concentrations, IS was added directly to the ultrafiltrate that was reconstituted in mobile phase prior to injection into the LC-MS/MS.

6.7.2 Albumin Assay

Albumin concentrations were measured in each plasma sample using an automated EasyRA® chemistry analyzer (Medica Corporation, Bedford, MA) with a concentration range of 4 to 70 g/L and limit of detection of 0.2 g/L. The EasyRa® automated albumin analyzer utilizes a colorimetric test that quantifies plasma albumin based on color intensity.¹⁰⁶ The reagent contains bromocresol green, which when bound to albumin, produces a blue-green color detected at a wavelength of 600 nm. The intensity of the blue-green color produced is proportional to the amount of albumin in the sample. Plasma study samples were thawed and mixed by inversion to ensure uniform reconstitution prior to transfer to the EasyRa® analyzer. A previous study by Cuhadar *et al.* demonstrated that up to seven repeated freeze-thaw cycles did not produce significant changes from baseline serum albumin concentrations as determined by a bromocresol green assay.¹⁰⁷ A new albumin Medica chemistry calibrator and reagent wedges were used on each day of sample analysis. To ensure accuracy and consistency, each sample was analyzed in duplicate.

6.8. Surgical Site Infection Follow-Up

Study subjects were monitored for the development of SSI during hospitalization using the CDC criteria for SSI surveillance.¹⁶ The CDC classifies SSIs based on the involved tissues and time of occurrence after surgery. Superficial incisional SSIs are defined as involving the skin or subcutaneous tissue of the surgical incision and occurring within 30 days after surgery.¹⁶ Deep incisional SSIs affect fascia and muscle layers, while organ/space SSIs involve any part of the body beyond the fascia and muscle layers. In cardiac surgery, the surveillance period for deep incisional and organ/space SSIs is extended to 90 days.¹⁶ Hallmark signs of SSIs include purulent drainage from the affected site, spontaneous dehiscence or deliberate reopening of the surgical wound and organisms identified from aseptically obtained fluid or tissue specimens.¹⁶

For cases of SSI, the date of occurrence, classification (i.e., superficial, deep or organ/space) and microbiology culture results, if available, were documented. The treatment for SSI, including name, dose, route and frequency of antimicrobial administration was detailed, as available. For study subjects who provided consent for postoperative follow-up, telephone interviews were conducted at 30 and 90 days after surgery to document the occurrence of clinically significant SSIs, defined as SSIs requiring systemic antimicrobial therapy. As telephone follow-up relied on study subject recall of SSI events, open-ended questions were posed to gather pertinent information related to the event. For SSIs that occurred after hospital discharge, patient description of the affected area was used to classify the wound.

7. OBJECTIVE 1. CEFAZOLIN CONCENTRATIONS

7.1. Methods

Study subjects, surgery characteristics and cefazolin AP were characterized using descriptive statistics. For continuous variables, mean and SD were reported for normally distributed data, otherwise median and interquartile range (IQR) were used. Categorical variables were presented as number per group and percentage of total population. For data analysis, cefazolin doses were normalized for body weight (i.e., mg/kg). Subjects classified with Class II or III obesity ($\text{BMI} \geq 35 \text{ kg/m}^2$)¹⁰⁸, cefazolin doses were normalized as mg/kg_{DW} using a dosing weight (DW) per Equation 2:

$$DW = IBW + 30\% (TBW - IBW) \quad \text{Equation 2}$$

Where ideal body weight (IBW) is 50 kg + 2.3 kg for every inch over 5 feet for males and 45 kg + 2.3 kg for every inch over 5 feet for females and total body weight (TBW) is the actual patient weight in kg.¹⁰⁹

The formula is based on a 30% correction factor for the Vd of drug in adipose tissue. The basis for the correction factor is the approximate 30% water content of adipose tissue, into which hydrophilic compounds such as cefazolin distribute. The use of total or ideal body weights to normalize cefazolin doses (i.e., mg/kg) would result in significant under- or over-estimations of cefazolin exposure, respectively. The decision to apply the dosage adjustment to subjects with Class II or III obesity was because physiological changes described in obesity are more

pronounced in the severely obese. Ultimately, the use of Equation 2 allowed for more accurate comparisons of cefazolin exposure between subjects.

Total cefazolin concentrations at peak, intraoperative trough and closure sampling times were characterized. Due to the importance of closure concentrations in providing effective AP¹¹⁰, total cefazolin concentrations at wound closure were analyzed compared to a target of ≥ 40 mg/L. In our study, a target cefazolin concentration of ≥ 40 mg/L was selected based on 80% protein binding, thus yielding an estimated target free concentration of ≥ 8 mg/L. At study start, cefazolin protein binding had been described in a small number of patients undergoing cardiac surgery with CPB.^{68,70,70,72} These studies reported a wide range of values, from 40 to 80% for cefazolin protein binding during cardiac surgery.^{68,71,71,72} The 80% protein binding estimate used in our study was based on the results published Hutschala *et al.* that cefazolin concentrations in subcutaneous interstitial fluid (i.e., free concentrations) were 22.6% of those in plasma.⁶⁸ Additionally, based on other studies of cefazolin protein binding available at the start of our study, 80% protein binding represented a conservative estimate of 20% for free, pharmacologically active cefazolin concentrations.

Our target of 8 mg/L for free cefazolin concentration is consistent with the CLSI susceptible breakpoint for *Enterobacteriaceae* spp. prior to its reduction to 2 mg/L in 2010 to better identify extended-spectrum β -lactamase (ESBL) producing organisms.⁷⁴ In addition, 8 mg/L was the susceptible breakpoint for cefazolin against staphylococci before ceftazidime testing for methicillin resistance became the default for anti-staphylococcal cephalosporins. Based on surgical pathogens

isolated from Canadian sites, the MIC₅₀ and MIC₉₀ of cefazolin are < 0.5 mg/L and 1 mg/L for MSSA, 2 mg/L and 8 mg/L for *E. coli*, and 2 mg/L and > 64 mg/L *Klebsiella* spp., respectively.¹¹¹ Based on data from the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the MIC₅₀ and MIC₉₀ of cefazolin against *S. epidermidis* are < 2 mg/L and < 32 mg/L, respectively.¹¹² Cephalosporins exhibit time-dependent activity, with maximum killing achieved at concentrations four times the MIC.¹¹³ At four times the MIC of susceptible skin flora (MICs ≤ 2 mg/L), a free cefazolin concentration of 8 mg/L was selected as it represented the start of bactericidal activity. As such, our target of 8 mg/L was expected provide reasonable AP coverage for the most likely SSI pathogens. However, it is important to acknowledge that achieving this target does not guarantee effectiveness against all possible cardiac SSI pathogens, as cefazolin does not have activity against all potential pathogens, including MRSA and *P. aeruginosa*.

Univariate analysis was conducted to identify associations between study subject-, surgery-, and cefazolin prophylaxis-related variables and the achievement of target total cefazolin concentration of ≥ 40 mg/L at wound closure. The tested variables included gender, age, body weight, obesity, Clcr, Charlson comorbidity index, type of cardiac surgery, net fluid balance, duration of surgery, duration of CPB, preoperative cefazolin dose, total (preoperative plus intraoperative) cefazolin dose and intraoperative complications.

The Student's t-test and Mann-Whitney U-test were used to assess differences between two continuous data sets. The parametric Student's t-test was applied to data with Gaussian distributions, while the non-parametric Mann-Whitney U-test was used for other data.¹¹⁴ For

differences between two groups of categorical data, the Pearson's Chi-square or Fisher's exact test were utilized, as appropriate. The Fisher's exact test was used in cases where the frequency in any cell was less than five, while for larger sample sizes the Pearson's Chi-square was utilized. Statistical analyses were conducted utilizing SYSTAT 12 (Systat Software Inc., San Jose, USA).

To further elucidate independent risk factors for below-target cefazolin closure concentrations, multivariate analysis of significant variables ($P < 0.1$) was conducted utilizing a backward, stepwise binary logistic regression model. Logistic regression determines odds ratios when exploring multiple explanatory variables. While similar to linear regression, logistic regression is utilized in the presence of a binomial response variable, such as closure concentrations above or below the target of 40 mg/L.¹¹⁵ By examining multiple variables, logistic regression enables the identification of independently associated variables for the response. In the backward, stepwise approach, plausible explanatory variables identified as significant in univariate analysis are included in the model. From this initial model, variables are removed one by one, in order of least significance. Once further elimination of variables does not result in an improved model, the remaining variables are considered to be independent possible explanatory variables for the response. Models were evaluated using Akaike information criterion (AIC) and Bayesian information criterion (BIC). The AIC and BIC were utilized to compare models and select the model of best-fit. The AIC and BIC are measures of model fit, such that lower values indicate the best model.¹¹⁶ However, the BIC penalizes the addition of variables more than the AIC, such that simpler models are preferred.

Classification and regression tree analysis (CART) was utilized to identify significant thresholds for increased risk of below-target closure concentrations. In this analysis, CART relied on a categorical outcome variable, i.e., above or below target closure concentrations. Predictor variables previously identified in the logistic regression analysis were utilized in the CART analysis. CART analysis conducted binary partitioning of the data until significant cut-offs in predictor variables were identified for the outcome of interest.¹¹⁷

7.2. Results

7.2.1. Study Subjects

A total of 60 study subjects were enrolled into the study. Of the 60 subjects, 55 completed the study and 4 were unable to participate as their surgeries were postponed beyond the study period. (Figure 2) One participant was removed from the study after developing signs and symptoms of an allergic reaction with the preoperative cefazolin dose. In that case, the allergic reaction was treated and the surgery proceeded with vancomycin administered for AP.

Characteristics of the study subjects are summarized in Table 2. The majority of subjects were male (38/55, 69.1%) and were classified as either overweight or obese, with ten subjects (18.2%) meeting the criteria for Class II or III obesity ($\text{BMI} \geq 35 \text{ kg/m}^2$). The most common comorbidities in the study population were hypertension, ischemic heart disease and diabetes mellitus. Sixty percent (33/55) of subjects had Charlson scores ≥ 3.0 with a predicted 10-year mortality rate of 59%. On the day of surgery, the majority of subjects (52/55, 94.5%) were classified by the anesthesiologist as ASA class IV, i.e., “a patient with severe systemic disease that is a constant

threat to life”.⁹⁶ Eight subjects (14.5%) were active smokers at the time of surgery, while 26 (47.3%) had a previous smoking history. Thirteen subjects (23.6%) received antimicrobial therapy within the three months prior to surgery, while six (10.9%) were hospitalized during that same time period.

7.2.2. Surgery Characteristics

The cardiac surgeries that study subjects underwent are detailed in Table 3. Of the 55 study participants, 26 (47.3%) underwent CABG alone, while 14 (25.5%) had a valve repair or replacement procedure. The remaining 15 (27.3%) subjects underwent CABG and/or valve repair or replacement along with another procedure, such as ascending aorta replacement, closure of patent foramen ovale (PFO) and left atrial appendage (LAA), atrial cryoablation or aortic dissection repair. Overall, 60% of the procedures performed included CABG with a median of 3 grafts performed per procedure. Grafts were most commonly harvested from the saphenous vein and the IMA (93.9% and 84.8%, respectively). Blood products, including platelets, fresh frozen plasma and red blood cells were administered in 30.9% (17/55) of cases. The median volume of administered blood products was 1500 mL (IQR 300, 2750). The blood products administered included platelets, red blood cells and fresh frozen plasma. IV albumin was not administered to any study subject. Fluid balance at the end of surgery was invariably positive at 3547 ± 1301 mL. The mean duration of surgery was 4.3 ± 1.7 hours with CPB for 2.2 ± 1.3 hours. Intraoperative complications occurred in 5.5% (3/55) of study subjects and re-exploration for postoperative bleeding was required in 9.1% (5/55) of cases. Most subjects were admitted to hospital on the day of surgery (47/51, 92.1%), and stayed for 5 days (IQR 4, 7) postoperatively.

7.2.3. Cefazolin Antimicrobial Prophylaxis

The cefazolin AP administered to study subjects is summarized in Table 4. The majority of study subjects (43/55, 78.2%) received a 2-gram preoperative cefazolin dose, while the remaining subjects received a 1-gram dose. The preoperative cefazolin dose was 21.4 ± 4.4 mg/kg_{DW}, where dosing weight was used in those with Class II or III obesity. The preoperative cefazolin dose was administered within 60 minutes before surgical incision in 52/55 (94.5%) of cases. Thirteen study subjects (23.6%) received only a preoperative cefazolin dose, while the majority (37/55, 67.3%) received one intraoperative dose and five (9.1%) received multiple intraoperative doses. Intraoperative doses were administered 3.9 ± 0.25 hours after the previous dose, consistent with the recommended intraoperative re-dosing interval of 4 hours. The total cefazolin dose, including preoperative and intraoperative doses was 3.3 ± 1.4 grams. Expressed as a function of subject weight, the total cefazolin dose was 39.7 ± 15.7 mg/kg_{DW}. The total dose normalized for surgery duration was 8.2 ± 2.2 mg/kg_{DW}/h.

7.2.4. Total Cefazolin Concentrations

A total of 136 blood samples were collected from the 55 subjects that participated in the study. This included 53 peaks, 30 intraoperative troughs and 51 closure samples (Figure 3). The remaining two samples were collected outside of the predefined sampling time points, including a “peak” sample that was collected more than 30 minutes after the preoperative dose and a “trough” sample collected more than an hour before the intraoperative dose. The total cefazolin concentrations at each sampling time are presented in Table 5. Total cefazolin concentrations in peak samples ranged from 91.1 to 225.1 mg/L with a median of 136.7 mg/L (IQR 115.6, 171.9).

The intraoperative trough concentrations ranged from 12.9 to 92.7 mg/L with a median of 46.7 mg/L (IQR 35.7, 52.4) and cefazolin closure concentrations ranged from 32.4 to 222.0 mg/L with a median of 89.4 mg/L (IQR 55.3, 140.9).

Forty percent (12/30) of intraoperative trough samples were below the target of 40 mg/L, along with 9.8% (5/51) of closure concentrations (Figure 4). Cefazolin concentrations were < 40 mg/L at some point during surgery (i.e., intraoperative trough or closure concentration) in 30.9% (17/55) of study subjects.

The results of univariate analysis of subject-, surgery, and prophylaxis-related variables associated with achievement of closure concentrations of ≥ 40 mg/L are detailed in Table 6. Female gender ($P = 0.037$), lower total body weight ($P < 0.001$), valve surgery ($P = 0.012$), shorter duration of surgery ($P < 0.001$), shorter duration of CPB ($P = 0.011$), a 1-gram preoperative dose ($P = 0.009$), lower preoperative cefazolin dose ($P = 0.045$), lower total cefazolin dose ($P = 0.002$) and lower total cefazolin dose for every hour of surgery ($P = 0.029$) were significantly associated with cefazolin closure concentrations below 40 mg/L.

To account for the small event rate of closure concentration below 40 mg/L ($n = 5$), two separate multivariate analyses were conducted, the first examining subject- and surgery-related factors and the second analyzing cefazolin prophylaxis-related variables. Prior to conducting multivariate analysis, potential correlations among variables such as duration of surgery and duration of CPB were investigated ($R^2 = 0.83$). In this case, duration of surgery was selected for inclusion in the

multivariate analysis as the more statistically significant and clinically relevant with regards to cefazolin AP. Of the subject- and surgery-related variables, lower body weight (OR = 1.22, 95% CI 1.02 – 1.46, $P = 0.027$) and shorter duration of surgery (OR = 1.07, 95% CI 1.001 – 1.15, $P = 0.045$) were independently associated with total cefazolin closure concentrations < 40 mg/L.

For the second multivariate analysis of prophylaxis-related variables, more comprehensive dosing variables normalized for body weight and time were developed. These included preoperative dose (mg/kg_{DW}), total cefazolin dose (mg/kg_{DW}) and total cefazolin dose per hour of surgery (mg/kg_{DW}/h). As in the previous analysis, potential correlations between variables were examined, such as total cefazolin dose (mg/kg_{DW}) and total cefazolin dose per hour of surgery (mg/kg_{DW}/h) ($R^2 = 0.65$). The latter was selected for inclusion in the multivariate as it incorporated both of the previously established independent risk factors of body weight and duration of surgery. Of the two significant preoperative cefazolin dose variables, the continuous mg/kg_{DW} variable was selected for inclusion into the multivariate analysis as it was deemed more informative for further analysis and clinical application. The second multivariate analysis of prophylaxis-related variables determined that only the total cefazolin dose per hour of surgery was significantly associated with below-target closure concentration (OR = 2.31, 95% CI 1.08 – 4.92, $P = 0.030$).

Of the three independent risk factors identified for below-target closure concentrations (lower body weight, shorter duration of surgery and lower total cefazolin dose per hour of surgery), only the total cefazolin dose per hour of surgery was modifiable through alterations to the AP regimen. As such, CART analysis was conducted to identify the threshold total cefazolin dose per hour of surgery required to meet the target closure concentrations. Based on this analysis, a total cefazolin

dose of 7.6 mg/kg_{DW} for every hour of cardiac surgery was identified as a significant threshold for maintaining target total closure concentrations above 40 mg/L.

7.3 Discussion

The variability in total cefazolin concentrations was one of the first observations noted after measurements were completed. Total intraoperative trough concentrations, which were collected immediately prior to an intraoperative cefazolin dose, ranged from 13 to 93 mg/L with 40% below the target of 40 mg/L. As all study subjects received intraoperative cefazolin doses four hours after the preoperative cefazolin dose, the variation in the measured concentrations cannot be explained by differences in the timing of cefazolin doses. Other potential sources of variability were examined with no association observed between intraoperative trough concentrations and either net fluid balance or CPB duration (Figures 5 and 6). Cefazolin trough concentrations were moderately correlated with Cl_{cr} (range 50 to 126 mL/min/72 kg) ($R^2 = 0.19$, Figure 7), as expected for a renally eliminated drug.³⁸ The most likely factor contributing to the significant variability in trough concentrations was the cefazolin dose, which ranged from 13.2 to 32.5 mg/kg_{DW}.

Significant variability was also observed in total cefazolin closure concentrations, which ranged from 32 to 222 mg/L. In addition to the influence of renal function and cefazolin dose, the time from the preceding cefazolin dose to wound closure would contribute to the variability in closure concentrations. In our study, nearly 10% of cefazolin concentrations at wound closure were below 40 mg/L, potentially increasing the risk of SSI. As previously stated, clinical practice guidelines recommend that serum and tissue concentrations remain above the MICs of potential SSI

pathogens for the entire duration of surgery.²⁵ The finding that almost one third of subjects had total cefazolin concentrations below 40 mg/L at some point during surgery suggested the need for further optimization of the AP regimen.

Our study identified that lower weight, shorter surgery duration and lower total intermittent cefazolin dose for every hour of surgery were independent risk factors for total cefazolin closure concentrations below the target of 40 mg/L. Patients with lower body weight received a lower dose of cefazolin for AP, resulting in below-target closure concentrations. Consistent with practice guidelines, cefazolin re-dosing occurred every 4 hours intraoperatively and as such did not occur during shorter surgeries. The lack of re-dosing combined with the short $t_{1/2}$ of cefazolin in patients with normal renal function may explain the lower cefazolin closure concentrations during shorter operations. Of the three independent predictors, total intermittent cefazolin dose for every hour of surgery was significant as the only modifiable risk factor for below-target closure concentrations. In our patient population with normal renal function (i.e., $\text{Clcr } 80 \pm 19 \text{ mL/min/72 kg}$), CART analysis identified a total intermittent cefazolin dose of 7.6 mg/kg_{DW} for every hour of surgery as a significant threshold.

Studies of alternative cefazolin AP regimens for cardiac surgery in adult patients are summarized in Table 7. Hutschala *et al.* and Andreas *et al.* used a relatively large preoperative cefazolin dose of 4 grams with re-dosing of 2 grams at skin closure.^{68,70,71} Regimens with re-dosing at particular stages such as skin closure can result in below-target concentrations due to variable durations of surgery. From our results for example, a 4-gram preoperative dose would have been sufficient in most patients for up to 6 hours, but re-dosing at skin closure would have occurred too late in 15%

of cases where the preoperative timing plus the duration of surgery exceeded 6 hours. Instead of using higher doses, Kosaka *et al.*, Odaka *et al.* and Hollis *et al.* describe AP regimens that added 1 gram of cefazolin to the CPB circuit in order to mitigate the effects of hemodilution with the priming fluid.^{65,72,118} The study by Asada also added cefazolin to the CPB circuit, but used a higher dose of 2 grams.¹¹⁹ The studies by Odaka and Hollis also utilized a shorter re-dosing interval of every 3 hours during surgery,^{72,118} while Trent Magruder *et al.* and Shoulders *et al.* re-dosed cefazolin every 2 hours during surgery.^{120,121} Finally, in the studies by Waltrip *et al.*, Adembri *et al.*, Trent Magruder *et al.* and Shoulders *et al.*, cefazolin was administered by continuous infusion to avoid the high peaks and low troughs characteristic of intermittent dosing.^{69,84,120,121} The retrospective analysis by Trent Magruder *et al.* reported significantly fewer SSIs of the venous harvest sites and the extremities in patients that received continuous infusions of cefazolin during cardiac surgery compared to those who received intermittent cefazolin dosing (0.3% versus 1.8%, $P = 0.03$).¹²⁰ However, the retrospective cohort study by Shoulders *et al.* failed to show a difference in overall SSIs between cefazolin AP administered by intermittent dosing versus continuous infusion (4.6% versus 1.7%, $P = 0.1$).¹²¹ Of note, the rate of superficial SSIs was lower with the latter (2.8% versus 0.4%, $P = 0.04$).¹²¹ Further study with a prospective design would be useful to confirm the potential clinical superiority of continuous infusions.

7.3.1. Knowledge Translation

Our study demonstrated that target closure concentrations of ≥ 40 mg/L were not achieved in nearly 10% of the studied subjects. Furthermore, it also identified a target dose necessary for achieving the target closure concentrations. Recognizing the clinical significance of these findings, further analysis was completed to translate these results to patient care.

Utilizing the 7.6 mg/kg_{bw}/h target total cefazolin dose per hour of surgery, a dosing schedule was developed to maintain target closure concentrations (Table 8). This dosing schedule suggested intermittent cefazolin doses (rounded to the nearest 250 mg) based on the patient's total body weight or dosing weight for patients with BMI ≥ 35 kg/m², as well as the dosing interval. The doses necessary to maintain target closure concentrations increased with body weight and dosing interval. It was apparent that a 1-gram cefazolin dose was inadequate even in patients with lower body weight when re-dosing occurs every 4 hours during surgery. Per our findings, a 1-gram dose in patients less than 70 kilograms would still require re-dosing every 2 hours during surgery. Based on 1-gram dosing increments, our data suggested that 2 grams of cefazolin be administered every 3 hours during surgery to achieve the aforementioned target total cefazolin dose per hour of surgery. Furthermore, a 3-gram cefazolin dose administered every 3 hours may be considered when the patient's total or dosing weight reaches 120 kg.

These findings were published in the Journal of Antimicrobial Chemotherapy (JAC)¹²² and subsequently generated a letter to the editor.¹²³ In their letter, Andreas *et al.* reported the cefazolin concentrations at wound closure using the AP regimen at their institution in which all patients received 4 grams of cefazolin preoperatively, followed by 2 grams at wound closure. Based on cefazolin concentrations measured in their previous studies, the authors reported that no patients experienced closure concentrations below 40 mg/L with the aforementioned AP regimen.¹²³ However, due to significant variability in cefazolin concentrations between patients, they reported borderline target attainment in certain patients. In our published reply and as outlined in Table 8, we demonstrated that a number of different AP regimens could be utilized to achieve target closure concentrations.¹²⁴ Our preference for fixed re-dosing intervals rather than re-dosing at wound

closure stemmed from the significant variation in surgery duration observed in our study. Per our results, a 4-gram preoperative dose would require re-dosing 6 hours later in patients with a dosing weight of 90 kg or more. In our study, 15% (8/55) of surgeries lasted longer than 6 hours and, in those cases, re-dosing at wound closure would have resulted in below-target closure concentrations. As such, a fixed re-dosing interval was preferred as it maintains target closure concentrations even during prolonged surgical procedures, which are not uncommon during cardiac surgery with CPB.

Based on our published findings, the cardiac surgery AP regimen at the St. Boniface Hospital was changed to cefazolin 2 grams preoperatively (3 grams if patient weighs more than 120kg) with re-dosing every 3 hours during surgery in patients whose Clcr was ≥ 50 mL/min/72 kg (Table 9). The postoperative AP regimen remained unchanged with re-dosing every 8 hours for 48 hours postoperatively. In December 2018, Alberta Health Services, an integrated health system providing health services to more than four million residents of the province of Alberta¹²⁵ published updated recommendations for AP regimens in adult patients.¹²⁶ In this document, a change to the cefazolin intraoperative re-dosing interval from every 4 hours to every 3 hours during cardiac surgery with CPB was recommended based on the published results of our study in JAC.

Next, simulation modeling was undertaken to examine whether changes to the AP regimen in patients with reduced renal function may be warranted. Our study excluded patients whose Clcr was below 50 mL/min/72 kg and thus direct evidence was not available for this patient population. However, our findings were used in simulations to extrapolate to this patient population based on the available evidence. Simulations were conducted to compare drug exposure with the newly

adopted cefazolin regimen between patients with normal and reduced renal function. The cefazolin drug exposure in a 24-hour period as represented by the area under the concentration time curve (AUC_{24h}) was predicted. A mean body weight of 85 kg and an AP regimen of 2 grams of cefazolin administered every 3 hours during surgery were used in the simulations. The above-mentioned body weight was chosen as it represented the mean body weight of all cardiac surgery patients at the St. Boniface Hospital between May and December of 2012. (S. Zelenitsky, University of Manitoba, private communication)

The AUC_{24h} was predicted for patients with Clcr of 10, 20, 30, 40, 50 and 100 mL/min/72 kg. In order to calculate AUC_{24h} , first the Clcr-adjusted half-life of cefazolin was determined (Equation 3), followed by the determination of the elimination rate constant (Equation 4).

$$t_{\frac{1}{2}}(\text{adjusted for Clcr}) = \frac{t_{1/2}(\text{normal renal function})}{[1 - fe \left(1 - \frac{Clcr}{100}\right)]} \quad \text{Equation 3}$$

Where Clcr is creatinine clearance, fe is fraction excreted unchanged in urine and $t_{1/2}$ is half-life

$$ke = \frac{0.693}{t_{1/2}} \quad \text{Equation 4}$$

Where ke is the elimination rate constant and $t_{1/2}$ is half-life

Postulating that the large volume of fluids administered during CPB would result in an expanded Vd of cefazolin, a conservative estimate of 15% increase in Vd from 0.15 to 0.17 L/kg was used to simulate CPB effects. The AUC was determined per Equation 5:

$$AUC_{24h} = \sum_{i=1}^n \left(\frac{C_{max_i}}{ke} - \frac{C_{min_i}}{ke} \right) \quad \text{Equation 5}$$

Where AUC_{24h} is the area under the concentration-time curve over a 24-hour period with a multiple-dose cefazolin AP regimen, and where n is the number of doses, C_{max} is the maximum concentration for a dose and C_{min} is the minimum concentration following a dose except for the last dose where C_{min} is the concentration at 24 hours.

The results of this simulation are presented in Figure 8 and demonstrate significantly higher cefazolin exposure in patients with reduced renal function if cefazolin was re-dosed every 3 hours during surgery without accounting for Cl_{cr} . The estimated exposure in patients with the most marked renal insufficiency ($Cl_{cr} = 10 \text{ mL/min/72 kg}$) was over 2-fold higher than that of a patient with Cl_{cr} of 50 mL/min/72 kg and 4-fold higher than that of a patient with Cl_{cr} of 100 mL/min/72 kg . As such, it was clear that an alternate dosing regimen was necessary for patients with reduced renal function.

In developing our recommendation for an alternative AP regimen, we aimed to combine target attainment and cefazolin exposure (i.e., AUC), while also considering simplicity and convenience in order to minimize the chance of dosing errors. As such, a regimen was proposed in which cefazolin would be administered as 2 grams (or 3 grams if $> 120\text{kg}$) within one hour prior to surgical incision, with a subsequent dose delivered after 3 hours and another dose 8 hours after the second dose. (Table 9) Thereafter, postoperative dosing would continue with the dosing interval based on Cl_{cr} such that patients with Cl_{cr} from 30 to 49 mL/min/72 kg would receive cefazolin every 8 hours for up to 48 hours postoperatively while those with Cl_{cr} from 11 to 29 mL/min/72 kg

kg would receive cefazolin every 12 hours for the same duration. Postoperative re-dosing of cefazolin was not recommended for patients with $\text{Clcr} \leq 10 \text{ mL/min/72 kg}$. The aforementioned AP regimen resulted in comparable cefazolin exposure in patients with $\text{Clcr} < 50 \text{ mL/min/72 kg}$ and patients with normal renal function. The $\text{AUC}_{24\text{h}}$ of patients with Clcr of 30 and 40 mL/min/72 kg more closely mirrored those in patients with $\text{Clcr} \geq 50 \text{ mL/min/72 kg}$ (Figure 9). While the $\text{AUC}_{24\text{h}}$ in patients with Clcr of 10 mL/min/72 kg was estimated to be 40% higher than that of a patient with Clcr of 50 mL/min/72 kg , these patients would not receive any further cefazolin doses and as such the exposure over the entire AP duration was expected to be similar. The proposed regimen for cefazolin AP for patients with renal insufficiency undergoing cardiac surgery with CPB was adopted into clinical practice at the St. Boniface Hospital.

8. OBJECTIVE 2. CEFAZOLIN PROTEIN BINDING

8.1. Methods

Free cefazolin peak, intraoperative trough and closure concentrations were characterized. The protein binding of cefazolin was calculated as the difference between the total and free measured cefazolin concentrations as a percentage of the total concentration. The percentage of protein binding for all samples was characterized. The overall associations between protein binding and patient demographic, health variables (i.e., gender, age, body weight, BMI, Clcr) and surgery characteristics (i.e., net fluid balance, duration of surgery and duration of CPB) were tested. For continuous variables (e.g., age), linear and non-linear correlations with protein binding were analyzed using the Pearson and Spearman's rho test, respectively. The parametric Pearson correlation coefficient (r) was utilized to quantify the strength of linear relationships between normally distributed variables, whereas the non-parametric Spearman's rho test was used to test monotonic relationships between variables.¹¹⁴ Correlations were described as weak, moderate or strong depending on the value of r . Weak correlations were those with r less than 0.3, while moderate and strong correlations had r between 0.3 and 0.7, and greater than 0.7, respectively. For categorical variables (e.g., gender), significant differences in protein binding between groups were analyzed using the Student's t-test.

Next, intra-subject variability in protein binding was investigated, as was the influence of sample timing relative to CPB, cefazolin concentration and albumin concentration. Intra-subject variability was assessed by comparing protein binding in paired samples collected from the same

subject. The paired, rather than the unpaired Student t-test was used for intra-subject analysis.¹¹⁴ The potential influence of sampling time with respect to CPB was examined by comparing protein binding in samples collected before starting CPB (pre-CPB), during CPB and after stopping CPB (post-CPB). The potential effect of cefazolin concentration on protein binding was determined by examining the association between free and total cefazolin concentrations. This association was described overall and separately for samples collected pre-CPB and those collected during/post-CPB. Deviations from linearity in the relationship between total and free cefazolin concentrations were suggestive of protein binding saturation. Finally, the influence of albumin concentrations on protein binding was tested overall and separately for samples collected pre-, during and post-CPB. Correlations were analyzed using the Pearson and Spearman's rho test, as appropriate.

8.2. Results

8.2.1. Free Cefazolin Concentrations

A total of 135 blood samples including 52 peaks, 30 intraoperative troughs and 51 closure samples were analyzed for free concentrations. Due to hemolysis of one peak sample, ultrafiltration and the subsequent measurement of free concentration could not be carried out. The free cefazolin concentrations at each sampling time point are presented in Table 5. Free cefazolin concentrations in peak samples ranged from 18.5 to 75.3 mg/L, with mean value of 37.8 ± 13.1 mg/L. The free intraoperative trough concentrations ranged from 4.4 to 29.4 mg/L with a median of 11.9 mg/L (IQR 9.6, 16.8), whereas free closure concentrations ranged from 4.9 to 99.9 mg/L with a median of 26.2 mg/L (IQR 12.2, 47.5).

8.2.2. Cefazolin Protein Binding

The protein binding of cefazolin during cardiac surgery with CPB is summarized in Table 10 where values ranged from 48.9 to 88.1% with a mean of $71.9 \pm 8\%$. Protein binding was similar in samples collected from males ($n = 93$) and females ($n = 42$) (73.0% (IQR 67.9, 76.5) versus 74.1% (IQR 68.0, 79.0), $P = 0.143$, Figure 10). There was no significant difference in protein binding between samples collected from obese ($n = 69$) and non-obese ($n = 66$) study subjects (72.2% (IQR 67.1, 76.6) versus 74.0% (IQR 68.8, 76.9), $P = 0.597$, Figure 11). There was no association between protein binding and subject age, weight, BMI, Clcr, net fluid balance or duration of surgery (Figure 12). There was a moderate correlation between protein binding and CPB duration, limited to samples collected during CPB ($R^2 = 0.24$, Figure 13).

The protein binding of cefazolin was influenced by CPB with mean values of $74.4 \pm 5.2\%$, $69.9 \pm 8.2\%$, and $70.4 \pm 8.6\%$ in samples collected pre-CPB, during CPB and post-CPB, respectively. This corresponded to an increase in free fraction from 25.6% pre-CPB to 29.7% during/post-CPB ($P = 0.002$, Figure 14). Intra-subject variability in protein binding was analyzed in 52 paired subject samples. Three subjects did not have a second sample available for analysis as two had samples collected during the distribution phase and one had a change in OR. The protein binding was significantly different between the paired samples ($74.8 \pm 5.4\%$ versus $69.6 \pm 9.3\%$, $P < 0.001$, Figure 15). The influence of CPB may explain the intra-subject variability in protein binding observed as the first set of samples were collected pre-CPB, while the second set was collected during or post-CPB.

When all the study samples were examined together, non-linear (i.e., saturable) protein binding was observed in the association between free and total cefazolin concentrations (Figure 16. A). Although initially the overall association between free and total cefazolin concentrations was deemed non-linear, on further analysis two distinct populations emerged; one consisting of samples collected pre-CPB and the other of samples collected during/post-CPB (Figure 16. B). This further examination demonstrated linear protein binding in samples collected pre-CPB, while non-linear protein binding was limited to samples during/post-CPB. Non-linear protein binding was observed over a wide concentration range of 13 to 222 mg/L, while linear protein binding was observed for pre-CPB samples with total concentration range from 91 to 225 mg/L.

The albumin concentrations in the 135 plasma samples collected during cardiac surgery ranged from 16 to 42 g/L (Table 11). The mean albumin concentration in all samples was 31 ± 5 g/L. Albumin was significantly higher pre-CPB (35 ± 3 g/L) compared with during CPB (27 ± 4 g/L, $P < 0.001$) or post-CPB (29 ± 3 g/L, $P < 0.001$). This difference was also significant when comparing albumin concentrations pre-CPB and during/post-CPB (35 g/L (IQR 33, 37) versus 29 g/L (IQR 27, 31), $P < 0.001$, Figure 17). Although there was a significant reduction in albumin concentrations during CPB, only a moderate correlation was observed between protein binding and albumin concentrations which was limited to samples collected during CPB ($R^2 = 0.12$, Figure 18).

8.3. Discussion

To our knowledge, this is the largest study to date to characterize the protein binding of cefazolin in adults undergoing cardiac surgery with CPB. The mean overall protein binding was $72 \pm 8\%$,

which is lower than the 80% that is typically referenced for cefazolin. This translates to a significant difference in free fraction in our study (i.e., 28%) compared to the generally accepted 20%. However, it is the free antimicrobial concentrations that are relevant for activity and an increase in free fraction doesn't directly translate to an increase in free concentrations as it is accompanied by increased drug clearance. This was confirmed in a recent study of ceftiofur PK in abdominal surgery by Boisson *et al.*¹²⁷ The authors found that albumin concentrations decreased with a corresponding increase in ceftiofur free fraction over time during prolonged abdominal surgeries (median 6.5 hours). However, the reduction in albumin concentrations was also associated with a decrease in total drug exposure (AUC_{total}), but not in free drug exposure (AUC_{free}).¹²⁷ Of note, although the free fraction of ceftiofur was higher, the free concentrations remained constant. This observation led to the conclusion that dose adjustments were not necessary despite the observed changes in albumin and free fraction.¹²⁷ Our findings were similar with an observed decline in albumin concentrations during surgery, although albumin was only moderately correlated to protein binding in our study. Our findings were consistent with their observations that decreasing albumin concentrations during surgery were accompanied by lower AUC_{total} but unchanged AUC_{free} . In our study, albumin and total cefazolin concentrations decreased, while free cefazolin concentrations remained relatively stable (Figure 19). An increase in free fraction results in an increase in total drug CL, without affecting free drug CL, which is why an increase in free fraction may be accompanied by a reduction in total, but not free concentrations.

The protein binding of cefazolin in our study can be compared to that in other surgical populations as summarized in Table 12. The mean protein binding of 72% in our study is in the middle of the range of median protein binding (i.e., 61% to 87%) reported in the non-cardiac surgical population.

Studies by Van Kralingen and Douglas in adults receiving cefazolin AP for abdominal surgery reported protein binding of 79% and 87%, respectively. These results are higher than those in our study and closer to the standard 80%.^{128,129} Meanwhile, studies by Naik in those undergoing major urological or spinal surgeries and Allegaert in pregnant women undergoing *in utero* surgery found protein binding closer to our own results at 69% and 75%, respectively.^{130,131} The physiological changes that take place during pregnancy, including expanded Vd and reduction in albumin may have resulted in a reduction in protein binding from the typical 80% to the 75% observed in the study by Allegaert. Indeed, the median albumin concentration in the patients studied by Allegaert *et al.* was 36 g/L.¹³⁰ Smits *et al.* reported the protein binding of cefazolin in neonates undergoing various surgical procedures.¹³² The median protein binding in neonates of 61% was lower than our results and was also the lowest value reported in non-cardiac surgery.¹³² This difference was not unexpected as neonates possess physiological characteristics unique from adults, including expanded extracellular fluid volume, reduced glomerular filtration rate and hypoalbuminemia, all of which affect may affect the protein binding of cefazolin.¹³²

None of the previously mentioned studies required the use of CPB, which had a prominent effect on protein binding in our study. As such, we also compared our results to those of studies of cefazolin protein binding in cardiac surgery with CPB.(Table 12) The protein binding of cefazolin in these studies was variable, ranging from less than 40% to 81%.^{68,70–72,89,119} The protein binding in our study was higher than those in three of the previous studies. In two studies by Andreas *et al.*, the protein binding of cefazolin in patients undergoing CABG was 40% and 57%.^{70,71} In a similar patient population, Hollis *et al.* reported protein binding ranging from 53% to 75%.⁷² The variability in protein binding among these studies may be explained by the small sample size of 8

to 10 patients which also may have made their findings more sensitive to outliers. The results of our study were more consistent with the two more recent larger trials of cardiac surgery patients.^{89,119} The study by De Cock *et al.* included 56 pediatric patients and reported a median protein binding of 72% (range 64% to 77%) compared to our median of 72% (range 68% to 77%).⁸⁹ The study by Asada *et al.* of 27 adult patients did not report an overall protein binding value and instead reported the protein binding before incision, before and during CPB and at wound closure, which ranged from 55% to 79%.¹¹⁹

Our findings demonstrated that protein binding is not a static process during cardiac surgery with CPB, which is an important consideration when assessing the adequacy of AP regimens. Similar to the findings by Asada and De Cock, we observed a difference in protein binding depending on the time of sample collection with respect to CPB, such that protein binding was higher pre-CPB than during/post-CPB (74% versus 70%, $P = 0.002$). In the former, the median cefazolin protein binding before, during and after CPB was 79%, 55% and 75%, respectively.¹¹⁹ Significant PK alterations take place during cardiac surgery with CPB, some of which could impact protein binding.^{79,81} Drug adsorption to the surface and subsequent sequestering in the CPB apparatus has been described for other antimicrobials such as vancomycin.⁷⁹ Denaturation of plasma proteins can also occur due to contact with the tubing or other parts of the CPB apparatus. The priming fluid administered at the start of CPB decreases the plasma protein concentrations by up to 50% which may reduce protein binding by reducing availability of binding sites.¹³³ Since cefazolin binds to albumin¹³⁴, the relationship between albumin concentrations and cefazolin protein binding was examined. Similar to protein binding, albumin concentrations were significantly lower during/post CPB compared to pre-CPB (35 g/L (IQR 33, 37) versus 29 g/L (IQR 27, 31), $P <$

0.001). Although CPB had an effect on both albumin concentrations and protein binding, no significant relationship was observed between albumin concentrations and protein binding. This suggested that CPB is an independent driver of the observed changes in both albumin and protein binding.

Our study also demonstrated the presence of protein binding saturation during/post-CPB. Cefazolin protein binding saturation has previously been reported in various patient populations including adults, children and pregnant women.^{89,130,132,135,136} However, only the study by De Cock examined a population of patients undergoing cardiac surgery with CPB. Similar to the conclusions by De Cock *et al.*, our results demonstrated that cefazolin protein binding is saturable during cardiac surgery with CPB. However, in our study it was determined that the saturation was limited to samples collected during/post-CPB.

The characterization of protein binding of antimicrobials is of clinical relevance. As free drug concentrations are pharmacologically active, the extent of protein binding of an antimicrobial is an important aspect to estimating its efficacy. Due to the dynamic nature of the protein binding described in our study, it would be inappropriate to model the activity of cefazolin based on a single estimate of protein binding. The most appropriate approach would be to measure both total and free concentrations and determine protein binding directly. However, if that is not possible, estimates accounting for the range of protein binding observed and accounting for differences in protein binding with respect to CPB would be the next best approach.

When combined with other PK parameters, knowledge of protein binding and its variability in the cardiac surgery population allows for more accurate predictions of free cefazolin concentrations

over time during cardiac surgery. As such, our findings will enable the construction of more accurate PK models of cefazolin in this patient population. Combining PK models with Monte Carlo simulations of target attainment can enable researchers to test the ability of AP regimens to meet PD targets. As such, optimization of AP can be carried out without the need for extensive testing in human subjects and various AP regimens can be studied and compared simultaneously.

9. OBJECTIVE 3. CEFAZOLIN POPULATION PHARMACOKINETICS

9.1 Methods

Population-pharmacokinetic (pop-PK) modeling was conducted to characterize PK variables, including the V_d and k_e of cefazolin during cardiac surgery with CPB. Pop-PK modeling was the approach selected for the identification and measurement of variability in PK parameters in the studied population utilizing sparse sampling within subjects. The pop-PK analysis was carried out using Pmetrics software (Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, CA) with the nonparametric adaptive grid (NPAG) algorithm.^{137–139} The advantage of nonparametric pop-PK modeling is the lack of assumption regarding the distribution of PK parameters.¹⁴⁰ This is in contrast to parametric modeling such as NONMEM, which assumes that PK parameters have normal or log-normal distributions. As such, nonparametric methods enable the identification of discrete subpopulations within a larger population, for example fast and slow drug metabolizers. The NPAG algorithm “calculates the maximum likelihood estimate of the population distribution with respect to all distributions.”¹⁴¹ As it provides exact, compared to approximate maximum likelihoods, outliers and sub-groups can be readily identified.¹⁴¹

The analysis was carried out using a one-compartment model of cefazolin with first-order processes. The variables incorporated into the model were cefazolin dose, time of cefazolin administration, time of blood sample collection, the cefazolin concentration measured in the blood sample, as well as study subject age, gender and Cl_{cr} . Estimates of assay error for the total cefazolin assay developed for this study were included in the modelling process as a polynomial,

$SD = \gamma (0.2188 + 0.0342x + 0.0004x^2)$. The x represents the cefazolin concentration and γ (γ) signifies the “extra process noise related to the observation, including mis-specified dosing and observation times.”¹³⁸ Gamma values between 1 and 5 are considered acceptable in terms of model specification and data ‘noise’. In our model, the gamma was 2.3. The fit of the model was assessed by visual inspection of the observed concentration versus model-predicted concentration plot, the coefficient of determination of the linear regression of the aforementioned plot, as well as the log-likelihood value. The AIC and BIC were also utilized to assess model fit, as previously described.¹¹⁶ The pop-PK estimates of ke and Vd were described, and the overall CL and $t_{1/2}$ were calculated per Equations 6 and 7.

$$CL = ke \times Vd \quad \text{Equation 6}$$

$$t_{\frac{1}{2}} = \frac{0.693}{ke} \quad \text{Equation 7}$$

The pop-PK model was then utilized to calculate individual study subject’s values for ke and Vd . Using this information, CL and $t_{1/2}$ were calculated for each study subject. Subsequently, subject- and surgery-related variables were tested for association with the individual model-predicted PK parameters. These variables included subject weight, net fluid balance at the end of surgery, the duration of surgery and the duration of CPB.

9.2. Results

The pop-PK model was based on data from 55 subjects who had a mean age, weight and Clcr of 65 ± 10 years, 90 ± 17 kg and 80 ± 19 mL/min/72 kg, respectively. The pop-PK model included measured cefazolin concentrations from 136 blood samples, including 53 peaks, 30 intraoperative troughs and 51 closure samples.

The pop-PK parameters for k_e and V_d were 0.35 ± 0.07 h⁻¹ and 0.14 ± 0.05 L/kg, respectively. (Figure 20) The overall calculated $t_{1/2}$ and CL were 2.0 h and 0.05 L/kg/h (0.8 mL/kg/min). The final pop-PK model converged after 118 runs with a strong correlation between the observed and model-predicted cefazolin concentration values ($R^2 = 0.73$). (Figure 21. A) The bias and precision of the model were 0.6 mg/L and 6.3 mg/L, respectively. When the data was examined using the maximum *a posteriori* Bayesian estimation step, the coefficient of determination was 83% with a bias and precision of -0.2 mg/L and 0.8 mg/L, respectively. (Figure 21. B)

The individual values for k_e and V_d were determined for each study subject. One subject was removed from further analysis after being identified as an extreme outlier using the 1.5 x IQR rule. The individual values for k_e ranged from 0.27 to 0.63 h⁻¹ with a mean value of 0.35 ± 0.06 h⁻¹. (Figure 22. A) The V_d ranged from 7.3 to 20.6 L, with a mean value of 12.2 ± 3.0 L. (Figure 22. B) The mean calculated $t_{1/2}$ was 2.0 ± 0.3 h and median CL was 3.9 L/h (IQR 3.3, 4.7). (Figure 23)

Results of covariate analysis demonstrated a strong correlation between study subject weight and Vd ($R^2 = 0.67$, Figure 24) while there was no association between Vd and the net fluid balance at the end of surgery. (Figure 25) A moderate correlation was observed between Clcr and ke ($R^2 = 0.24$, Figure 26), while there was no association between ke and either duration of surgery or duration of CPB. (Figure 27) Finally, a moderate correlation was observed between cefazolin CL and both study subject age ($R^2 = 0.16$, Figure 28. A) and creatinine clearance ($R^2 = 0.20$, Figure 28. B).

9.3. Discussion

Using pop-PK modeling, our study described pertinent PK parameters of cefazolin during cardiac surgery with CPB. Our results demonstrated a strong correlation between study subject weight and Vd. Total body water increases with body weight and as such the distribution space of hydrophilic drugs, such as cefazolin, is also increased. The moderate correlation between Clcr and ke was likely due to the limited range of Clcr in our study. As we excluded subjects with $\text{Clcr} < 50 \text{ mL/min/72 kg}$, the entire range of renal function was not represented in our study and as such the comprehensive relationship between Clcr and ke could not be described by our findings. The observation that CL was higher in younger study subjects is likely due to an age-related decline in renal function. The correlation between age and creatinine clearance in our study is shown in Figure 29 ($R^2 = 0.44$)

The results of studies examining cefazolin PK in healthy volunteers are summarized in Table 13. The $t_{1/2}$ in our study was 2.0 h while in healthy volunteers (21 to 42 years of age) it ranged from 1.2 h to 2.0 h after IV administration.^{75,77,78} Our $t_{1/2}$ estimate was at the upper end of the $t_{1/2}$ range

from healthy volunteers, which may be expected given the age and comorbidities of our study population. This suggests that at best CPB had modest effects on prolonging the elimination rate in our study population. The mean Vd of cefazolin in individual study subjects (i.e., $12.2 \text{ L} \pm 3.0 \text{ L}$) was higher compared to the 6.9 to 10.4 L in healthy volunteers^{75,77,78} The body weight range of the volunteers was only reported in the study by Rattie *et al.* where it ranged from 57 to 100 kg, compared to 60 to 125 kg in our analysis.⁷⁷ As such, it is not clear if a difference in body weight of the studied populations could have led to the observed difference in Vd. Another possible contributing factor for the observed difference may be the significant volume of fluids administered during cardiac surgery with CPB. The increased Vd may be a function of the net positive fluid balance at the end of surgery of $3.5 \text{ L} \pm 1.3 \text{ L}$. Additionally, the systemic inflammatory response generated by CPB may result in capillary leak syndrome, which may impact the volume of distribution of drugs.¹⁴² This phenomenon has been described in sepsis, where capillary leak syndrome and the resultant edema, along with IV fluids contribute to a substantial increase in Vd of hydrophilic antimicrobials, including β -lactams.¹⁴³ In our study, the Vd was only slightly higher than that observed in healthy adults, which may suggest a modest influence of the systemic inflammatory response on Vd by CPB as compared to sepsis.

Other studies of cefazolin PK during surgery are summarized in Table 13. Most of the data in adults is in non-cardiac surgeries, while data in cardiac surgery is largely derived from pediatric patients. The studies by Koopman *et al.*, Douglas *et al.* and Naik *et al.* examined the PK of cefazolin in adults undergoing major surgery.^{129,131,144} However, Koopman *et al.* reported free drug CL and Vd, which limits the comparison of their results to others.¹⁴⁴ The parameter estimates for CL in the studies by Douglas and Naik were 3.0 L/h and 4.7 L/h, respectively.^{129,131} The pop-PK-

derived CL in our study of 0.05 L/kg/h or 4.5 L/h for an average 90 kg patient was consistent with these results. Interestingly, the mean Vd of 0.14 L/kg (12.6 L for an average 90 kg patient) in the study by Douglas *et al.* in patients undergoing abdominal aortic aneurysm repair was the same as in our study.¹²⁹ The median weights of the studied subjects were similar in the study by Douglas 88 kg (IQR 81, 95) and in our own 88 kg (IQR 79, 100). As in our study, patients in the Douglas study received large volumes of IV fluids perioperatively (median 3.2 L, IQR 2.6, 4.0).¹²⁹ These similarities may explain the similarities in Vd between our study and that of Douglas *et al.* Both of the studies by Douglas and Naik identified Clcr as a covariate for cefazolin CL, whereas a moderate correlation between Clcr and CL was observed in our analysis as explained above.^{129,131}

The studies by Van Kralingen, Brill and Palma examined cefazolin PK in obese adults undergoing gastrointestinal surgery. On initial examination, the Vd reported by Van Kralingen and Palma were consistent to that in our study at 13.0 L and 9.88 L, respectively.^{128,145} However, the study subjects in the studies by Van Kralingen and Palma had mean weights of 151 ± 35 kg and 131 ± 25 kg, respectively compared to 90 ± 17 kg in our analysis. As such, although the Vd in liters was comparable, the Vd expressed as L/kg would have been smaller in those studies than in our own. Similar to our result, both Van Kralingen and Brill reported that Vd correlated with body weight.^{128,136} The pop-PK model in the study by Brill included data from both morbidly obese and non-obese subjects and as the overall weight of the entire group was not described, further comparisons are limited.¹⁴⁵ The CL reported by Brill *et al.* of 22.2 L/h was based on unbound drug and as such not comparable to our results, while those reported by Van Kralingen and Palma were more consistent with our results at 4.2 L/h and 4.88 L/h, respectively.^{128,136,145} In our study, as in the studies of Van Kralingen and Brill, body weight was strongly correlated with Vd. However,

the two aforementioned studies identified age as a covariate for cefazolin CL, whereas a moderate correlation was observed between those variables in our results.^{128,136}

The PK of cefazolin in adults undergoing cardiac surgery with CPB was studied by Miller *et al.*, Lehot *et al.* and Lanckohr *et al.* The study by Miller *et al.* was especially interesting as it compared PK parameters in eight patients before, during and after cardiac surgery with CPB.⁸² The results demonstrated an increase in both $t_{1/2}$ and Vd, with a reduction in CL during surgery. In comparison, the PK parameters are quite similar before and after surgery. During surgery, the $t_{1/2}$ and Vd were 11.0 ± 8.1 h and 0.26 ± 0.07 L/kg, respectively, while CL was 1.6 ± 1.0 L/h.⁸² These results were higher than the estimates determined by our pop-PK analysis despite the fact that the studied population was younger (59 ± 7 years) and had a lower body weight (71 ± 16 kg). However, the study subjects in Miller *et al.* had lower Clcr, ranging from 35 to 79 mL/min/72 kg, with a mean of 59 ± 14 mL/min/72 kg, compared to 80 ± 19 mL/min/72 kg in our study. The reduced Clcr would have resulted in a reduction in elimination rate, which explains the longer $t_{1/2}$ and lower CL. The difference in Vd (0.26 L/kg versus 0.14 L/kg), however, may be due to the effect of an outlier on the overall estimate. While seven out of the eight studied patients had a body weight between 53 and 76 kg, a single patient weighed 108 kg. In a study with a small sample size, this would have a vast impact on the mean estimate of Vd.

In the studies by Lehot and Lanckohr, the Vd in adults undergoing cardiac surgery with CPB was 0.14 ± 0.03 L/kg and 15.8 L, respectively which was comparable to the 0.14 L/kg (12.6 L in 90 kg patient) in our study.^{83,146} The $t_{1/2}$ reported by Lehot *et al.* of 3.9 ± 2.1 h was longer than in our own study.⁸³ Interestingly, the study subjects in the Lehot study had a similar Clcr (75 ± 26

mL/min/72 kg) to ours.⁸³ As such, we would have expected the $t_{1/2}$ to be similar to our results. The longer $t_{1/2}$ may be due to the small sample size (10 subjects) in their study, which may have been more sensitive to outliers. This explanation is supported by the large standard deviation in the $t_{1/2}$ estimate in the study by Lehot. The CL of 0.06 L/kg/h in the study by Lehot was similar to that of our pop-PK model of 0.05 L/kg/h (4.5 L/h for 90 kg subject) and also consistent with that reported by the larger study by Lanckohr *et al.* of 5.23 L/h.^{83,146}

As previously mentioned, the bulk of the cefazolin PK data during cardiac surgery with CPB is in children. The studies by De Cock *et al.* and Cies *et al.* examined cefazolin PK in children ranging from newborns to teenagers.^{89,147} De Cock reported overall PK estimates for all subjects ranging from 6 days to 15 years, while Cies reported them separately by age, making a direct comparison of the results from these two studies difficult. Furthermore, in the study by Cies, cefazolin was mixed directly into the CPB priming fluid which may have resulted in a slower cefazolin CL.¹⁴⁷ A comparison of the results of the studies by De Cock and Cies to our own is limited as children are physiologically unique from adults. The central and peripheral Vd reported by the De Cock study were 1.9 L and 2.4 L, respectively, the sum of which (4.3 L) is much smaller than the 12.6 L in our study, but is not unexpected as our study population was much larger physically (median body weight of 8 kg versus 88 kg).⁸⁹ The CL reported in the De Cock study was also lower (1.6 L/h versus 4.5 L/h) which may be explained by differences in body weight and drug clearing capabilities between adults and children.

10. OBJECTIVE 4. CEFAZOLIN PHARMACODYNAMICS

10.1. Methods

PD analysis was carried out in study subjects that had evaluable closure concentrations and had consented to SSI follow-up after hospital discharge (Figure 2). Study subjects were monitored for SSI development using the CDC criteria for SSI surveillance previously described. The SSI events were described, including SSI classification (i.e., superficial, deep or organ/space), microbiology culture results and the prescribed treatment. The prevalence of infection at 30 and 90-days post-surgery was determined.

The study subjects, as well as their surgery and AP characteristics were characterized using descriptive statistics. For data analysis, cefazolin doses were normalized for body weight (i.e., mg/kg). For subjects with BMI ≥ 35 kg/m², cefazolin doses were normalized as mg/kg_{DW} as previously described in Objective 1. Univariate analysis was used to examine potential subject-, surgery- and prophylaxis-related risk factors for SSI. Subject-related variables included gender, age, weight, BMI, Clcr, diabetes and Charlson comorbidity index. Surgery-related variables tested were type of surgery, duration of surgery, albumin and glucose concentrations, net fluid balance, surgery complications and length of hospital stay. Prophylaxis-related risk factors assessed included the preoperative cefazolin dose, timing of preoperative cefazolin administration, intraoperative re-dosing during surgery, total cefazolin dose during surgery and total and free cefazolin concentrations at wound closure. Comparisons were made using two-tailed Student's t-test, Mann Whitney U-test, or Fisher's exact test, as appropriate.

Significant variables ($P < 0.1$) were included in multivariate logistic regression analysis to assess for association with developing SSI following cardiac surgery. Area under receiver operating curves (AUROC) and Hosmer-Lemeshow goodness-of-fit test were used to evaluate the multivariate models. The Hosmer-Lemeshow chi-square test assesses model fit by comparing the “observed frequencies of cases and controls in the sample with their expected values under the model”.¹⁴⁸ A non-significant Hosmer-Lemeshow statistic denotes that the number of cases and controls predicted by the model were not significantly different from the observed number of cases and controls, i.e., the model is empirically consistent. To examine the predictive power of the model, AUROC was utilized. AUROC is the area under the receiver operator curve, on a graph of sensitivity versus specificity. AUROC values describe the “likelihood that a case will have a higher predicted probability of the event than a control across the range of criterion values investigated.”¹⁴⁸ An AUROC value of 0.5 denotes a model that lacks predictive power, while an AUROC value of 0.7 or greater is generally considered an acceptable model.¹⁴⁸

CART analysis was utilized to identify significant thresholds for increased risk of SSI for continuous variables. In this study, the CART analysis relied on the categorical outcome variable presence or absence of SSI. Predictor variables previously identified in multivariate logistic regression analysis were utilized in the CART analysis which conducted binary partitioning of the data until significant cut-offs for presence of SSI were identified.

10.2. Results

Eight episodes of superficial SSI were documented in 44 subjects who had follow-up at 30 days (18.2%, 8/44). No deep incisional or organ/space SSIs were reported in 42 patients contacted at 90 days. As such, the PD study was conducted based on the 30-day SSI data and was completed in the study subjects that also had evaluable cefazolin closure concentrations ($n = 40$)

The characteristics of the study subjects included in the PD analysis are detailed in Table 14. The majority of the subjects were male (25/40, 62.5%) with a mean age and weight of 65 ± 10 years and 88 ± 16 kg, respectively. The Clcr ranged from 50 to 126 mL/min/72 kg with a mean of $80 + 18$ mL/min/72 kg. Four subjects (10%) smoked actively at time of surgery, while nineteen (47.5%) had a previous smoking history. The most common comorbidities were hypertension (31/40, 77.5%), ischemic heart disease (22/40, 55.0%) and diabetes mellitus (13/40, 32.5%). The median Charlson Comorbidity Index and ASA scores were 3.0 (IQR 2.0, 4.0) and 4.0 (IQR 4.0, 4.0), respectively. In the three months prior to surgery, twelve subjects (30%) received antimicrobial therapy and three (7.5%) were hospitalized. Two subjects (5%) had undergone a previous cardiac surgery.

The cardiac surgery characteristics of the PD study subjects are detailed in Table 15. Of the 40 subjects, 18 (45.0%) underwent CABG alone, while 12 (30.0%) had valve repair or replacement procedures. The remaining 10 subjects (25.0%) had CABG and/or valve repair or replacement along with another type of procedure. Fifty-five percent (22/40) of surgeries involved CABG with a median of 3 grafts (IQR 2, 4). The saphenous vein was used for grafting in 95.4% (21/22) of CABG procedures, and the IMA in 81.8% (18/22) of these cases. Twelve subjects (30.0%)

received blood products during surgery with a median 1000 mL (IQR 300, 1800) transfused. The mean net balance at the end of surgery 3571 ± 1214 mL. The duration of surgery was 4.1 ± 1.2 hours with CPB accounting for 2.0 ± 0.9 hours. Intraoperative complications occurred in one subject (2.5%) and three study subjects (7.5%) required re-exploration for postoperative bleeding. The duration of the postoperative and total hospital stay were 5 days (IQR 4, 7) and 6 days (IQR 5, 8), respectively.

The cefazolin AP characteristics of the PD study subjects are summarized in Table 16. The majority of study subjects (30/40, 75.0%) received 2-gram preoperative cefazolin doses, while the remaining subjects received a 1-gram dose. The preoperative cefazolin dose was 23.5 ± 5.4 mg/kg_{DW}, where dosing weight was used in those with Class II or III obesity. The preoperative cefazolin dose was administered within 60 minutes before surgical incision in 38/40 (95.0%) of cases. Ten study subjects (25.0%) received only a preoperative cefazolin dose, while the majority (29/40, 72.5%) received one intraoperative dose and one subject (2.6%) received two intraoperative doses. The total cefazolin dose expressed as a function of subject weight was 41.4 ± 14.7 mg/kg_{DW}. The total dose normalized for surgery duration was 8.9 ± 2.3 mg/kg_{DW}/h. The total cefazolin closure concentrations ranged from 32.4 to 222.0 mg/L with a median of 88.5 mg/L (IQR 50.4, 139.5). The free cefazolin closure concentrations ranged from 4.9 to 99.9 mg/L with a median of 26.4 mg/L (IQR 11.4, 40.9). The total and free cefazolin closure concentrations in the study subjects included in the PD analysis are presented in Figure 30.

In the 40 evaluable study subjects, eight cases of superficial sternal SSI were identified. Two SSI cases occurred during hospitalization, while six were reported after hospital discharge. Of the two

SSI cases which occurred during hospitalization, one was an infected saphenous vein graft harvesting site. A wound swab of the infected site identified Gram-negative rods and non-hemolytic streptococcus. The second case was a sternal wound infection with *S. epidermidis* cultured on a wound swab. Both subjects were treated with PO amoxicillin-clavulanic acid. All of the subjects who reported SSI after hospital discharge were prescribed systemic antimicrobial therapy for the SSI, but only four subjects recalled the name of the antimicrobial prescribed. The prescribed therapies included PO doxycycline, amoxicillin and cephalexin, as well as IV vancomycin. A microbiology culture of the wound was ordered in only one of the affected subjects and it identified the presence of MRSA in the sternal wound. In addition to systemic antimicrobial therapy, two of the six affected subjects also required surgical wound drainage and re-stitching.

The results of the univariate analysis of subject-, surgery, and prophylaxis-related variables and the development of SSI are detailed in Table 17. BMI ≥ 35 kg/m² ($P = 0.082$), longer duration of surgery ($P = 0.045$), lower total cefazolin closure concentration ($P = 0.042$) and lower free cefazolin closure concentration ($P = 0.066$) were significantly associated with SSI development. The identification of BMI as a significant variable in univariate analysis signalled that weight had a possible effect on the risk of SSI. As such, the continuous variable subject weight was included in multivariate analysis over the less informative BMI variable. The variables subject weight, duration of surgery, and total and free cefazolin closure concentrations were included in multivariate analysis. Longer duration of surgery (OR = 1.02, 95% CI 1.0002 – 1.034, $P = 0.027$) and lower total cefazolin closure concentrations (OR = 0.97, 95% CI 0.942 – 0.998, $P = 0.038$) were independently associated SSI. The OR for postoperative SSI was 2.9 for every hour increase in surgery duration and 1.3 for every 10% decrease in total

cefazolin closure concentrations. The AUROC was 0.789 (95% CI 0.583–0.996) and the Hosmer-Lemeshow goodness-of-fit test was non-significant ($P = 0.21$). The probability of SSI based on the logit function of total cefazolin concentrations at wound closure stratified for length of surgery is shown in Figure 31. Significant thresholds for increased probability of postoperative infection identified by CART analysis were duration of surgery greater than 346 minutes (SSI rates of 60.0% versus 14.3%) and cefazolin closure concentrations less than 104 mg/L (SSI rates of 30.4% versus 5.9%).

10.3. Discussion

To our knowledge, this was the first study to assess the cefazolin concentrations achieved during cardiac surgery with CPB using the guideline-recommended re-dosing schedule. Furthermore, the independent association between total cefazolin closure concentrations and SSI was the first direct connection between antimicrobial concentrations and risk of SSI in cardiac surgery. The first study investigating antimicrobial concentrations during cardiac surgery and SSI occurrence examined cephalothin AP in prosthetic valve replacement surgery.⁶⁴ In that study, Goldmann *et al.* noted that patients with lower serum cephalothin concentrations at surgical wound closure were more likely to develop SSI.⁶⁴ However, due to the correlation between antimicrobial concentrations at wound closure and the duration of surgery, an independent association between lower cephalothin closure concentrations and SSIs could not be established.

Previous studies examining cefazolin PD relied on surrogate markers, rather than clinical outcomes. Previously, activity was inferred based on the ability of an AP regimen to maintain

concentrations above a specific target, usually the MIC of a common SSI pathogen. Other than the abovementioned Goldmann study, no other study has examined the association between antimicrobial concentrations and SSIs in cardiac surgery. The study of cefazolin AP in cardiac surgery patients by Kosaka *et al.* reported a higher SSI occurrence in patients with Clcr > 50 mL/min, compared to those with lower Clcr.⁶⁵ As cefazolin is renally cleared, this finding may potentially be indicative of an association between lower antimicrobial concentrations and SSIs, although the evidence is indirect. In comparison, our study examined the PD of the guideline-recommended cefazolin regimen and directly linked cefazolin concentrations to a clinically relevant outcome, i.e., the development of SSI.

The link between antimicrobial concentrations and SSIs has been established for other surgeries. The importance of closure concentrations with respect to AP efficacy was first established in a landmark study of gentamicin AP in colorectal surgery by Zelenitsky *et al.*¹¹⁰ In addition to established risk factors for SSI such as diabetes mellitus ($P = 0.02$), the presence of a stoma ($P = 0.04$) and patient age ($P = 0.05$), lower gentamicin concentration at wound closure ($P = 0.02$) was one of the principal independent risk factor for SSI at 30 days post-surgery.¹¹⁰ This study established the significance of antimicrobial concentrations at wound closure for AP efficacy, even after controlling for established risk factors for infection.

Our results are consistent with the findings by Zelenitsky *et al.* as we identified cefazolin closure concentrations as an independent risk factors for SSI following cardiac surgery. The absence of a correlation between closure concentrations and surgery duration ($R^2 = 0.08$) further confirmed the independence of the association between cefazolin concentrations and SSI. Furthermore, even with

varying surgery duration, the probability of SSI was reduced with increasing cefazolin concentrations, as described in Figure 31. Cardiac surgery is one of the few surgical procedures in which AP is extended beyond the end of surgery, for up to 48 hours postoperatively. It is thus even more notable that the significance of closure concentrations was detected even in the presence of prolonged postoperative AP.

The identification of total closure concentrations as opposed to free closure concentrations as the independent risk factor for SSI was an unexpected finding. Free concentrations are considered the most relevant to the efficacy of antimicrobials as protein-bound antimicrobials are pharmacologically inactive. As previously described in Objective 2, cefazolin protein binding was dynamic and at times non-linear during cardiac surgery with CPB. Protein binding was significantly higher pre-CPB, compared to during/post-CPB, and non-linear protein binding was also observed during/post-CPB. Given the dynamic protein binding observed, total cefazolin concentrations may have been a more constant and thus significant variable in our model.

Although a link between antimicrobial concentrations at other time points during surgery, e.g., intraoperative troughs, and the risk of SSI has not been established, it is plausible that low antimicrobial concentrations at any point during surgery may affect AP efficacy. This has been alluded to in the joint ASHP/IDSA/SIS/SHEA AP guidelines which state that the goal of AP is to ensure “adequate serum and tissue concentrations during the period of potential contamination”²⁵, understood as from beginning to end of surgery. SSIs are most commonly caused by the patient’s endogenous flora, although exogenous bacteria from OR personnel, the OR environment or surgical instruments have been implicated as well.¹⁴⁹ The risk of SSI increases when bacterial

contamination exceeds 10^5 organisms per gram of tissue, although smaller bacterial inocula may lead to infection in the presence of foreign materials.¹⁴⁹ However, SSI development depends on the interplay between the size of the bacterial inoculum, the virulence of the contaminating bacteria and the competence of host defences. AP aims to prevent infection by reducing the bacterial burden at the operating site.

The efficacy of AP has traditionally been inferred based on the ability of a regimen to achieve and maintain antimicrobial concentrations above the MICs of common SSI pathogens during surgery. As previously discussed, the target of 8 mg/L was selected as it was the CLSI susceptibility breakpoint for *Enterobacteriaceae* spp. prior to its reduction for ESBL detection and it was also the last susceptibility breakpoint for anti-staphylococcal cephalosporins. As such, cefazolin concentrations of at least 8 mg/L would exceed the MIC of susceptible bacteria. In comparison, CART analysis of cefazolin PD in our study identified a significant threshold of total cefazolin closure concentrations greater than 104 mg/L (29 mg/L free cefazolin based on previously measured 72% protein binding) for AP in cardiac surgery with CPB.

There are several factors which may explain this difference. First, CART analysis identifies the most significant value based on the study data distribution, and therefore is not a single absolute threshold to the exclusion of others. Furthermore, this finding may also be indicative of bactericidal concentrations. β -lactam antimicrobials exhibit time-dependent bactericidal action, with maximum bactericidal activity achieved at four times MIC (i.e., 32 mg/L) which is consistent to the threshold identified in CART analysis.¹¹³ As such, our results suggest that PD targets may

need to be elevated in order to target bactericidal rather than bacteriostatic antimicrobial concentrations in order to prevent SSIs.

11. LIMITATIONS

The research reported in this thesis was not without limitations. The target total cefazolin closure concentration of 40 mg/L was chosen based on an estimated cefazolin protein binding of 80%. However, subsequent analysis demonstrated that protein binding during cardiac surgery with CPB was dynamic, with an overall mean of 72%. As such, our target closure concentrations may have yielded higher free concentrations than we had anticipated in some subjects. Another limitation is the relatively small number of events ($n = 5$) for closure concentrations below 40 mg/L which was used in the multivariate analyses. However, our analyses tested physiologic and dosing variables inevitably related to closure concentrations.

A potential limitation of our protein binding analysis was the inability to maintain the temperature of the study samples equivalent to that at the time of sample collection. However, to limit temperature variation, whole blood samples were separated into plasma and ultrafiltrate immediately after collection, thus avoiding the potential effects of freezing and thawing on protein binding. Moreover, as the ultrafiltration was performed at the same temperature for all samples, the effect of temperature is expected to be the same for all groups and therefore cannot explain our observation that protein binding differs in during/post-CPB versus pre-CPB samples. Another potential limitation is the lack of measurement of pH levels in our samples. Although pH levels can affect protein binding, particularly in the presence of calcium ions, all the samples were processed immediately after collection, thus minimizing CO₂ loss associated with sample storage which can lead to an alkaline shift from physiological pH.^{99,150}

A potential limitation of the pop-PK analysis was the inability to describe the PK of cefazolin specifically during CPB. However, due to the large sample size of our study and the robust nonparametric modeling approach used in this analysis, the results of our analysis describe the overall parameters of cefazolin during cardiac surgery with CPB, which are of clinical importance.

A potential limitation of the PD analysis was the smaller sample size available for this analysis. While lower cefazolin closure concentrations were associated with SSI independent of surgery duration, the sample size likely explains the lack of significance for other established risk factors for infection, such as diabetes.²⁵ Despite this, the principles of PD and significance of cefazolin closure concentration were evident. Furthermore, although the importance of postoperative antimicrobial concentrations in SSI prevention has not been established, we cannot discount the presence of such effects on SSI development. The assessment of SSIs following hospital discharge was based on study subject recall during phone follow-up, which could have potentially led to recall bias. However, open ended questions were posed and answers clarified to minimize this risk. Although the retrospective design of the PD analysis may be viewed as a limitation, there are considerable ethical barriers to conducting prospective PD studies where a range of drug concentrations including those that are suboptimal are required. Of note, however, our primary clinical outcome, SSI, was based on prospective follow-up at 30 days post-surgery.

Finally, as our study included subjects with normal renal function (i.e., $\text{Clcr} \geq 50 \text{ mL/min/72 kg}$), the results of our study may not be applicable to patients with reduced renal function. Although a simulation based on our study results was carried out to estimate the cefazolin exposure in cardiac

surgery patients with reduced renal function, a validation study in this patient population would be prudent to confirm these findings.

12. CONCLUSIONS

This thesis assessed the guideline-recommended cefazolin AP in adults undergoing elective cardiac surgery with CPB. The studied AP regimen did not maintain concentrations above 40 mg/L throughout surgery in all study subjects. Almost 10% of closure concentrations and 40% of intraoperative trough concentrations were below target. As almost one-third of the study subjects experienced below-target concentrations at some point during surgery, optimization of cefazolin AP was necessary to meet the guideline recommendation of maintaining adequate antimicrobial concentrations from beginning to end of surgery. Lower body weight, shorter duration of surgery and lower total cefazolin dose per hour of surgery were independent risk factors for below-target closure concentrations. CART analysis identified a threshold cefazolin dose of 7.6 mg/kg_{DW} per hour of surgery to maintain target closure concentrations. Based on this finding, alternative regimens were proposed and a new cefazolin AP regimen adopted at the St. Boniface Hospital. The new regimen consisted of 2 grams of cefazolin (3 grams if ≥ 120 kg) administered within one hour before surgical incision and re-dosed every 3 hours intraoperatively and every 8 hours for up to 48 hours postoperatively in patients with $\text{Cl}_{\text{cr}} \geq 50$ mL/min/72 kg.

The protein binding of cefazolin was dynamic throughout cardiac surgery with CPB. Although the overall protein binding was 72%, protein binding was significantly higher in samples collected pre-CPB compared to those collected during/post-CPB. Furthermore, protein binding saturation was identified and determined to be limited to samples collected during/post-CPB. Pop-PK modeling was used to describe cefazolin PK parameters. The k_e and V_d were 0.35 ± 0.07 h⁻¹ and 0.14 ± 0.05 L/kg, respectively and the calculated $t_{1/2}$ and CL were 2.0 h and 0.05 L/kg/h,

respectively. Compared to healthy volunteers, patients undergoing cardiac surgery with CPB had a similar k_e , but an expanded V_d . The finding of an expanded V_d may be explained by the differences in body weight between the studied populations, the fluids administered during surgery, as well as the systemic inflammatory response generated during CPB. The characterization of cefazolin PK parameters, including protein binding enables the accurate modeling of the time-course of cefazolin during cardiac surgery with CPB. This will enable future researchers to more accurately predict the efficacy of AP regimens in this patient population without the need for extensive studies in humans.

In the PD analysis, longer surgery duration and lower total cefazolin closure concentrations were independently associated with SSI. These results were the first to demonstrate a link between cefazolin closure concentrations and risk of SSI in cardiac surgery with CPB. Significant thresholds for increased SSI risk were surgery duration exceeding 346 minutes and total closure concentrations below 104 mg/L. The latter finding suggests that bactericidal concentrations of cefazolin may be necessary in order to prevent SSI following cardiac surgery.

In conclusion, this pharmacokinetic/pharmacodynamic study of cefazolin AP in cardiac surgery with CPB contributed to the scientific knowledge regarding cefazolin AP in cardiac surgery with CPB and led to the optimization of AP in this special patient population.

13. FUTURE DIRECTIONS

This thesis has contributed to the knowledge and understanding of the PK and PD of cefazolin AP in cardiac surgery with CPB. It specifically identified that guideline-recommended AP did not meet target closure concentrations in every patient, identified the non-linear protein binding of cefazolin during/post-CPB, described pop-PK parameters of interest and identified risk factors for SSI following cardiac surgery. The work of this thesis provides a foundation for future research, including some potential studies listed below:

1. In the design of the new cefazolin AP regimen, the pharmacokinetic parameter of closure concentrations was used as a surrogate for the clinical endpoint of SSI infections. As such, it would be important to assess whether the optimization of this surrogate endpoint resulted in an improvement in the clinical endpoint. This could be done by determining the 30-day SSI prevalence with the new regimen and comparing it to that of the old regimen.
2. Based on our results, simulations were carried out to determine whether changes to the AP regimen were required for patients with $\text{Clcr} < 50 \text{ mL/min/72 kg}$. As the simulation model was constructed from data originating from patients with $\text{Clcr} \geq 50 \text{ mL/min/72 kg}$, it would be prudent to validate these recommendations in the population of interest. This could be carried out by collecting blood samples (e.g., intraoperative trough and wound closure) in the target population and measuring cefazolin concentrations to ensure that target closure concentrations are being achieved.

3. Another important study would involve the identification of pathogens causing cardiac SSIs in Canadian hospitals. In this thesis, the description of the cardiac surgery SSI pathogens relied mainly on data from the United States. The CDC regularly conducts and publishes the results of national surveillance efforts, while the comparable Canadian data is not available. Conducting a surveillance study to determine the prevalence and susceptibility of pathogens at the hospital, provincial and national levels would enable researchers and clinicians to ensure that AP regimens are targeted to the appropriate pathogens. In addition, it would allow the tracking of changes in epidemiology and susceptibility of pathogens over time to ensure continued AP efficacy.

4. Monte Carlo simulations could be conducted to assess the efficacy of different cefazolin AP regimens in cardiac surgery with CPB. Using the pop-PK parameters identified in this study, more accurate simulations of cefazolin concentrations during surgery could be made. Comparing these concentrations to PD targets would enable researchers to predict the efficacy of various AP regimens more rapidly and without the need for human volunteers.

14. TABLES

Table 1. Summary of recommendations for systemic antimicrobial prophylaxis (AP) in cardiac surgery from North American, European and global guidelines on AP in surgery.

Guideline, Year reference	Antimicrobial	Dose	Timing pre- incision	Re-dosing during surgery	Duration post-surgery
North America					
STS, 2006 and 2007 ^{51,52}	Cefazolin	2 g	≤60 min	1 g q 3–4 h	≤48 h
ACCF, AHA, 2011 ⁵³	Cefazolin or Cefuroxime	1 g 1.5 g	30–60 min	1 g if surgery >3 h	N/R
ASHP, IDSA, SIS, SHEA, 2013 ²⁵	Cefazolin or Cefuroxime	2 g, 3 g if ≥120kg 1.5 g	≤60 min	2 or 3 g q 4 h or extensive blood loss	N/R
NIH, 2014 ⁵⁵	Cefazolin (based on surgery, SSI pathogens)	2 g, 3 g if ≥120kg	≤60 min	2 or 3 g every 2 t _{1/2} or extensive blood loss	24 h
CDC, 2017 ⁵⁶	N/R	N/R	N/R	N/R	none
Europe					
RCSI, RCPI, 2012 ⁵⁷	N/R	N/R	≤60 min	surgery >4 h or blood loss >1500 mL	24–48 h
SIGN, 2014 ⁵⁸	N/R	N/R	≤60 min	surgery >4 h or blood loss >1500 mL	≤48 h
EACTS, 2017 ⁵⁹	Cefazolin or Cefuroxime	N/R	≤60 min	every 2 t _{1/2} or extensive blood loss	24–48 h
NICE, 2019 ⁶⁰	N/R	N/R	At anesthesia induction	every 1 t _{1/2}	N/R
Global					
WHO, 2016 ⁶¹	N/R	N/R	≤120 min	N/R	none
GAIS, WSES, 2017 ⁶²	N/R	N/R	30–60 min	every 2 t _{1/2} or blood loss >1500 mL	none

ACCF, American College of Cardiology Foundation; AHA, American Heart Association; ASHP, American Society of Health-System Pharmacists; CDC, Centers for Disease Control and Prevention; EACTS, European Association for Cardio-Thoracic Surgery; GAIS, Global Alliance for Infection in Surgery; IDSA, Infectious Diseases Society of America; NICE, National Institute for Health and Care Excellence; NIH, National Institutes of Health; RCPI, Royal College of Physicians in Ireland; RCSI, Royal College of Surgeons of Ireland; SHEA, Society for Healthcare Epidemiology of America; SIGN, Scottish Intercollegiate Guidelines Network; SIS, Surgical Infection Society; STS, Society of Thoracic Surgeons; t_{1/2}, half-life of antimicrobial; WHO, World Health Organization; WSES, World Society of Emergency Surgery

Table 2. Study subjects (*n* = 55).

Male	38 (69.1%)
Age (years)	64.9 ± 10.4
Weight (kg)	89.7 ± 16.5
BMI (kg/m ²)	30.9 ± 5.3
BMI classification	
Normal (18.5 – 24.9 kg/m ²)	3 (5.5%)
Overweight (25 – 29.9 kg/m ²)	25 (45.5%)
Class I obesity (30 – 34.9 kg/m ²)	17 (30.9%)
Class II obesity (35 – 39.9 kg/m ²)	6 (10.9%)
Class III obesity (≥ 40 kg/m ²)	4 (7.3%)
Serum creatinine (μmol/L)	74 ± 16
Creatinine clearance (mL/min/72 kg)	80 ± 19
Hemoglobin A1C preoperative (%)	6.2 ± 0.9
Blood glucose postoperative (μmol/L)	8.1 ± 1.4
Albumin preoperative (g/L)	40 ± 2
Smoker (current/past)	8 (14.5%) / 26 (47.3%)
Co-morbidities	
Hypertension	40 (72.7%)
Ischemic heart disease	34 (61.8%)
Diabetes mellitus	16 (29.1%)
Myocardial infarction	11 (20.0%)
Peripheral vascular disease	3 (5.5%)
Heart failure	2 (3.6%)
Chronic obstructive pulmonary disease (COPD)	1 (1.8%)
Charlson comorbidity index	3 (2 – 4)
ASA Classification	4 (4 – 4)
Previous cardiac surgery	2 (3.6%)
Antibiotics within 3 months	13 (23.6%)
Hospitalization within 3 months	6 (10.9%)

Data are presented as n (%), mean ± standard deviation or median (interquartile range)

ASA, American Society of Anesthesiologists

Adapted from: Calic D, Ariano RE, Arora RC, et al. Evaluation of cefazolin antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass. J Antimicrob Chemother. December 2017. doi:10.1093/jac/dkx439

Table 3. Cardiac surgeries (*n* = 55).

Coronary artery bypass grafting (CABG)	26 (47.3%)
Valve repair or replacement	14 (25.5%)
Mixed/other procedure ¹	15 (27.3%)
Procedures involving CABG (<i>n</i> = 33)	
Number of grafts	3 (2, 4)
Saphenous vein	31 (93.9%)
Internal mammary artery	28 (84.8%)
Blood products administered during surgery (mL) (<i>n</i> = 17)	1500 (300, 2750)
Net fluid balance (mL)	3 547 ± 1 301
Duration of surgery (min)	258 ± 99
Duration of CPB (min)	129 ± 78
Intraoperative complications / Re-exploration	3 (5.5%) / 5 (9.1%)
Postoperative hospital stay (days)	5 (4 – 7)
Total hospital stay (days)	6 (5 – 8)

Data are presented as n (%), mean ± standard deviation or median (interquartile range)

¹ Combination of CABG, valve or another procedure e.g., aortic root replacement

Table 4. Cefazolin prophylaxis administered to study subjects preoperatively and during cardiac surgery with CPB ($n = 55$).

Preoperative cefazolin dose	
1 gram	12 (21.8%)
2 grams	43 (78.2%)
Dose (mg/kg _{DW})	21.4 ± 4.4
Timing prior to incision	35 ± 14
≤ 30 min	21 (38.2%)
30 – 60 min	31 (56.4%)
> 60 min	3 (5.5%)
Intraoperative cefazolin doses	
1 intraoperative dose	37 (67.3%)
2 intraoperative doses	4 (7.3%)
3 intraoperative doses	1 (1.8%)
Timing since previous dose (min) ($n = 48$)	232 ± 15
Total number of doses administered	
Preoperative dose only	13 (23.6%)
Preoperative and ≥ 1 intraoperative dose	42 (76.4%)
Total preoperative and intraoperative cefazolin dose	
grams	3.3 ± 1.4
mg/kg _{DW}	39.7 ± 15.7
mg/kg _{DW} /h	8.2 ± 2.2

Data are presented as n (%), mean ± standard deviation or median (interquartile range)

Table 5. Total and free cefazolin concentrations during cardiac surgery with CPB.

	Mean \pm SD	Median (IQR)	Range
Total cefazolin concentrations (mg/L)			
Peaks ($n = 53$)	145.2 \pm 36.6	136.7 (115.6, 171.9)	91.1 – 225.1
Intraoperative troughs ($n = 30$)	46.5 \pm 17.5	46.7 (35.7, 52.4)	12.9 – 92.7
Closure samples ($n = 51$)	103.0 \pm 55.7	89.4 (55.3, 140.9)	32.4 – 222.0
Free cefazolin concentrations (mg/L)			
Peaks ($n = 52$)	37.8 \pm 13.1	37.3 (27.4, 44.8)	18.5 – 75.3
Intraoperative troughs ($n = 30$)	14.0 \pm 6.5	11.9 (9.6, 16.8)	4.4 – 29.4
Closure samples ($n = 51$)	34.7 \pm 27.7	26.2 (12.2, 47.5)	4.9 – 99.9

IQR, interquartile range; SD, standard deviation

Table 6. Univariate analysis of subject-, surgery- and cefazolin prophylaxis-related variables with regards to a target total cefazolin closure concentration ≥ 40 mg/L.

Variable	Total Cefazolin Closure Concentration		P value
	<40 mg/L (n = 5)	≥40 mg/L (n = 46)	
Subject			
Female	4 (80%)	13 (28%)	0.037
Age (years)	68.4 ± 9.5	64.8 ± 9.9	0.459
Weight (kg)	73.1 ± 6.1	91.3 ± 16.9	<0.001
Obese (BMI ≥ 30 kg/m²)	2 (40%)	23 (50%)	1.000
Creatinine clearance (mL/min/72 kg)	82.6 ± 26.2	79.9 ± 18.2	0.834
Charlson comorbidity index	3.0 ± 1.2	3.1 ± 1.6	0.917
Surgery			
CABG surgery only	1 (20%)	24 (52%)	0.349
Valve surgery only	4 (80%)	9 (20%)	0.012
Mixed Surgery	0 (0%)	13 (28%)	0.311
Net fluid balance (mL)	3350 ± 579	3634 ± 1352	0.406
Duration of surgery (min)	191 ± 15	260 ± 84	<0.001
Duration of CPB (min)	98.4 ± 9.5	126.6 ± 66.2	0.011
Cefazolin Prophylaxis			
Preoperative cefazolin dose only	3 (60%)	10 (22%)	0.098
Preoperative dose 1 gram	4 (80%)	8 (17%)	0.009
Preoperative cefazolin dose (mg/kg _{DW})	16.2 ± 4.5	21.8 ± 4.2	0.045
Total cefazolin dose (mg/kg _{DW})	21.9 ± 7.6	40.1 ± 13.8	0.002
Total cefazolin dose for every hour of surgery (mg/kg _{DW} /h)	5.8 ± 1.7	8.2 ± 2	0.029
Intraoperative complications	0 (0%)	2 (4%)	1.000

Data are presented as n (%), mean \pm standard deviation

Adapted from: Calic D, Ariano RE, Arora RC, et al. Evaluation of cefazolin antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass. J Antimicrob Chemother. December 2017. doi:10.1093/jac/dkx439

Table 7. Cefazolin prophylaxis for adult patients undergoing cardiac surgery described in the literature.

Author, Year ^{reference}	Cefazolin Regimen
Lehot, 1990 ⁸³	25 mg/kg preop and q8h for 48 h after end of CPB
Fellinger, 2002 ⁶⁶	1 g preop and 1 g after start of CPB
Waltrip, 2002 ⁸⁴	1 g preop and at end of CPB 2 g preop, then CI of 20 mg/min (1.2 g/h) during surgery 3 g preop, then CI of 15 mg/min (0.9 g/h) during surgery
Caffarelli, 2006 ⁶⁷	1 g preop and at wound closure
Hutschala, 2007 ⁶⁸	4 g preop and 2 g at skin closure
Adembri, 2010 ⁶⁹	2 g preop, 1 g at end of CPB and at 9 and 15 h after CPB dose 2 g preop, then CI of 1 g over 6 h (0.17 g/h) for 18 h
Kosaka, 2012 ⁶⁵	2 g preop, 1 g in priming fluid and q6h for up to 24 h
Andreas, 2013 ⁷⁰	4 g preop and 2 g at skin closure
Andreas, 2015 ⁷¹	4 g preop and 2 g at skin closure
Odaka, 2015 ¹¹⁸	1 g preop and in priming fluid (discretionary re-dosing) 1 g preop and q3h during surgery and q8h for 48 h (Clcr ≥ 50) or 1 g preop and q4h during surgery and q12h for 48 h (Clcr 10 – 50)
Hollis, 2015 ⁷²	2 g preop, 1 g in priming fluid and 2 g q3h during surgery
Trent Magruder, 2015 ¹²⁰	2 g preop, q2h during surgery and q8h for 24 h 2 or 3 g preop, then CI of 1 g/h during surgery (adjusted for renal function) and 2 g q8h for 24 h
Lanckohr, 2016 ¹⁴⁶	2 g preop, at start of CPB and at 4 h after CPB dose
Shoulders, 2016 ¹²¹	2 or 3 g preop and q2h during surgery and 1 g q8h for 24 h (adjusted for body weight and renal function) 2 or 3 g preop, then CI of 0.33–1 g/h during surgery (based on renal function) and 1 g q8h for 24 h (adjusted for body weight and renal function)
Asada, 2018 ¹¹⁹	1 g preop and q4h. 2 g added to priming fluid

Preop, preoperatively; CPB, cardiopulmonary bypass; CI, continuous infusion; Clcr, creatinine clearance

Adapted from: Calic D, Ariano RE, Arora RC, et al. Evaluation of cefazolin antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass. J Antimicrob Chemother. December 2017. doi:10.1093/jac/dkx439

Table 8. Cefazolin doses required to maintain closure concentration above 40 mg/L based on significant threshold of 7.6 mg/kg_{DW} for every hour of surgery identified in current study.

Total Body Weight or Dosing Weight ¹	Dosing Interval			
	Q2H	Q3H	Q4H	Q6H
70 kg	1000 mg	1500 mg	2250 mg	3250 mg
80 kg	1250 mg	1750 mg	2500 mg	3750 mg
90 kg	1250 mg	2000 mg	2750 mg	4000 mg
100 kg	1500mg	2250 mg	3000 mg	4500 mg
110 kg	1750 mg	2500 mg	3250 mg	5000 mg
120 kg	1750 mg	2750 mg	3750 mg	5500 mg

Based patient population with normal renal function (Cl_{cr} >50; mean 80 ± 19 mL/min/72 kg)

¹ Where dosing weight is applied for BMI ≥ 35 kg/m² as discussed in Methods.

Adapted from: Calic D, Ariano RE, Arora RC, et al. Evaluation of cefazolin antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass. J Antimicrob Chemother. December 2017. doi:10.1093/jac/dkx439

Table 9. Recommended cefazolin prophylaxis regimen for adult patients undergoing cardiac surgery with CPB.

Clcr¹	Cefazolin 2 g (or 3 g if ≥ 120 kg) preoperatively and during surgery	Cefazolin 2 g (or 3 g if ≥ 120 kg) after surgery, not to exceed 48h
≥ 50	< 1 hour prior to incision + Q3H during surgery	then q8h
30 - 49	< 1 hour prior to incision + dose after 3 hours + dose after 8 hours ²	then q8h
11- 29		then q12h
≤ 10		none required

¹ Clcr (mL/min/72 kg) = $\frac{(140 - \text{age}) \times 80}{\text{sCr } (\mu\text{mol/L})}$ (x 0.85 if female)

² Timing may be during or after surgery depending on duration of surgery

Table 10. Protein binding of cefazolin in samples collected during cardiac surgery with CPB.

	Protein Binding (%)	<i>P</i> value
Overall (<i>n</i> = 135 samples)	71.9 ± 8	—
Gender		
Male (<i>n</i> = 93)	73.0 (67.9, 76.5)	0.143
Female (<i>n</i> = 42)	74.1 (68.0, 79.0)	
Obesity		
Obese (BMI ≥ 30 kg/m ² , <i>n</i> = 69)	72.2 (67.1, 76.6)	0.597
Non-obese (BMI < 30 kg/m ² , <i>n</i> = 66)	74.0 (68.8, 76.9)	
Cardiopulmonary bypass (CPB) [†]		
Pre-CPB (<i>n</i> = 52)	74.4 ± 5.2	0.005 ¹
During CPB (<i>n</i> = 23)	69.9 ± 8.2	0.811 ²
Post-CPB (<i>n</i> = 60)	70.4 ± 8.6	0.004 ³
During/Post-CPB (<i>n</i> = 83)	70.3 ± 8.4	0.002 ⁴

Data are presented as mean ± standard deviation or median (interquartile range).

[†]Bonferroni correction applied

¹ P-value for difference in means between Pre-CPB and During CPB

² P-value for difference in means of During-CPB and Post-CPB

³ P-Value for difference in means of Pre-CPB and Post-CPB

⁴ P-value for difference in means of Pre-CPB and During/Post-CPB

Table 11. Albumin concentrations in samples collected during cardiac surgery with CPB.

	Albumin Concentration (g/L)	<i>P</i> value
Overall (<i>n</i> = 135)	31 ± 5	—
Cardiopulmonary bypass (CPB) [†]		
Pre-CPB (<i>n</i> = 52)	35 ± 3	< 0.001 ¹
During CPB (<i>n</i> = 23)	27 ± 4	0.0156 ²
Post-CPB (<i>n</i> = 60)	29 ± 3	< 0.001 ³
During/Post-CPB (<i>n</i> = 83)	28 ± 3	< 0.001 ⁴
Gender		
Male (<i>n</i> = 93)	31 ± 4	0.217
Female (<i>n</i> = 42)	30 ± 5	

Data are presented as mean ± standard deviation

[†]Bonferroni correction applied

¹ P-value for difference in means between Pre-CPB and During CPB

² P-value for difference in means of During-CPB and Post-CPB

³ P-Value for difference in means of Pre-CPB and Post-CPB

⁴ P-value for difference in means of Pre-CPB and During/Post-CPB

Table 12. Studies of cefazolin protein binding in surgical prophylaxis.

Author, Year reference	Type of Surgery	Subject (age)	% Protein Bound*
<i>Cardiac Surgery with CPB</i>			
Hutschala, 2007 ⁶⁸	Aortic valve replacement	7 adults (60 ± 13 y)	77% – 81%
Andreas, 2013 ⁷⁰	CABG with LIMA	8 adults (69 ± 11 y)	40 ± 40%
Andreas, 2015 ⁷¹	CABG with LIMA	9 adults (67 ± 16 y)	57 ± 18%
Hollis, 2015 ⁷²	CABG	10 adults (62 ± 6 y)	53% – 75%
De Cock, 2017 ⁸⁹	Cardiac surgery	56 children (6 d – 15 y)	72% (64% – 77%)†
Asada, 2018 ¹¹⁹	Cardiac surgery	27 adults (70 ± 12 y)	55% – 79%**
<i>Other Surgeries</i>			
Allegaert, 2009 ¹³⁰	<i>In utero</i> surgery	30 pregnant females	75% (59% – 86%)†
Van Kralingen, 2011 ¹²⁸	Bariatric surgery	20 obese adults (44 ± 11 y)	79% (74% – 82%)†
Douglas, 2011 ¹²⁹	AAA repair	12 adults (70 y, IQR 66, 76)	87% (74% – 90%)
Smits, 2012 ¹³²	Various surgeries	40 neonates (9 d, IQR 1, 108)	61% (27% – 90%)†
Naik, 2017 ¹³¹	Urological and spinal surgery	20 adults	69% (44 – 80%)

Data presented as mean ± standard deviation or median (range)

AAA, abdominal aortic aneurysm; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; IQR, interquartile range; LIMA, left internal mammary artery

*Protein binding determined by ultrafiltration, except in ⁶⁸ where microdialysis was used

**Median protein binding depended on time of sampling

† Saturable protein binding reported

Table 13. Studies of cefazolin pharmacokinetics in populations of interest.

Author, Year ^{reference}	Study Population (# samples)	PK Method; # of Compartments	PK parameters	Covariates-PK variable
<i>Healthy Volunteers</i>				
Kirby, 1971 ⁷⁵	17 adult males (219)	Manual methods; 1-compartment	$t_{1/2}$ 1.8 ± 0.2 h Vd 10 ± 0.4 L/1.73m ² CL 62 ± 6 mL/min/1.73m ²	N/A
Rattie, 1975 ⁷⁷	40 adult males (300)	Manual methods; 2-compartment	$t_{1/2}$ 1.4 ± 0.2 h Vd 6.94 L	N/A
Lavillaureix, 1975 ⁷⁸	20 adults* (160)	Manual methods; 1-compartment	$t_{1/2}$ 1.8 h Vd 8.6 ± 1.0 L CL 3.1 ± 0.2 L/h	N/A
<i>Non-Cardiac Surgery</i>				
Koopman, 2007 ¹⁴⁴	46 Adults Orthopedic, general or spinal surgery (46)	NONMEM 5; 1-compartment	Vd _(free drug) 79.2 L CL _(free drug) 23.5 L/h	Age-CL Weight-CL
Van Kralingen, 2011 ¹²⁸	20 obese adults (BMI >35 kg/m ²) Gastric surgery (39)	KinFit; non-compartmental	Vd 3.0 ± 3.1 L CL 4.2 ± 1.0 L/h	Age-CL Weight-Vd
Douglas, 2011 ¹²⁹	12 adults AAA repair surgery (109)	Manual methods; Non-compartmental	Vd 0.14 L/kg (IQR 0.11, 0.15) CL 3.0 L/h (IQR 1.7, 3.9)	Cardiac output-CL Clcr-CL

Brill, 2014 ¹³⁶	15 Morbidly obese and non-obese adults GI surgery (309)	NONMEM 6.2; 2-compartment	V ₁ 8.94 L V ₂ 8.31L CL 22.2 L/h	Age-CL Weight-V ₁
Van Hasselt, 2014 ¹⁵²	94 pregnant women, <i>In utero</i> surgery or caesarean sections	NONMEM 7.1; 2-compartment	Empirical model: V ₁ 33.1 L V ₂ 12.8 L CL 7.14 L/h Semi-physiological model: V ₁ 14.1 L V ₂ 17.1 L CL 8.52 L/h	Empirical model: Gestational age-CL Semi-physiological model: Clcr -CL
Naik, 2017 ¹³¹	20 adults Major urological or spinal surgery (318)	Pmetrics; 2-compartment	V ₁ 5.7 ± 2.4 L CL 4.7 ± 1.1 L/h	Clcr -CL Weight -Vd
Palma, 2018 ¹⁴⁵	9 obese adults Bariatric surgery (211)	Monolix 4.3.3; 2-compartment	V ₁ 9.9 L V ₂ 6.8 L CL 4.9 L/h	None
<i>Cardiac Surgery with CPB</i>				
Miller, 1980 ⁸²	8 adults (136**)	Manual methods	t _{1/2} 11.0 ± 8.1 h Vd _{ss} 0.26 ± 0.07 L/kg CL 1.6 ± 1.0 L/h	None
Lehot, 1990 ⁸³	10 adults (150)	Manual methods; 1-compartment	t _{1/2} 3.9 ± 2.1 h Vd 0.24 + 0.05 L/kg CL 1.05 ± 0.49 mL/kg/min	N/A

Lanckohr, 2016 ¹⁴⁶	24 adults (168)	NONMEM version 7.2; 1-compartment	Vd 15.8 L CL 5.23 L/h	CPB-Vd Albumin-Vd Clcr-CL
De Cock, 2017 ⁸⁹	56 children (494)	NONMEM version 7.2; 2-compartment	V ₁ 1.93 L V ₂ 2.39 L CL 1.56 L/h PB 72% (IQR 64, 77)	eGFR-CL Weight-CL Albumin-PB
Cies, 2019 ¹⁴⁷	41 children (492)	Pmetrics; 1-compartment	Birth – 6 m: Vd 0.598 ± 0.26 L/kg CL 0.009 ± 0.006 mL/min/kg 7 m– 3 y: Vd 0.786 ± 0.15 L/kg CL 0.01 ± 0.005 mL/min/kg 4 – 16 y: Vd 3.4 ± 0.94 L/kg CL 0.007 ± 0.004 mL/min/kg	Weight-Vd
Ingrande, 2019 ¹⁵⁷	10 infants (74)	NONMEM 7.3 and PLTTools; 2-compartment	CL 0.624 L/h V ₁ 0.314 L V ₂ 2.19 L	Age-V ₁

AAA, abdominal aortic aneurysm, BMI, body mass index; CL, clearance; Clcr, creatinine clearance, CPB, cardiopulmonary bypass; eGFR, estimated glomerular filtration rate; GI, gastrointestinal; IM, intramuscular; IV, intravenous; IQR, interquartile range PB, protein binding; $t_{1/2}$ = half-life; V₁, volume of distribution of the central compartment; V₂, volume of distribution of the peripheral compartment; Vd, volume of distribution; Vd_{ss}, volume of distribution at steady state

*Subjects with varying degrees of renal dysfunction were studied as well, but data presented in table limited to healthy volunteers

** Results reported in table limited to those during surgery

Table 14. Study subjects included in pharmacodynamic analysis ($n = 40$).

Male	25 (62.5%)
Age (years)	65.3 \pm 10.4
Weight (kg)	88.1 \pm 16.3
BMI (kg/m ²)	30.7 \pm 5.4
Serum creatinine (μ mol/L)	73 \pm 16
Creatinine clearance (mL/min/72 kg)	80 \pm 18
Hemoglobin A1C preoperative (%)	6.3 \pm 1.0
Blood glucose postoperative (μ mol/L)	8.3 \pm 1.5
Smoker (current/past)	4 (10.0%) / 19 (47.5%)
Co-morbidities	
Hypertension	31 (77.5%)
Ischemic heart disease	22 (55.0%)
Diabetes mellitus	13 (32.5%)
Myocardial infarction	6 (15.0%)
Peripheral vascular disease	2 (5.0%)
Heart failure	1 (2.5%)
Chronic obstructive pulmonary disease (COPD)	1 (2.5%)
Charlson comorbidity index	3 (2 – 4)
ASA Classification	4 (4 – 4)
Previous cardiac surgery	2 (5.0%)
Antibiotics within 3 months	12 (30.0%)
Hospitalization within 3 months	3 (7.5%)

Data are presented as n (%), mean \pm standard deviation or median (interquartile range)

ASA, American Society of Anesthesiologists

Table 15. Cardiac surgeries of study subjects included in the pharmacodynamic analysis (*n* = 40).

Coronary artery bypass grafting (CABG)	18 (45.0%)
Valve repair or replacement	12 (30.0%)
Mixed/other procedure ¹	10 (25.0%)
Procedures involving CABG (<i>n</i> = 22)	
Number of grafts	3 (2, 4)
Saphenous vein	21 (95.4%)
Internal mammary artery	18 (81.8%)
Blood products administered during surgery (mL) (<i>n</i> = 12)	1000 (300, 1800)
Net fluid balance (mL)	3 571 ± 1 214
Duration of surgery (min)	243 ± 71
Duration of CPB (min)	121 ± 51
Intraoperative complications / Re-exploration	1 (2.5%) / 3 (7.5%)
Postoperative hospital stay (days)	5 (4 – 7)
Total hospital stay (days)	6 (5 – 8)

Data are presented as *n* (%), mean ± standard deviation or median (interquartile range)

¹ Combination of CABG, valve or another procedure e.g., aortic root replacement

Table 16. Cefazolin prophylaxis administered to study subjects included in pharmacodynamic analysis preoperatively and during surgery ($n = 40$).

Preoperative cefazolin dose	
1 gram	10 (25.0%)
2 grams	30 (75.0%)
Dose (mg/kg _{DW})	23.5 ± 5.4
Timing prior to incision (min)	35 ± 14
≤ 30 min	14 (35.0%)
30 – 60 min	24 (60.0%)
> 60 min	2 (5.0%)
Intraoperative cefazolin doses	
1 intraoperative dose	29 (72.5%)
2 intraoperative doses	1 (2.5%)
3 intraoperative doses	0 (0.0%)
Total number of doses administered	
Preoperative dose only	10 (25.0%)
Preoperative and ≥ 1 intraoperative dose	30 (75.0%)
Total preoperative and intraoperative cefazolin dose	
Mg/kg _{DW}	41.4 ± 14.7
Mg/kg _{DW} /h	8.9 ± 2.3

Data are presented as n (%), mean \pm standard deviation

Table 17. Univariate analysis of subject-, surgery- and cefazolin prophylaxis-related variables with regards to development of surgical site infection (SSI) at 30 days post cardiac surgery.

Variable	no SSI (n = 32)	SSI (n = 8)	P value
Subject			
Male	20 (62.5%)	5 (62.5%)	1.00
Age (years)	66 ± 9	63 ± 12	0.47
Weight (kg)	86.2 ± 14.4	95.7 ± 21.8	0.28
Body mass index ≥ 35 kg/m ²	3 (9.4%)	3 (37.5%)	0.082
Creatinine clearance (mL/min/72 kg)	78.7 ± 16.5	86.5 ± 25.0	0.42
Diabetes mellitus	9 (28.1%)	4 (50.0%)	0.40
Charlson comorbidity index	3.0 (2.0, 4.0)	3.0 (2.5, 3.0)	0.75
Surgery			
CABG with or without other procedure	22 (68.8%)	6 (75.0%)	1.00
Duration of surgery (min)	266 ± 60	324 ± 100	0.045
Albumin-end of surgery (g/L)	31.5 ± 3.0	30.9 ± 1.7	0.45
Glucose-end of surgery (µmol/L)	8.2 ± 1.5	8.7 ± 1.5	0.39
Fluid balance (mL)	3,422 ± 1188	4,169 ± 1207	0.15
Surgery complications	3 (9.4%)	1 (12.5%)	1.00
Hospital stay following surgery (days)	5.0 (4.0, 6.3)	4.5 (4.0, 7.3)	0.58
Cefazolin Prophylaxis			
Preoperative dose (mg/kg)	23.6 ± 5.3	22.9 ± 6.1	0.77
Timing of preoperative dose (min prior to incision)	34.3 ± 13.9	36.3 ± 6.1	0.55
Received dose(s) during surgery	23 (71.9%)	7 (87.5%)	0.65
Total cefazolin dose per hour of surgery (mg/kg)	9.0 ± 2.3	8.6 ± 2.5	0.64
Cefazolin closure concentration (mg/L)			
total	105.9 ± 57.8	70.4 ± 35.6	0.042
free	35.5 ± 27.9	21.6 ± 14.8	0.066

Data are presented as number (%), mean ± standard deviation or median (interquartile range)

Adapted from: Zelenitsky S, Calic D, Arora RC, et al. Antimicrobial prophylaxis for patients undergoing cardiac surgery: Intraoperative cefazolin concentrations and sternal wound infections. *Antimicrob Agents Chemother* November 2018. Copyright © American Society for Microbiology, *Antimicrobial Agents and Chemotherapy*, 62, 2018, e01360-18. DOI 10.1128/AAC.01360-18.

15. FIGURES

Figure 1. Predicted time course of cefazolin concentration and intraoperative sampling times during cardiac surgery with CPB. Cefazolin dosing (blue), sample collection times (red) and significant surgical time points of incision, cardiopulmonary bypass (CPB) and wound closure are also depicted.

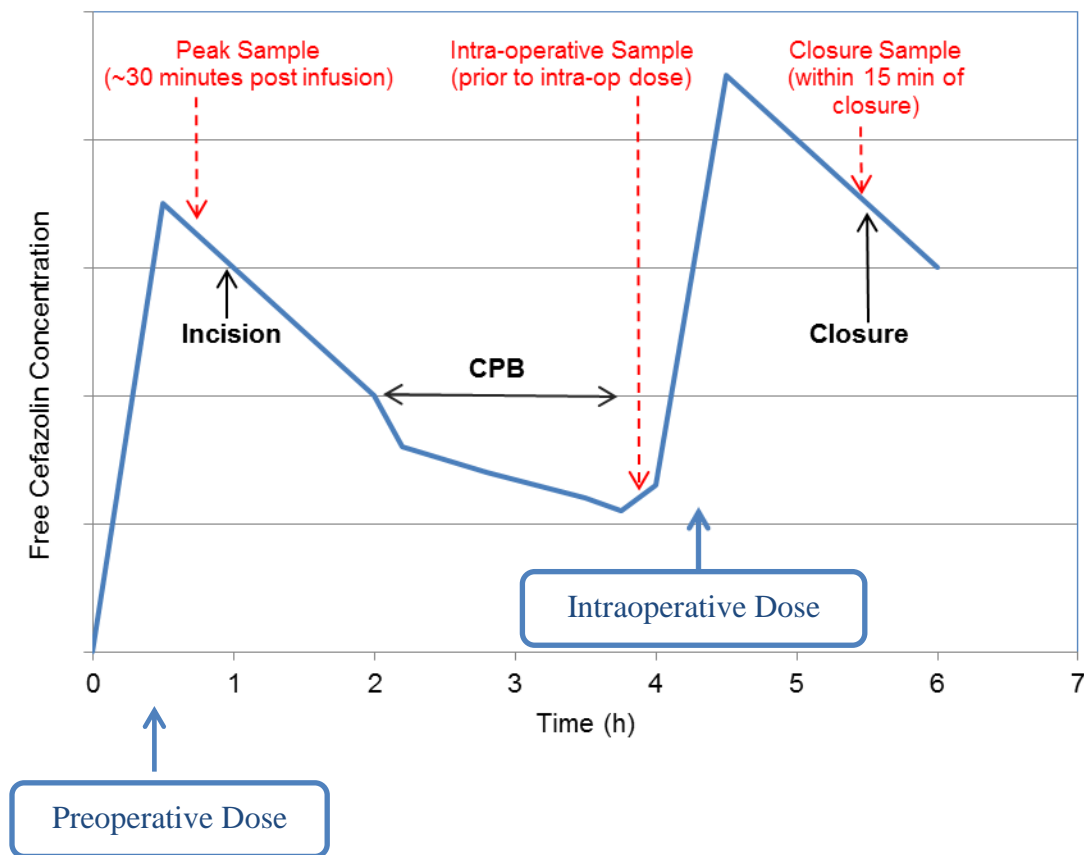


Figure 2. Study flow diagram

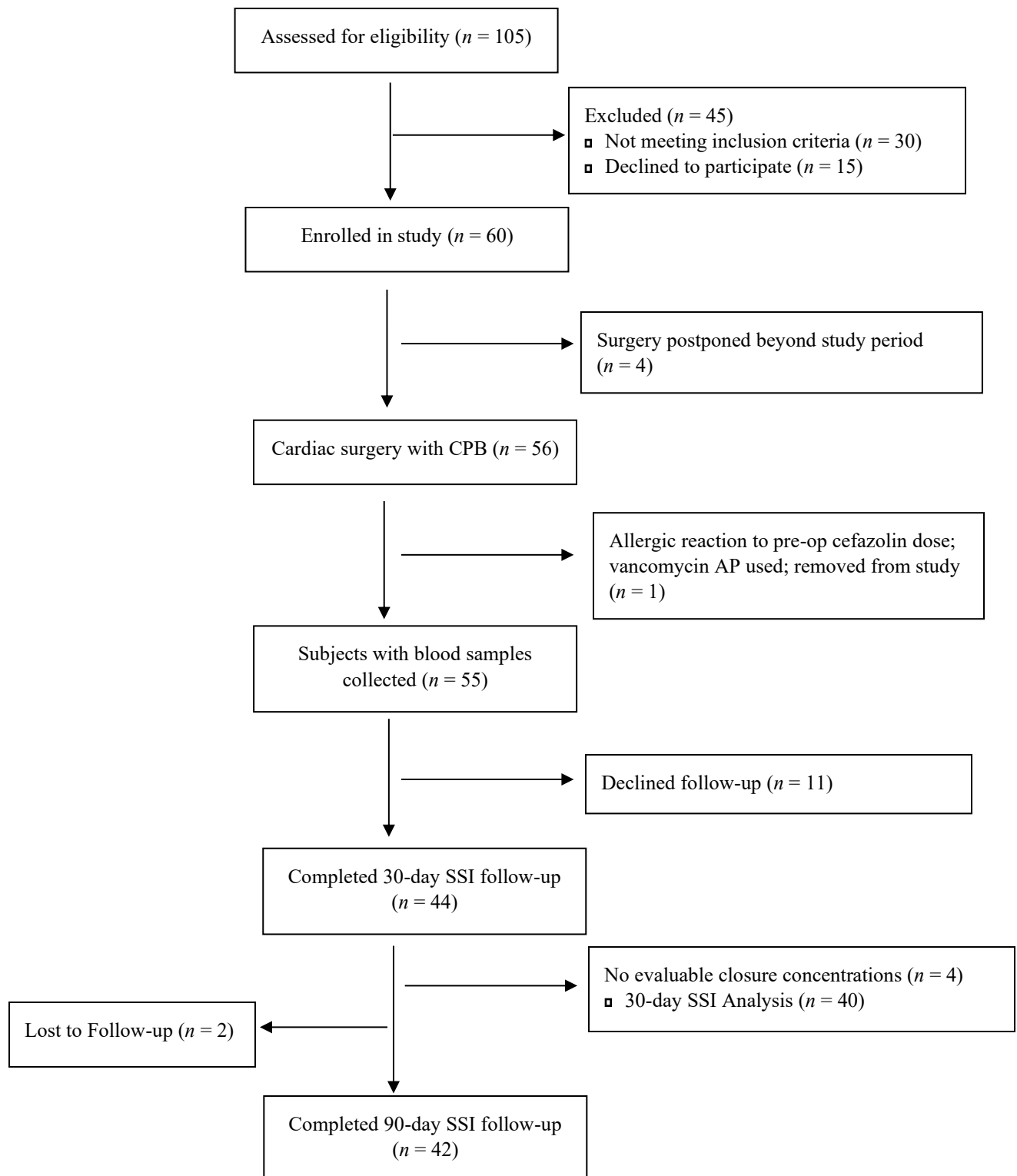
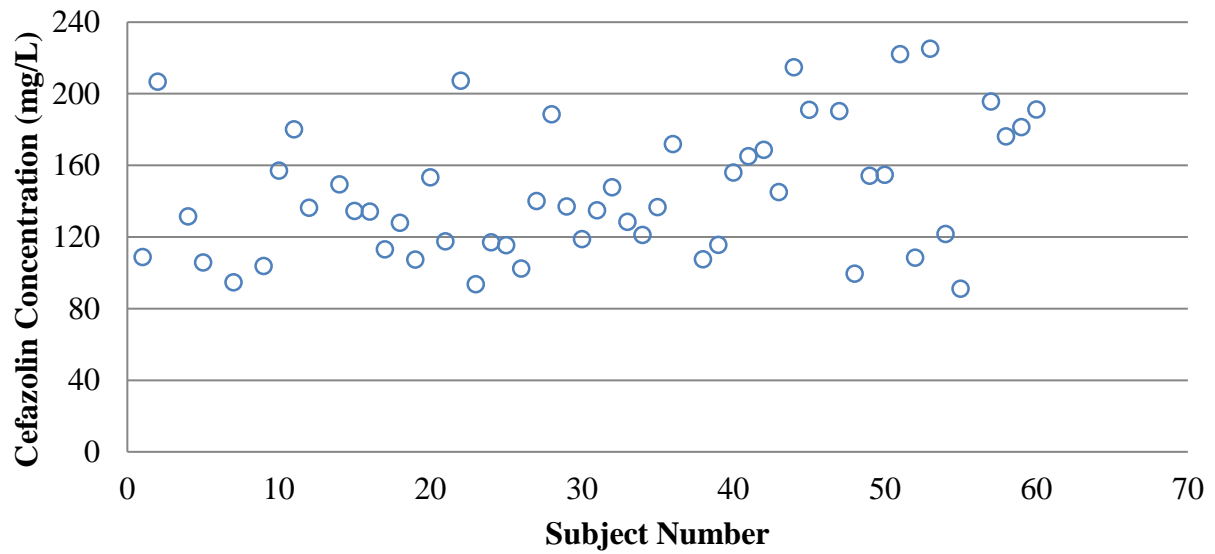


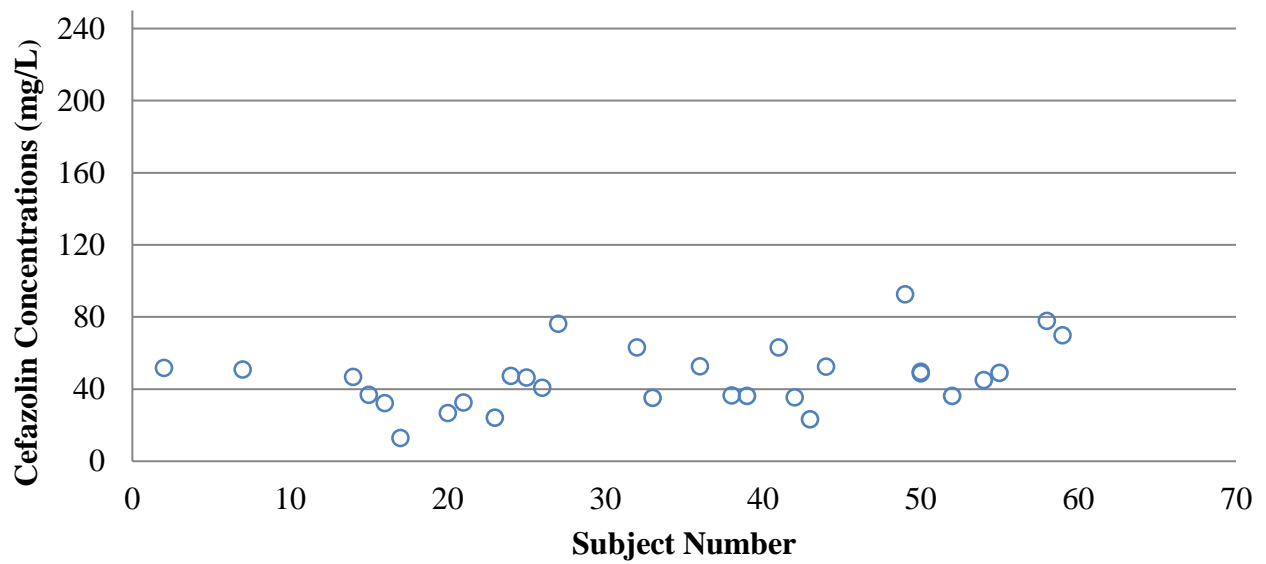
Figure 2. Flow Diagram Adapted from The CONSORT Flow Diagram.¹⁵⁴

Figure 3. Scatterplots of total cefazolin A) peak concentrations ($n = 53$), B) intraoperative trough concentrations ($n = 30$) and C) closure concentrations ($n = 51$).

A)



B)



C)

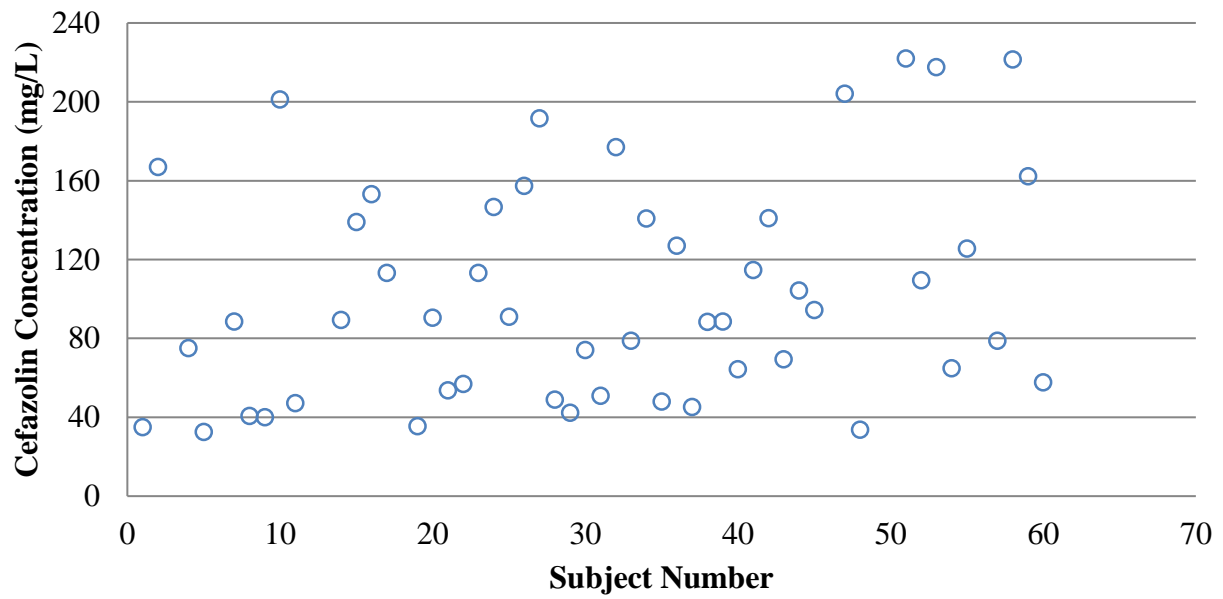
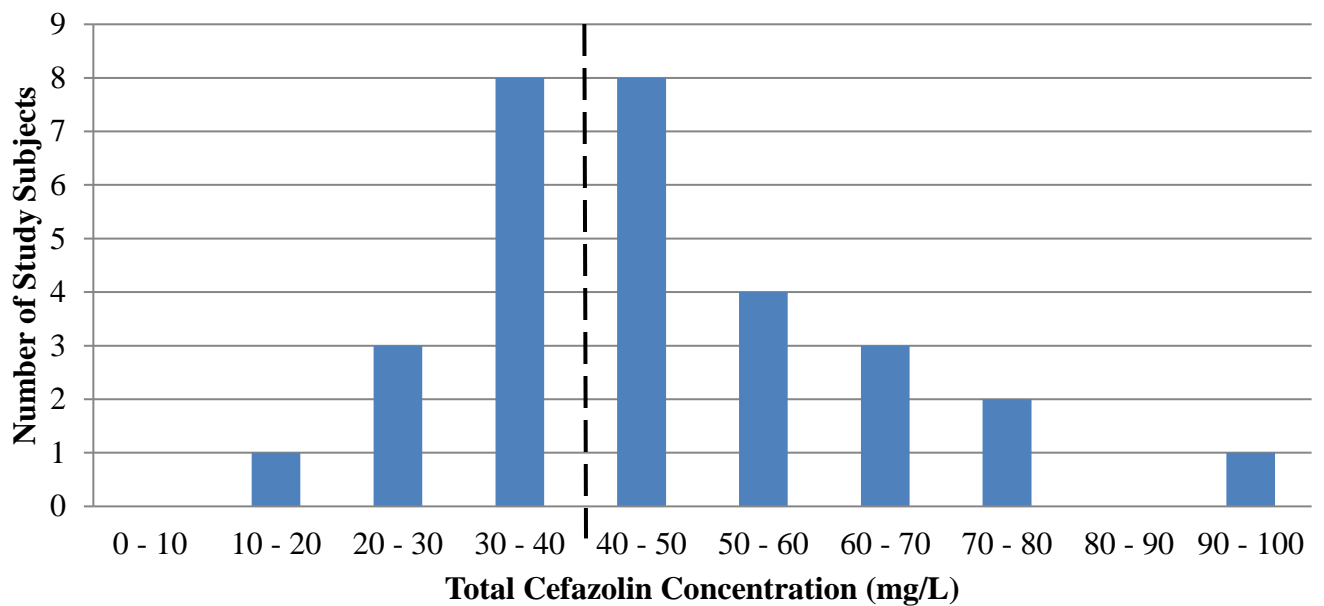


Figure 3. C) Adapted from: Calic D, Ariano RE, Arora RC, et al. Evaluation of cefazolin antimicrobial prophylaxis during cardiac surgery with cardiopulmonary bypass. J Antimicrob Chemother. December 2017. doi:10.1093/jac/dkx439

Figure 4. Distribution of total cefazolin A) intraoperative trough concentrations ($n = 30$) and B) closure concentrations ($n = 51$). Dashed line represents target concentration of 40 mg/L.

A)



B)

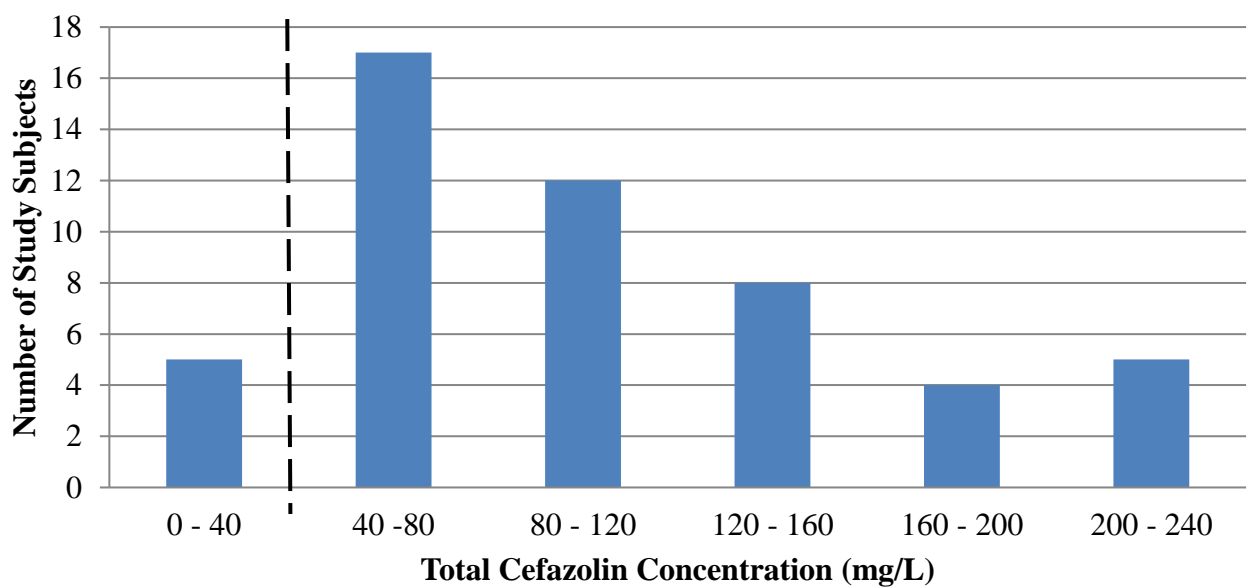


Figure 5. Total cefazolin intraoperative trough concentrations versus net fluid balance.

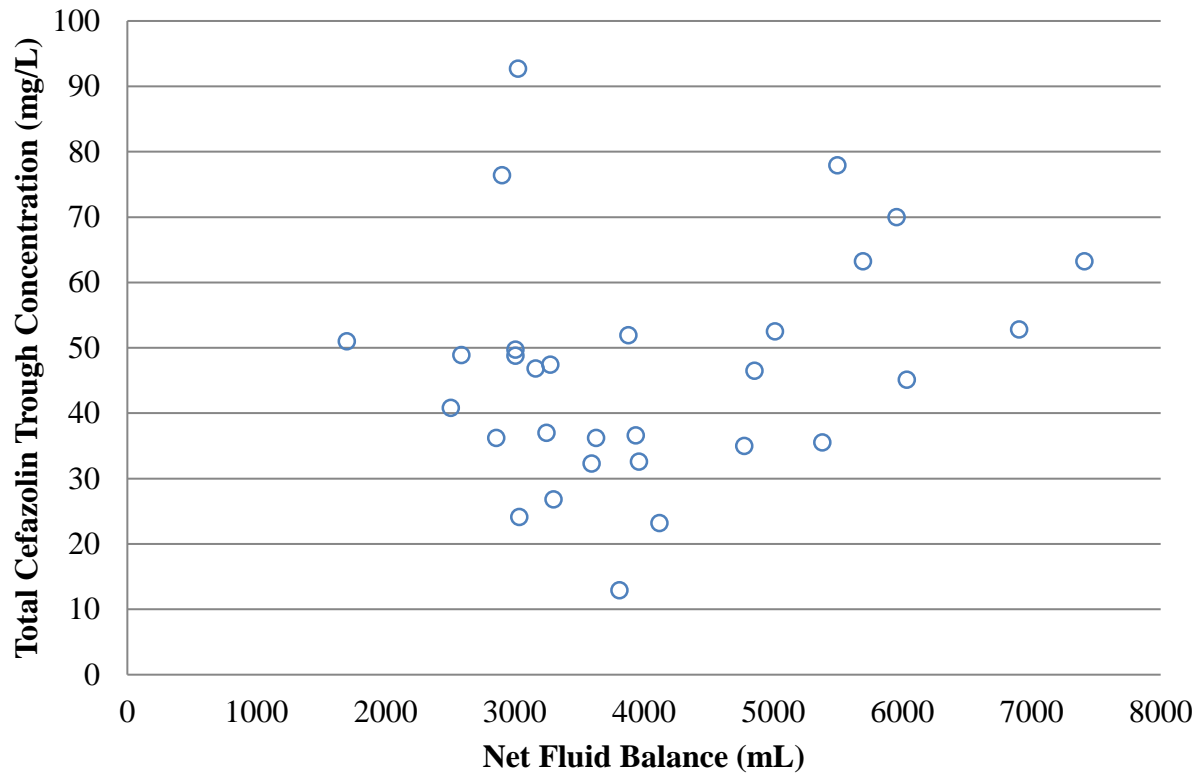


Figure 6. Total cefazolin intraoperative trough concentrations versus cardiopulmonary bypass duration.

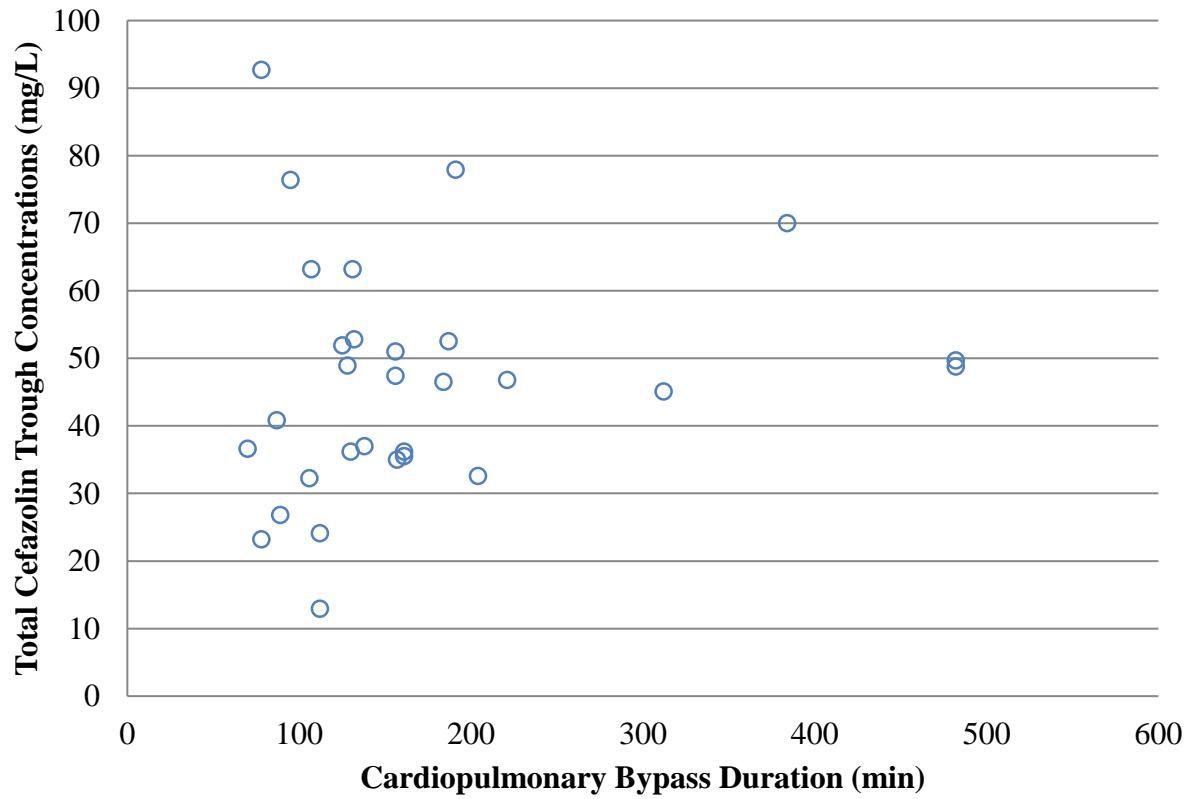
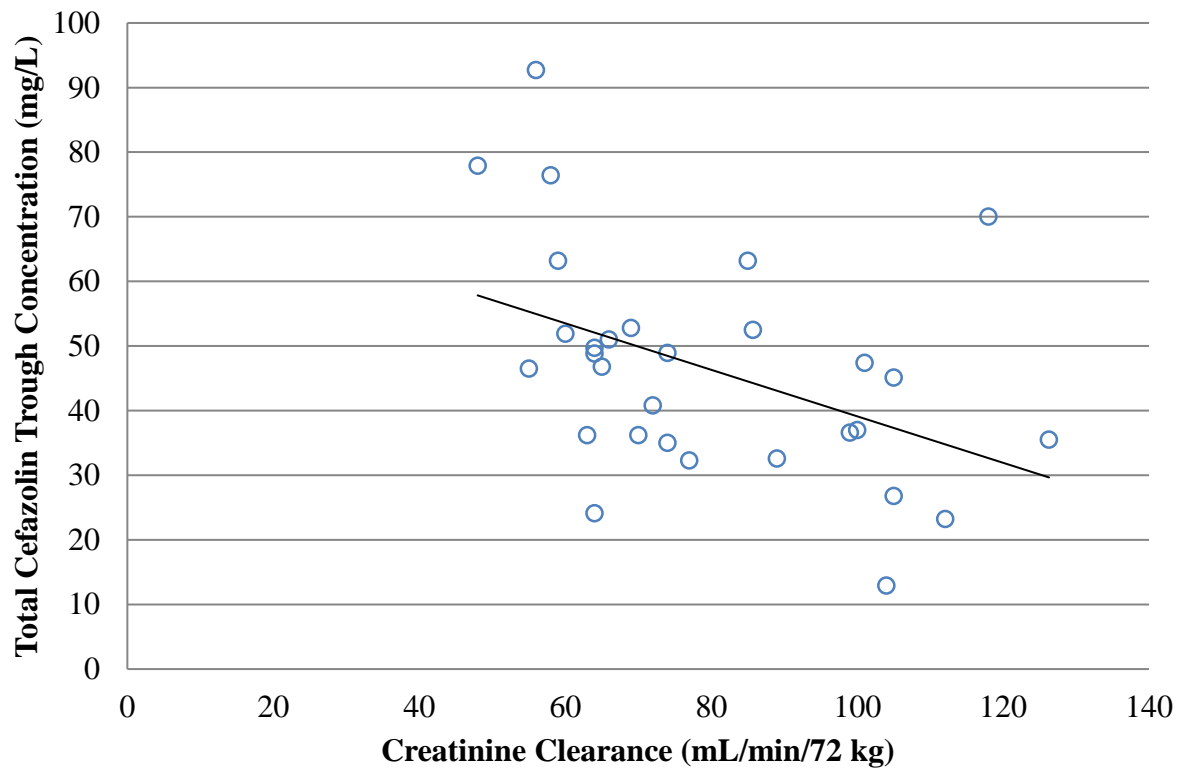
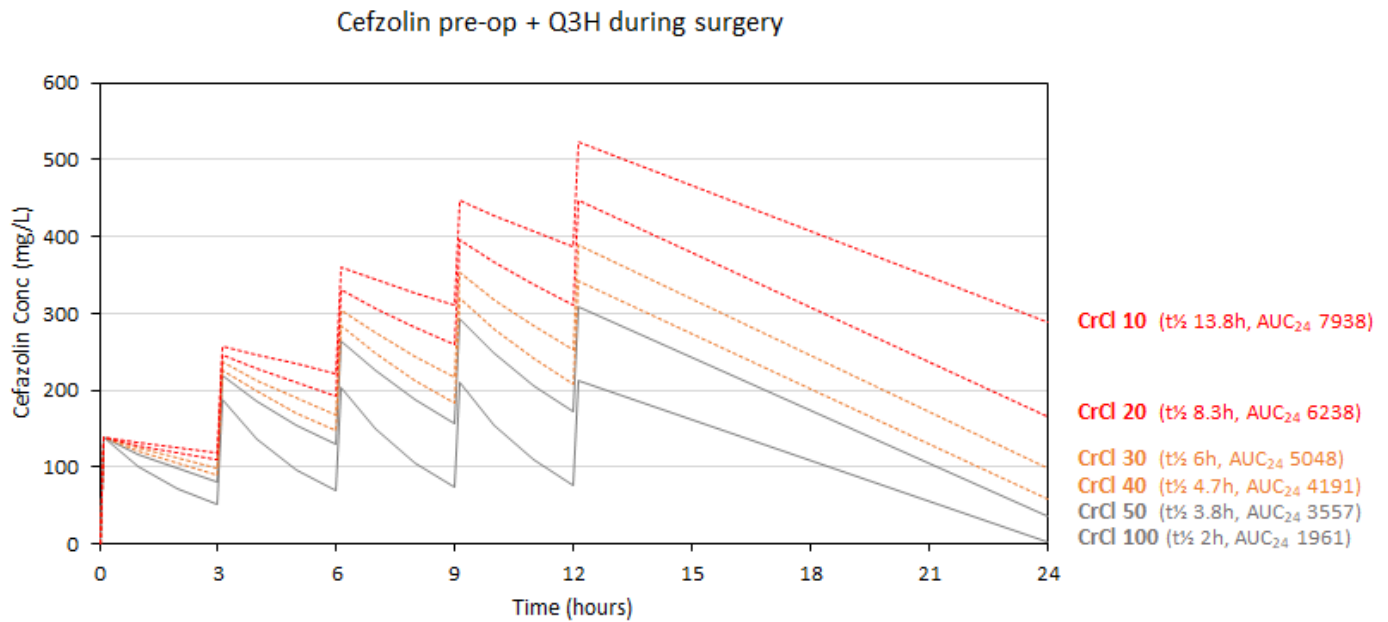


Figure 7. Total cefazolin intraoperative trough concentrations versus creatinine clearance.



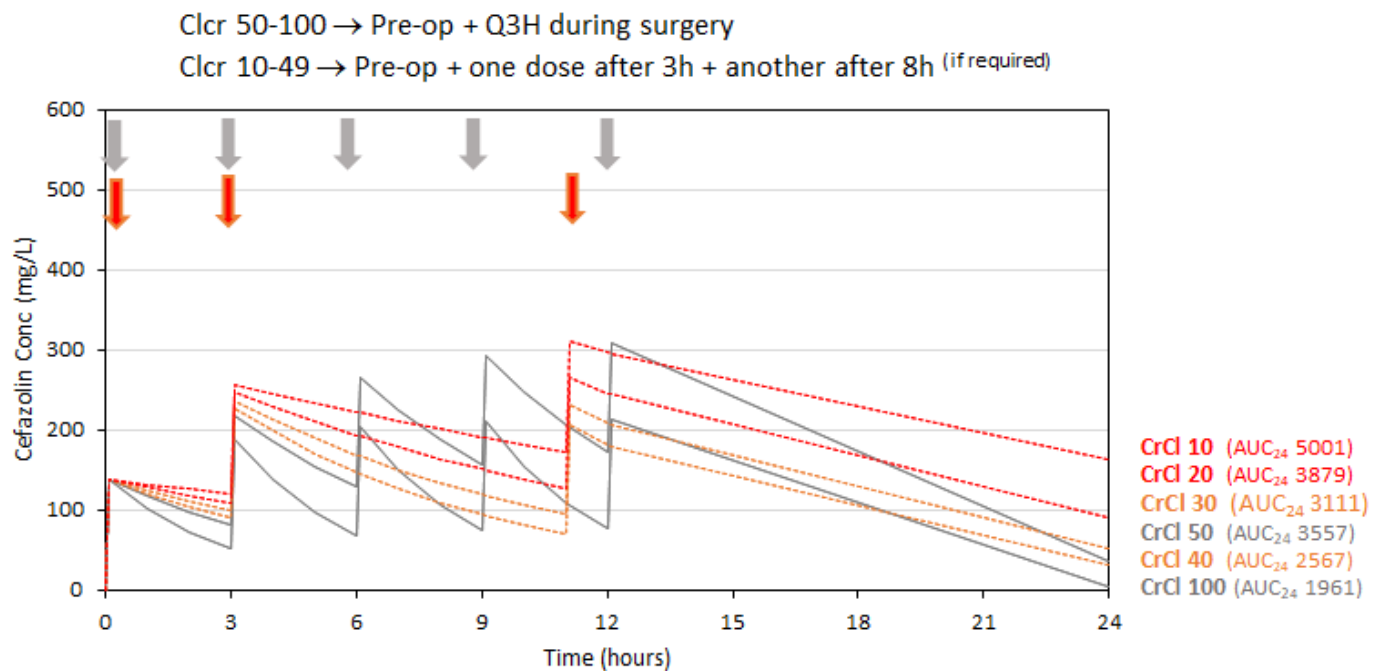
Trough Concentration = - 0.36 x (Creatinine Clearance) + 75.1; $R^2 = 0.19$

Figure 8. Simulated cefazolin drug exposure in a scenario where cefazolin is administered every 3 hours during cardiac surgery with CPB regardless of patient renal function. Gray lines represent creatinine clearance ≥ 50 mL/min/72 kg as per our original study.



AUC_{24} , area under the concentration time curve; Clcr, creatinine clearance; $t_{1/2}$, half-life

Figure 9. Simulated cefazolin drug exposure using renal-adjusted cefazolin regimen for patients with $\text{Clcr} < 50 \text{ mL/min/72 kg}$. Grey arrows represent cefazolin AP regimen in which cefazolin is dosed every 3 hours during surgery for patients with $\text{Clcr} \geq 50 \text{ mL/min}$. Red arrows represent preoperative dose, one dose after 3 hours and one dose after 8 hours for patients with $\text{Clcr} 10 - 49 \text{ mL/min}$.



AUC_{24} , area under the concentration time curve; Clcr , creatinine clearance; $t_{1/2}$, half-life

Figure 10. Cefazolin protein binding in samples collected from male ($n = 93$ samples) and female study ($n = 42$ samples) subjects undergoing cardiac surgery with CPB. Dashed line connects median values.

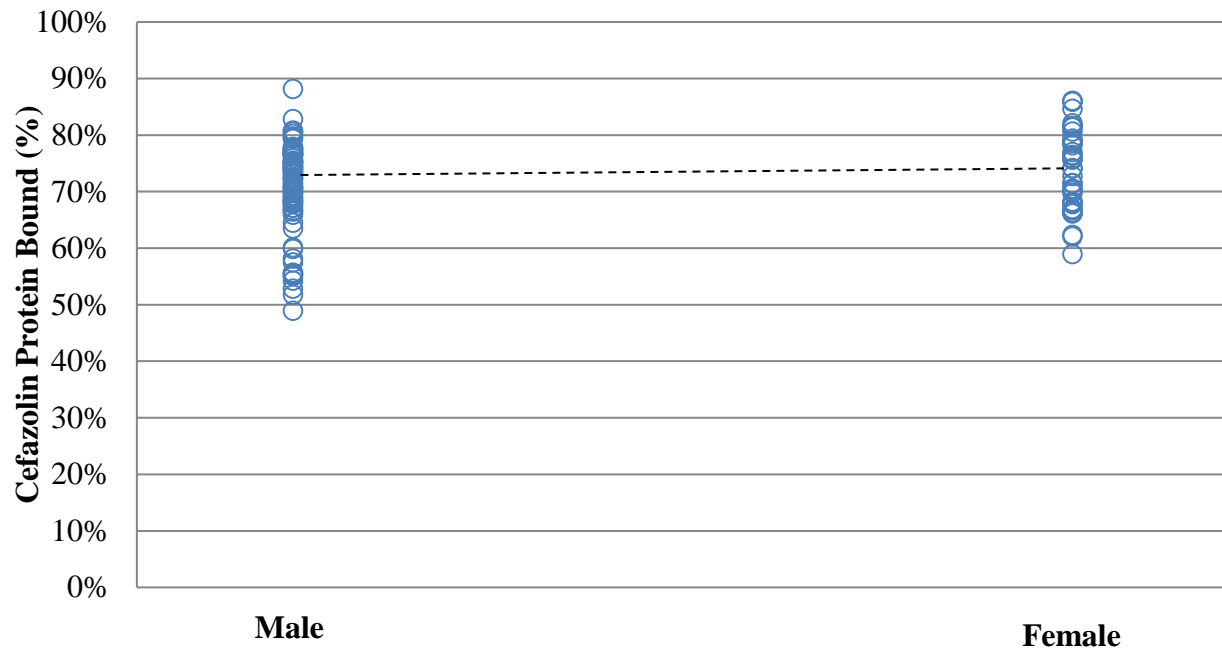


Figure 11. Cefazolin protein binding in samples collected from obese ($\text{BMI} \geq 30 \text{ kg/m}^2$, $n = 69$) and non-obese study subjects ($\text{BMI} < 30 \text{ kg/m}^2$, $n = 66$). Dashed line connects median values.

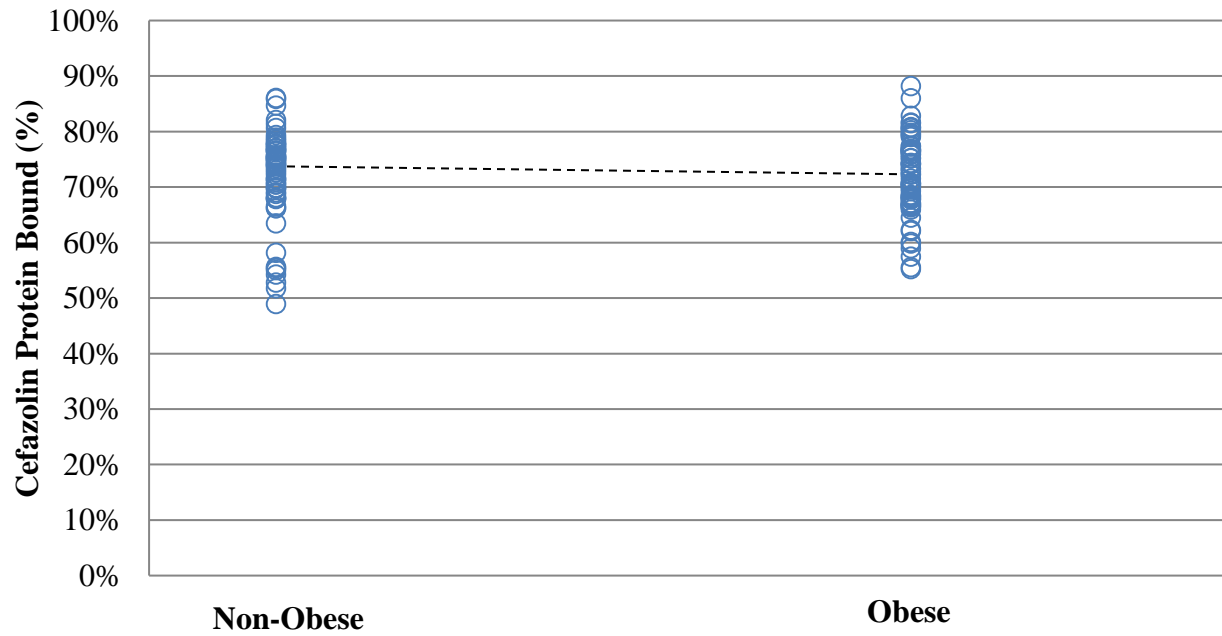
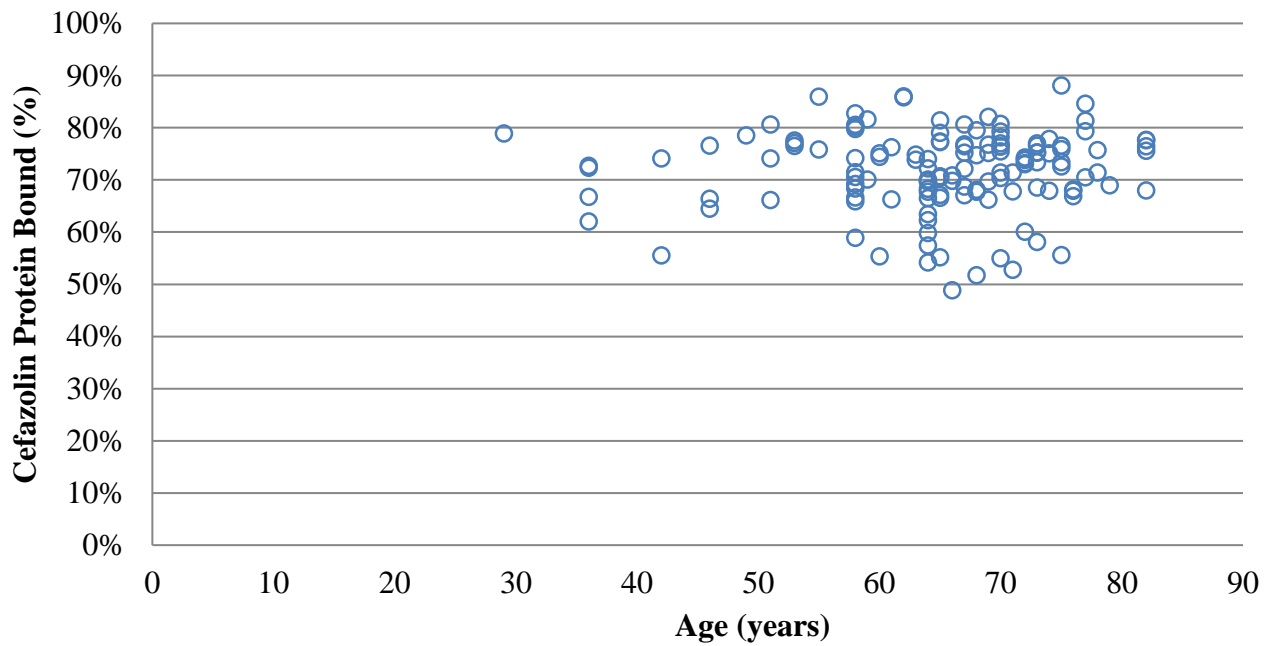
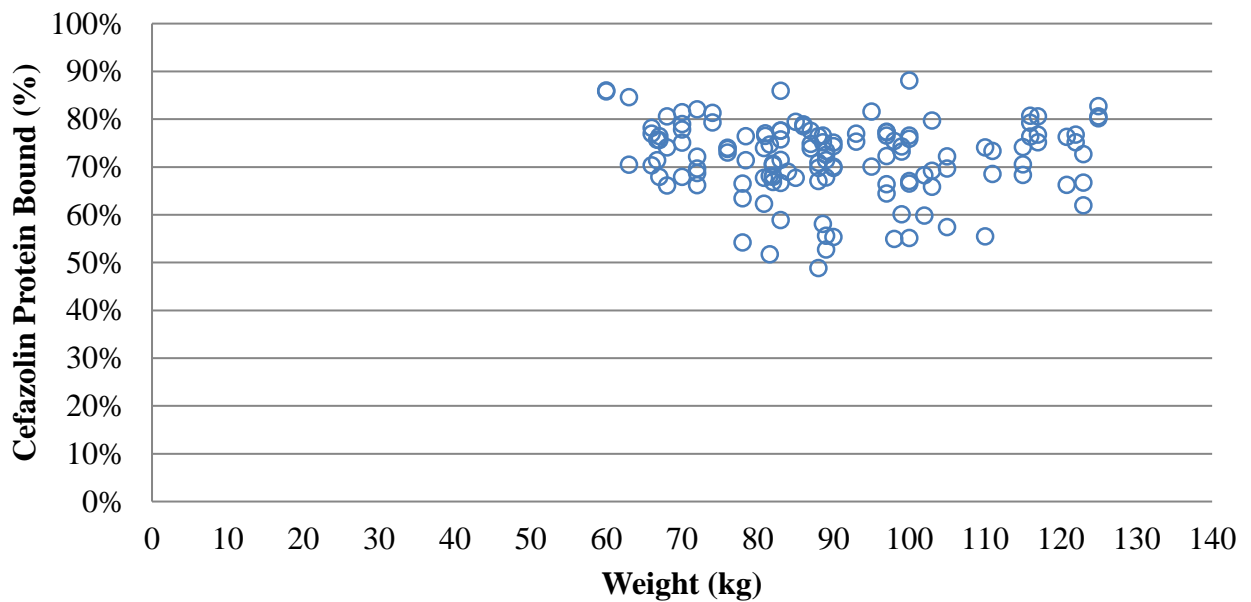


Figure 12. Cefazolin protein binding versus A) age, B) weight, C) body mass index, D) creatinine clearance, E) net fluid balance and F) duration of surgery.

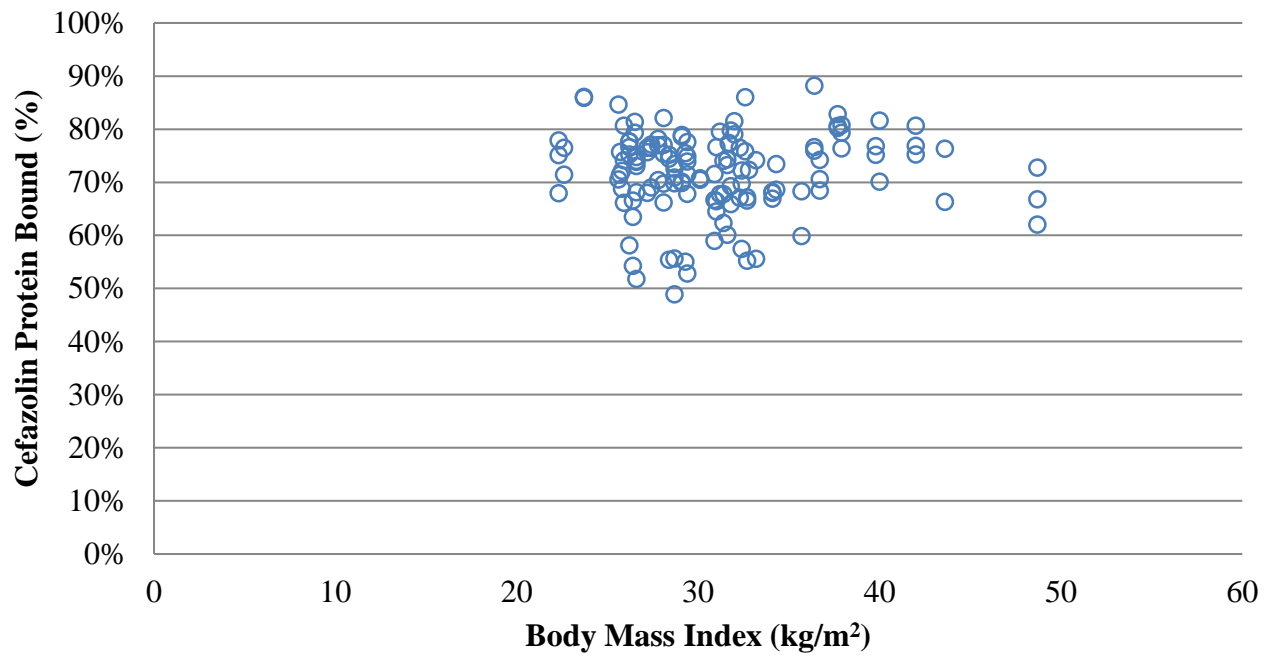
A)



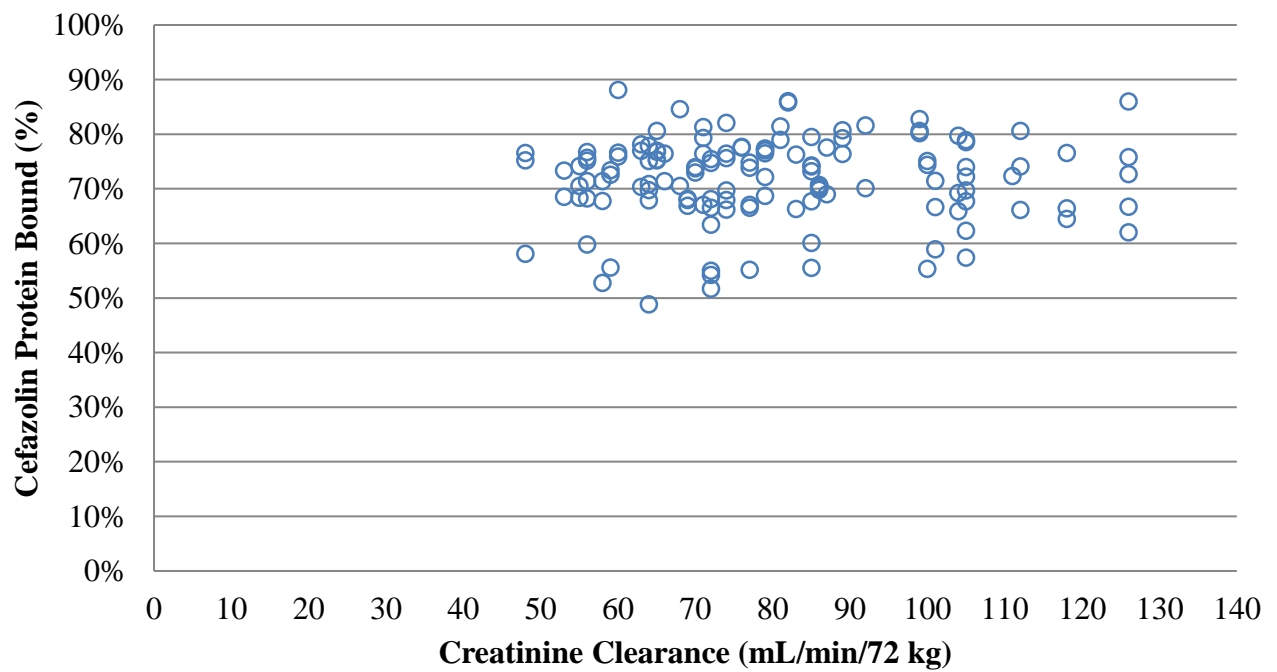
B)



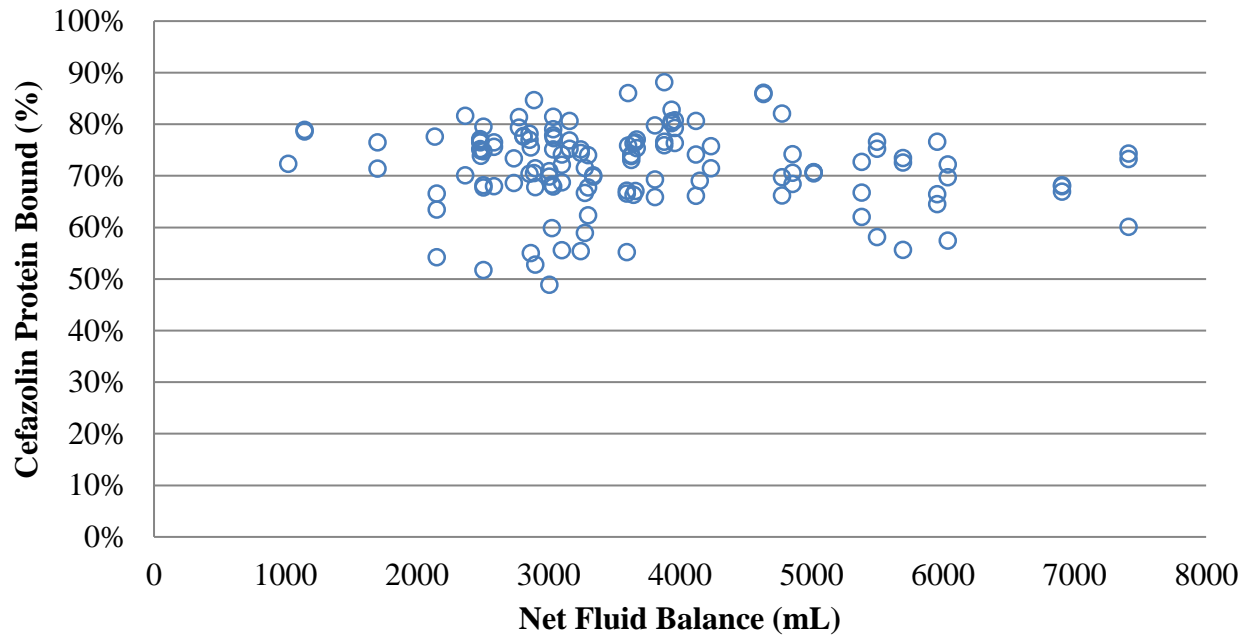
C)



D)



E)



F)

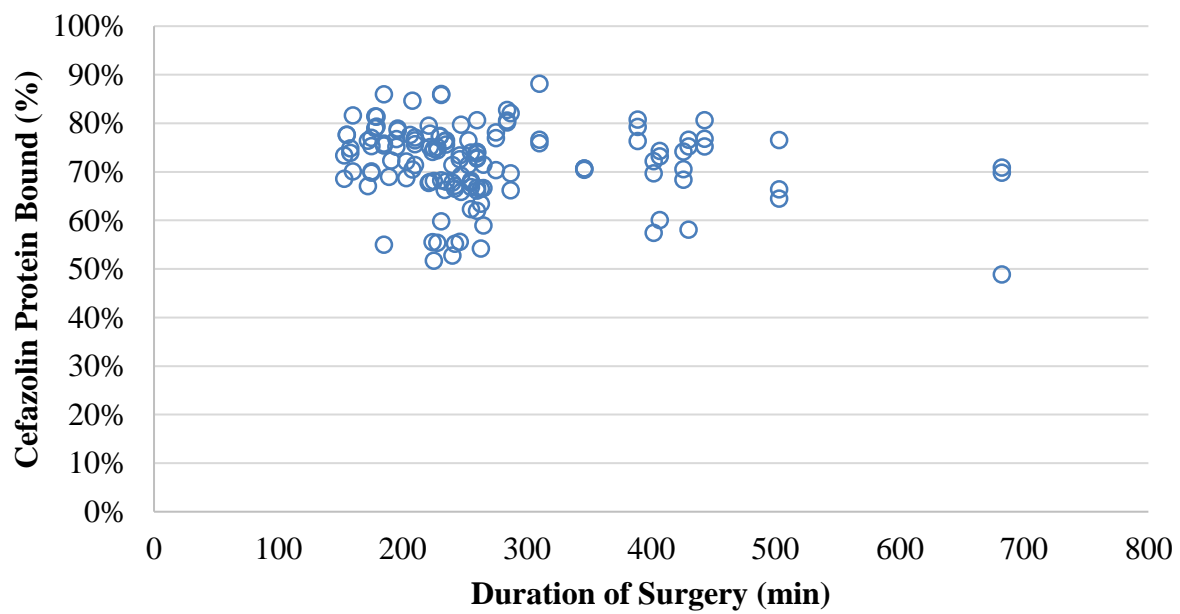
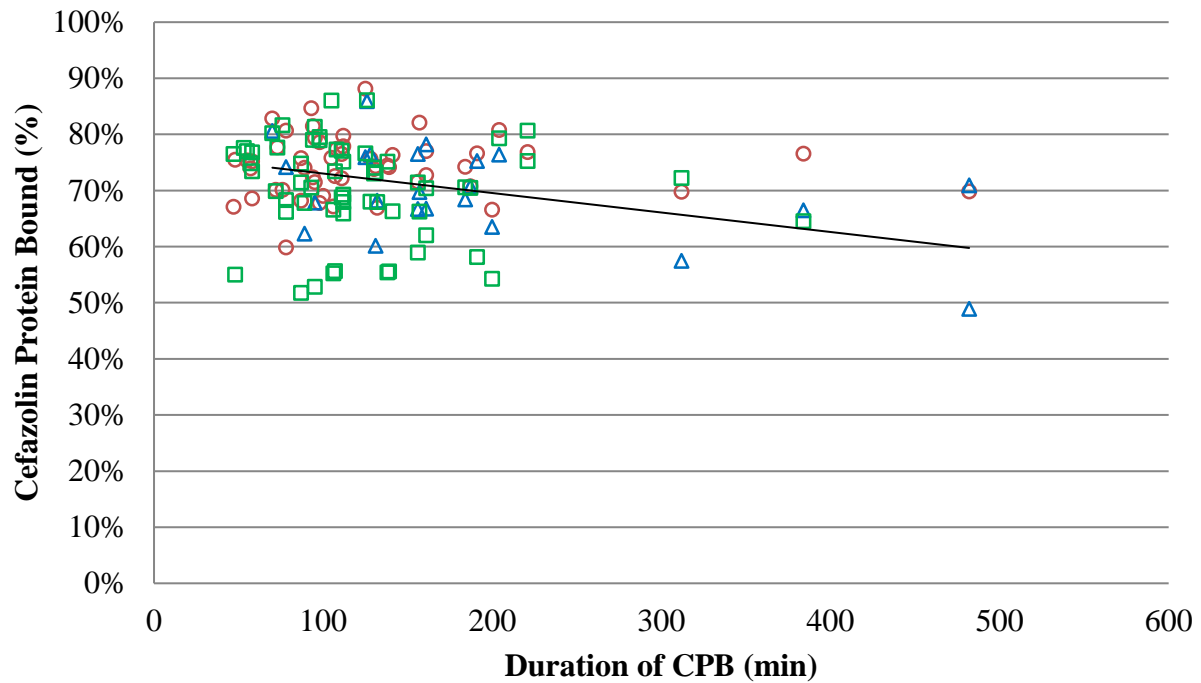


Figure 13. Association between cefazolin protein binding and duration of CPB in samples collected before starting CPB (red circle, $n = 52$), during CPB (blue triangle, $n = 23$), and post-CPB (green square, $n = 60$).



During CPB: Cefazolin Protein Bound = - 0.003 x (CPB minutes) + 0.765; R² = 0.24

Figure 14. Cefazolin protein binding in samples collected pre-CPB ($n = 52$) and during/post-CPB ($n = 83$). Dashed line connects mean values.

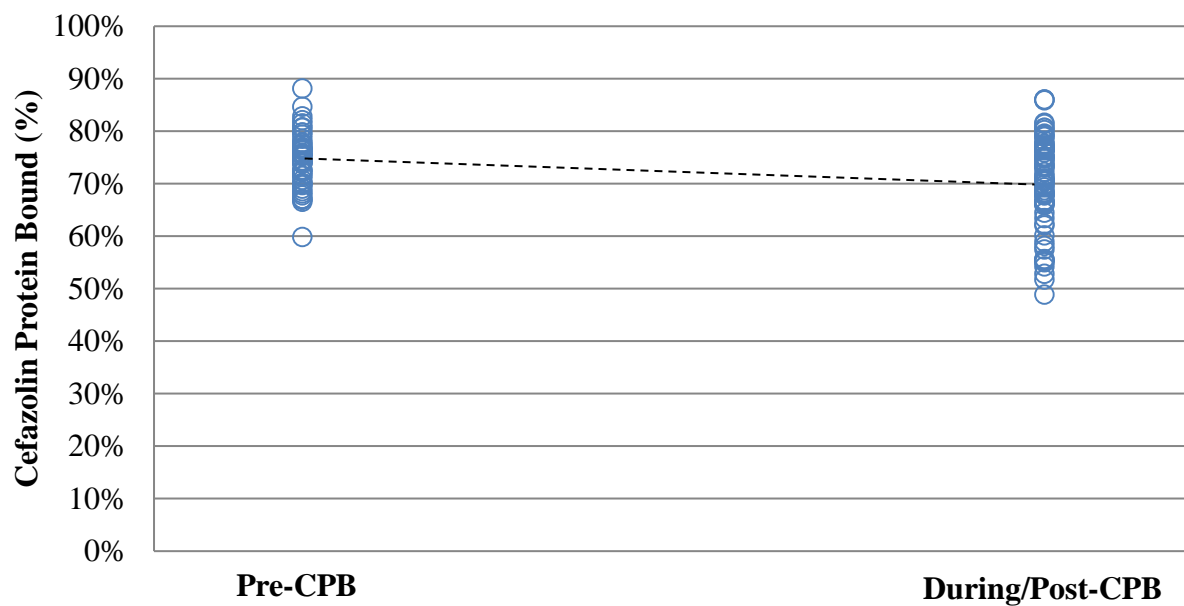


Figure 15. Cefazolin protein binding in paired samples ($n = 52$) collected from the same patients during cardiac surgery.

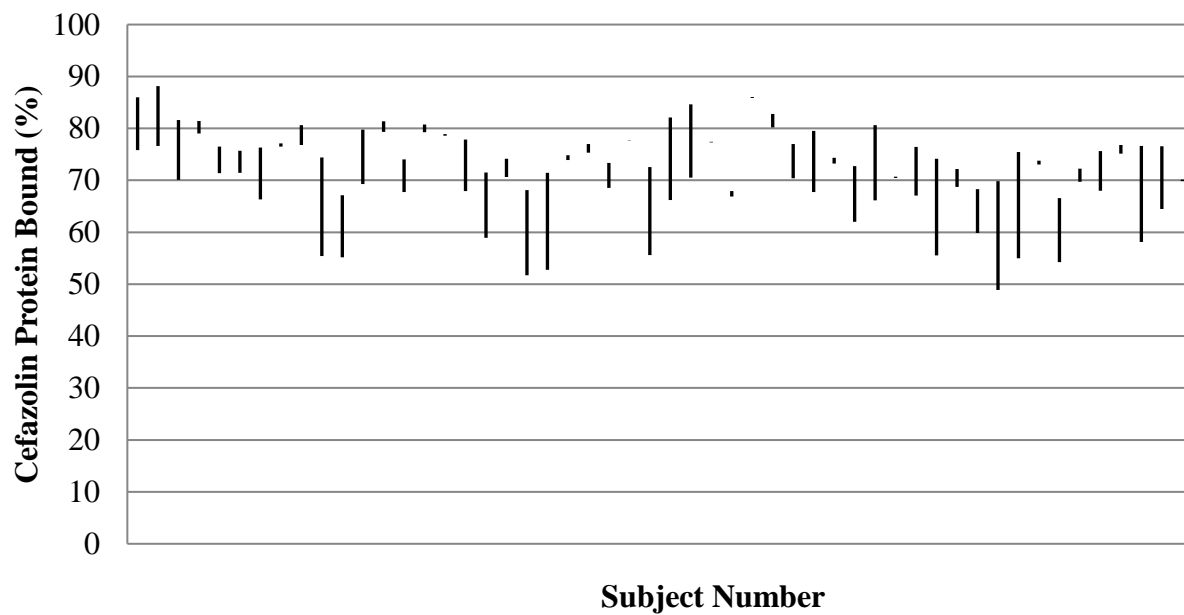
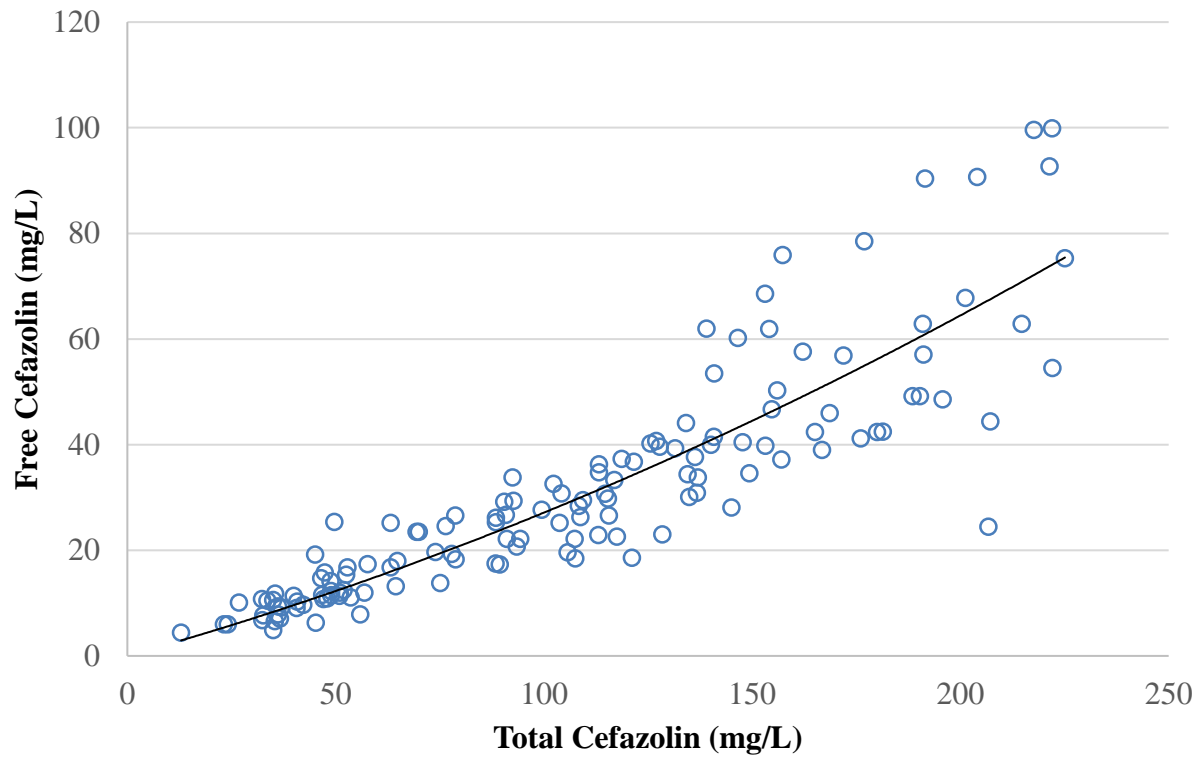


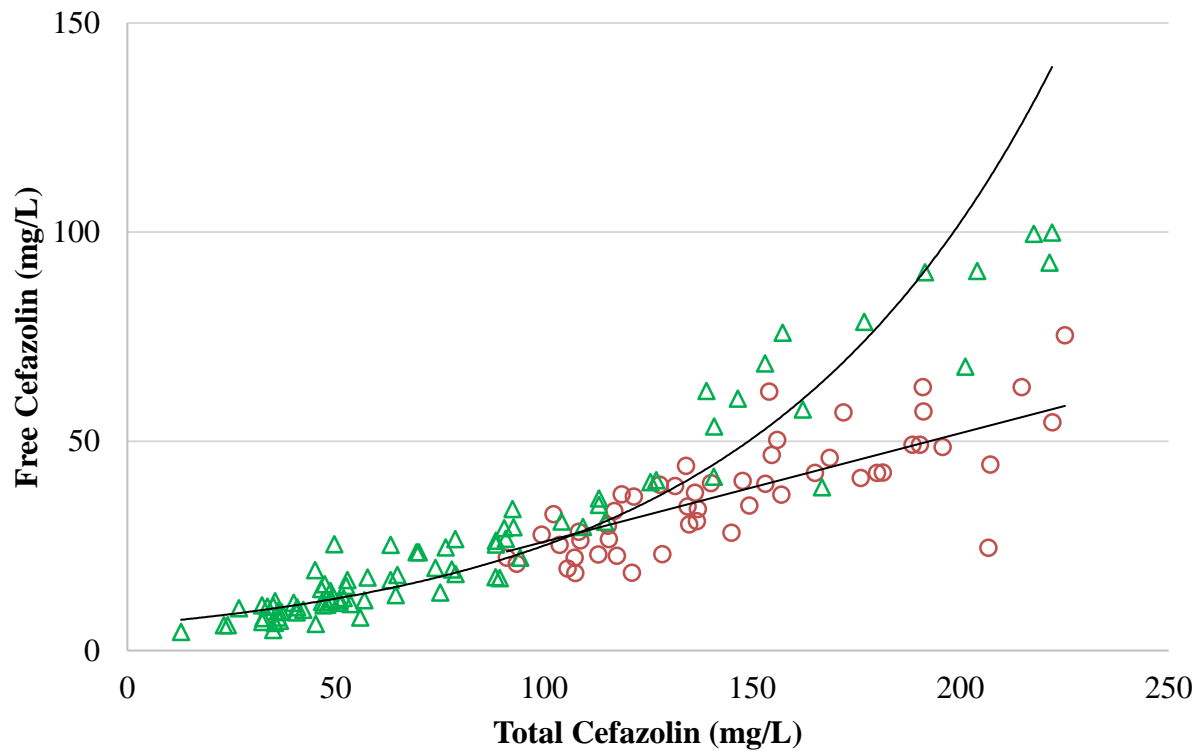
Figure 16. Free versus total cefazolin concentrations in A) all samples collected during cardiac surgery with CPB and B) in samples collected before starting cardiopulmonary bypass (CPB) (red circle, $n = 52$) and during /post-CPB (green triangle, $n = 83$).

A)



$$\text{Free Cefazolin} = 0.0005 \times (\text{Total Cefazolin})^2 + 0.2202 \times (\text{Total Cefazolin}); R^2 = 0.78$$

B)



Pre-CPB: Free Cefazolin = $0.26 \times \text{Total Cefazolin}$; $R^2 = 0.61$

During/Post-CPB: Free Cefazolin = $6.14 e^{(0.014 \times \text{Total Cefazolin})}$; $R^2 = 0.88$

Figure 17. Albumin concentrations (g/L) in samples collected pre-CPB ($n = 52$) and during/post-CPB ($n = 83$). Dashed line connects median values.

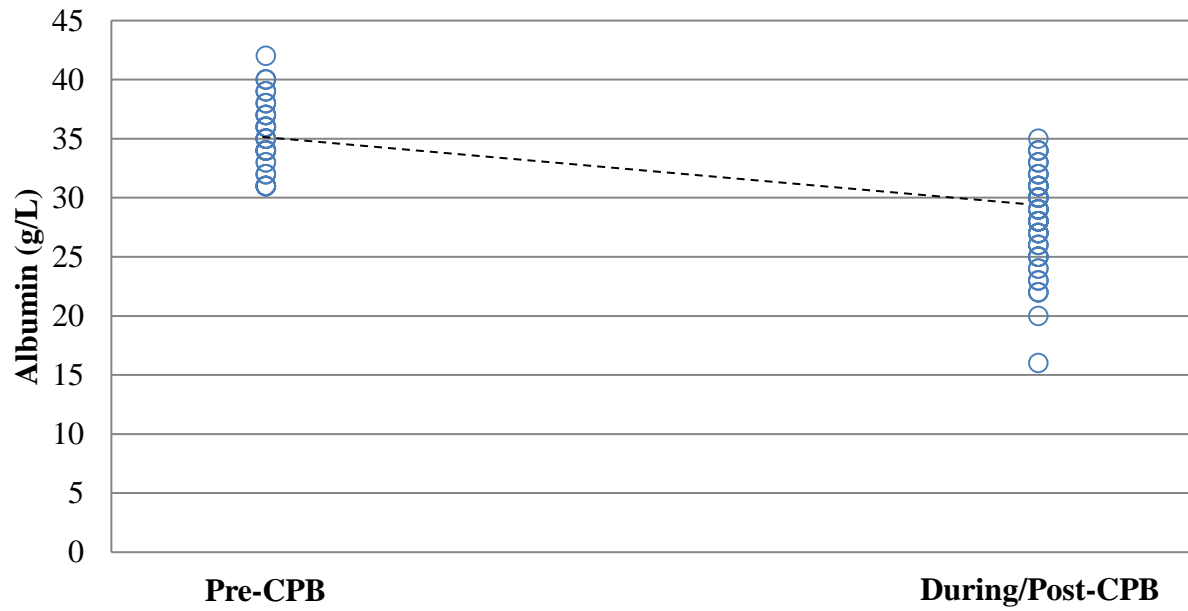
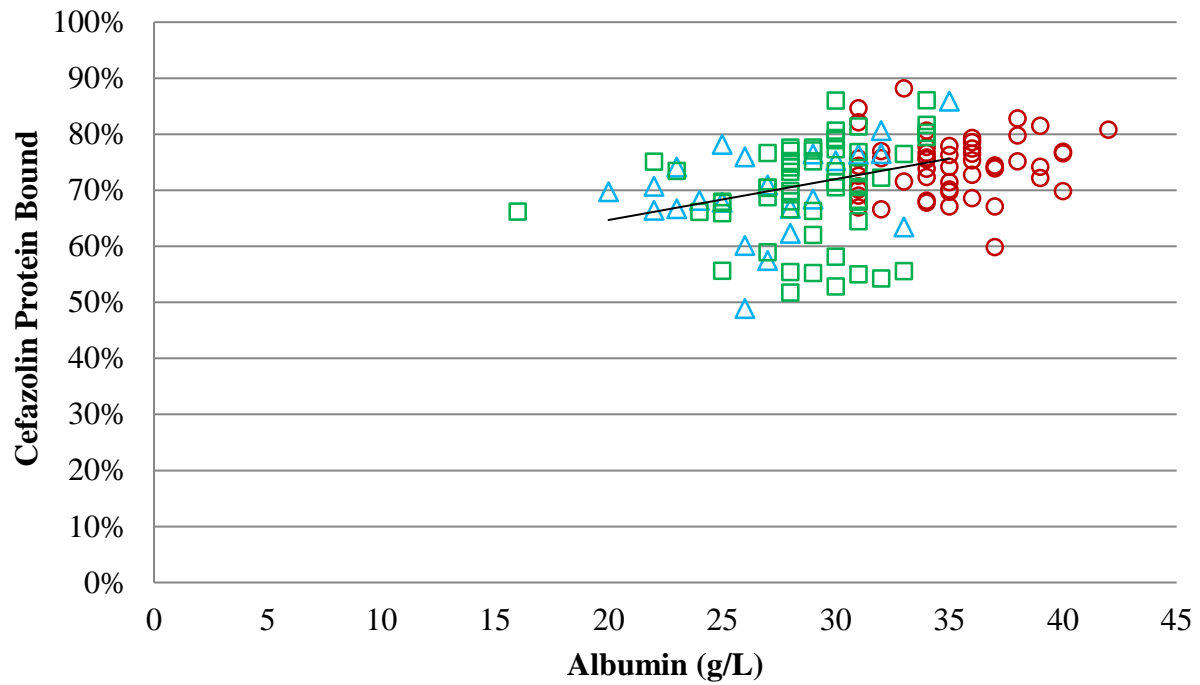
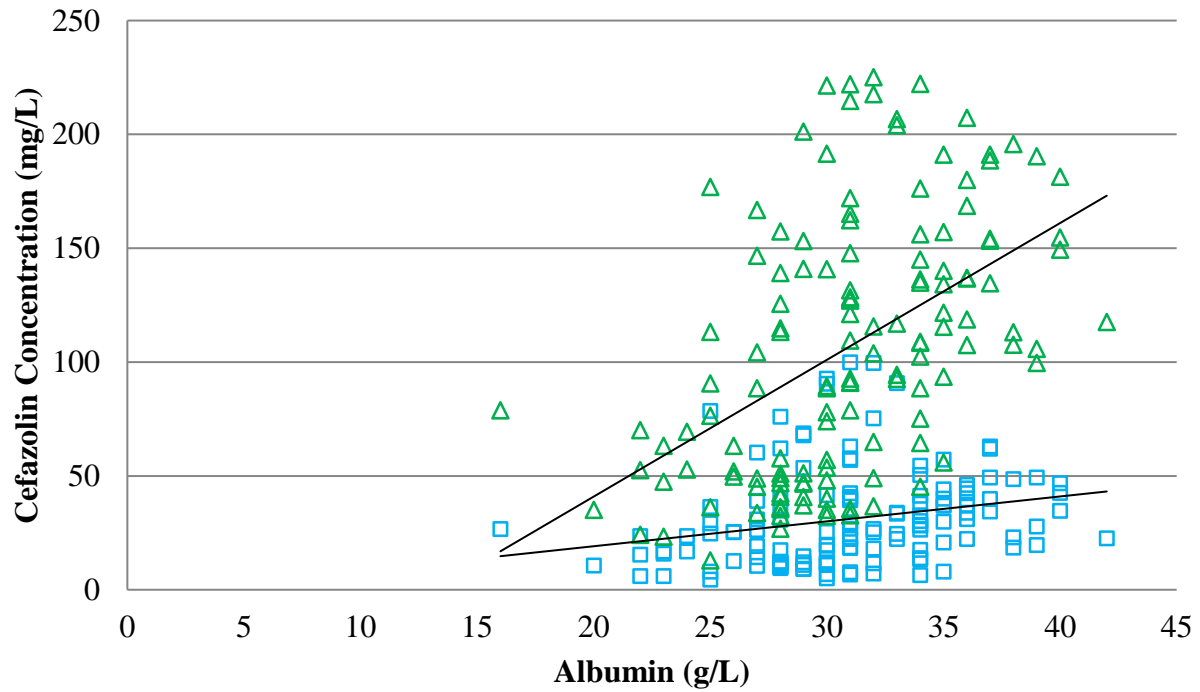


Figure 18. Cefazolin protein binding versus albumin concentrations in samples before starting cardiopulmonary bypass (CPB) (red circle, $n = 52$), during CPB (blue triangle, $n = 23$), and post-CPB (green square, $n = 60$).



During CPB: Cefazolin Protein Bound = $0.007 \times \text{Albumin} + 0.5$; $R^2 = 0.12$

Figure 19. Total (green triangle) and free (blue square) cefazolin concentrations versus albumin concentrations.



$$\text{Total Cefazolin} = 6.0 \times \text{Albumin} - 79.2; R^2 = 0.24$$

$$\text{Free Cefazolin} = 1.1 \times \text{Albumin} - 2.8; R^2 = 0.06$$

Figure 20. The distribution of the elimination rate constant (left panel) and volume of distribution (right panel) for the population-pharmacokinetic model of 55 subjects undergoing cardiac surgery with CPB.

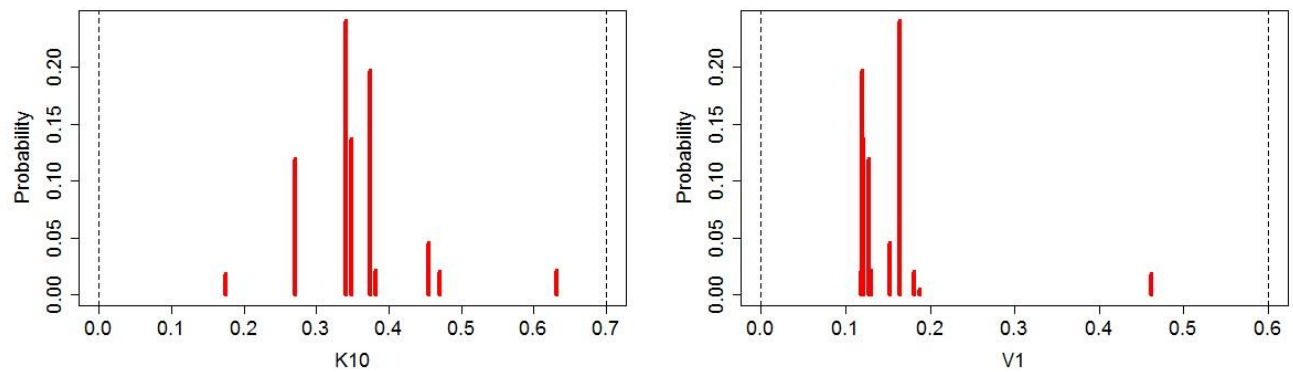
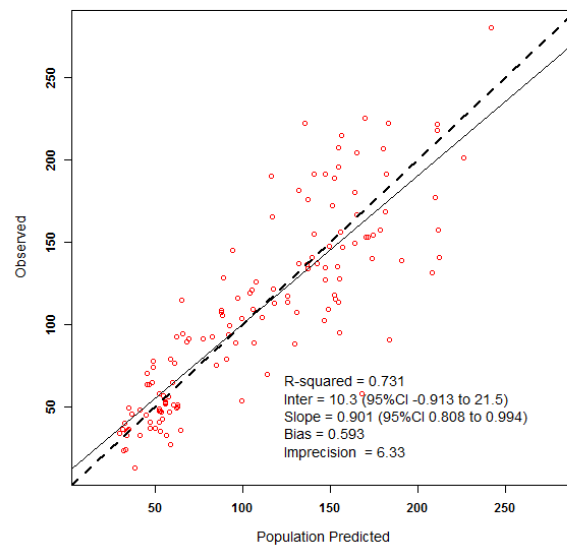


Figure 21. Diagnostic plots for the population-pharmacokinetic model of cefazolin in subjects undergoing cardiac surgery with CPB. A) Observed cefazolin concentrations versus population predicted concentrations and B) Observed cefazolin concentrations versus individual predicted concentrations.

A)



B)

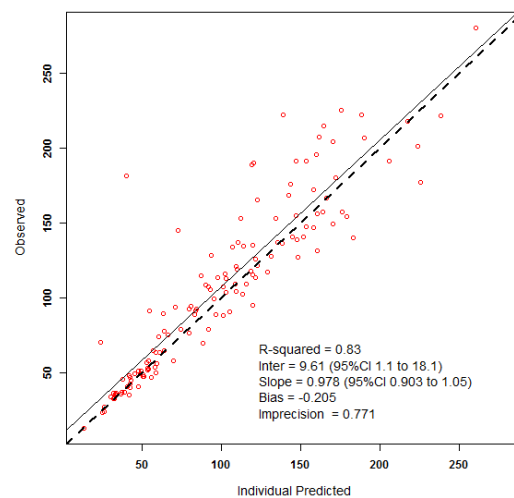
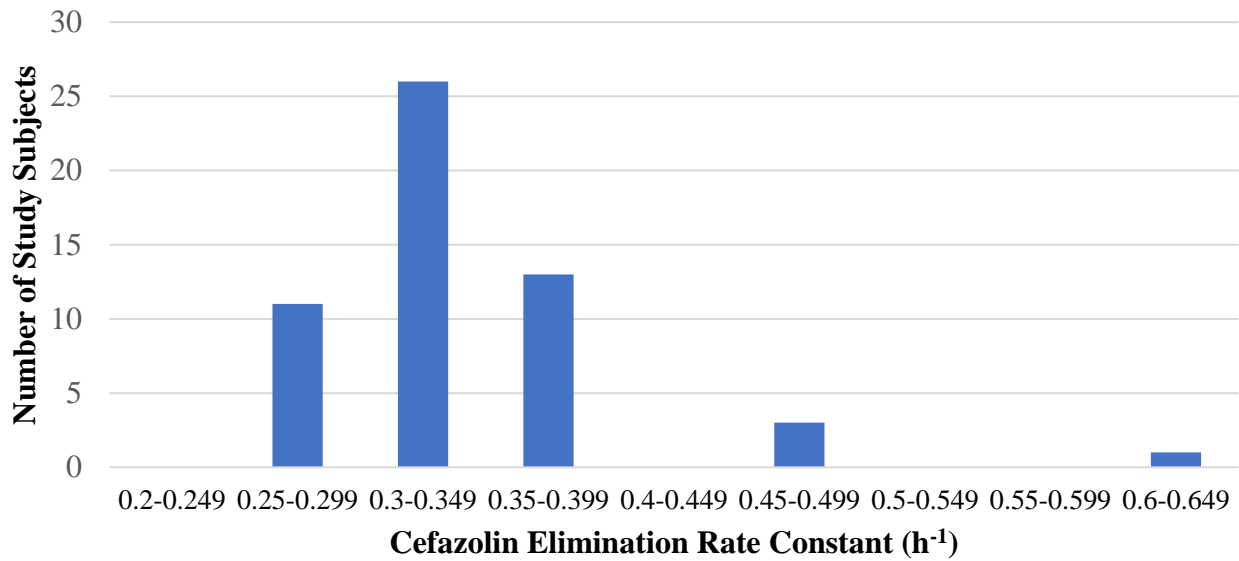


Figure 22. The distribution of the individual Bayesian posterior values for the cefazolin elimination rate constant (A) and volume of distribution (B) of 54 subjects undergoing cardiac surgery with CPB.

A)



B)

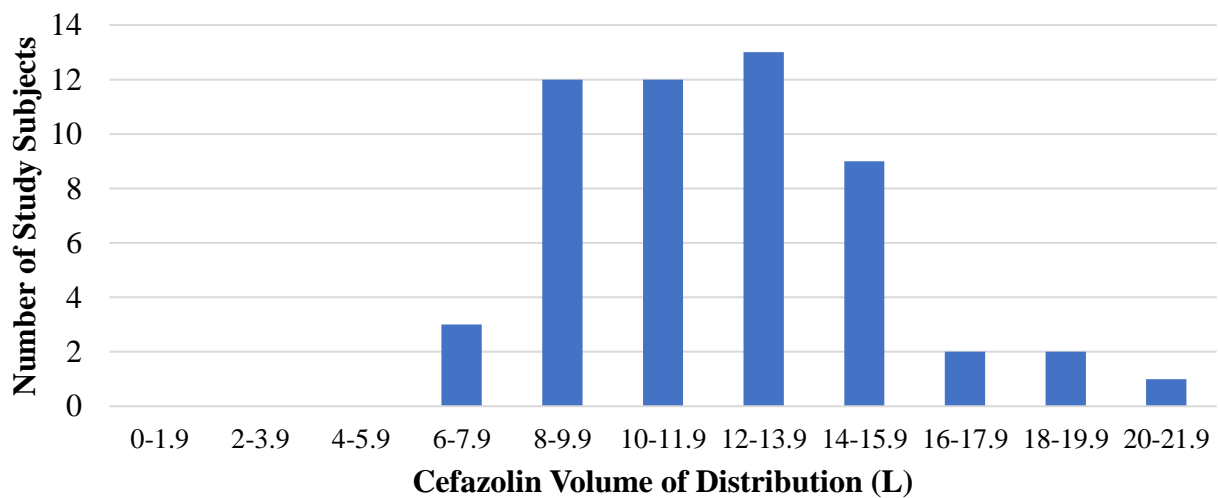
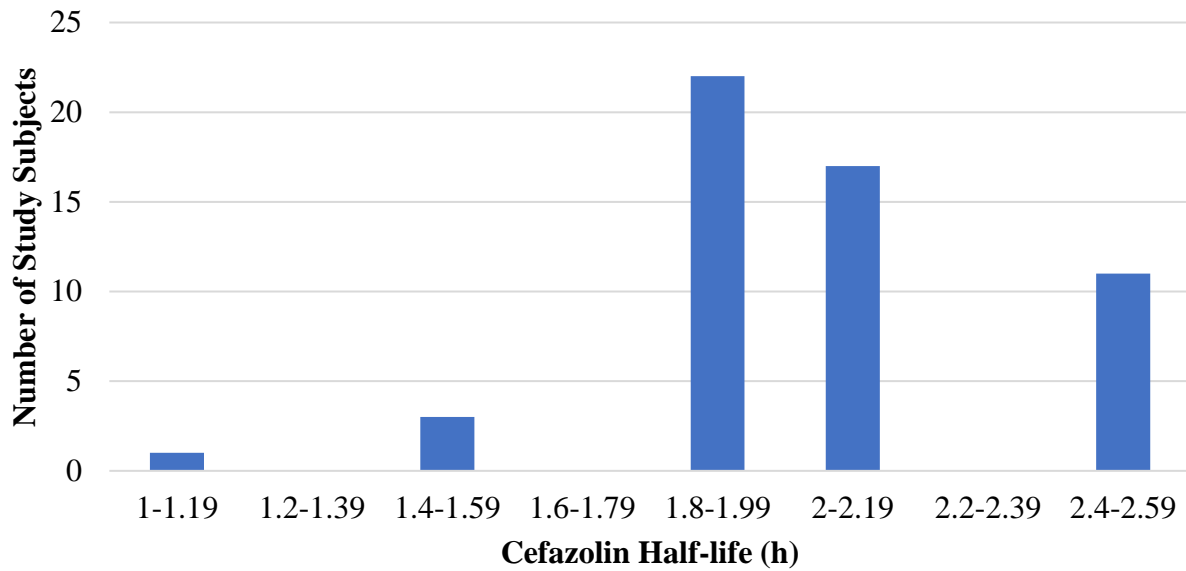


Figure 23. The distribution of the individual Bayesian posterior values for the cefazolin half-life (A) and clearance (B) of 54 subjects undergoing cardiac surgery with CPB.

A)



B)

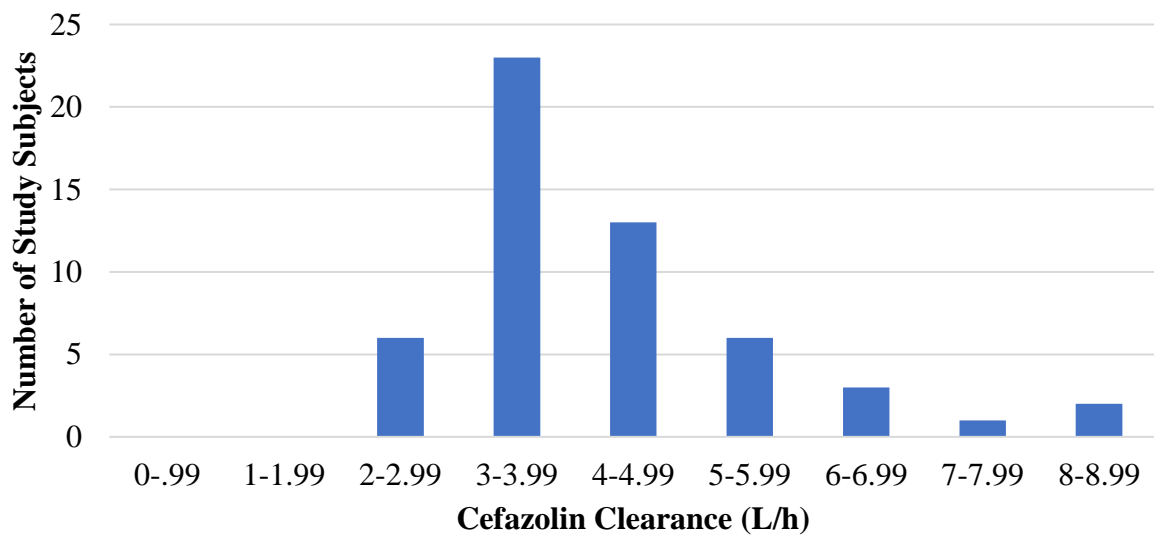
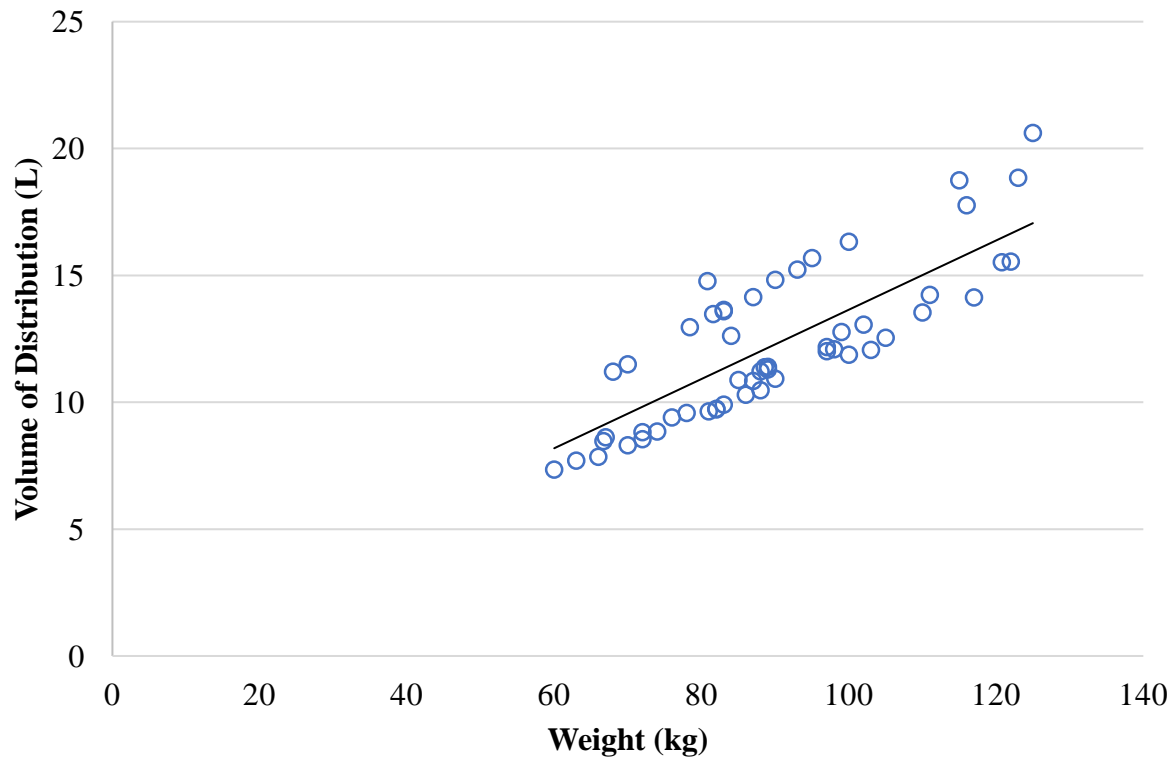


Figure 24. Cefazolin volume of distribution versus study subject weight.



Volume of Distribution = $0.137 \times \text{Weight}$; $R^2 = 0.67$

Figure 25. Cefazolin volume of distribution versus net fluid balance at end of cardiac surgery with CPB.

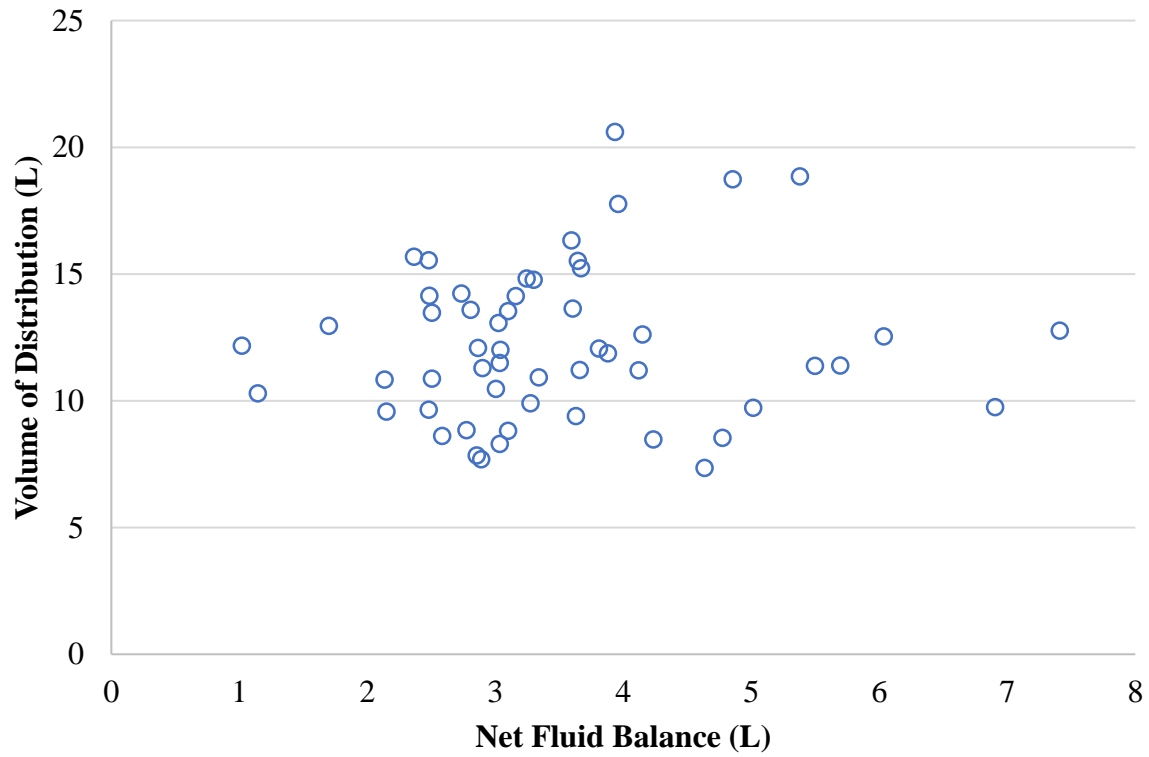
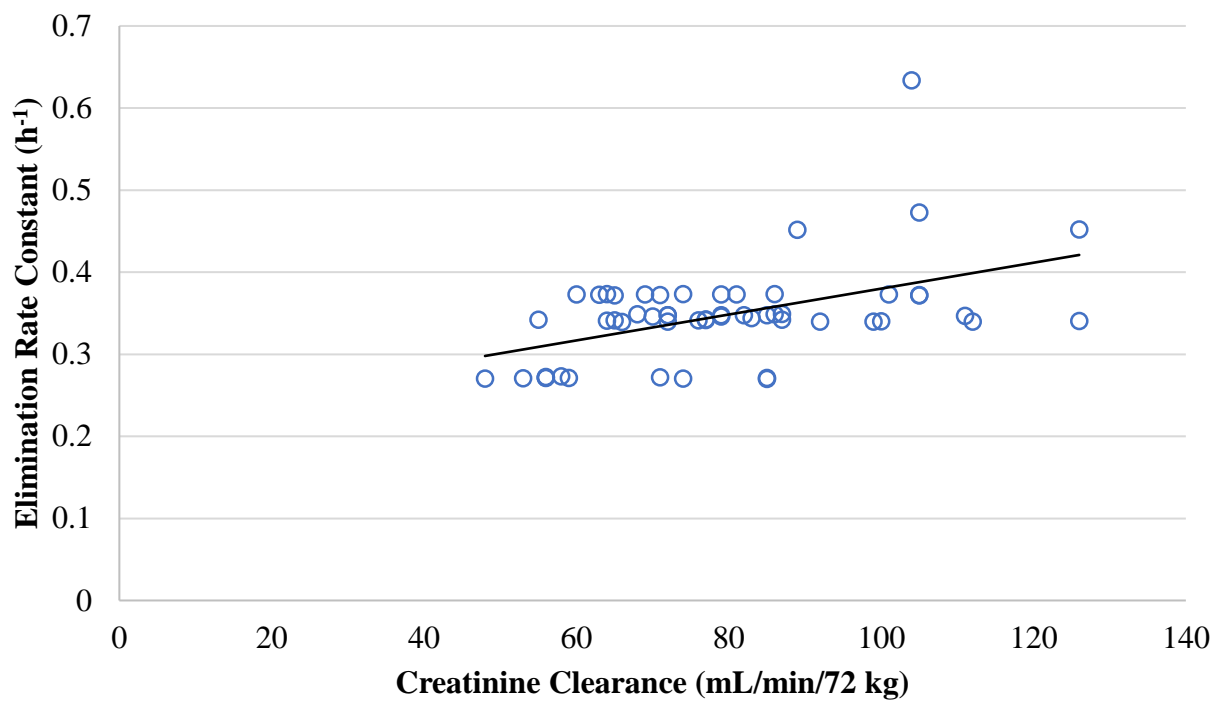


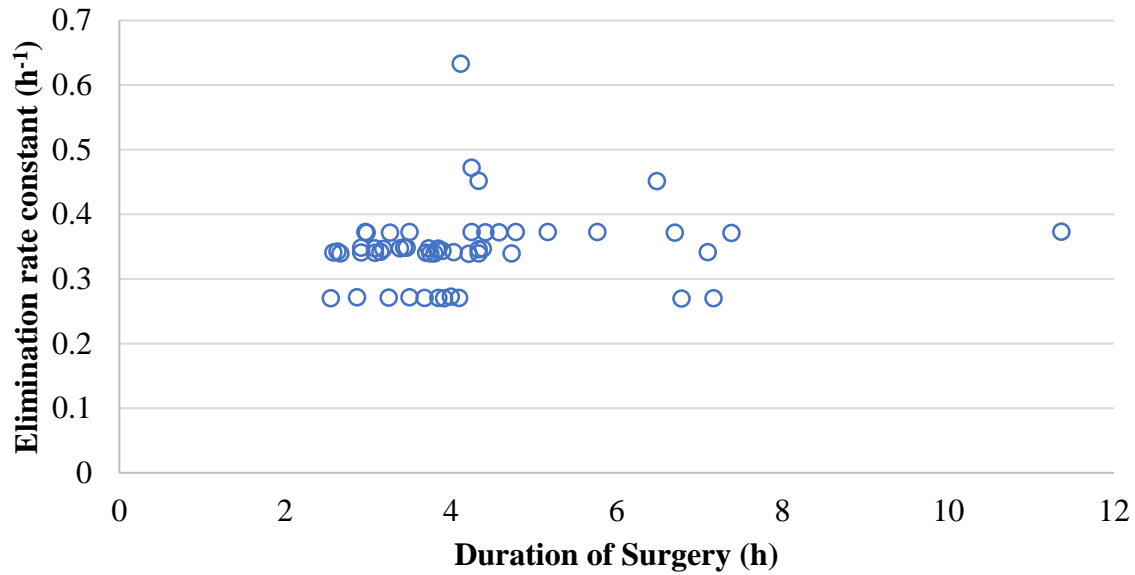
Figure 26. Cefazolin elimination rate constant versus study subject creatinine clearance.



Elimination rate constant = $0.002 \times \text{Clcr} + 0.22$; $R^2 = 0.24$

Figure 27. Cefazolin elimination rate constant versus A) duration of surgery and (B) duration of cardiopulmonary bypass.

A)



B)

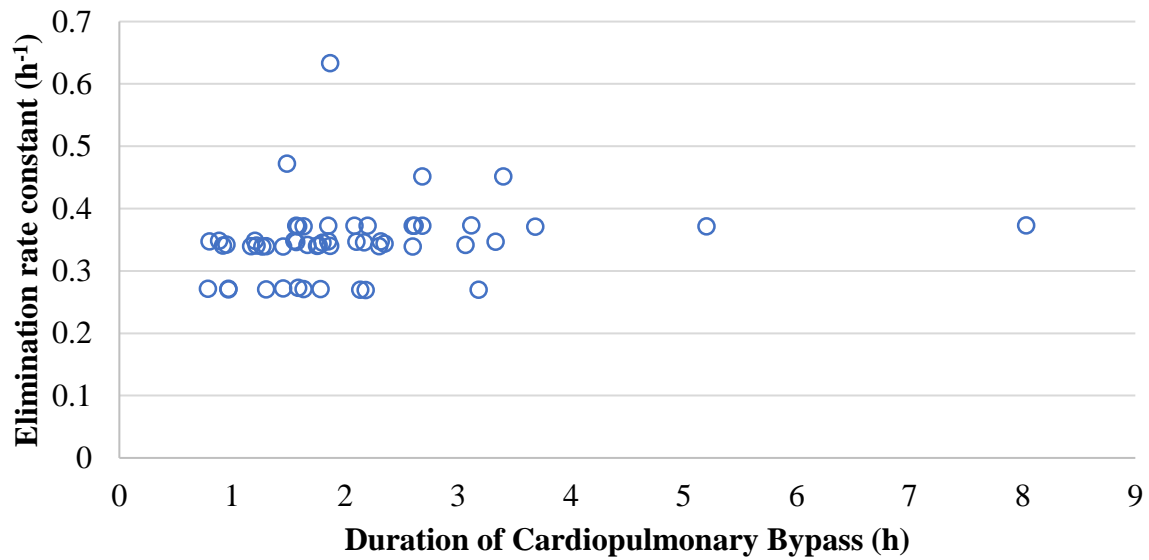
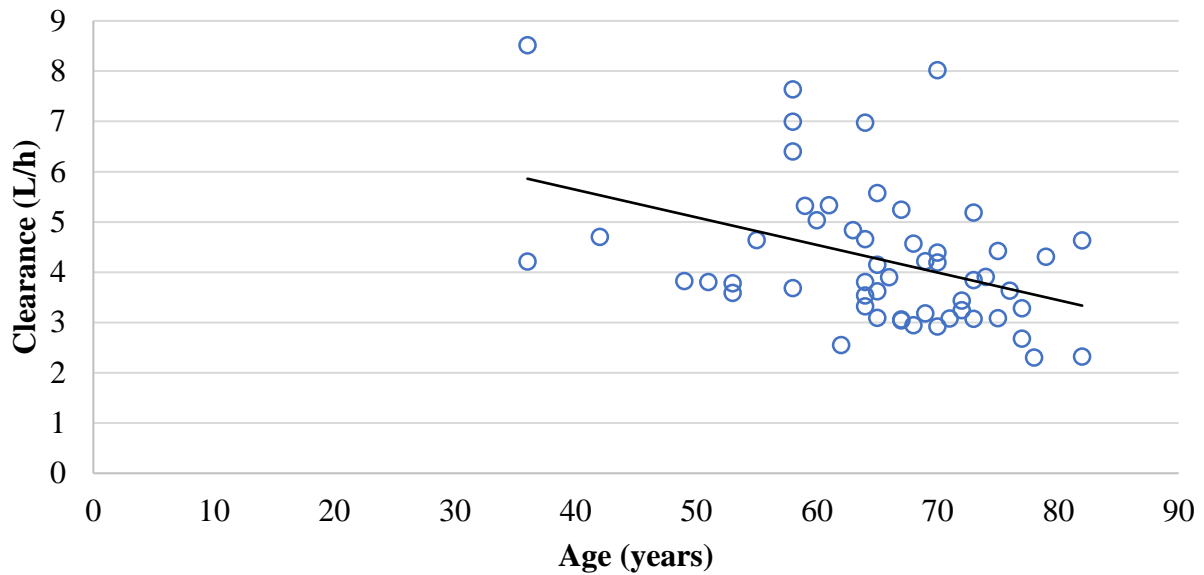


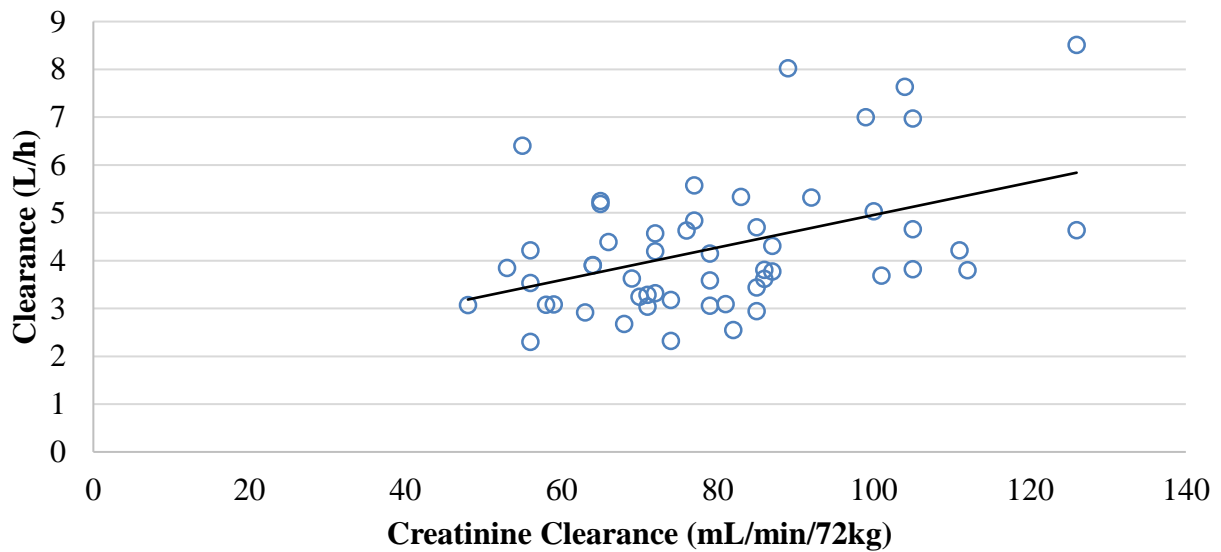
Figure 28. Cefazolin clearance versus A) study subject age and B) creatinine clearance.

A)



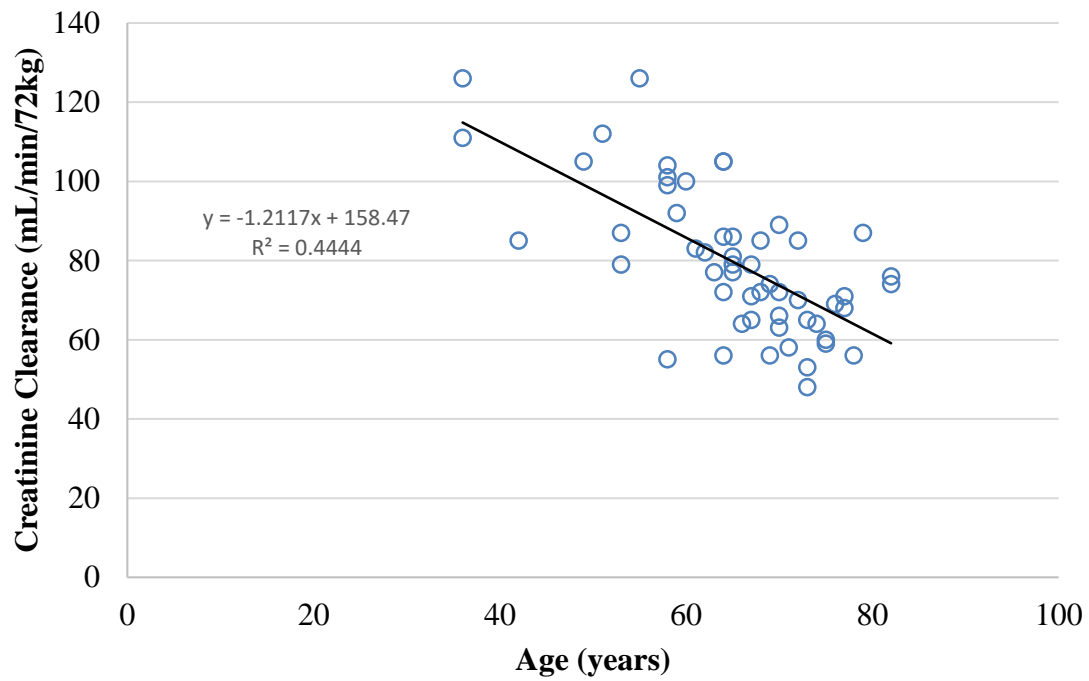
$$\text{Clearance} = -0.06 \times \text{Age} + 7.84; R^2 = 0.16$$

B)



$$\text{Clearance} = 0.03 \times \text{Clcr} + 1.56; R^2 = 0.20$$

Figure 29. Study subject creatinine clearance versus age.



Creatinine clearance = $-1.21 \times \text{Age} + 158.47$, $R^2 = 0.44$

Figure 30. A) Total and B) free cefazolin closure concentrations in study subjects included in pharmacodynamic analysis ($n = 40$). Solid circles represent cases of surgical site infection.

A)

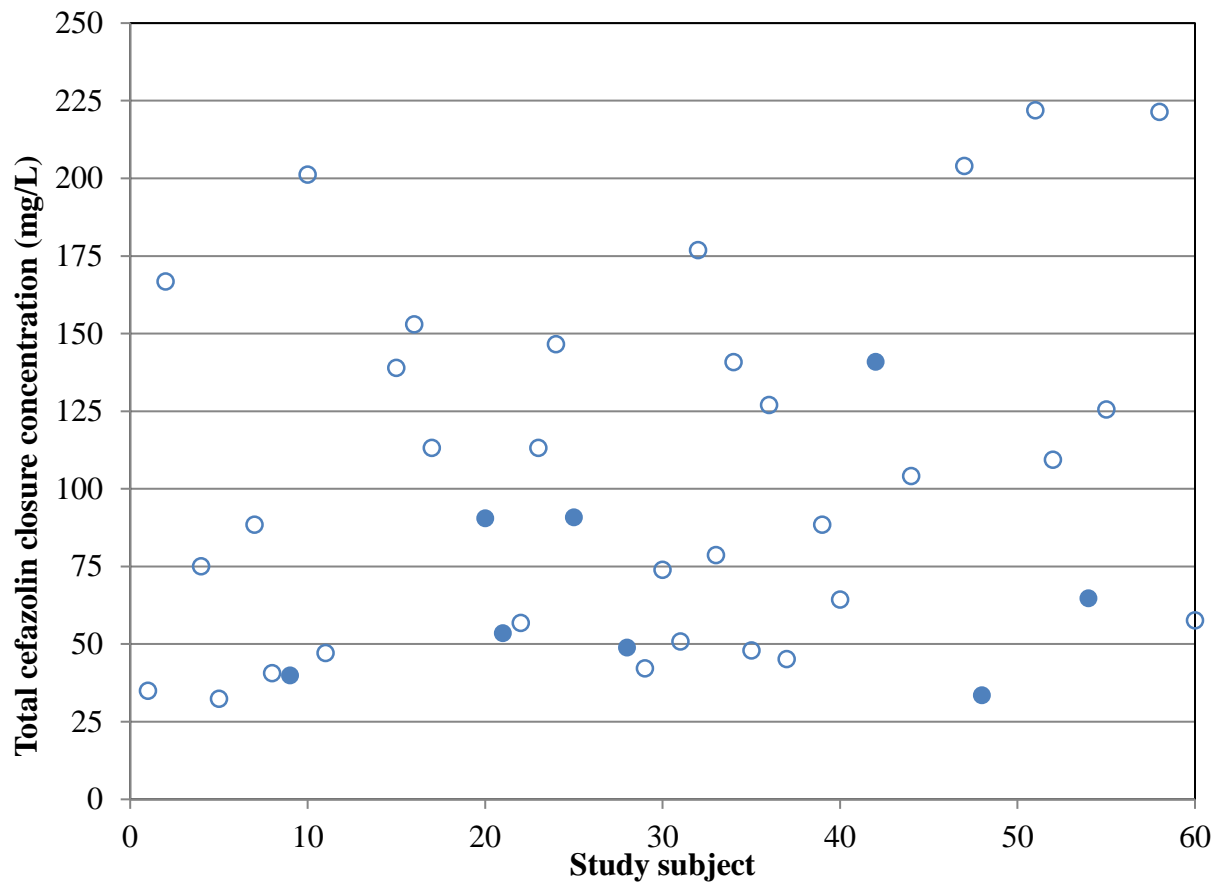


Figure 30. A) Adapted from: Zelenitsky S, Calic D, Arora RC, et al. Antimicrobial prophylaxis for patients undergoing cardiac surgery: Intraoperative cefazolin concentrations and sternal wound infections. *Antimicrob Agents Chemother* November 2018. Copyright © American Society for Microbiology, *Antimicrobial Agents and Chemotherapy*, 62, 2018, e01360-18. DOI 10.1128/AAC.01360-18.

B)

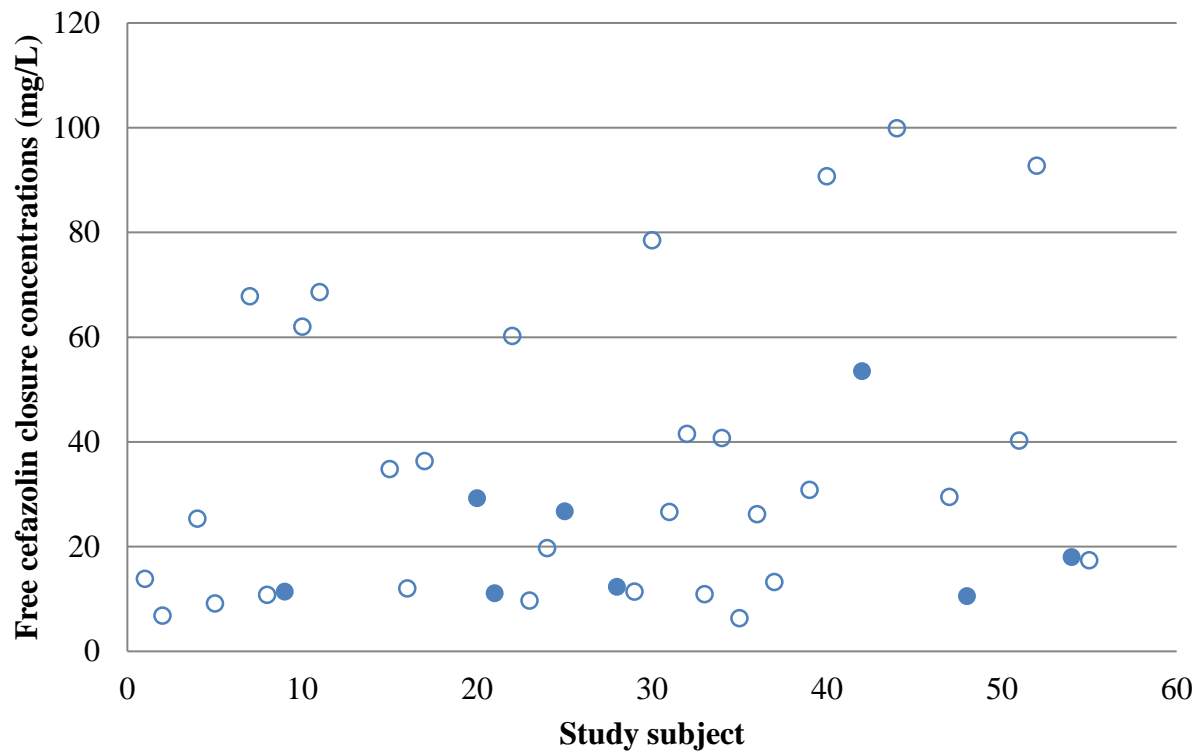
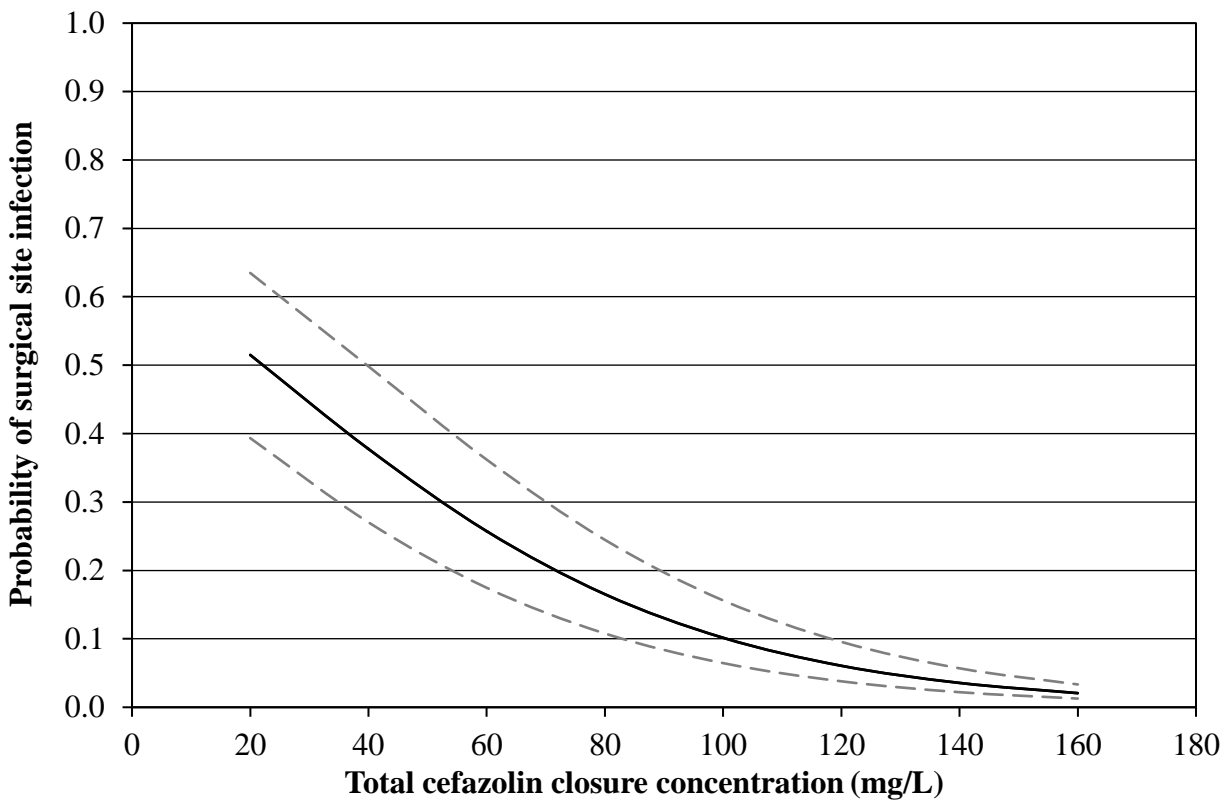


Figure 31. Probability of surgical site infection based on the logit function of total cefazolin closure concentration stratified for duration of surgery. [where solid line represents the median duration of surgery (230 minutes), lower hatched line represents the 25th percentile (200 minutes) and upper hatched line represents the 75th percentile (260 minutes)]



Originally published: Zelenitsky S, Calic D, Arora RC, et al. Antimicrobial prophylaxis for patients undergoing cardiac surgery: Intraoperative cefazolin concentrations and sternal wound infections. *Antimicrob Agents Chemother* 2018.

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APPENDIX A. University of Manitoba Health Research Ethics Board Certificate of Final Approval for New Studies



BANNATYNE CAMPUS
Research Ethics Board

P126 - 770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Telephone 204-789-3255
Fax 204-789-3414

HEALTH RESEARCH ETHICS BOARD (HREB) CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES Full Board Review

PRINCIPAL INVESTIGATOR: Dr. S. Zelenitsky	INSTITUTION/DEPARTMENT: UofM / Pharmacy	ETHICS #: H2014:142
HREB MEETING DATE: April 28, 2014	APPROVAL DATE: May 7, 2014	EXPIRY DATE: April 28, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: Is the recommended Cefazolin Prophylaxis Adequate in Cardiac Surgery?
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: UofM Internal Funds	

Submission Date(s) of Investigator Documents: April 4 and May 1, 2014	REB Receipt Date(s) of Documents: April 4 and May 2, 2014
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THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
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Protocol:

Protocol

April 4, 2014

Consent and Assent Form(s):

Research Participant Information and Consent Form

05/01/14

Other:

CERTIFICATION

The University of Manitoba (UM) Health Research Board (HREB) has reviewed the research study/project named on this **Certificate of Final Approval** at the **full board meeting** date noted above and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM HREB.

HREB ATTESTATION

The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in

QUALITY ASSURANCE

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. This amendment is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval.** **A Bannatyne Campus Annual Study Status Report** must be submitted to the HREB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the HREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the HREB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM HREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report.**

Sincerely,



John Arnett, PhD, C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary Telephone: (204) 789-3255/ Fax: (204) 789-3414

APPENDIX B. St. Boniface Hospital Research Review Committee Approval Form



Hôpital St-Boniface Hospital

409 Taché Ave, Winnipeg MB Canada R2H 2A6

Research Review Committee Approval Form

Principal Investigator: Dr. S. Zelenitsky
RRC Reference Number: RRC/2014/1408
Date: July 16, 2014
Protocol Title: Is the Recommended Cefazolin Prophylaxis Adequate in Cardiac Surgery?

The following is/are approved for use:

- Protocol, Version dated April 4, 2014
- Research Participant Information and Consent Form, Version date July 1, 2014
- Study Data Collection Sheet, Version dated April 4, 2014

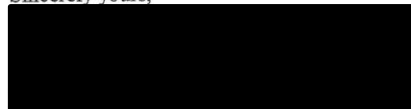
The above was approved by Dr. B. Ramjiawan, Co-Chairperson, Research Review Committee (RRC), St. Boniface Hospital, on behalf of the Committee. As the recommendations by the Research Review Committee have been met, final approval is now granted.

As a reminder any changes to the study Protocol and/or Informed Consent Form must be reported to the Research Review Committee along with any other documents required as per Standard Operating Procedures for Clinical Investigators. The Research Review Committee must be notified regarding discontinuation or study closure.

Should you require assistance during any stage of your research project, please do not hesitate to contact the St. Boniface Hospital Office of Clinical Research (204-258-1044).

The Research Review Committee wishes you much success with your study.

Sincerely yours,



Co-Chairperson, Research Review Committee
St. Boniface Hospital

Please quote the above reference number on all correspondence.

Inquiries should be directed to the RRC Secretary

Telephone: (204) 235-3623 Fax: (204) 237-9860

N1004 - 409 Taché, Winnipeg, MB, Canada R2H 2A6

BR/ar

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Espoir et guérison
Hope and Healing

APPENDIX C. Research Participant Information and Consent Form



Hôpital St-Boniface Hospital



UNIVERSITY
OF MANITOBA

Faculty of Pharmacy

Apotex Centre
750 McDermot Avenue
Winnipeg, Manitoba
Canada R3E 0T5
Telephone (204) 474-9306
Fax (204) 474-7617

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE:

Is the Recommended Cefazolin Prophylaxis Adequate in Cardiac Surgery?

Principal Investigator (researcher):

Dr. Sheryl Zelenitsky, PharmD, Professor

Faculty of Pharmacy, University of Manitoba

750 McDermot Avenue, Winnipeg, Manitoba R3E 0T5

Phone: [REDACTED]

zelenits@umanitoba.ca

Co-Investigators (researchers):

Ms. Divna Calic, B.Sc. Pharm, M.Sc. (student)

Faculty of Pharmacy, University of Manitoba

750 McDermot Avenue, Winnipeg, Manitoba R3E 0T5

Phone: [REDACTED]

umcalic@myumanitoba.ca

Dr. Rob Ariano, PharmD, Clinical Pharmacist Department of

Pharmacy, St. Boniface General Hospital Phone: [REDACTED]

rariano@sbgh.mb.ca

Dr. Rakesh Arora, MD, Associate Professor Faculty of

Medicine, University of Manitoba Phone: [REDACTED]

arora@cc.umanitoba.ca

Dr. Hilary Grocott, MD, Professor

Faculty of Medicine, University of Manitoba

Phone: [REDACTED]

hgrocott@sbgh.mb.ca

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study researchers. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or your doctor before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

Purpose of Study

Just before heart surgery, patients are given an intravenous antibiotic called cefazolin to help prevent infection at the surgical site. This is referred to as antibiotic prophylaxis. **The purpose of this study is to determine if the standard dose of cefazolin is adequate throughout the surgery.**

This study will include a total of 50 study patients also known as participants.

Study procedures

The standard antibiotic prophylaxis in cardiac surgery involves giving an intravenous dose of cefazolin just prior to and, in some cases, another dose during surgery. This will not change for this study.

Individuals with known allergies to penicillin or penicillin-related antibiotics who are prescribed an alternative to cefazolin will not be eligible to participate in this study.

During this study, 2 to 3 blood samples will be collected during surgery. The amount of cefazolin in the blood samples will be measured. Certain patient health, medical and surgical information will be collected from hospital charts/records. Study participants will be followed in hospital and contacted by phone 30 and 90 days after surgery to document if infection occurred at the surgical incision site.

If you take part in this study, you will have the following procedures:

On the day of surgery, you will receive the standard cefazolin prophylaxis, as prescribed by your surgeon. For study participants, 2 to 3 blood samples will be collected during surgery including:

Sample A – 30 minutes following the first dose of	
cefazolin	Sample B – just prior to a second dose, if given
during surgery	Sample C – within 15 minutes of the end of
surgery	

Blood samples will be drawn from an existing catheter/tube in your blood vessel or from the bypass machine by a doctor present during your surgery. Since blood samples will be collected from an existing device, there are no additional needles or pokes required for this study. Six milliliters (6 mL) or just over 1 teaspoon of blood will be collected for each sample. The blood samples will be labeled with a study number (without your name) and stored in a secured freezer unit at the Faculty of Pharmacy, University of Manitoba. Batches of samples will be thawed and processed, and cefazolin levels will be measured in a laboratory at the Faculty of Pharmacy, University of Manitoba.

Certain personal health information (listed below) will be collected from your hospital chart/records. This information will be used to help explain the cefazolin levels in your blood during surgery, as well as the differences in levels observed between individuals. We hope to use this information to identify the best cefazolin dose to prevent infection in patients undergoing heart surgery.

Your information that will be collected includes: study number, age (without birth date), gender, height, weight, BMI, smoking status, surgical history, chronic diseases (e.g., diabetes, lung disease, heart disease), medications and laboratory data. Information related to your surgery including the type of operation, timing, amounts of fluids

given or lost will be recorded. Cefazolin prophylaxis including dose and timing will be detailed, along with any other antibiotics given after surgery. The information will be documented on paper forms and transferred to computer files which will be password protected.

You will be assigned a study number which will be used on all study samples, forms and files. Your name, birth date, phone number and other identifying information will not appear. One paper copy of a master list linking you to your study number will be kept in a secure cabinet in the principal researcher's locked office.

During your hospital stay, you will be followed to document any infection that may occur at your surgical incision site. At 30 and 90 days after surgery, one of the study researchers (Ms. Calic or Dr. Zelenitsky) will contact you by telephone to ask if any infection occurred at your surgical incision site after leaving the hospital. This will only be done if you agree.

Participation in the study will be for the duration of your hospitalization or for 90 days from the day of surgery if you agree to telephone follow-up.

You can stop participating and withdraw from the study at any time. However, if you decide to withdraw, you are encouraged to talk to the study researchers first.

Risks and Discomforts

The expected risk to study participants is minimal. Blood samples will be drawn from an existing catheter/tube in your blood vessel or from the bypass machine during your surgery.

As listed above, certain personal health information will be collected for the study. All precautions will be taken to respect and keep your personal information confidential. Despite these efforts however, absolute confidentiality cannot be guaranteed.

Benefits

There may not be direct benefit to you from participating in this study. We hope to use the cefazolin levels from all participants to identify the best cefazolin dosing for preventing infection in patients undergoing heart surgery.

Costs

All the procedures, which will be performed as part of this study, will be paid for by the researchers.

Payment for participation

You will not receive payment or reimbursement for taking part in this study.

Alternatives

You do not have to participate in this study to receive treatment for your condition. Please talk to your surgeon about your treatment

Confidentiality

Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. All paper and electronic records will be kept in a secure area and only accessed by study personnel. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed.

Your personal information may be disclosed if required by law. The University of Manitoba Research Ethics Board or the St. Boniface General Hospital Research Review Board may inspect and/or copy your research records (including personal health information collected) for quality assurance and data analysis. If any of your research medical records are copied for the above, your name and all identifying information will be removed.

The overall findings of this research study may be published or presented in public forums. Your participation, name and other identifying information will not be used or revealed.

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your care at this centre. If the study researchers feel that it is in your best interest to withdraw you from the study, they will remove you without your consent.

Medical Care for Injury Related to the Study

Injury or illness resulting from this study is unlikely. However, if physical injury should occur as a result of collecting the blood samples, the necessary medical treatment will be available by the study doctor at no additional cost to you.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study researchers:

Ms. Divna Calic at [REDACTED]

or

Dr. Sheryl Zelenitsky at [REDACTED]

For questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus **Research Ethics Board Office at (204) 789-3389.**

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Ms. Calic, Dr. Zelenitsky or other study researcher. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statements or implied statements. Any relationship (such as employer, supervisor or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board or St. Boniface General Hospital Research Review Board for quality assurance purposes.

By signing this consent form, I have not waived any of the legal rights that I have as a research study participant.

I agree to be contacted at 30 and 90 days after surgery for follow-up for this study:

(This is not a requirement to participate in this study.)

Yes ___ No ___ (if yes, please provide phone number: _____)

Upon study completion, I wish to receive a summary of the study results.

Yes ___ No ___

Participant signature _____ **Date** _____

Participant printed name _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed name _____ **Date** _____

Signature _____ **Role** _____

APPENDIX D. Study Data Collection Sheet

STUDY DATA COLLECTION SHEET

Study #: _____

DEMOGRAPHICS & MEDICAL HISTORY

Date of Admission: _____ Date of Discharge: _____ Duration of Stay: _____

Age: _____ Gender: _____ Male _____ Female Ht: _____ Wt (kg): _____ BMI: _____

Smoker: _____ No _____ Yes

Antibiotics within 3 months: _____ No _____ Yes Hospitalized within 3 months: _____ No _____ Yes

Previous Surgery: _____ No _____ Yes, specify: _____

Co-Morbidities:

- ☐ COPD ☐ chronic bronchitis ☐ emphysema
- ☐ ischemic heart disease
- ☐ myocardial infarction
- ☐ congestive heart failure
- ☐ hypertension
- ☐ cerebrovascular disease _____
- ☐ peripheral vascular disease _____
- ☐ dementia
- ☐ diabetes mellitus ☐ medication controlled ☐ insulin dependent
- ☐ connective tissue disease _____
- ☐ autoimmune disease _____
- ☐ malignancy ☐ solid tumor ☐ lymphoma ☐ leukemia ☐ multiple myeloma
- ☐ chemotherapy _____
- ☐ chronic steroids _____
- ☐ other immune suppressant/modulator _____
- ☐ HIV/AIDS _____
- ☐ Hepatitis B or C _____
- ☐ Other _____
- Charlson index: _____

Medications on Admission:

Allergies:

SURGERY

Date of Surgery: _____ Pre-op stay (d): _____ Post-op stay (d): _____

Type of Surgery: _____

Aortic Balloon: _____ No _____ Yes

Grafts number/type (if applicable): _____

Stent placement (if applicable): _____

ASA Score: _____

Labs Date / Time	/	/	/	/	/	/
Serum Creatinine (μmol/L)						
Albumin (g/L)						
Fasting blood glucose						

Events	Time
Pre-op cefazolin (_____ g) infusion started / stopped	/
Incision	
CBP pump started / stopped (duration)	/ ()
Closure	
Intra-op cefazolin (_____ g) infusion started / stopped	
Post-op cefazolin (_____ g)	

Fluid Balance	Volume
Priming solution	
Other fluids	
Blood lost	
Blood replaced	
Urine output	
Net Balance	

Intra-operative complications: __

SSI SURVEILLANCE

SSI during hospitalization (documentation of any of following)

- ☐ purulent drainage from incision or drain,
- ☐ organisms isolated from fluid or tissue cultures of areas manipulated during surgery, or
- ☐ spontaneous dehiscence or deliberate re-opening of wound by a physician along with one of: fever >38°C, localized swelling, redness, heat, or pain/tenderness.

Date: _____

Type of SSI: _____

SSI pathogen/s: _____

Treatment:

SSI Follow-up at 30 d by: _____ Date: _____

SSI treatment/hospitalization: _____ No _____ Yes, specify:

SSI Follow-up at 90 d by: _____ Date: _____

SSI treatment/hospitalization: _____ No _____ Yes, specify:

Other post-operative complications: _____
