

# **Preventing Infection in Total Knee Arthroplasty**

## **Intraosseous Administration of Prophylactic Antibiotics**

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

The University of Auckland

June 2017

This thesis is for examination purposes only and is confidential to  
the examination process

## **Abstract**

### **Aims**

The aim of the research underpinning this thesis was to investigate the use of prophylactic antibiotics by intraosseous regional administration (IORA) in total knee arthroplasty (TKA) patients. The research aimed to identify which organisms cause periprosthetic joint infection (PJI), the relative importance of PJI as a failure mechanism in TKA, compared the tissue concentrations of cefazolin and vancomycin achieved via the IORA route versus systemic administration in primary and revision TKA, and whether IORA provides more effective prophylaxis in a murine model of TKA.

### **Methods**

Six studies are presented. Two studies retrospectively reviewed patients who underwent TKA across three public hospitals in Auckland to identify the mechanisms of TKA failure and the causative organisms in PJI. Three prospective randomised clinical trials compared tissue concentrations of cefazolin and vancomycin achieved with IORA versus systemic administration in both primary and revision TKA. Antibiotic prophylaxis by IORA is given as a bolus injection into a tibial intraosseous cannula below an inflated thigh tourniquet, immediately before skin incision. Subcutaneous fat and bone samples were taken during the procedure and antibiotic concentrations were measured using high-performance liquid chromatography. Finally, the effectiveness of antibiotic prophylaxis delivered by IORA was investigated in a mouse model of TKA.

**Findings**

The most common reason for revision following TKA over 15 years was PJI, and the most common infecting organisms were coagulase-negative staphylococci (CoNS). Ninety-two percent of CoNS strains were resistant to cefazolin, the antibiotic typically used for prophylaxis in TKA. The mean tissue concentrations of antibiotics in subcutaneous fat and bone were 4–10 times higher with IORA than with systemic administration in both primary and revision TKA. These differences were consistent across all sample time points. In the murine model of TKA, vancomycin delivered via IORA afforded the most effective prophylaxis against PJI.

**Conclusions**

PJI is the dominant cause of failure in modern TKA, and most infections are caused by bacteria resistant to commonly used prophylactic agents. When antibiotic prophylaxis is delivered via IORA, markedly higher tissue antibiotic concentrations are achieved. IORA has the potential to enhance the effectiveness of prophylaxis to prevent PJI in TKA.



## **Dedication**

For Maria, Emi, and Clara

## **Acknowledgements**

I would like to thank Irene Zeng for her superb assistance with the statistical analysis and Grant Moore and Mei Zhang for their tireless analysis of the laboratory data. I also thank the Centre for Clinical Research and effective practice (CCRep) and Te Whanau Awhina Charitable Trust for their funding support and Vidicare (San Antonio, TX, USA) for supplying the intraosseous needles and support without charge for all the studies included in this thesis and its funding support for the revision TKA study.

I am profoundly grateful to my supervisor, Rocco Pitto, who first encouraged my interest in this research topic, and to my colleagues who assisted with the studies, including John Mutu-Grigg, Paul Pavlou, and Brendan Coleman. I also thank Mark Clatworthy and Andrew Williams, two dedicated surgeons whom I worked for as a junior doctor and who inspired me to choose knee surgery as a speciality.

I would particularly like to acknowledge Kelly Vince, whose encouragement when I suggested the idea of IORA provided the impetus for the first study in this thesis, and whose generous invitation to a closed meeting of the North American Knee Society provided the inspiration to further the project. I am also grateful to Mark Spangehl and Henry Clarke at the Mayo Clinic for agreeing to a rather unlikely project before I had even arrived for my fellowship and for driving it forward.

Finally, I would like to thank my parents Anthony and Carol for their love and support, and my wife Maria and children Emilia and Clara, who forgave the hours needed to complete this project, and to whom it is dedicated.



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## Abbreviations

ASA	American Society of Anesthesiologists
AUC	area under the concentration-time curve
BMI	body mass index
CFU	colony forming units
CI	confidence interval
C <sub>max</sub>	maximum serum concentration
CoNS	coagulase-negative staphylococci
CTD	connective tissue disease
CV	coefficient of variation
HPLC	high-performance liquid chromatography
IORA	intraosseous regional administration
IV	intravenous
IVRA	intravenous regional administration
LS-MS/MS	liquid chromatography coupled with tandem mass spectrometry
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	methicillin-sensitive <i>Staphylococcus aureus</i>
NZJR	New Zealand Joint Registry
PJI	periprosthetic joint infection
RCT	randomised controlled trial
SD	standard deviation
THA	total hip arthroplasty
TKA	total knee arthroplasty
VRE	vancomycin-resistant enterococci

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 2 - Higher cefazolin concentrations with intraosseous regional prophylaxis in TKA', published in 2013 in Clinical Orthopaedics and Related Research (volume 471, pages 244–249).

Nature of contribution by PhD candidate	First and Lead author, Concept and Study design, protocol development, conduct of clinical trial, data analysis, manuscript preparation
Extent of contribution by PhD candidate (%)	80%

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The undersigned hereby certify that:

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Chapter 3 - 'Antibiotic resistance in early periprosthetic joint infection in the Auckland region and its implications for prophylaxis', aspects of which are presented in an article entitled 'Antibiotic Resistance in Early Periprosthetic Joint Infection' by Ravi S, Zhu M, Luey C, and Young SW and published in the Australian and New Zealand Journal of Surgery in December 2016 (volume 12, pp 1014–1018) Note the % contribution below relates to the published article rather than the thesis chapter.

Nature of contribution by PhD candidate	Senior and lead author, concept and Study design, protocol development, data analysis, manuscript preparation
Extent of contribution by PhD candidate (%)	40%


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Chapter 4 - 'Higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in total knee arthroplasty'. This chapter contains a modified version of an article entitled 'The Mark Coventry Award: Higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in TKA' published in 2014 in Clinical Orthopaedics and Related Research (volume 472, pages 57–65). published in 2013 in Clinical Orthopaedics and Related Research (volume 471, pages 244–249).	
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Chapter 5 - Regional Intraosseous Administration of Prophylactic Antibiotics is More Effective Than Systemic Administration in a Mouse Model of TKA. This chapter contains a modified version of an article entitled 'Regional intraosseous administration of prophylactic antibiotics is more effective than systemic administration in a mouse model of TKA' published in 2015 in Clinical Orthopaedics and Related Research (volume 473, pages 3573–3584).	
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Nature of contribution by PhD candidate	Senior and lead author, concept and study design, protocol development, data analysis, manuscript preparation
Extent of contribution by PhD candidate (%)	45%

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Nature of contribution by PhD candidate	Frist and lead author, concept and Study design, funding application, protocol development, conduct of clinical trial, data analysis, manuscript preparation
Extent of contribution by PhD candidate (%)	65%


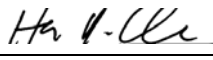


### CO-AUTHORS

Name	Nature of Contribution
Mark Spangehl	Protocol development, funding app, conduct of clinical trial, manuscript review
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Mei Zhang	Sample processing and analysis, manuscript review
Rocco Pitto	Protocol review, Manuscript review
Grant Moore	Sample processing and analysis, manuscript review

### Certification by Co-Authors

The undersigned hereby certify that:

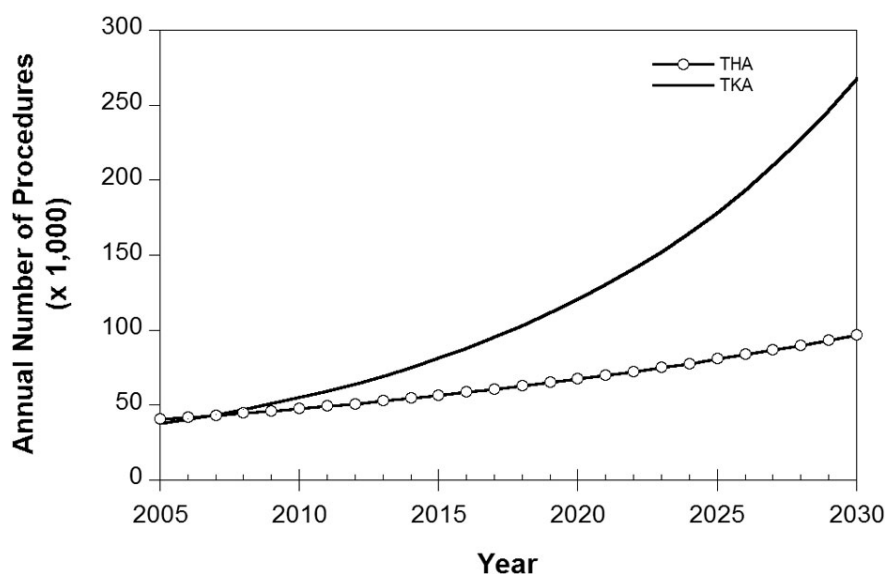
- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- ❖ that the candidate wrote all or the majority of the text.

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## Chapter 1 Introduction

### 1.1 Deep infection in arthroplasty

Total knee arthroplasty (TKA) remains one of the most successful interventions in medicine. Patients who are facing lifelong crippling pain due to knee arthritis now have the opportunity to return to near normal function. Recognising the effectiveness of TKA and its positive impact on people's lives, the New Zealand government instigated a "Government Joint Initiative Scheme" in 2004 to increase funding for District Health Boards and to reduce waiting times. Approximately 6000 knee replacements are performed each year in New Zealand, and this number is expected to increase with the continuing aging of our population (Figure 1.1).<sup>1-3</sup>



**Figure 1.1** *Projected number of revision total hip and knee arthroplasties in the USA from 2005 to 2030. Reproduced from Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am. 2007;89(4):780-5.<sup>2</sup>*

Despite the success of total knee replacement surgery, deep infection remains the most feared complication for both patient and surgeon. A joint prosthesis acts as a

foreign body when infection is present, allowing bacteria to form a 'biofilm' on its surface.<sup>4</sup> This biofilm 'shields' the bacteria both from the body's immune system and from antibiotic therapy, making an infection extremely difficult to treat. On average, a patient with a deep infection following a knee replacement spends an extra 21 days in hospital, undergoes an additional five operations, receives prolonged (>6 weeks) intravenous antibiotic therapy, and may eventually require removal of the prosthesis in order to eradicate the infection.<sup>5,6</sup> When compared with a non-infected TKA procedure, deep infection increases the readmission rate by four-fold, the mean hospital stay by six-fold, and increases the cost per patient by five-fold.<sup>6</sup> Overall, the total cost of treating a knee periprosthetic joint infection (PJI) has been estimated to be at least \$NZ 130,000<sup>6,7</sup>, and even after successful treatment the patient is often left with residual pain and compromised function.

When arthroplasty procedures were first developed in the 1960s, infection rates in the range of 9.5% to 11% were reported.<sup>8,9</sup> Five decades on, despite concerted efforts, infections after primary TKA continue to be reported at a rate of 0.86% to 2.5%.<sup>10-13</sup> Further, there is evidence that the incidence of infection after TKA and total hip replacement have been increasing in recent decades.<sup>7,14</sup>

### 1.1.1 Classification of deep infection

In 1975, Coventry<sup>15</sup> proposed a classification system for periprosthetic infections of the hip that was later refined by Fitzgerald et al<sup>16</sup>. This system has been extended to TKA and remains in regular use.<sup>13</sup> An infection occurring within 3 months of surgery is considered 'acute' and one arising within 3 months to 2 years is considered 'delayed', and both are considered likely to be caused by intraoperative

contamination. Deep infection occurring after 2 years is classified as 'late' and the infecting organisms likely reach the knee joint via haematogenous spread.

Most infections are classified as early or delayed. In a series of 71 infected arthroplasties, 29% of the infections arose early (within the first 3 months), 52% were delayed (3 months to 2 years), and only 19% occurred after 2 years.<sup>13</sup> In the delayed group, 26 of 39 (67%) infections arose between 3 and 12 months after surgery. Such classification is arbitrary, given that not all haematogenous infections present late and not all infections due to intraoperative contamination present early. Tsukayama et al proposed an alternative system<sup>17</sup> that categorises deep infection as: class A ('acute'), an infection occurring within 4 weeks after the index operation; class B ('chronic'), an infection occurring more than 4 weeks postoperatively; or class C ('haematogenous'), an infection considered to be of haematogenous origin due to confirmed or suspected seeding of the implant from a remote site.

### 1.1.2 Origin of deep infection

Bacterial seeding of a knee arthroplasty can occur during the surgical procedure or later via haematogenous spread from a distant site. It is often difficult to definitively identify the origin of the bacteria causing an infection, but the majority of early and delayed infections after TKA are believed to be due to bacterial contamination at the time of surgery.<sup>18</sup> Many authors also argue that late infections arising more than 2 years after surgery are not necessarily haematogenous in origin, and may be due to intraoperative contamination. *Staphylococcus aureus* can invade intracellularly, and can lie dormant inside osteoblasts and cause late reactivation of infection.<sup>19</sup>

Additionally, low-virulence organisms, such as coagulase-negative staphylococci (CoNS), can cause subclinical low-grade periprosthetic infection, which may be

diagnosed late. In a 15-year prospective review of experience at a specialist orthopaedic hospital, Phillips et al found that seven (26%) of 27 cases of deep infection due to CoNS first manifested more than 2 years after surgery.<sup>13</sup> This finding, together with the observation that most deep infections occur soon after surgery, suggests that intraoperative contamination is a major cause of deep infection following TKA.

## **1.2 Intraoperative contamination**

Even with strict sterile surgical technique, 23% to 63% of joint replacement procedures show bacterial contamination within the operative field.<sup>20-22</sup> Further, intraoperative contamination has been associated with subsequent deep infection. In a study where four intraoperative cultures were taken from instruments and bone samples during 100 total hip arthroplasty (THA) procedures, Knobben et al identified bacterial contamination in 36% of cases and found an association between bacterial contamination and subsequent deep infection.<sup>23</sup> Seventeen percent of patients (6/36) with intraoperative contamination developed deep infection compared with 1.5% (1/64) without contamination ( $p=0.008$ ).

This finding is supported by animal studies. Craig et al investigated the effect of contamination by *S. aureus* on the risk of subsequent deep infection in a rabbit model of total knee replacement.<sup>24</sup> In their study, 10 knees each were inoculated with 0,  $10^2$ ,  $10^3$ , or  $10^4$  colony-forming units (CFU) of *S. aureus* and 10 knees were injected with saline. Deep infection developed in 4/10 knees when  $10^2$  CFU of *S. aureus* were introduced and in 7/10 knees when  $10^3$  or  $10^4$  CFU of *S. aureus* were introduced. No evidence of deep infection was found in the knees injected with saline. These findings



suggest that there is a dose-response relationship between bacterial contamination and subsequent deep infection after TKA.

CoNS are the organisms most commonly identified in studies of intraoperative contamination, followed by *S. aureus*. Using bacterial swabs, Jonsson et al measured contamination rates in 90 THA and TKA procedures and found that 28 operations (31%) were contaminated by CoNS, 9 (10%) by *S. aureus*, and 4 (4%) by other species.<sup>21</sup> In a similar study, Byrne et al reported a contamination rate of 23% in 80 primary TKA and total hip replacement procedures and that 71% of positive swabs grew CoNS.<sup>22</sup> Further, Davis et al reported finding contamination in the operative field in 63% of 100 primary TKA and THA procedures, with CoNS identified as the contaminating organism in 76% of specimens.<sup>20</sup>

The above findings correspond with clinical data showing staphylococcal species (CoNS and *S. aureus*) to be the most common causative organisms associated with deep infection in arthroplasty procedures. In a series of 121 infected TKA procedures, Nickinson et al reported the most frequent organisms to be CoNS (49%) and *S. aureus* (13%).<sup>12</sup> Similarly, in a study of 71 infected TKA and THA procedures, Phillips et al reported that the causative organism was CoNS in 36% of infections and *S. aureus* in 25%.<sup>13</sup> Further, in a series of 70 patients with infected TKA or THA, Kilgus et al grew CoNS from 26 joints (37%) and *S. aureus* from 32 joints (45%).<sup>25</sup> The observation that the organisms causing intraoperative contamination are also the ones that most commonly cause deep infection provides further evidence of causality, and it seems clear that contamination is a prerequisite for subsequent deep infection.

### 1.3 Prevention of deep infection

Deep infection following arthroplasty can be thought of as a balance between the number of bacteria contaminating the wound during surgery and the body's ability to eradicate these organisms before infection occurs (Figure 1.2). Therefore, factors that increase the risk of contamination, such as a prolonged operating time<sup>26-30</sup>, can be expected to increase the risk of infection. Similarly, factors that impair the host immune system, such as diabetes mellitus and other medical comorbidities, also increase the risk of deep infection.<sup>26,27,31</sup> With this concept in mind, surgeons use two main strategies to minimise the risk of infection following arthroplasty, i.e., reducing bacterial contamination intraoperatively with measures such as laminar flow ventilation and wearing of body exhaust gowns, and protecting the patient should contamination occur, mainly by use of prophylactic antibiotics.



**Figure 1.2** *The risk of postoperative infection can be conceptualised as a balance between the number of bacteria contaminating the surgical site and the ability of the host to eradicate them.*

### 1.3.1 Reducing contamination

Following development of the 'germ theory' by Louis Pasteur, Joseph Lister introduced the concept of antisepsis in surgery.<sup>32</sup> In a 1867 paper, he reported that careful cleaning and decontamination of the operating room environment and surgical instruments using carbolic acid decreased the mortality rate following limb amputation from 45% to 15%. Throughout the remainder of the 19th century and the early 20th century, a number of improvements on Lister's practices were introduced, including heat sterilisation of instruments, wearing of sterile gowns, caps, and gloves, and use of surgical masks.<sup>33</sup> All these practices aimed to reduce the number of bacteria contaminating the wound.

Development of hip arthroplasty in the 1960s by John Charnley saw a further doubling of efforts to reduce contamination. A prosthesis implanted in a joint cavity acts as a foreign body to which contaminating bacteria can adhere, so the threshold at which contamination of the wound site can produce a clinical infection is lower. In his early series, Charnley reported deep infection rates as high as 9.5%.<sup>9</sup> Infecting bacteria formed a biofilm on the surface of the prosthesis and were very difficult to eradicate unless the implant was removed, leaving the patient with a non-functional joint. Thus, the presence of a prosthesis in arthroplasty surgery was associated with higher infection rates and more severe clinical consequences. Charnley introduced two measures in an effort to reduce the risk of contamination, i.e., a 'clean-air operating enclosure' and wearing of body exhaust suits by surgeons in the operating room.<sup>34</sup>

The clean-air operating enclosure incorporates a laminar flow system that ventilates the operating theatre using filtered air in an effort to reduce the risk of contamination

from air passing over the wound. The body exhaust suit uses intake and outtake tubing to create negative pressure inside the surgeon's gown, which removes shed skin particles (and the bacteria they contain) from the operative area.<sup>34-36</sup> A large randomised trial in the 1980s found that use of a clean-air enclosure and body exhaust gowns reduced deep infection rates to less than 1%.<sup>37</sup> Modern versions of laminar flow ventilation and body exhaust suits remain in use in arthroplasty surgery today.<sup>38</sup>

### 1.3.2 Prophylactic antibiotics

Despite the above measures, some degree of contamination occurs during most if not all arthroplasty procedures.<sup>20-22</sup> The second strategy used to prevent such contamination leading to clinical infection is antibiotic prophylaxis.

The concept of preoperative antibiotic prophylaxis was pioneered by John Burke in 1961.<sup>39</sup> In a landmark study, Burke performed a series of experiments in guinea pigs to investigate the prophylactic effect of timing of antibiotic administration on surgical incisions contaminated by *S. aureus*. When prophylactic antibiotics were given within one hour before bacterial inoculation of the wound, no inflammatory response occurred. These wounds were not clinically or microscopically different from wounds inoculated with dead bacteria. This observation suggested that prophylactic antibiotics aid in the killing of contaminating bacteria, preventing progression of contamination to clinical infection. Burke also found that an inflammatory response occurred when antibiotics were administered one hour after inoculation and that the inflammatory response was identical to that in an untreated control group when antibiotics were administered more than 3 hours after inoculation. Burke concluded that prophylactic antibiotics were effective if present in the tissues at an adequate concentration from

the time of incision until the time of closure, i.e., when wound contamination was occurring.

#### **1.4 Effectiveness of prophylactic antibiotics**

Subsequent clinical studies in orthopaedic patients demonstrating that prophylactic antibiotics dramatically reduced infection rates in arthroplasty supported Burke's findings. In 1977, Ericson et al randomised 118 patients undergoing THA to receive preoperative cloxacillin or placebo and reported infection rates of 0% in the group receiving antibiotics and 14% in the control group.<sup>40</sup> In a subsequent multicentre randomised trial of 2137 patients undergoing THA, Hill et al reported an infection rate of 3.3% in patients who received placebo compared with 0.9% when prophylactic cefazolin was used.<sup>41</sup> Other randomised trials in a variety of orthopaedic procedures demonstrated conclusively that prophylactic antibiotics reduce the incidence of surgical site infection.<sup>42-45</sup>

It should be emphasised that the aim of prophylactic antibiotics is not to sterilise already contaminated tissues, but to act as an adjunct to modulate bacterial contamination to a level that will not overwhelm host immune defences. Thus, adequate concentrations of antibiotic need to be present in the tissues for the entire period the wound is open and at risk of contamination.

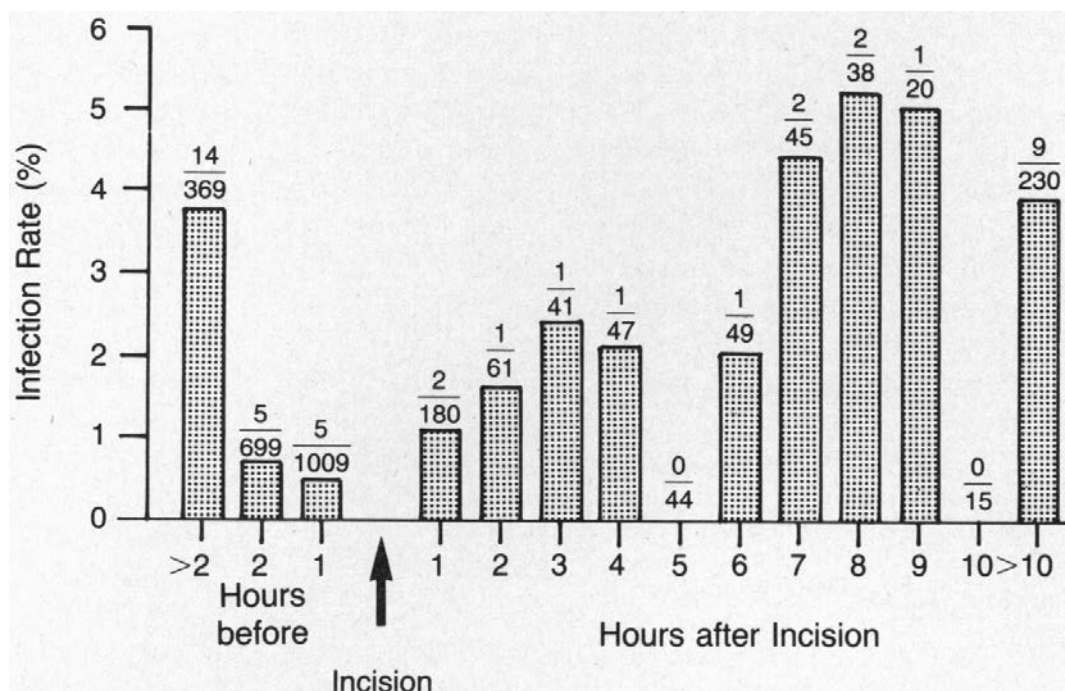
##### **1.4.1 Choice of prophylactic antibiotic therapy**

Prophylactic antibiotics need to be effective against the bacteria most likely to cause contamination during surgery. As shown in the studies discussed in Section 1.2.1, contaminants during arthroplasty procedures are typically Gram-positive cocci such as *S. aureus* and CoNS.

A first-generation or second-generation cephalosporin such as cefazolin or cefuroxime has a suitable spectrum of activity that includes cover for staphylococci and other Gram-positive bacteria. These agents have also been shown to penetrate tissue at the operative site, including bone, soon after intravenous administration.<sup>46,47</sup> In addition, the cost of the early-generation cephalosporins is low and adverse effects are rare, so they have become the recommended prophylactic antibiotic agents for arthroplasty procedures.<sup>45,48</sup> Vancomycin is recommended as an alternative in cases of cephalosporin allergy because its spectrum of activity includes Gram-positive cocci.<sup>48</sup>

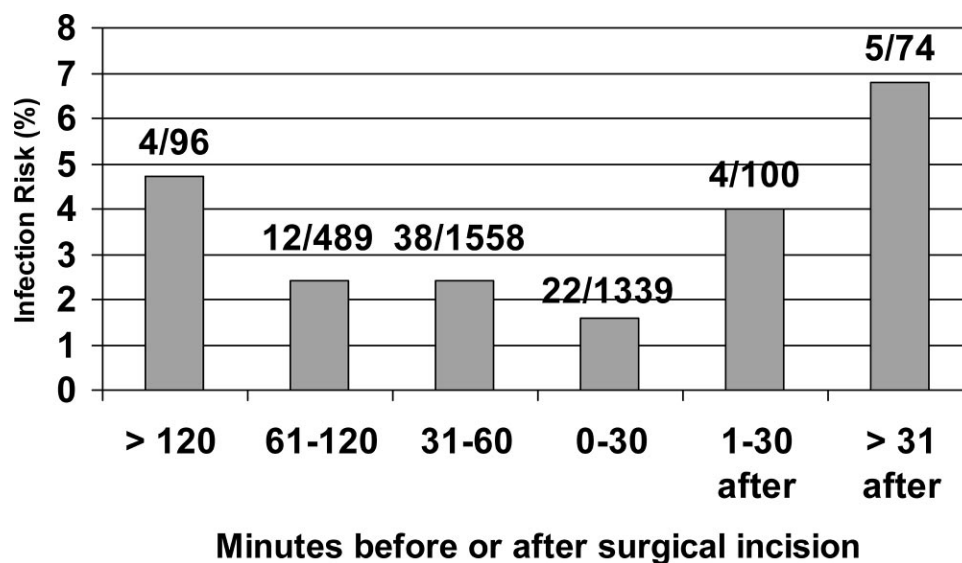
#### 1.4.2 Timing of antibiotic prophylaxis

Burke's original animal experiments suggested that antibiotics were most effective when administered immediately prior to surgery.<sup>39</sup> This has been proven in subsequent clinical studies. In 1992, Classen et al investigated 2847 patients undergoing elective surgical procedures and found that infection rates were lowest in patients who received antibiotics in the 2 hours prior to incision (Figure 1.3).<sup>49</sup> Higher rates of infection were seen in patients who received antibiotics more than 2 hours before incision (relative risk 6.7), within 3 hours after incision (relative risk 2.4), or more than 3 hours after incision (relative risk 5.8).



**Figure 1.3** Surgical wound infection rate according to the temporal relationship between prophylactic antibiotic administration and start of surgery. The number of infections and the number of patients appear as the numerator and denominator, respectively. Reproduced from Classen DC, Evans RS, Pestotnik SL, Horn SD, Menlove RL, Burke JP. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. *N Engl J Med.* 1992;326(5):281-6.<sup>49</sup>

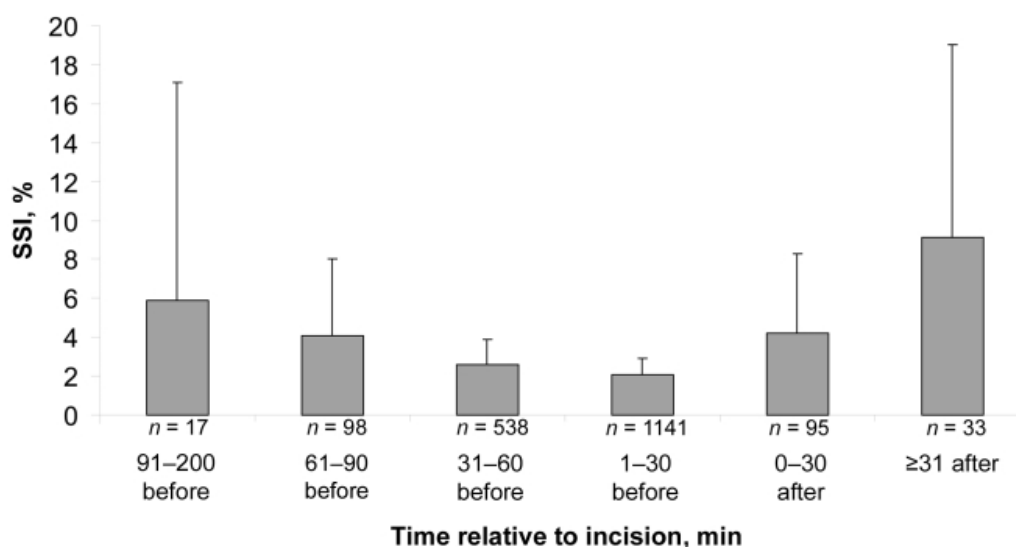
More recently, Steinberg et al reported on 113 surgical site infections in 4472 randomly selected cardiac, hip/knee arthroplasty, and hysterectomy cases performed at 29 hospitals.<sup>50</sup> When antibiotics requiring long infusion times (such as vancomycin) were excluded, the infection rate was 1.6% if prophylactic antibiotics were administered within 30 minutes prior to incision and 2.4% if administered 31 to 60 minutes prior to incision (odds ratio 1.74). They found that the risk of infection increased as the time interval between preoperative administration of prophylactic antibiotics and incision increased and when the antibiotic was first administered after incision (Figure 1.4).



**Figure 1.4** Risk of surgical site infection based on timing of the perioperative antibiotic dose (omitting vancomycin and fluoroquinolones). Annotation shows the numbers of infections/operations for each time interval. Reproduced from Steinberg JP, Braun BI, Hellinger WC, Kusek L, Bozikis MR, Bush AJ, et al; Trial to Reduce Antimicrobial Prophylaxis Errors (TRAPE) Study Group. Timing of antimicrobial prophylaxis and the risk of surgical site infections: results from the Trial to Reduce Antimicrobial Prophylaxis Errors. *Ann Surg* 2009;250(1):10–16.<sup>50</sup>

Similar findings have been reported in patients undergoing THA. Van Kasteren et al investigated 1922 patients undergoing THA at any one of 11 hospitals participating in the Dutch Surgical Prophylaxis and Surveillance project and found an overall rate of infection (superficial and deep) of 2.6%.<sup>51</sup> Patients receiving prophylaxis within 30 minutes before surgery had the lowest risk of surgical site infection. The highest risk of infection was found in patients who received prophylaxis after the incision (odds ratio 2.8, p=0.07; Figure 1.5).





**Figure 1.5** Association between timing of administration of prophylaxis and incidence of SSI following total hip arthroplasty. Abbreviation: SSI, surgical site infection. Reproduced from van Kasteren ME, Manniën J, Ott A, Kullberg BJ, de Boer AS, Gyssens IC. Antibiotic prophylaxis and the risk of surgical site infections following total hip arthroplasty: timely administration is the most important factor. *Clin Infect Dis.* 2007;44(7):921-7.<sup>51</sup>

Based on these findings and those of other studies, the American Academy of Orthopaedic Surgeons, Centers for Disease Control, and Surgical Care Improvement guidelines recommend that prophylactic antibiotics be infused completely within one hour before the surgical incision.<sup>48</sup> In view of the extended infusion time required, vancomycin should be started within 2 hours before incision.

The findings of these studies support Burke's original hypothesis that, to be effective, prophylactic antibiotics should be present in the tissue at adequate concentrations when contamination is occurring, i.e., from the time of the surgical incision until the time of closure. If antibiotics are administered too early, tissue concentrations may have fallen to levels that are no longer protective when the risk of contamination is

highest. If administered too late, bacterial adhesion may already have occurred, and antibiotics are less able to modulate contamination to a level that does not overwhelm host immune defences.

#### 1.4.3 Duration of antibiotic prophylaxis

Many studies have investigated duration of treatment with prophylactic antibiotics across surgical specialties and two consistent findings are reported. First, the preoperative dose appears to be the most important in reducing infection rates. Second, extending the duration of treatment beyond 24 hours postoperatively does not confer any additional benefit in terms of reducing the risk of infection and may in fact promote resistance.<sup>52</sup> The current American Academy of Orthopaedic Surgeons guidelines state that ‘prophylactic antibiotics should be discontinued within 24 hours of surgery’.<sup>53</sup>

In a 1998 meta-analysis of randomised trials comparing single-dose versus multiple-dose prophylaxis in major surgery, McDonald et al found no advantage of multiple doses over a single-dose regimen (odds ratio 1.04; 95% confidence interval [CI] 0.86–1.25).<sup>54</sup> Similarly, in a 2007 study, Slobogean et al pooled the results of seven randomised controlled trials involving 3808 patients with closed fractures undergoing surgical fixation or arthroplasty.<sup>55</sup> When compared with multiple doses of prophylactic antibiotics, administration of a single preoperative dose did not increase the risk of infection (risk ratio 1.24, 95% CI 0.60–2.60; pooled risk difference 0.005, 95% CI 0.011–0.021).

Similar findings have been reported in patients undergoing arthroplasty. In a prospective, double-blind, randomised controlled trial, Mauerhan et al compared rates

of deep wound infection in 1354 patients undergoing primary THA or TKA in whom prophylactic antibiotics were continued for 24 hours or 3 days.<sup>56</sup> In the THA group, the prevalence of deep wound infection was 0.5% (1/187) in those treated with antibiotics for 24 hours and 1.2% (2/168) in those receiving the 3-day regimen; in the TKA group, the rates were 0.6% (1/178) and 1.4% (3/207), respectively. The authors concluded that there was no significant difference between the two regimens.

The above findings emphasise the importance of the preoperative dose of antibiotics, which again supports Burke's original theory that the key to effective prophylaxis is adequate antibiotic concentrations in the tissues from the time of incision until the time of closure, i.e., while contamination is occurring. An extended duration of antibiotic prophylaxis does not appear to improve efficacy, and may in fact cause harm by promoting development of resistant organisms.<sup>57</sup>

#### 1.4.4 Antibiotics for prophylaxis versus treatment of infection

Use of antibiotics for prophylaxis should be distinguished from their use for treatment of an established infection. The skin is an important barrier against bacteria, and during surgery this barrier is compromised until the wound is closed. Prophylactic antibiotics support the body's innate (non-specific) immune system to prevent bacterial colonisation, i.e., before bacterial adhesion and other virulence factors can overcome these initial immune defences and cause infection.<sup>45</sup> Once an infection is established, antibiotics act instead to assist the body's adaptive (antigen-specific) immune response to eradicate the infection.

This difference explains the clinical finding that a well-timed preoperative antibiotic dose is most important for prophylaxis whereas a prolonged duration of antibiotic therapy is required when treating an infection.

### **1.5 Improving the effectiveness of prophylactic antibiotics**

Despite the use of prophylactic antibiotics, infection rates following primary TKA continue to be reported at a rate of 0.86%–2.5%.<sup>10-13</sup> As outlined in Section 1.4.2, prophylactic antibiotics are less effective if administered more than one hour before the surgical incision, suggesting that they afford the most effective prophylaxis when the tissue concentration is maximal. Typically, the maximum dose of an antibiotic is limited by the risk of systemic side effects.

#### 1.5.1 Tissue antibiotic concentrations

Quintiliani and Nightingale proposed that, for effective prophylaxis, tissue antibiotic concentrations of at least 4–5 times the minimum inhibitory concentration (MIC) for a bacterial strain should be achieved in a patient with normal host defences.<sup>58</sup> In animal models of infection, saturation of the killing rate when using a cephalosporin occurs at around 5 times the MIC.<sup>59</sup> However, studies using animal models of treatment of established infection and higher multiples (64 times the MIC) are known to lead to earlier initiation of bacterial killing, which may be more important in prophylaxis where the goal is to prevent initial bacterial adhesion and colonisation.<sup>59,60</sup>

Studies using systemic prophylactic cefazolin in TKA have reported concentrations of 4.7–16.1  $\mu\text{g/g}$  in bone tissue samples.<sup>46,61-63</sup> When prophylactic antibiotics were introduced in the 1970s, typical MIC<sub>90</sub> levels for CoNS species were 0.5–1.0  $\mu\text{g/mL}$ .<sup>47,64</sup> In the early studies, the measured tissue concentrations of prophylactic

antibiotics were well in excess of these MIC<sub>90</sub> levels, indicating that systemic cephalosporins provide adequate prophylaxis.<sup>47,62,63</sup>

### 1.5.2 Problem of increasing antibiotic resistance

In a 2011 report, Yamada found that MIC<sub>90</sub> levels for cefazolin were higher than 100  $\mu\text{g/mL}$  for over half of reported CoNS species.<sup>61</sup> This led to concern that tissue concentrations of antibiotics administered systemically may no longer be adequate to cover CoNS. By definition, the highest MIC<sub>90</sub> for cefazolin-sensitive CoNS is 8  $\mu\text{g/mL}$ . Moller compared hospital CoNS isolates from 1964 to 1986, and found the percentage of methicillin-resistant (and therefore also cefazolin-resistant) isolates increased from 2% to 58% over this 22-year period. More recent studies show that 60% to 90% of CoNS isolates currently causing orthopaedic infections are resistant to cephalosporins.<sup>12,61,65</sup>

The rate of methicillin resistance in *S. aureus* species has also increased. Data from intensive care units in the USA for 1992 to 2003 show that the proportion of methicillin-resistant *S. aureus* infections increased from 35.9% to 64.4%, representing an increase of 3.1% per year.<sup>66</sup> Similar findings have been reported for arthroplasty infections. Bjerke-Kroll et al retrospectively reviewed all hip and knee arthroplasty infections performed at their institution over a 14-year period and found a statistically significant increase in the proportion of methicillin-resistant *S. aureus* infections from 11% in 1998 to 48% in 2010 (incidence rate ratio 1.11,  $p=0.019$ ).<sup>67</sup> Similarly, in a study of 898 cases of infected TKA and THA from the ENDO-Klinik in Germany and 772 cases from the Rothman Institute in the USA, Aggarwal et al reported that the causative organism was CoNS in 39.3% and 20.2% of cases, respectively, and *S. aureus* in 13% and 31% of cases.<sup>68</sup> The incidence of methicillin-resistant *S. aureus*

was 12.8% in the European centre and 48.1% in the US centre (odds ratio 6.27, 95% CI 3.39–12.31;  $p < 0.0001$ ).

Although regional variation exists, cephalosporin resistance has increased markedly in the two bacterial strains most likely to cause infection in TKA. This has implications for both the choice of prophylactic agent and the clinical outcome, given that infections in TKA caused by resistant organisms are associated with higher rates of treatment failure and reinfection.<sup>69,70</sup>

### **1.6 Regional administration of antibiotic prophylaxis**

Prophylactic antibiotics are typically given systemically via the intravenous route. The antibiotic distributes throughout the systemic circulation, including the tissues around the surgical site. Tissue concentrations achieved at the surgical site are a function of the antibiotic dose and the total volume of distribution in the body.

Prophylactic antibiotics can also be administered ‘regionally’. Regional administration involves intravenous injection of a drug below an inflated tourniquet. The tourniquet interrupts circulation to the limb, so that the distribution of a medication injected below the tourniquet is restricted to that limb. Gustav Bier pioneered this technique in the early 1900s as a means of administering local anaesthetic agents to provide regional anaesthesia to the limb, allowing procedures to be carried out with the patient awake and leaving the major organs unaffected.<sup>71</sup> This technique was largely forgotten until a publication by Charles Holmes, a New Zealand anaesthetist, in *The Lancet* in 1963, which revived worldwide interest.<sup>72,73</sup> The ‘Bier’s block’ remains in widespread use today, particularly in closed reduction of wrist fractures<sup>72</sup> (Figure 1.6).

TKA is routinely performed using a tourniquet, which reduces blood loss and improves visualisation for the surgeon. While typically used to administer local anaesthetic agents, the regional route can be used to administer any medication, including prophylactic antibiotics. Four previous studies have investigated 'regional' administration of antibiotics during TKA, injecting the antibiotic into a foot vein after the tourniquet is inflated.<sup>74-77</sup> Using this method, tissue concentrations of antibiotic in the knee were 10 times higher than those achieved by systemic administration and without increased risk of systemic side effects.



**Figure 1.6** *The 'Bier's block' technique used to provide anaesthesia to a limb. A tourniquet is inflated around the upper arm and an intravenous cannula is inserted into the hand. A local anaesthetic agent is then injected into the cannula, which is prevented from entering the systemic circulation by the inflated tourniquet. Image of regional intravenous anaesthesia courtesy of Mr Arifnajafov.*

(<https://commons.wikimedia.org/w/index.php?curid=14837004>)

### 1.6.1 Studies of intravenous regional antibiotic prophylaxis

Intravenous regional administration (IVRA) of prophylactic antibiotics in TKA involves cannulation of a foot vein to obtain venous access below the tourniquet. Using this method, Hoddinott et al compared 750 mg of intravenous regional cefuroxime with 1 g of systemic cefamandole and reported that the mean levels of cefuroxime in bone ( $133.1 \mu\text{g/mL}$ ) and fat ( $88.4 \mu\text{g/mL}$ ) were significantly higher following regional administration than those of cefamandole ( $9.1 \mu\text{g/mL}$  and  $9.8 \mu\text{g/mL}$ , respectively) following systemic dosing ( $p < 0.001$ ).<sup>77</sup>

In a series of three studies, de Lalla et al investigated IVRA for antibiotic prophylaxis with teicoplanin in TKA. Teicoplanin is a glycopeptide antibiotic similar to vancomycin and is approved for use in Europe and New Zealand but not in the USA. These investigators first performed a randomised controlled trial in which 24 patients undergoing TKA were randomised to receive 800 mg of systemic intravenous teicoplanin or 400 mg of IVRA teicoplanin via a foot vein 2.5 hours preoperatively.<sup>76</sup> Teicoplanin concentrations in tissue samples (skin, subcutaneous tissue, bone, synovium) were 2–10 times higher with the IVRA route than with the systemic intravenous route.

In a subsequent clinical study by de Lalla et al, no deep infections occurred during 2 years of follow-up in 161 patients undergoing TKA who received 400 mg of teicoplanin via the IVRA route.<sup>75</sup> In the final study by this group, patients undergoing TKA received 800 mg of systemic intravenous teicoplanin ( $n=5$ ) or 200 mg of teicoplanin via the IVRA route ( $n=15$ ) 2.5 hours preoperatively.<sup>74</sup> Teicoplanin concentrations in tissue samples (skin, subcutaneous tissue, bone, synovium) were found to be two times higher in the patients treated via the IVRA route. The authors



pointed out that these higher concentrations were achieved despite a much lower IVRA dose, thus protecting the patient from systemic side effects, in particular renal toxicity.

Miller et al subsequently explored IVRA of prophylactic antibiotics during elbow surgery.<sup>78</sup> Using an arm tourniquet, they injected prophylactic antibiotics into a hand vein, comparing 1 g of cefazolin injected systemically with 1 g of cefazolin given by IVRA 20 minutes before surgical incision. The cefazolin concentrations achieved in bone were 41 times higher and those achieved in fat were 133 times higher in the IVRA group when compared with the systemic group (1484  $\mu\text{g/g}$  versus 35.8  $\mu\text{g/g}$  in bone and 1422.7  $\mu\text{g/g}$  versus 10.7  $\mu\text{g/g}$  in fat;  $p < 0.05$ ). The extremely large differences in tissue concentrations seen in this study reflect the smaller regional volume of distribution in the upper limb.

### 1.6.2 Potential advantages of regional administration

IVRA has a number of potential advantages when administering prophylactic antibiotics in patients undergoing TKA. First, far higher tissue concentrations can be reached at the site of the open surgical wound, which maximises the effectiveness of antibiotic prophylaxis. In contrast, the tissue antibiotic concentrations that can be achieved via the systemic route are limited by dose-dependent toxicity to major organs. While the possibility of local toxic effects on osteocytes and other cells must be considered<sup>79,80</sup>, regionally administered prophylaxis can maximise local tissue concentrations while limiting systemic toxicity.<sup>81</sup> Second, regional administration may make surgeons more comfortable with single-dose prophylaxis, given that concentrations greater than the MIC have been reported in drain fluid for up to 21 hours following a surgical procedure.<sup>77</sup> This is presumably secondary to the depot

effect of the high initial tissue concentrations achieved using this route. While it is known that prolonging antibiotic treatment for many days confers no additional benefit<sup>82</sup>, many surgeons continue administration of antibiotics for 24 hours following surgery<sup>18,82,83</sup>. Single-dose prophylaxis confers significant economic benefit, with savings of up to \$US7.7 million per 100,000 patients reported.<sup>84</sup>

Finally, by requiring injection following preparation and draping, optimal timing of prophylactic antibiotic administration is ensured, which, as outlined above, is important for efficacy. In a study of 34,133 Medicare surgical inpatients, Bratzler et al found that only 55.7% of eligible patients received prophylactic antibiotics within one hour prior to incision.<sup>85</sup> In patients undergoing hip or knee arthroplasty, Rosenberg et al reported that optimum timing of prophylactic antibiotic administration was achieved on only 65% of occasions.<sup>86</sup> Achieving optimum timing is even more difficult with antibiotics such as vancomycin or teicoplanin that require prolonged infusion times. In a study of 1610 surgical patients given vancomycin as prophylaxis, Bull et al reported appropriate timing of administration in just 22% of cases.<sup>87</sup>

### 1.6.3 Regional prophylaxis via the intraosseous route

Studies of regional administration of antibiotic prophylaxis in the lower limb have required cannulation of a foot vein to deliver the antibiotics to the vascular system below the level of the thigh tourniquet. Cannulating a foot vein is difficult, time-consuming, and often unsuccessful, particularly in obese patients, so the uptake of IVRA in clinical practice has been limited.<sup>74</sup> The skin of the foot is also known to have higher bacterial skin counts than other parts of the body<sup>88</sup>, and is normally covered in sterile drapes during TKA.

Intraosseous administration offers an alternative to intravenous cannulation. The metaphysis of a long bone contains ‘venous sinusoids’ that form a honeycomb-like structure containing a dense network of blood vessels.<sup>89</sup> Unlike veins in the skin, the blood vessel walls at these sites are supported by bone, so are not prone to collapse. The contents of an injection into this area of bone travel directly to the circulation, as would those of an injection into a vein. Commonly used in children, this technique has become popular in adults in recent years as a reliable method of achieving rapid access to the circulation in emergency and intensive care settings.<sup>90-94</sup> The intraosseous injection route is effective in adults and children<sup>95</sup>, and both fluids and medications can be given using this method. The pharmacokinetics of agents injected via the intraosseous route are similar to those achieved by peripheral or central intravenous administration.<sup>96</sup> The intraosseous route is particularly popular in the military setting, where rapid and reliable access to the systemic circulation in the field is paramount.<sup>90,93</sup>

Regional anaesthesia via the intraosseous route has also been reported to be effective in orthopaedic surgery<sup>97</sup>, and regional administration of antibiotics by this route is a well validated method of treating limb infection in horses<sup>98-102</sup>. However, prior to the research underpinning this thesis, intraosseous regional administration (IORA) of prophylactic antibiotics in TKA had not been reported.

#### 1.6.4 Safety of the intraosseous route

Use of the intraosseous route for administration of fluids and medications is well established. In 1947, Heinild et al investigated the intraosseous route in over 1000 paediatric patients and reporting a 95% success rate in administering fluids, blood products, and a variety of medications.<sup>103</sup> The technique has been particularly popular

in paediatric patients, in whom intravenous access is more difficult, but is as effective as the intravenous route in adult patients.<sup>96</sup>

Three papers have investigated regional (with tourniquet) intraosseous administration of antibiotics in horses to treat limb infections. Scheuch et al compared intraosseous versus intravenous infusion of the antibiotic amikacin in 21 horses, and found similar tissue levels using these two routes.<sup>104</sup> Mattson et al found effective tissue concentrations of gentamicin after IORA in 12 horses, and recommended this as a form of treatment for infection.<sup>105</sup> Similarly, Rubio-Martínez et al compared regional intravenous versus intraosseous administration of vancomycin in 12 horses and found equivalent tissue concentrations using these routes.<sup>101</sup> None of the above studies reported any complications using the intraosseous route.

Regional (with tourniquet) intraosseous infusion of local anaesthetic agents has also been investigated in humans, and is a variation of the well-known 'Bier's block' that uses intravenous access. Waisman et al reported on 109 patients who received local anaesthetic agents in an upper or lower limb via IORA to allow surgical procedures to be performed on the limb with the patient awake.<sup>97</sup> The procedure was successful in 106 of 109 patients. The three failures included incorrectly positioning of the needle in one patient and inadequate anaesthesia in two patients (the latter attributed by the authors to infusion of an insufficient volume of medication). No other complications were reported. This study provided evidence of the safety of intraosseous injection below a tourniquet (i.e., regional administration using the intraosseous route) in a large number of patients.

Four main complications of intraosseous infusion have been reported in adults<sup>96</sup>, all of which relate to technical error or prolonged infusion in an emergent setting:

### 1) *Extravasation of fluid*

This complication occurs when infusion of fluid or medication is commenced with the needle not placed correctly (i.e., placed outside the bone). With modern intraosseous needles, the risk of this complication is reduced by monitoring the patient closely, particularly at the intraosseous needle insertion site, and using intraosseous needles of an appropriate length to prevent overpenetration of the bone. The reported success rates for insertion of modern intraosseous kits range from 94% to 100%.<sup>90,94,106</sup>

### 2) *Compartment syndrome*

Compartment syndrome associated with incorrect needle placement has occasionally been described following intraosseous infusion in case reports.<sup>107-110</sup> Compartment syndrome occurs if the tip of the needle is placed into soft tissue rather than into bone and a prolonged infusion of fluids is commenced. Large published series of intraosseous infusions have not reported this complication, suggesting that it is very rare.<sup>96</sup>

### 3) *Fracture*

There have been isolated reports in the literature of bone fracture following intraosseous needle placement.<sup>96,111</sup> This is thought to relate to use of excessive force with manual needle placement in paediatric patients, and has not been reported with modern powered needle drivers.<sup>96</sup>

### 4) *Infection/osteomyelitis*

Infection following intraosseous needle placement is rare. A meta-analysis of 4359 intraosseous needle insertion attempts reported a 0.6% incidence of infection.<sup>112</sup> It is recommended that needles be removed after 24 hours to reduce the risk of this

complication<sup>96</sup>. However, in the setting of IORA for antibiotic prophylaxis, the intraosseous needle is removed immediately following injection.

#### 1.6.5 Fat embolus following intraosseous injection

A theoretical risk of intraosseous injection is embolisation of bone marrow fat caused by the increased intraosseous pressures during injection. This is a concern when considering IORA antibiotics in TKA, considering that patients are often elderly with limited cardiopulmonary reserve. However, there have been no recorded clinical cases of fat emboli following intraosseous injection in adults or children.<sup>96</sup>

The risk of fat embolisation has been evaluated in animal studies. In 1942, Wile and Schamberg reported fat emboli in the pulmonary arterioles in five of seven rabbits that received repeated intraosseous infusions over 5 days.<sup>113</sup> Fat embolism was thought to be responsible for the sudden death of one of these animals. In contrast, Plewa et al found no fat emboli following intraosseous infusion in a study performed in 16 piglets.<sup>114</sup> The animals were bled slowly (20 mL/kg over 20 minutes) and the blood was reinfused via the intraosseous route 10 minutes later. After 48 hours, lung samples were examined histologically and no fat or inflammation was found. However, given that the samples were examined 48 hours after intraosseous infusion, it is possible that fat emboli in these healthy animals may have cleared by that stage.<sup>115</sup>

Orlowski et al used a canine model to investigate the risk of fat emboli after intraosseous infusion of medications in dogs via the distal femur.<sup>116</sup> The dogs received various medications, including epinephrine, atropine, and lidocaine, or normal saline (controls), in ten groups each containing three dogs. The animals were sacrificed 4

hours following intraosseous infusion for examination of lung tissue. Fat and bone marrow emboli were found in all lung sections, varying in mean number from 0.9 emboli per mm<sup>2</sup> of lung in dogs receiving the medications to 0.3 emboli per mm<sup>2</sup> of lung in the controls. There was no significant difference in the number of emboli between the dogs that received medication and the controls ( $p=0.07$ ). Despite the presence of fat emboli, the authors found no clinical effect on the ventilation-perfusion relationship when evaluating arterial blood gases. They concluded that although fat emboli are common following intraosseous infusion, they are not of any immediate clinical importance and do not result in adult respiratory distress syndrome.

Hasan et al investigated the effects of volume, pressure, and rate of administration of intraosseous fluid on fat emboli in 30 piglets.<sup>115</sup> Group 1 received a fluid bolus under 300 mmHg of pressure, group 2 received the same bolus of fluid by free flow under gravity, group 3 received fluid over 20 minutes, and group 4 received fluid over 7 minutes. Histological examination of lung specimens from the upper and lower lobes revealed fat emboli (1–3 per high-power field) in approximately 30% of samples. There was no statistically significant difference in frequency of emboli between the four groups. The authors concluded that fat embolism is common; however, the method of intraosseous fluid administration did not influence the number of emboli, and the clinical relevance of such emboli is unclear.

In a well-designed animal study, Fiallos et al assessed the incidence of fat emboli after cardiopulmonary resuscitation and intraosseous infusion of resuscitation drugs in 33 mixed-breed piglets<sup>117</sup>. Hypoxic cardiac arrest was induced and followed by chest compressions and mechanical ventilation for a minimum of 30 minutes. The animals

were divided into five groups and subjected to hypoxemic arrest, cardiopulmonary resuscitation, and intraosseous administration of resuscitation drugs and fluid. Lung samples were compared with those in a control group in which no intraosseous infusion was administered. Histological analysis showed no difference in the number or distribution of fat emboli between any of the experimental groups and the control group. The authors concluded that intraosseous infusion during cardiopulmonary resuscitation did not increase the incidence of fat or bone marrow emboli.

In summary, animal studies have yielded conflicting findings but it appears that subclinical fat emboli may occur following intraosseous infusion. However, there have been no documented cases of the clinical syndrome of fat embolism after intraosseous infusions in infants or children.<sup>96</sup> Further, two studies of intraosseous infusion involving 150 adult patients did not report fat embolism as a complication<sup>95,97</sup>. This suggests that, if present, the risk of clinically significant fat embolus following IORA antibiotics is low.

## **1.7 Study aims**

The aims of the research underpinning this thesis were:

- To compare the tissue concentrations of a standard antibiotic (cefazolin) achieved via the IORA route with those achieved via systemic administration of the same dose of cefazolin as antibiotic prophylaxis in patients undergoing TKA
- To identify the organisms causing deep PJI in the Auckland region and their antibiotic sensitivities



- To compare the tissue concentrations of a more systemically toxic antibiotic (vancomycin) delivered by low-dose IORA with those achieved by standard-dose systemic administration in TKA
- To assess whether either of these strategies might provide more effective prophylaxis using a murine model of TKA
- To identify the causes of failure in modern TKA and the relative importance of infection as a mechanism
- To investigate delivery of antibiotic prophylaxis by IORA in patients undergoing revision TKA and at high risk of infection

### **1.8 Thesis structure**

This thesis has been formatted to include published work in accordance with the 2011 University of Auckland PhD thesis regulations. Chapter 1 of the thesis is the present introductory chapter, which provides the context for the subsequent work. Chapters 2–7 report results and each comprises the following:

- A brief introduction
- Results in the form of either a published article or a manuscript for submission
- A discussion providing critical evaluation of the work and a perspective on the impact of the work since publication (where applicable).

Chapter 8 is an overall summary of the research and includes a discussion of future directions and perspectives on how this research may influence clinical practice in the future. Chapter 9 contains appendices and Chapter 10 contains the references used in the preceding chapters.

## **Chapter 2 Higher cefazolin concentrations with intraosseous regional prophylaxis in total knee arthroplasty**

### **2.1 Preface**

Regional administration of prophylactic antibiotics in total knee arthroplasty (TKA) via the intravenous route in a foot vein was first reported by Hoddinott et al in 1993.<sup>77</sup> While the intraosseous route is well established for administration of fluids and medication, use of this route for regional delivery of prophylactic antibiotics in TKA has not been previously reported.

The following section contains a modified version of a manuscript entitled ‘Higher cefazolin concentrations with intraosseous regional prophylaxis in TKA’, published in 2013 in *Clinical Orthopaedics and Related Research* (volume 471, pages 244–249). *Clinical Orthopaedics and Related Research* has a 2016 impact factor of 3.127.

The paper was presented at the 2011 closed meeting of the Knee Society in London, ON, Canada, and was published as part of the Knee Society Symposium. The paper also received the 2011 Royal Australasian College of Surgeons Louis Barnett Prize and the Auckland Orthopaedic Society Research Award in November 2010.

### **2.2 Higher cefazolin concentrations with intraosseous regional prophylaxis in TKA**

#### 2.2.1 Title page

Higher cefazolin concentrations with intraosseous regional prophylaxis in TKA

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The institution of one of the authors (MZ) received funding from the Centre for Clinical Research and effective practice (CCRep), a charitable trust with no relationship to the subject of this article.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.

Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical

principles of research, and that informed consent was obtained from all patients included in the study.

Procedures and sample collection were performed at Middlemore Hospital. Samples were analysed at Canterbury Health Laboratories.

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### 2.2.2 Abstract

*Background* Prophylactic antibiotics reduce the risk of deep infection after primary total knee arthroplasty. However, conventional systemic dosing may not provide adequate tissue concentrations against more resistant organisms, such as coagulase-negative staphylococci. Regional intravenous administration of antibiotics after tourniquet inflation achieves far higher tissue concentrations but requires cannulation of a foot vein. The intraosseous route may offer a rapid and reliable method of regional administration.

*Purpose* To compared tissue concentrations of cefazolin achieved with systemic versus regional intraosseous administration.

*Methods* Twenty-two patients undergoing primary total knee arthroplasty were randomised into two groups. Group 1 received 1 g of cefazolin systemically 10 minutes before tourniquet inflation. Group 2 received 1 g of cefazolin via the

intraosseous route in 200 mL of normal saline through a tibial cannula after tourniquet inflation and before skin incision. Subcutaneous fat and femoral bone samples were taken at set intervals during the procedure and antibiotic concentrations were measured using a validated technique involving high-performance liquid chromatography.

*Results* The overall mean tissue concentration of cefazolin in subcutaneous fat was 186  $\mu\text{g/g}$  in the intraosseous group and 11  $\mu\text{g/g}$  in the systemic group. The mean tissue concentration in bone was 130  $\mu\text{g/g}$  in the intraosseous group and 11  $\mu\text{g/g}$  in the systemic group. These differences were consistent across all sample time points throughout the procedure.

*Conclusions* Intraosseous regional administration can achieve concentrations of antibiotic in tissue an order of magnitude higher than systemic administration. Further work is required to determine if this translates into increased efficacy in preventing infection, particularly against coagulase-negative staphylococci.

*Level of Evidence* Level I; prospective randomised study

### 2.2.3 Introduction

Periprosthetic joint infection (PJI) is one of the most devastating complications of total knee arthroplasty (TKA). Despite concerted efforts to reduce infection rates, the reported incidence of PJI after primary TKA continues to be between 0.86% and 2.5%<sup>10-13</sup>. Coagulase-negative staphylococci (CoNS) cause up to 49% of PJI, and there is evidence showing that the causative role of these organisms is increasing<sup>10-13</sup>.

The majority of early postoperative infections result from intraoperative contamination of the surgical site.<sup>18</sup> Even with strict aseptic technique, bacterial contamination occurs in most if not all arthroplasty procedures.<sup>20</sup> Prophylactic antibiotics reduce the risk of contamination progressing to overt clinical infection and their efficacy in orthopaedic surgery is well established.<sup>41,82,118</sup> For antibiotic prophylaxis to be effective, the concentration of antibiotic in the tissues must exceed the minimum inhibitory concentration (MIC) for organisms that commonly cause infection during the period between skin incision and wound closure.<sup>39</sup> The MICs of cephalosporins are relatively high for CoNS, which are one of the most common causes of infection post TKA.<sup>61</sup> Conventional systemic dosing of prophylactic cephalosporins may not provide adequate tissue concentrations against these organisms.<sup>61,76</sup>

Regional administration of medication using a tourniquet achieves higher tissue concentrations than systemic administration by limiting distribution of the drug to the targeted limb. Some authors have used a foot vein to administer prophylactic antibiotics in TKA. With this approach, substantially higher tissue concentrations of antibiotic can be achieved at the surgical site without systemic side effects.<sup>74-77</sup> (Table 2.1) However, cannulation of a foot vein is difficult, time-consuming, and may compromise sterility. An alternative means of regional administration is intraosseous cannulation. Since its first reported use over 70 years ago<sup>89</sup>, the intraosseous route has gained popularity as a rapid and reliable method of accessing the circulation<sup>96</sup>. The aim of this study was to compare tissue concentrations of cefazolin achieved by the regional intraosseous route with those achieved by the systemic route in patients undergoing primary TKA.

**Table 2.1** Papers investigating regional administration of prophylactic antibiotics in TKA.

<b>Reference</b>	<b>Comparison</b>	<b>Outcomes</b>
Hoddinott et al <sup>77</sup>	Compared 1000 mg IV cefamandole versus 750 mg regional cefuroxime through a foot vein in the same 5 patients	Mean concentrations of cefuroxime in bone (133 mg/L) and fat (88 mg/L) were higher than those of cefamandole in bone (9 mg/L) and fat (10 mg/L); p<0.001
de Lalla et al <sup>76</sup>	RCT in 24 patients comparing 800 mg IV teicoplanin 2.5 hours preoperatively versus 400 mg teicoplanin through a foot vein	Tissue samples (skin, subcutaneous tissue, bone, synovium) 2–10 times higher through the regional route
de Lalla et al <sup>75</sup>	Clinical study of 160 patients (205 knees) undergoing TKA, 400 mg teicoplanin through a foot vein	One superficial infection; no deep infections at 2-year follow-up
Lazzarini et al <sup>74</sup>	5 patients with 800 mg IV teicoplanin 2.5 hours preoperatively versus 15 patients with 200 mg teicoplanin through a foot vein	Tissue samples (skin, subcutaneous tissue, bone, synovium) 2 times higher through the regional route

Abbreviations: IV, intravenous; RCT, randomised controlled trial; TKA, total knee arthroplasty

#### 2.2.4 Patients and methods

Patients undergoing primary TKA at a single institution were eligible for enrolment in this prospective, randomised controlled trial. The inclusion criteria were age 55–85 years and a primary diagnosis of osteoarthritis. We excluded patients with previous compartment syndrome, allergy to an antibiotic used in the study, abnormal renal or liver function, recent antibiotic treatment (within the past week), or a body mass index (BMI)  $>35 \text{ kg/m}^2$ .

From March to August 2010, we considered 32 patients undergoing primary TKA for osteoarthritis for enrolment in this trial. Ten patients were excluded (eight with a BMI  $>35 \text{ kg/m}^2$ , one who refused to provide consent, and one on oral antibiotics for a recent nasal infection), leaving 22 patients who were randomised to a systemic group or an intraosseous group using computer-generated random allocations placed in numbered, opaque, sealed envelopes (Table 2.2). We randomised patients in the preoperative area to allow appropriate setup in the operating room.



**Table 2.2** Patient demographic and procedural characteristics

Variable	Intraosseous group (n=11)	Systemic group (n=11)
Sex		
Male	6	4
Female	5	7
Age, years	71.8 (56–87)	65.3 (48–83)
Body mass index (kg/m <sup>2</sup> )	27.7 (22.1–35)	29.1 (23.1–35)
Tourniquet time (minutes)	84 (44–135)	82 (43–113)
Procedure length (minutes skin to skin)	74 (37–122)	76 (39–110)
American Society of Anesthesiologists score	2.2	2.1

The data are shown as the mean with range in parentheses.

Based on the data published by Hoddinott et al<sup>77</sup> showing a mean ( $\pm$  standard deviation) cephalosporin concentration in fat tissue across 5 time points of  $88 \pm 88 \mu\text{g/mL}$  with regional administration versus  $11 \pm 9 \mu\text{g/mL}$  with systemic administration, an a priori power analysis calculated that 11 patients in each arm would provide more than 80% statistical power to detect the expected difference of  $77 \mu\text{g/mL}$  in subcutaneous fat concentrations between two groups at the 5% significance level. This sample size also provided adequate statistical power ( $>90\%$ ) to detect a difference in mean bone concentrations between the two groups, assuming the mean ( $\pm$  standard deviation) bone concentrations to be  $133 \pm 101 \mu\text{g/mL}$  and  $11 \pm 9 \mu\text{g/mL}$  for regional and systemic administration, respectively. As discussed later, it is difficult to quantify what clinical effect such a difference would have on infection

rates. However, antibiotic concentrations below the MIC for a particular organism are unlikely to provide effective prophylaxis against that organism<sup>39</sup>. CoNS causes up to 49% of TKA infections<sup>12</sup>, and the MIC of cefazolin is  $>32 \mu\text{g/mL}$  for at least 68% of CoNS isolates at our institution. Assuming a tissue concentration distribution similar to that of the cephalosporins used in the study by Hoddinott et al<sup>77</sup>, such isolates would not be covered by the tissue concentrations seen with systemic dosing ( $11 \mu\text{g/mL}$ ); however, tissue concentrations with regional dosing ( $88\text{--}133 \mu\text{g/mL}$ ) would provide effective prophylaxis against such isolates, suggesting the differences used in our power analysis are clinically relevant.

Patients in both groups received 1 g of systemic cefuroxime 10–30 minutes before tourniquet inflation. All patients underwent limb exsanguination and tourniquet inflation to 300 mmHg before routine preparation and draping. The tourniquet remained inflated for the entire procedure. Patients in the systemic group received 1 g of cefazolin systemically through a forearm vein 10–30 minutes before tourniquet inflation. Patients in the intraosseous group received 1 g of cefazolin through an EZ-IO intraosseous cannula (Vidacare, San Antonio, TX, USA; FDA-approved) placed in the medial aspect of the proximal tibia after draping and before skin incision. The cefazolin was administered as a bolus in 200 mL of normal saline following the recommendations of Waisman et al<sup>97</sup>. In the intraosseous group, the incision was made immediately (within 1 minute) following antibiotic injection.

We took samples of subcutaneous fat and femoral cancellous bone at four stages during the procedure. The first subcutaneous fat sample was taken immediately after skin incision, and both bone and fat samples were taken at the time of the distal

femoral cut, trialling of components, and immediately before closure. Times were recorded for each sample (Table 2.3), which were approximately 0.5–1 cm<sup>2</sup> in size.

We rinsed the samples in normal saline to remove excess blood and stored them at -90°C until analysis. Bone samples were crushed with pliers, finely cut further with a scalpel, weighed, and then immersed in phosphate-buffered saline (pH 7.3) for 15 hours at 4°C. The fat samples were finely cut with a scalpel and then treated in the same way as the bone samples. The immersed bone or fat tissue suspension was vortexed for 30 seconds and centrifuged at 15,000 g for 10 minutes. We transferred the supernatant to a clean tube and perchloric acid was added to precipitate the proteins. After centrifugation at 15,000 g for 5 minutes, 50 µL of clear supernatant was injected into the high-performance liquid chromatography (HPLC) system. A validation study of the extraction and HPLC technique was carried out using bone and tissue samples spiked with known concentrations of cefazolin. We analysed all samples in duplicate.

Means, standard deviations, and 95% confidence limits were calculated for the cefazolin concentrations in the different tissue samples. The tissue samples were pooled according to the surgical steps at which they were taken. Coefficients of variation (CVs) for concentration levels were also summarised at each surgical step for the comparison between the two drug administration routes. We used repeated measures analysis of covariance to compare the average level of cefazolin across time between groups adjusted by BMI, age, and length of the surgical procedure. The Shapiro-Wilk test was applied to assess the normality of the residuals.

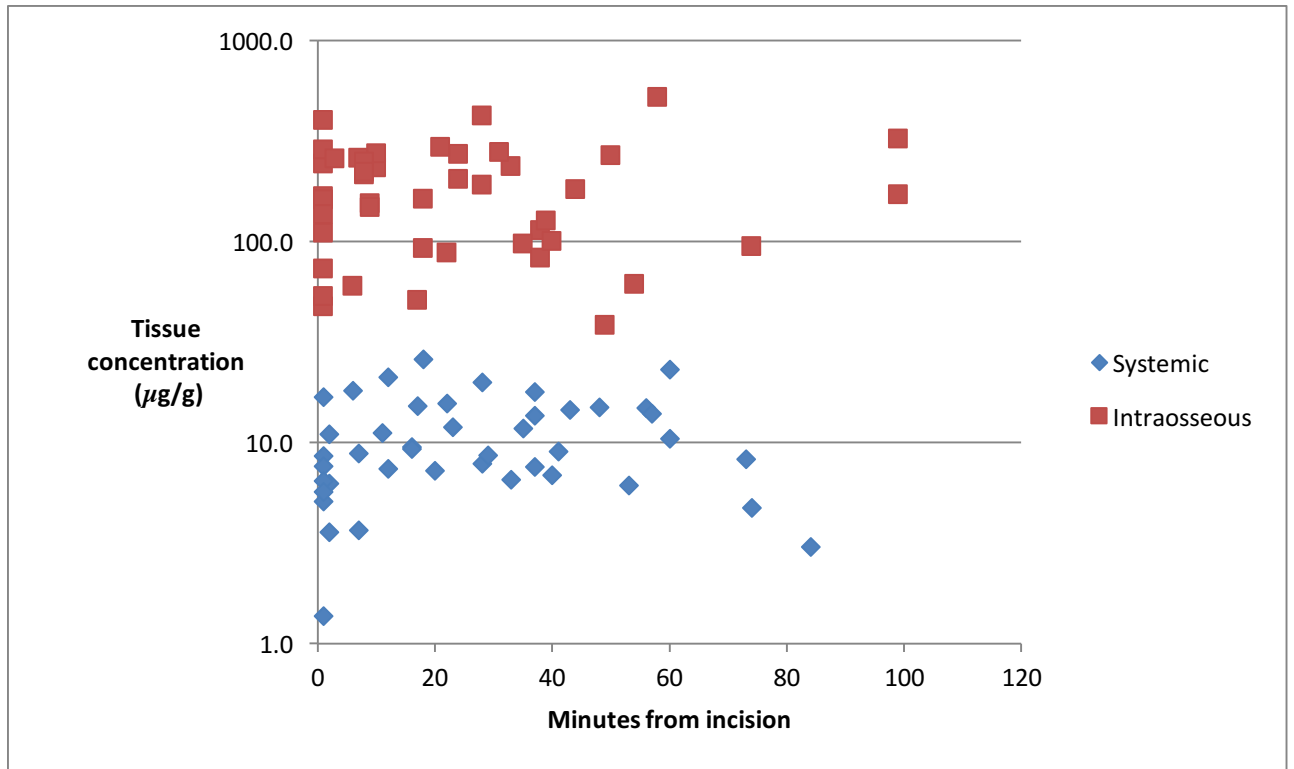
**Table 2.3** Mean tissue concentrations of cefazolin at each sample point

Sampling point	Intraosseous			Systemic		
	Time (minutes)	Concentration (µg/g)	95% CI	Time (minutes)	Concentration (µg/g)	95% CI
Subcutaneous fat 1	1.2 (0.6)	175.3 (110)	102–250	1.3 (0.4)	7.2 (4.3)	4.2–10.3
Subcutaneous fat 2	11 (5.1)	193.0 (79.8)	140–247	14 (6.6)	12.8 (6.6)	8.4–17.2
Subcutaneous fat 3	30 (11.1)	206.3 (127)	121–292	35 (12.3)	11.2 (4.1)	8.4–14.0
Subcutaneous fat 4	56 (23.2)	169.1 (120)	88–250	54 (17.3)	11.3 (6.2)	7.1–15.4
Bone 1	11 (5.1)	75.4 (74.2)	26–125	14 (6.6)	9.2 (2.6)	7.4–10.9
Bone 2	30 (11.1)	165.6 (216)	21–311	35 (12.3)	14.1 (8.2)	8.6–19.6
Bone 3	56 (23.2)	148.8 (105)	79–219	54 (17.3)	10.8 (4.6)	7.7–13.8

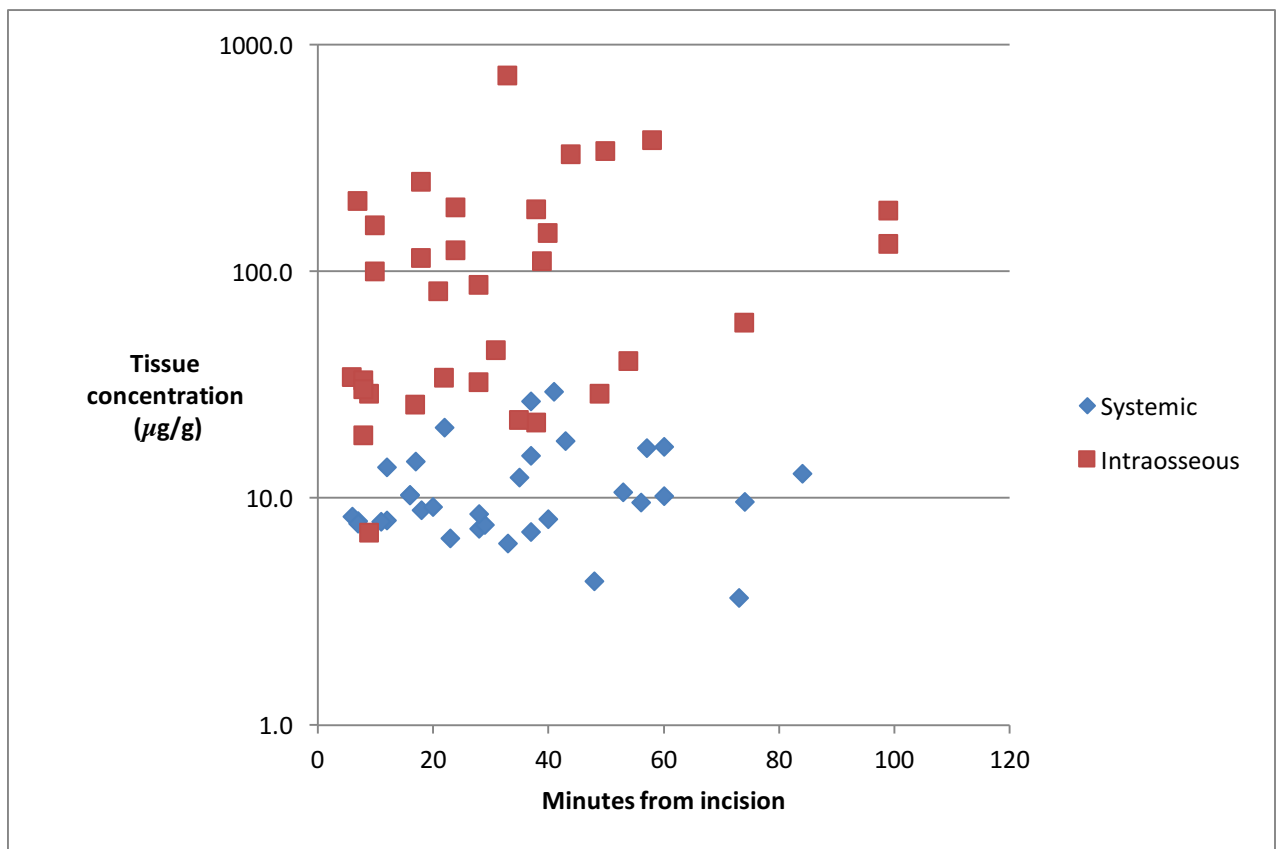
The data are shown as the mean with the standard deviation in parentheses. Time is given as minutes after the surgical incision. Differences in mean tissue concentrations between the two groups were statistically significant ( $p < 0.001$ ) for all comparison points. Abbreviation: CI, confidence interval

### 2.2.5 Results

The mean tissue concentration of cefazolin in subcutaneous fat at the different collection points ranged from  $175 \pm 110 \mu\text{g/g}$  to  $206 \pm 127 \mu\text{g/g}$  in the intraosseous group and from  $7.2 \pm 4.3 \mu\text{g/g}$  to  $12.8 \pm 6.6 \mu\text{g/g}$  in the systemic group (Figure 2.1, Table 2.3). The mean tissue concentration in bone ranged from  $75 \pm 74 \mu\text{g/g}$  to  $165.6 \pm 216 \mu\text{g/g}$  in the intraosseous group and from  $9.2 \pm 2.6 \mu\text{g/g}$  to  $14.1 \pm 8.2 \mu\text{g/g}$  in the systemic group (Figure 2.2). The overall mean tissue concentration of cefazolin in subcutaneous fat was  $186 \mu\text{g/g}$  in the intraosseous group and  $10.6 \mu\text{g/g}$  in the systemic group ( $p < 0.01$ ). The mean tissue concentration in bone was  $130 \mu\text{g/g}$  in the intraosseous group and  $11.4 \mu\text{g/g}$  in the systemic group ( $p < 0.01$ ). The concentration was noted to be more variable with the intraosseous route for both subcutaneous fat samples (CV 0.62–0.71 versus 0.37–0.56) and bone samples (CV 0.7–1.0 versus 0.3–0.6). Repeated measures analysis of covariance showed no association between tissue concentrations and age, BMI, sex, or length of the surgical procedure. No complications were seen in either group during the early postoperative period or at one-year follow-up.



**Figure 2.1** Tissue concentrations of cefazolin in subcutaneous fat for each sample.



**Figure 2.2** Tissue concentrations of cefazolin in femoral bone for each sample.

### 2.2.6 Discussion

Early studies of systemic cefazolin for surgical prophylaxis reported bone and soft tissue concentrations that were adequate to prevent infection<sup>46,47,62</sup> and assumed MIC<sub>90</sub> levels of 0.5–1.0 µg/mL for CoNS. However, over recent decades, resistance of CoNS to cephalosporins has increased markedly, and current MIC<sub>90</sub> values for these agents are as high as 100 µg/mL for half of reported species<sup>61</sup>. This increase in resistance coincides with clinical data reporting a rise in the number of CoNS causing deep prosthetic infections<sup>12</sup>. Regional delivery of antibiotics may offer better protection against CoNS by achieving higher tissue concentrations (Table 2.1); however, cannulation of a foot vein is difficult, time-consuming, and may compromise sterility. This study demonstrates that the more convenient intraosseous route is an effective alternative for regional delivery of antibiotic prophylaxis.

There are a number of limitations to this study. Firstly, while fluids and medications administered via the intraosseous route are reported to have pharmacokinetics similar to those of intravenous administration<sup>96</sup>, use of this route for regional administration is not as well studied. However, the tissue concentrations of cephalosporins in our study are comparable with those seen with intravenous regional administration<sup>77</sup>, suggesting the effectiveness of the two routes are similar. Secondly, we excluded patients with a high BMI to minimise the effect of this variable and because many authors recommend a higher systemic cefazolin dose in heavier patients<sup>61</sup>. We used a relatively high BMI cut-off of >35, so some of the patients in our systemic group may have been underdosed. However, Yamada et al<sup>61</sup> found a mean bone concentration of only 16 µg/g in TKA patients with a mean BMI of 25 given 2 g of cefazolin systemically, suggesting that a higher systemic dose would be unlikely to alter our

findings. A higher intraosseous dose could be considered for obese patients, but given the much smaller volume of distribution in regional administration, 1 g of cefazolin is still likely to provide extremely high tissue levels. Finally, although we saw no complications with this technique in our study, the number of study participants was small. Potential complications with intraosseous infusions include fluid extravasation with compartment syndrome related to incorrect needle placement in emergency situations<sup>96</sup>. Needle site infection has been reported rarely<sup>96</sup>, and correlates with the length of time the needle is left in situ. Subclinical fat emboli have been seen histologically in animal studies<sup>115</sup>, but no cases of fat embolism after intraosseous infusion have been reported in humans.

We found that intraosseous regional administration of prophylactic antibiotics in TKA provides tissue concentrations 10–15 times higher than those achieved by systemic administration. Our findings are similar to those of previous studies in TKA that used intravenous regional administration of other cephalosporins (Table 2.1). Hoddinott et al<sup>77</sup> compared 1 g of intravenous regional cefuroxime with 1 g of systemic cefamandole and found tissue concentrations 5–30 times higher with regional administration. During elbow surgery, Miller et al reported bone cefazolin concentrations 41 times higher and fat concentrations 133 times higher than those achieved by systemic dosing, reflecting the smaller regional volume of distribution in the upper limb<sup>78</sup>.

Do such high levels lead to increased efficacy? Nickinson et al reported that 49% of TKA infections were due to CoNS and that over 55% of CoNS strains were methicillin-resistant<sup>12</sup>. In 1990, Friedman et al<sup>46</sup> reported that the MIC<sub>90</sub> of cefazolin for resistant strains of CoNS was 64  $\mu\text{g/mL}$ , and in 2011 Yamada et al<sup>61</sup> reported an



MIC<sub>90</sub> of 100 µg/mL. Similar to previous studies<sup>46,47,62</sup>, we found systemic dosing provided tissue concentrations of cefazolin (mean 10.6 µg/g in fat, 11.4 µg/g in bone) that were well below these levels. In contrast, the regional intraosseous route provided mean cefazolin concentrations of 185.9 µg/g in fat and 129.9 µg/g in bone. Such levels have a plausible theoretical advantage by providing greater activity against organisms such as CoNS for which the MICs of cefazolin are typically high.

Whether such high cefazolin levels improve efficacy against more sensitive (lower MIC) bacterial strains is less clear. While antibiotics such as aminoglycosides and fluoroquinolones exhibit concentration-dependent killing, for beta-lactam antibiotics such as cefazolin ‘time above MIC’ is the most important factor. Animal models of infection suggest that saturation of the killing rate occurs at cephalosporin concentrations 4–5 times the MIC<sup>59</sup>. By definition, the highest MIC<sub>90</sub> for cefazolin-sensitive CoNS is 8 µg/g<sup>61</sup>, so while the intraosseous regional route will ensure tissue concentrations are at least 5 times this level, any clinical advantage may well be small. However, higher beta-lactam concentrations (64 times MIC) are known to promote earlier initiation of bacterial killing<sup>59</sup>, which may be more important in prophylaxis, where the goal is to prevent the initial bacterial adherence and colonisation.

Regional intraosseous antibiotic administration is used in the treatment of equine limb infection<sup>101,104,105</sup>; however, only one study has investigated the use of regional intraosseous medications in humans. Waisman et al<sup>97</sup> reported on 109 patients given local anaesthetic in 140 mL of saline via the regional intraosseous route before upper or lower limb surgery. Two patients had inadequate anaesthesia, which the authors attributed to an insufficient volume (80 mL) infused in these patients. In our study, we

chose a higher volume of 200 mL because during regional administration the circulation has effectively ceased and distribution relies on the volume of fluid to ‘push’ the medication through the vasculature of the limb. We felt this volume would ensure the antibiotic is present in the tissues at incision, which occurs immediately after intraosseous injection. Our data showed very high antibiotic levels in the first tissue sample, and it is possible a smaller volume may be adequate.

For regional delivery of antibiotics in TKA, the main advantages of intraosseous over foot vein cannulation are reliability and speed. The proximal tibia is already exposed during TKA, and modern intraosseous cannulation system kits offer rapid access<sup>94</sup>. The average time for cannulation and injection in this study was under 2 minutes, so the difference in overall tourniquet time between the two groups was minimal.

In summary, we have developed a technique for administering intraosseous regional antimicrobial prophylaxis prior to TKA that can achieve tissue levels an order of magnitude higher than with systemic administration. Further work is required to confirm whether this translates into increased efficacy in preventing infection, particularly if caused by CoNS.

#### 2.2.7 Acknowledgments

We thank Irene Zeng MSc (Hons) for her assistance with the statistical analysis, Grant Moore BSc (Hons) for his assistance with the laboratory analysis, and the charitable trust Clinical Research and effective practice (CCRep) for its funding support. We also thank Vidicare (San Antonio, TX, USA) for supplying the intraosseous needles without charge.

## 2.3 Discussion of article

### 2.3.1 Contribution and significance

The primary finding of this paper was that the intraosseous route could be used effectively to deliver regional antibiotic prophylaxis in TKA. Insertion of an intraosseous needle was simple, rapid, and reproducible. Previously, regional administration of prophylactic antibiotics in TKA required cannulation of a foot vein, which can be time-consuming and is not always successful.<sup>75</sup> The intraosseous technique therefore makes regional prophylaxis in TKA practical for routine use.

The tissue concentrations in fat were extremely high in the first sample taken immediately following skin incision, suggesting that the antibiotic was rapidly distributed through the limb when administered by intraosseous injection. This is important because one of Burke's original criteria for effective prophylaxis was adequate tissue concentrations from the time of incision until the time of closure.<sup>39</sup> Intraosseous injection of the prophylactic antibiotic into the tibia occurs below a tourniquet, so there is no circulation to distribute the antibiotic through the vascular tree of the limb. Therefore, distribution relies on diffusion, which may not occur rapidly enough to provide adequate tissue levels when the skin is first incised and contamination starts to occur. However, this study showed high concentrations from the very first sample. This may have been facilitated by injection of a large volume of fluid (200 mL) that allowed rapid diffusion of the antibiotic<sup>97</sup>.

Similarly, antibiotic concentrations in both fat and bone were consistently high in the intraosseous regional administration (IORA) group across all patients and samples. This suggests that the intraosseous route was reliable and effective in this group of adult patients with knee arthritis and supports the findings of previous animal

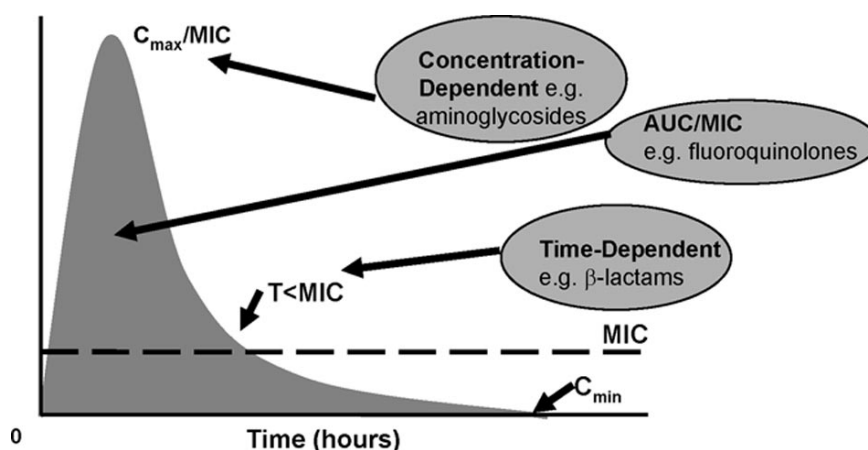
studies<sup>99,102,105,119</sup> and a human study<sup>96,120</sup> showing that intraosseous administration is equivalent to intravenous administration in both adult and paediatric patients.

### 2.3.2 Effect of high cefazolin concentrations on efficacy

This paper demonstrates that IORA achieves very high tissue concentrations of cefazolin, but whether this would reduce the rate of periprosthetic joint infection (PJI) is unclear. Given that the reported rates of PJI following TKA are between 0.86% and 2.5%<sup>10-13</sup>, adequately powering a study to detect a reduction in PJI rates would be difficult. However, knowledge of the pharmacokinetics and pharmacodynamics of cefazolin may offer insights into the likely effects of the higher concentrations seen with IORA.

In 1976, Shah et al proposed that antibiotics could be grouped based on their patterns of bactericidal activity as either concentration-dependent or time-dependent.<sup>121</sup>

Concentration-dependent antibiotics kill bacteria more effectively at higher antibiotic concentrations, and the pharmacokinetic-pharmacodynamic parameters associated with efficacy are  $C_{\max}/MIC$  or  $AUC/MIC$  (Figure 2.3). In contrast, time-dependent antibiotics kill at a similar rate and extent once the concentration is above a certain threshold, and the parameter associated with efficacy is time above MIC. In this second pattern, increases in the antibiotic concentration beyond this point typically do not augment antibacterial activity, and thus the effectiveness relates to the duration of exposure<sup>59,122,123</sup>.



**Figure 2.3** Pharmacokinetics and pharmacodynamics of antibiotics on a concentration versus time curve and the three parameters (circled) associated with bacterial killing.

Abbreviations: AUC, area under the curve; MIC, minimum inhibitory concentration.

Reproduced from Roberts JA, Kruger P, Paterson DL, Lipman J. Antibiotic resistance—What's dosing got to do with it? *Crit Care Med.* 2008;36(8):2433–2440.<sup>124</sup>

While such classifications are highly dependent on the specific antibiotic molecule and bacterial pathogen being studied, in general aminoglycosides and fluoroquinolones are considered concentration-dependent, whereas beta-lactam antibiotics such as cefazolin are considered time-dependent<sup>122</sup>. Therefore, the high concentrations of cefazolin seen with the IORA technique in this study may offer limited additional benefit in reducing the incidence of PJI following TKA.

However, classification of the pharmacodynamics of cefazolin as time-dependent or concentration-independent is based on animal models of treatment of established infection, typically mouse models of pneumonia<sup>125,126</sup>. In this setting of established infection, antibiotics serve to support the body's immune system in eradicating the infection. In contrast, surgical prophylaxis aims to prevent the initial bacterial colonisation that leads to PJI. The humoral (antibody-mediated) immune response

does not occur until at least 7 days following contamination of an arthroplasty site.<sup>127</sup> Therefore, prophylactic antibiotics serve to support the innate immune system by preventing initial bacterial adherence before activation of avoidance mechanisms such as formation of biofilm. In the presence of an implant, as few as 100 colony-forming units of *Staphylococcus aureus* are sufficient to establish PJI<sup>127</sup>, and clinical manifestations of infection may not appear until months or years following surgery<sup>128</sup>.

Given that the antibiotic effect requires direct contact between an antibiotic molecule and a bacterium, such contact is likely to occur earlier in the presence of higher antibiotic concentrations. This is supported by animal studies showing that higher tissue concentrations lead to earlier initiation of bacterial killing, even for time-dependent antibiotics.<sup>59</sup> This may be more important in the setting of prophylaxis, so the high cefazolin levels seen with IORA may increase the chances of contaminating bacteria being eradicated before colonisation occurs. In addition, *S. aureus* is known to form aggregates in the presence of synovial fluid.<sup>129</sup> Such aggregates are protective and render these bacteria insensitive to the cefazolin concentrations typically achieved after a 2 g intravenous dose<sup>129</sup>, and higher concentrations may be help to overcome this bacterial defence mechanism.

Such considerations are theoretical, and currently there are no clinical data on the relationship between the tissue concentration of cefazolin and the efficacy of prophylaxis in arthroplasty surgery. However, it seems clear that any improved efficacy of prophylaxis using IORA would depend on the antibiotic used and that concentration-dependent antibiotics are likely to be more suited to IORA.

### 2.3.3 Antibiotic resistance

As noted previously, the two organisms causing contamination and early PJI in TKA are *S. aureus* and CoNS<sup>12,128,130</sup>. Resistance of both species to cephalosporins is increasing worldwide, including in Australasia. Peel et al reported on the organisms causing PJI in 9392 TKAs and THAs during one year using the Victorian Healthcare Associated Infection Surveillance System database.<sup>130</sup> Overall, 81% of PJIs were caused by *S. aureus* and CoNS. Methicillin resistance was present in 47% of *S. aureus* and 90% of CoNS isolates.

When methicillin resistance is present in contaminating bacteria, even the high concentrations of cefazolin achieved using IORA may not be sufficient to overcome this resistance and prevent colonisation and subsequent PJI, and alternative agents such as vancomycin may offer an advantage. However, the microbiology of bacteria PJI varies considerably by region, and local knowledge in New Zealand is currently lacking.

## Chapter 3 Antibiotic resistance in early periprosthetic joint infection in the Auckland region and its implications for prophylaxis

### 3.1 Introduction

The choice of prophylactic antibiotic is largely informed by the bacteria most likely to cause contamination and subsequent periprosthetic joint infection (PJI). Most guidelines in orthopaedics recommend cefazolin<sup>45,131</sup>, but also stipulate that antibiotics should be chosen to cover the pathogens most frequently encountered in a given geographic region<sup>130</sup>.

Little is known about the frequency of bacteria causing PJI following total knee arthroplasty (TKA) or the rates of antibiotic resistance in Auckland, New Zealand. PJI that occurs in the first 2 years following TKA surgery is most likely due to intraoperative contamination, unless an obvious haematogenous source exists<sup>16</sup>. The bacteria causing these infections are those most relevant to the choice of antibiotic prophylaxis.

In order for prophylactic antibiotics to be effective in reducing the risk of PJI, their spectrum of activity must cover the organisms likely to cause intraoperative contamination.<sup>39</sup> In TKA, antibiotics must therefore be effective against the two most common infective organisms, *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS).<sup>12,18,128</sup> In the 1960s, when arthroplasty procedures were first performed, methicillin resistance among CoNS strains was 2%.<sup>64</sup> Currently, 55%–75% of CoNS hospital isolates are methicillin-resistant.<sup>12,25,65,128,132</sup> Further, some regions have reported that up to 56% of *S. aureus* isolates from infected joint arthroplasties are methicillin-resistant (MRSA).<sup>74</sup> Methicillin resistance amongst staphylococci confers resistance to all beta-lactam antibiotics, including cefazolin.



The aim of the research outlined in this chapter was to identify the bacteria causing PJI following TKA in Auckland, along with rates of resistance and the implications for prophylaxis. Some aspects of this chapter have been addressed in a recent article on antibiotic resistance in early PJI published by our group in Auckland.<sup>133</sup>

### **3.2 Methods**

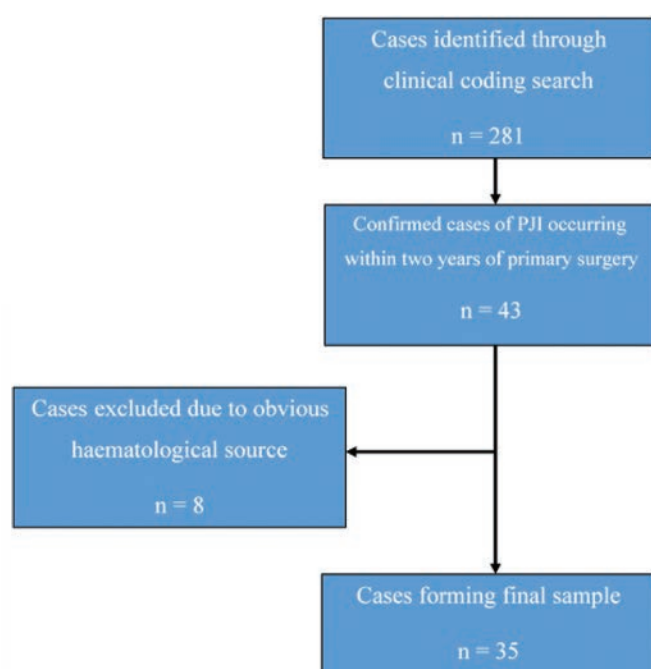
We conducted a multicentre retrospective review of 4009 primary arthroplasties (2157 knees, 1852 hips) performed between January 1, 2006 and December 31, 2008 at three tertiary referral hospitals in Auckland, New Zealand, and identified all PJIs that occurred within 24 months of primary implantation.

PJI was diagnosed according to the Infectious Diseases Society of America definition, which requires fulfilment of any one of the following criteria: sinus tract continuous with the prosthesis; periprosthetic purulence without another aetiology; acute inflammation on histology of a periprosthetic specimen; and at least two microbiological specimens with the same organism on either periprosthetic or blood culture samples.<sup>134</sup> The Coventry system, as subsequently modified by Fitzgerald et al<sup>15,16</sup>, was used to classify the PJIs. In this classification, infections with symptom onset within 2 years of the index procedure are assumed to be due to intraoperative contamination unless a clear haematogenous source exists.

PJIs were classified as haematogenous and excluded if there was an identifiable remote site of infection involving the same organism as that isolated from the arthroplasty or if the patient presented with acute signs of systemic infection (e.g., fever, rigors, and/or night sweats) and/or signs of local infection (e.g., acute joint

inflammation and/or rapid-onset pain) in a previously well-functioning implant more than 3 months after the index procedure.<sup>128</sup>

An initial clinical coding search was undertaken at each hospital using discharge summaries and theatre operating codes (ICD-9 and ICD-10). Appendix 1 shows the specific codes used in the search. Patient files were then screened manually to identify those who met the inclusion criteria and study definitions, after which 43 cases were identified as having occurred within 2 years of the primary surgery (Figure 3.1).



**Figure 3.1** Flow chart showing the number of cases enrolled after the exclusion criteria were applied. Abbreviation: PJI, periprosthetic joint infection

Demographic, operative, and laboratory data were collected from hospital records and cross-referenced with the New Zealand Joint Registry database. The capture rate was 100% against this database. A univariate analysis was performed, with frequency tabulation of the following: demographic criteria, infective organisms, and

susceptibility of cultured organisms to cefazolin. The infection profile at various time intervals (<6 weeks, 6–12 weeks, 3–12 months, and 12–24 months) was also evaluated.

### **3.3 Results**

Forty-three cases of PJI were identified as having occurred within 2 years after the primary arthroplasty. Eight cases occurring more than 3 months post implantation were classified as acute haematogenous in origin, leaving 35 cases of PJI for inclusion in the study (Figure 3.1). This gave an overall rate of PJI due to intraoperative contamination of 0.87% (0.7% for hips, 1.0% for knees).

The cohort consisted of 13 (37.1%) THA and 22 (62.9%) TKA infections. The median patient age was 70 years. The majority (71.4%) of the patients were European, and the most common indication for the primary procedure was osteoarthritis (82.9%). Twenty-seven patients (77.1%) had an immunosuppressive comorbidity, the most common of which was smoking (Table 3.1).

**Table 3.1** Patient demographic and clinical characteristics

Age, years	Mean	68	Joint	Hips	13
	SD	9.51		Knees	22
	Range	48–82	Indication	Osteoarthritis	29
Sex	Male	17		Rheumatoid arthritis	3
	Female	18	Fracture	3	
Ethnicity	European	25	Comorbidities	Any	27
	Pacific Islander	4		Diabetes mellitus	8
	Maori	3		Malignancy	5
	Indian	2		Smoking	18
Hospital	Chinese	1	Rheumatoid arthritis	4	
	ACH	3	Other CTD	3	
	MMH	20	Other IS condition/drug	6	
	NSH	12	Renal failure	5	

Abbreviations: ACH, Auckland City Hospital; CTD, connective tissue disorder; IS, immunosuppressive; MMH, Middlemore Hospital; NSH, North Shore Hospital; SD, standard deviation

Thirty of the 35 cases had at least one positive culture (Table 3.2). From these, 43 organisms were cultured, of which 33 (77%) were Gram-positive organisms. CoNS (15, 35%) and *S. aureus* (11, 26%) were the most common infective organisms.

**Table 3.2** Microbiological findings

Patient infection characteristics (n=30)		Cultured infective organisms (n=43)	
Monomicrobial	21	Coagulase-negative staphylococci	15
Polymicrobial	9	Methicillin-susceptible <i>Staphylococcus aureus</i>	10
		Methicillin-resistant <i>Staphylococcus aureus</i>	1
Gram-positive infection only	22	Streptococcus spp.	6
Gram-negative infection only	3	Corynebacterium spp.	1
Mixed Gram-positive and	5	Enteric Gram-negative spp.	8
Gram-negative infection		Pseudomonas spp.	2

Cefazolin susceptibility was available for 38 (88%) isolates (Table 3.3). Overall, 21 (55%) of the infective organisms were cefazolin-resistant. Methicillin resistance was present in 11 (92%) CoNS isolates and one (9%) *S. aureus* isolate. Information on prophylaxis was available for 25 (71%) patients. Cefazolin prophylaxis was used in 24 (96%) patients and gentamicin prophylaxis was used in the remaining patient.

**Table 3.3** Susceptibility of organisms to cefazolin (n=38)

Infective organism	Cefazolin-susceptible (n=17)	Cefazolin-resistant (n=21)
Coagulase-negative staphylococci	1	11
Methicillin-sensitive <i>Staphylococcus aureus</i>	10	0
Methicillin-resistant <i>Staphylococcus aureus</i>	0	1
Streptococcus spp.	4	0
Enteric Gram-negative	2	3
<i>Morganella morganii</i>	0	2
Pseudomonas spp.	0	2
<i>Serratia marcescens</i>	0	1
Corynebacterium spp.	0	1

Cumulatively, PJIs occurred by 6 weeks in 18 cases (infection rate 0.45%), 3 months in 22 cases (infection rate 0.55%), 12 months in 29 cases (infection rate 0.72%), and 24 months in 35 cases (infection rate 0.87%). CoNS and *S. aureus* remained the most common pathogens during the <6-week, 6–12-week, and 3–12-month time intervals, however, CoNS and streptococci were most common in the 12–24-month time interval.

### 3.4 Discussion

#### 3.4.1 Contribution and significance

This study is the first to focus on the microbiology of PJIs that are most likely due to intraoperative contamination and to be influenced by prophylactic antibiotics, in

contrast with late infections caused by haematogenous spread of bacteria from another site in the body. This study also provides important local data relevant to New Zealand, considering that the microbiology of infection can vary by geographic region<sup>68</sup>. We found that over 50% of organisms causing early PJI following primary total hip replacement or TKA were resistant to cefazolin, with the most resistance seen in Gram-positive organisms.

In contrast with previous studies on the microbiology of PJI<sup>12,67,68,135,136</sup>, we focused on PJIs most likely to be secondary to intraoperative contamination. Such PJIs can be expected to have a distinct bacterial profile, which will be influenced by the prophylactic antibiotic used. Our findings support those of Fulkerson et al, who found that the majority of PJIs occurring within 4 weeks of surgery were cefazolin-resistant.<sup>128</sup> This is important because the outcome of treatment of PJI due to resistant organisms may be worse; Kilgus et al reported an 18% success rate for two-stage revision TKA if infection was caused by methicillin-resistant bacteria compared with 89% if caused by methicillin-susceptible strains.<sup>25</sup>

### 3.4.2 Limitations

There are a number of limitations to this study. Firstly, it was retrospective, so the audit was reliant on the quality of the clinical coding searches performed at the hospitals. However, we cross-referenced the patients found on the New Zealand Joint Registry and had a capture rate of 100% against that database. Secondly, although we used the established Coventry classification to identify PJIs most likely due to intraoperative contamination, the true source of the bacteria causing PJI in an individual patient is difficult to identify with certainty. We found that a significant number of PJI cases were caused by hospital-acquired organisms, such as MRSA,

*Morganella morganii*, *Serratia marcescens*, and *Pseudomonas* spp. It is possible that such organisms cause PJI through either wound contamination or haematogenous spread in the early postoperative period instead of intraoperative contamination.<sup>137</sup> However, studies have shown that the initial preoperative prophylactic antibiotic dose is the most important, and that extended postoperative antibiotic regimens do not reduce the infection rate further.<sup>49,138</sup> This suggests that careful consideration of an appropriate prophylactic agent to prevent intraoperative contamination progressing to PJI remains important. Moreover, given that up to 26% of CoNS infections may present more than 2 years after surgery<sup>13</sup>, we recognise that this definition may exclude some indolent PJI cases caused by intraoperative contamination. Finally, our microbiological data apply to the Auckland region only, and different centres will have differing resistance profiles.

#### 3.4.3 Implications for prophylaxis

Prophylactic antibiotics function by preventing bacteria that contaminate the wound from surviving and progressing to cause PJI. Therefore, early PJI can be considered a ‘failure’ of prophylaxis. Cefazolin was the most commonly used prophylactic agent during our study period, so contamination with cefazolin-resistant organisms would be more likely to progress to PJI. Therefore, selection pressure is likely to explain the high rate of resistant Gram-positive organisms seen in this study. However, such resistant organisms are important in early PJI, and any attempt to reduce infection rates needs to consider this.

Resistance to cefazolin is mediated by alterations in the beta-lactam receptor in the bacterial cell wall to which cefazolin binds<sup>139</sup>. Such resistance may not be overcome by simply increasing the tissue concentrations of cefazolin, because binding between



the antibiotic molecule and its receptor is still compromised. Therefore, improving antibiotic efficacy against cefazolin-resistant organisms requires use of an alternative agent, such as vancomycin.

#### 3.4.4 Tissue concentrations of vancomycin and bacterial killing

Vancomycin is effective against cefazolin-resistant organisms, and in the context of IORA may also have the advantage of enhanced bacterial killing at higher tissue concentrations. This contrasts with the time-dependent killing seen with cefazolin, as discussed in Section 2.3.2.

While some early research suggested that vancomycin also exhibits time-dependent killing,<sup>140</sup> a number of animal studies have shown that the area under the concentration-time curve (AUC) divided by the MIC (AUC/MIC ratio) is the major pharmacodynamic parameter correlated with the therapeutic efficacy of vancomycin<sup>59,141</sup>.

Ebert et al demonstrated in a neutropenic mouse model that the AUC/MIC was the best predictor of the activity of vancomycin against methicillin-susceptible, methicillin-resistant, and glycopeptide intermediate-resistant *S. aureus*.<sup>141</sup> Further, in a mouse model of *Streptococcus pneumoniae* non-neutropenic peritonitis, Knudsen et al<sup>142</sup> demonstrated that the peak serum concentration divided by the MIC (peak/MIC) was the pharmacodynamic parameter most associated with the success of vancomycin treatment. This suggests that higher concentrations would provide more effective bacterial killing, and this may be due to the post-antibiotic effect of vancomycin, which is particularly enhanced by higher tissue concentrations<sup>141</sup>. Löwdin et al reported that when vancomycin concentrations were 2–4 times above the MIC, the

post-antibiotic effect increased from 0.2 to 2 hours for *S. aureus* and from 4.3 to 6.5 hours for *S. epidermidis*.<sup>143</sup>

There are also clinical data to support the relationship between higher vancomycin concentrations and greater efficacy. Moise-Broder et al assessed the relationship between the AUC/MIC for vancomycin and outcomes in 108 patients with MRSA pneumonia.<sup>144</sup> The odds of a successful clinical response in vancomycin-treated patients with an AUC/MIC  $\geq 350$  were approximately seven times better than for patients with AUC/MIC values  $< 350$ . While there are no specific data available in regard to arthroplasty, these pharmacodynamic studies support the hypothesis that a higher tissue concentration would enhance the prophylactic efficacy of vancomycin (Table 3.4).

**Table 3.4** Pharmacodynamic parameters and correlation with clinical efficacy and bacterial eradication.

Antibiotic class	Pharmacodynamic parameter correlating with efficacy	Pharmacodynamic parameter associated with bacterial eradication
Fluoroquinolones	$AUC_{0-24}/MIC$	$C_{max}:MIC$
Aminoglycosides	$C_{max}:MIC$	$C_{max}:MIC$
Carbapenems	$T > MIC$	$T > MIC$
Glycopeptides (e.g., vancomycin)	$AUC_{0-24}/MIC$	$T > MIC$ and $C_{max}:MIC$

These parameters are often derived from in vitro studies and may not be directly transferable to clinical settings. Abbreviations: AUC, area under the curve; MIC, minimum inhibitory concentration;  $C_{max}$ , maximum serum antibiotic concentration. Adapted from Roberts et al.<sup>124</sup>

### 3.4.5 Problems with systemic vancomycin prophylaxis

There are several issues concerning the use of systemic vancomycin as a prophylactic agent. Firstly, vancomycin carries the risk of toxicity to other organs. In particular, higher systemic concentrations of vancomycin have been associated with a greater risk of nephrotoxicity.<sup>145</sup> In a study of 1828 patients who underwent hip or knee arthroplasty, Courtney et al reported a higher rate of acute kidney injury in patients who received dual prophylaxis with vancomycin and cefazolin than in those who received cefazolin alone (13% versus 8%,  $p=0.002$ ).<sup>146</sup> Secondly, there is concern that routine use of vancomycin may promote further antibiotic resistance. Thirdly, rapid systemic administration of vancomycin can trigger release of histamine and subsequent 'red man syndrome' caused by dilation of blood vessels secondary to

histamine release. Therefore, vancomycin is typically infused over 1–2 hours, which can be difficult to arrange, particularly for the first patient on the operating list. The timing of prophylactic antibiotics is also important for efficacy, and the dose should be timed to finish within the 60 minutes preceding the surgical incision.<sup>13</sup> Achieving optimum timing of an infusion that needs to be started hours before surgery can be difficult in a busy operating room. Bull et al reviewed 18,342 arthroplasty procedures and found that vancomycin was given with appropriate timing in only 22% of cases compared with 77% of cases given a cephalosporin.<sup>87</sup> While data for orthopaedic procedures are limited, appropriate timing of vancomycin appears to be important in its effectiveness when administered as prophylaxis. Garey et al prospectively monitored 2048 patients undergoing cardiac surgery with vancomycin prophylaxis.<sup>147</sup> They reported that the infection rate was two times higher when the vancomycin infusion finished more than 60 minutes before surgery, and tissue concentrations would have started to fall below their peak at the time of incision.

Finally, the problem of achieving optimal timing of systemic vancomycin prophylaxis may be exacerbated by inadequate dosing. Because of the risk of systemic toxicity, there is a tendency to be cautious when administering vancomycin. This can lead to inadequate dosing, particularly in obese patients<sup>148</sup>. Catanzano et al reported that serum vancomycin concentrations were lower than 15 mg/L in 60% of 216 patients given systemic vancomycin prophylaxis prior to orthopaedic surgery.<sup>149</sup> Therefore, inadequate tissue concentrations when using systemic administration may be a reason why vancomycin prophylaxis has not been shown to reduce PJI following arthroplasty.<sup>150</sup>

### 3.4.6 Vancomycin prophylaxis using IORA

In summary, there are multiple reasons why vancomycin may be more suitable than cefazolin for IORA prophylaxis in TKA. These include the ability of vancomycin to provide coverage for cefazolin-resistant organisms and its enhanced ability to kill bacteria when present at higher concentrations.

Administration of prophylactic vancomycin via IORA has advantages over systemic vancomycin. It is easier to achieve optimal timing with an IORA bolus than with a prolonged systemic infusion and a lower dose can be used, reducing the risk of systemic toxicity. The lower more targeted dose may reduce subsequent development of resistance. These advantages led to further study of IORA using low-dose vancomycin as the prophylactic agent.

## **Chapter 4 Higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in total knee arthroplasty**

### **4.1 Preface**

Intraosseous regional administration may allow high tissue concentrations of prophylactic antibiotic to be achieved at the surgical site with a lower overall dose. This has particular appeal for antibiotics with known systemic toxicity, such as vancomycin<sup>146</sup>. Vancomycin also offers coverage of resistant strains of *Staphylococcus aureus* and CoNS, which are common causes of infection in TKA.

The following section contains a modified version of an article entitled ‘The Mark Coventry Award: Higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in TKA’ published in 2014 in *Clinical Orthopaedics and Related Research* (volume 472, pages 57–65). *Clinical Orthopaedics and Related Research* has a 2016 impact factor of 3.127. The paper received the 2014 Knee Society Mark Coventry award, presented in Chicago, IL, USA. This is the first time this award has been received by a New Zealand researcher. It was presented at the 2014 open meeting of the Knee Society in Chicago and published as part of the Knee Society Symposium. The paper also received a Top 5 award at the 2013 International Congress for Joint Replacement in New York in 2013.

## **4.2 Higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in TKA**

### 4.2.1 Title page

#### **The Mark Coventry Award**

Higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in TKA: a randomized trial

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The institutions of two of the authors (MZ, GAM) received funding from the Awhina Trust (Auckland, New Zealand), a charitable trust with no relationship to the subject of this article.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.

Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

Procedures and sample collection were performed at North Shore Hospital, Auckland, New Zealand. Sample analysis was performed at Canterbury Health Laboratories, Christchurch, New Zealand.

#### 4.2.2 Abstract

*Background* In response to increasing antibiotic resistance, vancomycin has been proposed as an alternative prophylactic agent in total knee arthroplasty (TKA). However, vancomycin requires a prolonged administration time, risks promoting



further antibiotic resistance, and can cause systemic toxicity. Intraosseous regional administration (IORA) is known to achieve markedly higher antibiotic concentrations than systemic administration and may allow the use of a lower vancomycin dose.

*Questions/purposes* We assessed whether low-dose IORA vancomycin can achieve tissue concentrations equal or superior to those of systemic administration in TKA and compared complications between patients treated with IORA and those treated with intravenous vancomycin.

*Methods* We randomised 30 patients undergoing primary TKA to receive 250 mg or 500 mg of vancomycin by IORA or 1 g of vancomycin by systemic administration. IORA was performed as a bolus injection into a tibial intraosseous cannula below an inflated thigh tourniquet immediately before skin incision. Subcutaneous fat and bone samples were taken during the procedure and antibiotic concentrations were measured.

*Results* The overall mean tissue concentration of vancomycin in subcutaneous fat was 14 µg/g in the 250 mg IORA group, 44 µg/g in the 500 mg IORA group, and 3.2 µg/g in the systemic group. Mean concentrations in bone were 16 µg/g in the 250 mg IORA group, 38 µg/g in the 500 mg IORA group, and 4.0 µg/g in the systemic group. One patient in the systemic group developed 'red man syndrome' during infusion.

*Conclusions* Low-dose IORA vancomycin results in tissue concentrations equal or superior to those achieved by systemic administration. IORA optimises timing of vancomycin administration, and the lower dose may reduce the risk of systemic side effects while providing equal or enhanced prophylaxis in TKA.

*Level of Evidence* Level I; therapeutic study.

### 4.2.3 Introduction

Prophylactic antibiotics dramatically reduce infection rates after arthroplasty.

Randomised trials during the 1970s reported deep infection rates of 1%–2% when prophylactic cephalosporins were administered compared with 7%–15% for placebo.<sup>41,118,151</sup>

However, due to increasing antibiotic resistance in recent decades, cephalosporins may no longer provide adequate prophylaxis.<sup>61</sup> To be effective, prophylactic antibiotics require a spectrum of activity that covers the organisms likely to cause contamination during the procedure.<sup>39</sup> In total knee arthroplasty (TKA), the two most common organisms causing infection are *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS).<sup>12,18</sup> Currently, 60%–90% of CoNS isolates are resistant to cephalosporins<sup>12,61</sup> and 33%–56% of *S. aureus* isolates from infected joint arthroplasties are methicillin-resistant (MRSA)<sup>74,152</sup>. Data from the National Nosocomial Infection Surveillance System showed that the rate of methicillin resistance in *S. aureus* infections rose from 35.9% to 64.4% between 1992 and 2003, representing an increase of 3.1% per year.<sup>66</sup>

Despite increasing methicillin resistance, the vast majority of MRSA and CoNS isolates remain sensitive to vancomycin<sup>12</sup>, leading many to propose it as an alternative prophylactic agent in TKA<sup>87,153-155</sup>. However, vancomycin has several disadvantages. Firstly, it requires a prolonged intravenous administration time because rapid infusion can cause red man syndrome, which consists of a pruritic erythematous rash related to histamine release.<sup>156</sup> A typical prophylactic dose of 1 g requires the infusion to be started a minimum of 1 hour before surgery, and failure to achieve this may lead to underdosing<sup>87</sup>. In a review of 18,342 arthroplasty procedures, vancomycin was given

with appropriate timing in only 22% of cases compared with 77% of cases given a cephalosporin.<sup>87</sup> Secondly, widespread use of vancomycin risks promoting further antibiotic resistance.<sup>157</sup> Finally, vancomycin can also cause renal and other systemic toxicity.<sup>146,156</sup>

We have validated intraosseous regional administration (IORA) of prophylactic antibiotics in TKA<sup>158</sup> and recorded markedly higher tissue concentrations of antibiotic than were achievable with systemic administration. IORA may allow lower vancomycin doses, thereby reducing systemic toxicity and avoiding the difficulties associated with prolonged preoperative infusion times. We hypothesised that lower doses of vancomycin via IORA could still achieve tissue concentrations equal or superior to those of systemic administration before TKA. We also sought to compare complications between patients treated with IORA and those treated with intravenous vancomycin.

#### 4.2.4 Patients and methods

Patients undergoing primary TKA at a single institution were eligible for enrolment into this prospective, randomised controlled trial. Inclusion criteria were age younger than 90 years and a primary diagnosis of osteoarthritis. Exclusion criteria were previous compartment syndrome, allergy to an antibiotic used in the study, abnormal cardiac or renal function, or concurrent nephrotoxic medications. From November 2011 to February 2012, 35 patients undergoing primary TKA for osteoarthritis were assessed for enrolment. Three patients were excluded (two with significant cardiac dysfunction [aortic stenosis, congestive heart failure] and one who refused to provide consent), leaving 32 patients who were randomised into three groups using computer-

generated random allocations placed in numbered, opaque, sealed envelopes (Table 4.1).

**Table 4.1** Patient demographic characteristics.

	<b>IORA vancomycin 250 mg (n=10)</b>	<b>IORA vancomycin 500 mg (n=10)</b>	<b>Systemic vancomycin 1 g (n=10)</b>
<b>Sex</b>			
<b>Male</b>	5	4	1
<b>Female</b>	5	6	9
<b>Age (years)</b>	70.8 (49–89)	71.7 (55–85)	71.4 (53–83)
<b>Body mass index</b>	32.2 (26–36.7)	30.0 (22–38)	34.8 (27–51)
<b>Tourniquet time (minutes)</b>	105 (88–135)	102 (82–130)	99 (74–116)
<b>Duration of procedure (minutes skin to skin)</b>	101 (85–130)	97 (78–128)	97 (74–114)
<b>ASA score</b>	2.7	2.2	2.7

Values are given as the mean with the range in parentheses. Abbreviations: ASA, American Society of Anesthesiologists; IORA, intraosseous regional administration

Patients were randomised in the preoperative area to allow appropriate setup in the operating room. Two patients were excluded post-randomisation due to technical errors (one patient was given an incorrect dose of systemic vancomycin and the intraosseous injection equipment was unavailable for another patient), leaving 30 patients available for analysis.

Data from a previous randomised trial comparing IORA and systemic administration of cefazolin<sup>158</sup> showed mean ( $\pm$  standard deviation) tissue concentrations of cefazolin in subcutaneous fat at different collection intervals ranging from  $175.3 \pm 110 \mu\text{g/g}$  to

206.3 ± 127 µg/g in the IORA group and from 7.2 ± 4.3 µg/g to 12.8 ± 6.6 µg/g in the systemic group. The mean tissue concentration in bone ranged from 75.4 ± 74.2 µg/g to 165.6 ± 216.1 µg/g in the IORA group and from 9.2 ± 2.6 µg/g to 14.1 ± 8.2 µg/g in the systemic group. Thus, the concentration of cefazolin was approximately 10 times higher when using IORA than when using systemic administration. Using these data, an a priori power analysis calculated that 10 patients in each arm would provide greater than 80% statistical power to detect the expected fold difference in subcutaneous fat and bone concentrations among the three groups at the 5% significance level when IORA doses 25% (250 mg) and 50% (500 mg) of the systemic dose (1 g) were used. While data on the pharmacodynamics of vancomycin when used for prophylaxis are lacking, in treatment models of infection, the area under the concentration-time curve (AUC) divided by the minimum inhibitory concentration (MIC) is the pharmacokinetic-pharmacodynamic parameter most predictive of efficacy. Therefore, further increases in tissue vancomycin concentrations are likely to enhance the effectiveness of prophylaxis, particularly when the MIC of vancomycin is ≥1 µg/mL, such as for MRSA and CoNS; this suggests the differences used in our power analysis are clinically relevant.

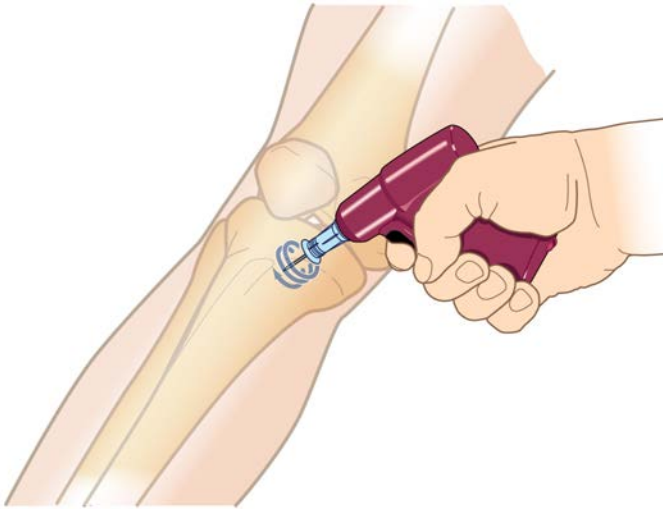
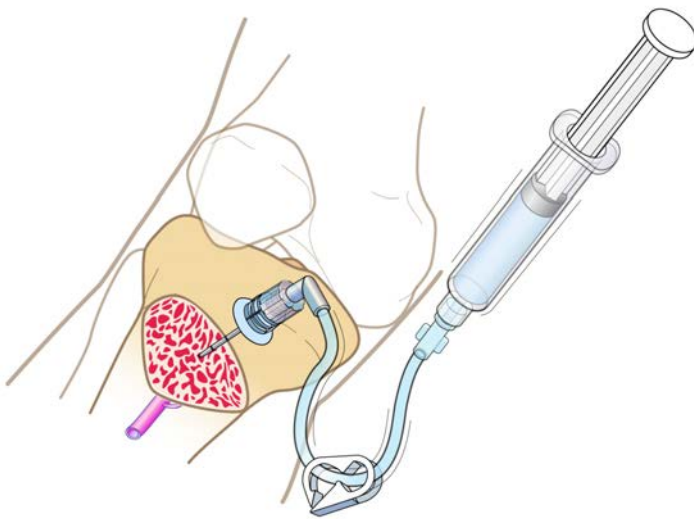
Because the study was investigating a new technique, all patients received standard prophylaxis with 1 g of systemic cefazolin 10–30 minutes before tourniquet inflation regardless of treatment allocation. All patients underwent limb exsanguination and tourniquet inflation to 250 mmHg before routine preparation and draping. The tourniquet remained inflated for the entire procedure. TKA was performed using an imageless computer navigation system (Stryker Orthopedics, Mahwah, NJ, USA).

The first group (250 mg IORA) received 250 mg of vancomycin in 200 mL of normal saline via IORA using an EZ-IO (Vidacare Corp, San Antonio, TX, USA; FDA-

approved) intraosseous cannula placed in the medial aspect of the proximal tibia at approximately the level of the tibial tubercle (Figure 4.1), after draping and before skin incision (video 1; supplemental materials are available with the online version of CORR®). The injection was administered as a bolus immediately after tourniquet inflation and the surgical incision was made immediately (<1 minute) thereafter. The second group (500 mg IORA) received 500 mg of vancomycin according to the same protocol, which has been previously described<sup>158</sup>. The third group (1 g systemic) received 1 g of vancomycin systemically through a forearm vein as a 1-hour infusion, starting 60–120 minutes before surgery.

Surgery was carried out under combined spinal and epidural anaesthesia in 27 patients, spinal anaesthesia with a femoral nerve block in two patients, and femoral nerve block alone in one patient. Patients were monitored for clinical signs of red man syndrome throughout the procedure, particularly after tourniquet deflation. An antihistamine was available for use if required.

Samples of subcutaneous fat and femoral cancellous bone (approximately 0.5 cm<sup>3</sup>) were taken at four points during the procedure. The first subcutaneous fat sample was taken immediately after skin incision, and subsequently both bone and fat samples were taken at the time of the distal femoral cut, at the time of trialling the components, and immediately before closure. Bone samples were taken from the distal femur using a curette. Collection times were recorded for each sample (Table 4.2). In addition, systemic blood samples were taken at the time of the final tissue sample (while the tourniquet was inflated), then at 1, 4, and 8 hours post-deflation and on the morning after the procedure. In previous animal studies of IORA vancomycin, peak systemic concentrations occurred 60–70 minutes after deflation of the tourniquet.<sup>102</sup>

**A****B**

**Figure 4.1** (A) Insertion of the intraosseous needle using a sterilised driver and (B) the needle in situ allowing injection of the antibiotic after tourniquet inflation and prior to skin incision. (Images from Vidacare Corp, San Antonio, TX, USA)

**Table 4.2** Mean tissue concentrations of vancomycin at each sample point.

Sample point	IORA vancomycin 250 mg		IORA vancomycin 500 mg		Systemic vancomycin 1 g	
	Time (min)	Concentration (µg/g)	Time (min)	Concentration µg/g	Time (min)	Concentration µg/g
Subcutaneous fat 1	0.3 (0.6)	<b>19.4</b> (11.7)	0.1 (0.3)	<b>50.4</b> (36)	0.1 (0.3)	<b>2.7</b> (1.0)
Subcutaneous fat 2	27 (9.0)	<b>17.0</b> (12.0)	24 (6.3)	<b>52.3</b> (67)	24 (7.0)	<b>4.4</b> (2.0)
Subcutaneous fat 3	52 (16.8)	<b>11.4</b> (9.1)	51 (6.9)	<b>32.0</b> (18.1)	54 (10.3)	<b>3.2</b> (1.4)
Subcutaneous fat 4	80 (19.7)	<b>8.1</b> (5.6)	83 (16.7)	<b>41.1</b> (36.5)	81 (11.1)	<b>2.4</b> (1.5)
Bone 1	27 (9.0)	<b>11.6</b> (7.9)	24 (6.3)	<b>20.7</b> (23.9)	24 (7.0)	<b>3.3</b> (2.4)
Bone 2	52 (16.8)	<b>19.2</b> (10.2)	51 (6.9)	<b>44.0</b> (66)	54 (10.3)	<b>5.3</b> (2.7)
Bone 3	80 (19.7)	<b>18.1</b> (11.0)	83 (16.7)	<b>50.0</b> (54.1)	81 (11.1)	<b>3.5</b> (2.1)

The data are presented as the mean and standard deviation. Times are given as minutes after surgical incision. Differences in mean tissue concentrations between the three groups were statistically significant ( $p < 0.001$ ) for all comparison points after adjustment for sex, age, and time from incision.



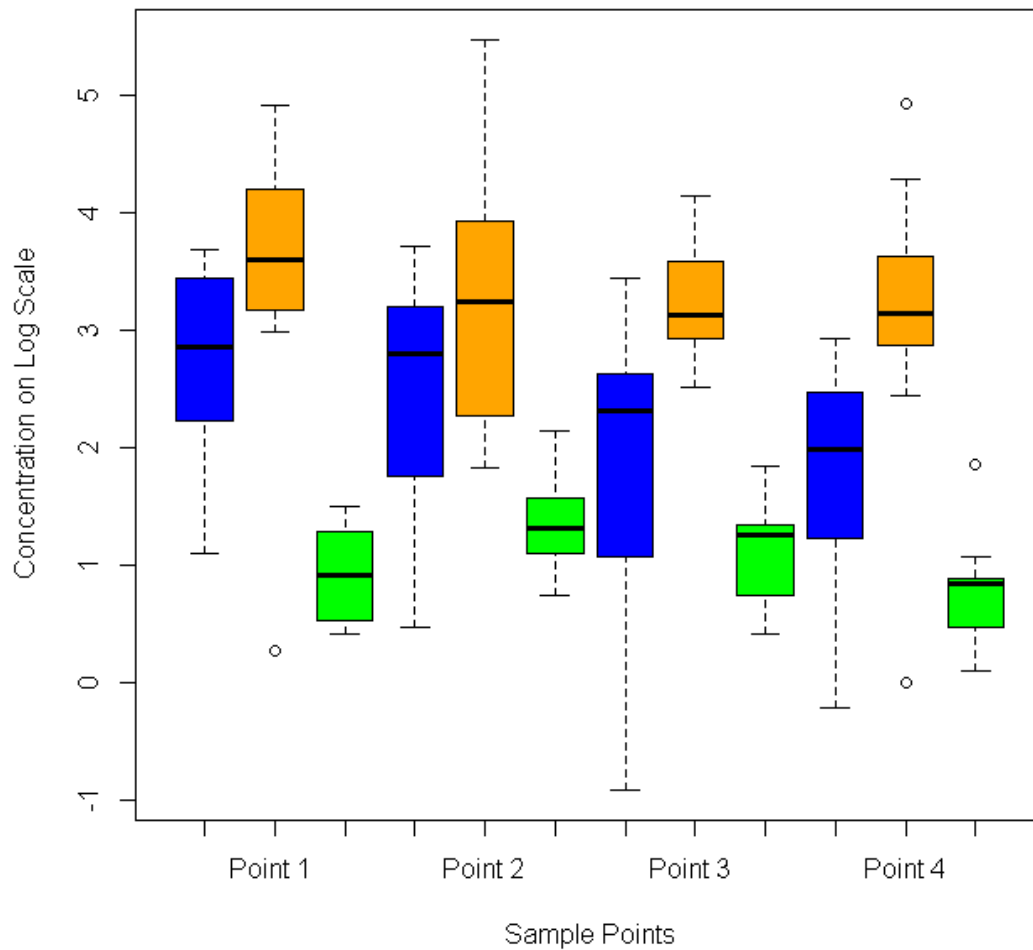
Tissue samples were rinsed in saline to remove excess blood and stored at  $-90^{\circ}\text{C}$  until analysis. Vancomycin concentrations were analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Each bone sample was crushed with pliers, finely cut further with a scalpel into small particles, then weighed and immersed in phosphate-buffered saline pH 7.3 (the ratio of bone/phosphate-buffered saline pH 7.3 was 1:5, w/v) at  $4^{\circ}\text{C}$  overnight to extract vancomycin from the bone. Each fat sample was finely minced with a scalpel, weighed, and then treated in the same way as the bone samples. The immersed tissue suspensions were vortexed and centrifuged to precipitate tissue particles. Fifty microliters of the supernatant were transferred to a 1.5 mL plastic centrifuge tube, and 25  $\mu\text{L}$  of internal standard (0.25  $\mu\text{g}/\text{mL}$  aminopterin) was added. The mixture was then vortexed, and 200  $\mu\text{L}$  of methanol were added to precipitate the proteins. After centrifugation at 15,000 g for 5 minutes, a 50  $\mu\text{L}$  aliquot of clear supernatant was mixed with 500  $\mu\text{L}$  of water and transferred to a 96-well plate autosampler. A 10  $\mu\text{L}$  volume was injected into the LC-MS/MS system. Vancomycin and the internal standard, aminopterin, were resolved on a Luna® C18(2) 5  $\mu\text{m}$ , 50 mm  $\times$  2.0 mm internal diameter column (Phenomenex, Inc, Torrance, CA, USA) using a gradient elution of 0.05% formic acid and methanol. The two compounds were detected using electrospray ionisation in the positive ion mode. The optimised precursor-to-product ion transitions monitored for vancomycin  $[\text{M} + 2\text{H}]^{2+}$  and aminopterin  $[\text{M} + \text{H}]^{+}$  were  $m/z$  725.6  $\rightarrow$  144.2 and  $m/z$  441.2  $\rightarrow$  294.2, respectively. The vancomycin and internal standard peaks were free of interference from endogenous substances present in blank bone and fat. The standard curve was linear over the concentration range of 0.05–50 mg/L ( $r > 0.999$ ), which encompasses clinical concentrations, bias was less than  $\pm 10\%$ , intraday and interday coefficients of variation were less than 10%, and the limit of quantification was 0.05 mg/L. Systemic

blood samples were analysed using homogeneous particle-enhanced turbidimetric inhibition immunoassay on a Dimension Vista® analyser (Siemens, Erlangen, Germany). All patient samples were analysed in duplicate, and laboratory analysis was carried out blinded to group allocation.

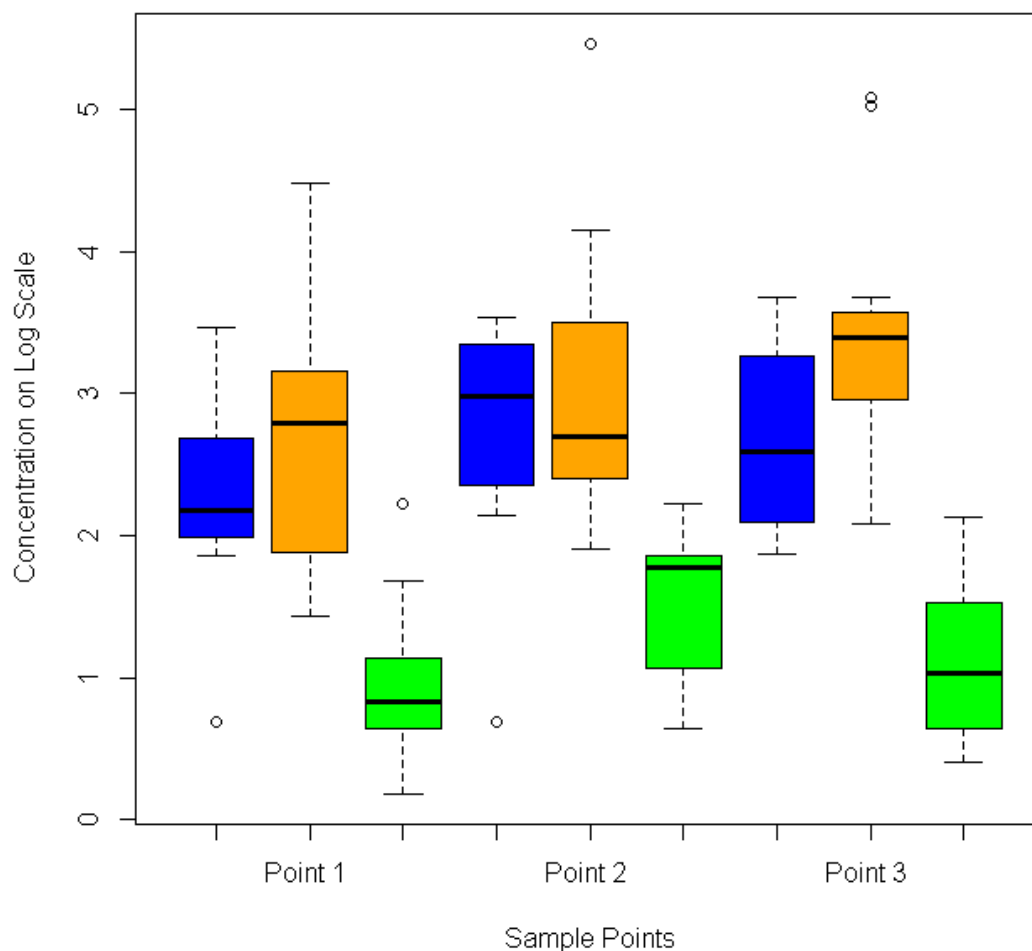
Means, standard deviations, and 95% confidence intervals were calculated for the concentrations in the different samples. The different tissue samples were pooled according to the surgical steps at which they were taken. Repeated measures analysis of variance was used to compare the average concentration across time between the groups adjusted by sex, age, and time from incision. The interaction between time from incision and group was also assessed. For those with serum vancomycin concentrations  $<0.8 \mu\text{g/mL}$ , a random imputation was applied assuming the mean  $\log(\text{concentration})$  was equal to 0.4 and the standard deviation was derived by the other records available at the same time point.

#### 4.2.5 Results

The overall mean tissue concentration of vancomycin in subcutaneous fat was higher in the 250 mg IORA group than in the 1 g systemic group ( $14 \mu\text{g/g}$  versus  $3.2 \mu\text{g/g}$ ;  $p < 0.001$ , Table 4.2) and was highest in the 500 mg IORA group ( $44 \mu\text{g/g}$ ) when compared with the other groups ( $p < 0.001$ , Figure 4.2). Similarly, the overall mean tissue concentration of vancomycin in bone was higher in the 250 mg IORA group than in the 1 g systemic group ( $16 \mu\text{g/g}$  versus  $4.0 \mu\text{g/g}$ ,  $p < 0.001$ ) and higher in the 500 mg IORA group ( $38 \mu\text{g/g}$ ) when compared with the other groups ( $p < 0.001$ , Figure 4.3).



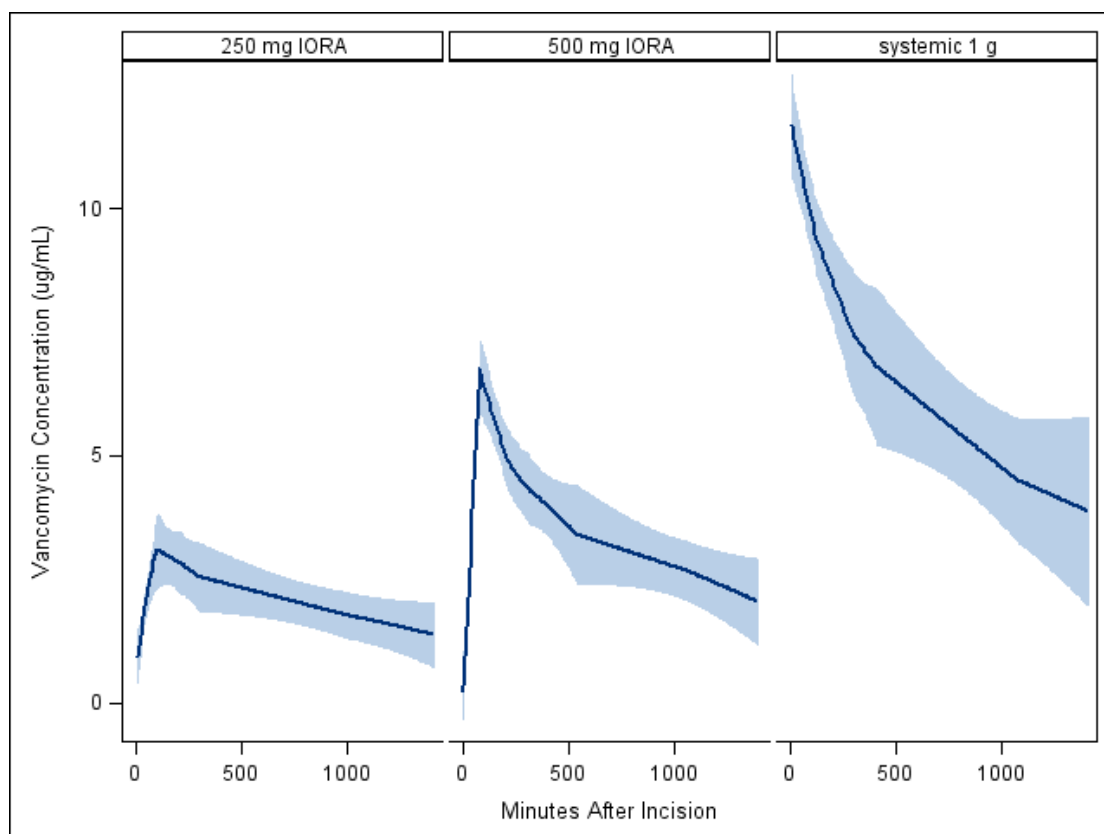
**Figure 4.2** Tissue concentrations of vancomycin in subcutaneous fat at each sample point. The 250 mg IORA vancomycin group is in blue, the 500 mg IORA vancomycin group is in orange, and the 1 g systemic vancomycin group is in green. Concentrations are shown on a log scale: 2 = 7  $\mu\text{g/g}$ , 4 = 55  $\mu\text{g/g}$ , and 6 = 403  $\mu\text{g/g}$ . Each box represents the median; the horizontal line in each box represents the 25% and 75% quartiles; the whiskers represent 1.5 times the interquartile range from the box.



**Figure 4.3** Tissue concentrations of vancomycin in bone at each sample point. The 250 mg IORA vancomycin group is in blue, the 500 mg IORA vancomycin group is in orange, and the 1 g systemic vancomycin group is in green. Concentrations are shown on a log scale: 1 = 3  $\mu\text{g/g}$ , 2 = 7  $\mu\text{g/g}$ , 3 = 20  $\mu\text{g/g}$ , 4 = 55  $\mu\text{g/g}$ , and 5 = 148  $\mu\text{g/g}$ . Each box represents the median; the horizontal line in each box represents the 25% and 75% quartiles; the whiskers represent 1.5 times the interquartile range from the box.

Twenty-five percent (16 of 63) of bone and fat tissue samples in the 1 g systemic group were less than 2.0  $\mu\text{g/g}$ , which is the typical MIC of vancomycin for CoNS. In comparison, 4% of samples (three of 70) in the 250 mg IORA group and 1% (one of

70) in the 500 mg IORA group were below this level. For patients in the IORA groups, vancomycin levels were either not detectable or only slightly raised in intraoperative systemic blood samples taken at a mean 86 minutes after injection, indicating generally successful functioning of the tourniquet (Table 4.3). After tourniquet deflation, peak vancomycin concentrations in systemic blood were lower in both IORA groups than in the 1 g systemic group (Figure 4.4).



**Figure 4.4** Loess graph showing the systemic blood concentrations of vancomycin with predicted confidence intervals in the three intervention groups.

**Table 4.3** Median systemic blood concentrations of vancomycin at each sample point.

<b>Systemic blood sample</b>	<b>IORA vancomycin 250 mg (µg/g)</b>	<b>IORA vancomycin 500 mg (µg/g)</b>	<b>Systemic vancomycin 1 g (µg/g)</b>	<b>p-value*</b>
<b>Intraoperatively</b>	0.7 (0.6, 1.06)	0.6 (0.6, 0.7)	11.4 (10.2, 13.4)	<0.001
<b>Postoperative hour 1</b>	2.7 (2.1, 3.5)	6.0 (5.3, 6.9)	9.4 (8.9, 11.1)	<0.001
<b>Postoperative hour 4</b>	2.6 (1.0, 4.0)	4.6 (4.2, 4.9)	7.0 (6.6, 7.8)	<0.001
<b>Postoperative hour 8</b>	1.4 (1.4, 1.4)	4.0 (2.9, 4.1)	5.3 (5.1, 5.8)	0.001
<b>Postoperative hour 20</b>	1.4 (1.0, 2.2)	2.4 (0.6, 2.7)	3.7 (2.9, 5.3)	<0.001

The data are presented as the median with the interquartile range in parentheses.

One patient in the 1 g systemic group developed red man syndrome, consisting of erythema, pruritus, and hot flushing, during infusion of vancomycin. The vancomycin infusion was stopped after 700 mg had been administered and the symptoms resolved. The patient remained haemodynamically stable and the procedure was carried out as normal. Tissue and blood samples for this patient were not included in the analysis. No clinical signs of red man syndrome were seen in any patient undergoing IORA; in particular, no signs were seen after tourniquet deflation. Minor transient decreases in systolic blood pressure (5–30 mmHg) were seen after tourniquet deflation in six patients in the 250 mg IORA group, five patients in the 500 mg IORA group, and seven patients in the 1 g systemic group. One patient in the 500 mg IORA group developed a deep vein thrombosis in a peroneal calf vein, which was seen on an ultrasound scan on day 3. He was treated with warfarin, and a repeat scan at 6 weeks showed resolution of the clot and the warfarin was discontinued. No deep or superficial infections occurred in either group. One patient in the 250 mg IORA group went on to have a TKA performed on the contralateral knee 2 months after participation in this study. The patient was given systemic prophylaxis with 1 g of cefazolin and developed a deep infection 4 weeks postoperatively, which eventually required two-stage revision surgery. The infecting organism was cefazolin-resistant CoNS.

#### 4.2.6 Discussion

Antibiotic resistance is an increasing problem, and rates of orthopaedic infection due to MRSA and resistant CoNS are rising<sup>155</sup>. This, together with the severe consequences of a deep infection, has led some authors to propose vancomycin as an alternative prophylactic agent in TKA<sup>153</sup>, particularly in centres where MRSA rates

are high<sup>154,155</sup>. However, vancomycin has several disadvantages, including systemic side effects, in particular nephrotoxicity and ototoxicity, potential promotion of further bacterial resistance, and a prolonged administration time. We previously investigated IORA as a method of maximising tissue concentrations of cefazolin in TKA.<sup>158</sup> This study explores the use of IORA to give a lower dose of a more toxic drug, potentially minimising its adverse effects. We hypothesised that lower doses of vancomycin via IORA could still achieve tissue concentrations equal or superior to those of systemic administration before TKA.

Although we saw no evidence of red man syndrome with IORA vancomycin, our study had the limitations of a small number of patients and exclusion of patients with significant cardiac disease. Red man syndrome is an anaphylactoid reaction caused by degranulation of mast cells that results in release of histamine. It is not an allergic reaction and is independent of preformed immunoglobulin E. It occurs in 30%–90% of healthy volunteers given vancomycin<sup>159</sup>; symptoms are usually mild and alleviated by use of an antihistamine. Its occurrence is related to both vancomycin dosage and rate of infusion; Polk et al<sup>160</sup> observed the reaction during systemic infusion of vancomycin 1 g in 82% of volunteers, but no reaction occurred when a 500 mg dose was used. Healy et al<sup>161</sup> noted symptoms in eight of 10 volunteers (80%) given 1 g of vancomycin over one hour, but in only three of 10 volunteers (30%) given the same dose over 2 hours. The absence of red man syndrome in patients who received IORA vancomycin in our study is likely due to both the lower doses used and the depot effect of the high tissue concentrations causing antibiotic to be released gradually into the systemic circulation after tourniquet deflation<sup>100</sup>. However, until data on a larger number of patients are available, we recommend that patients receiving vancomycin



by IORA be monitored closely after tourniquet deflation and an antihistamine be available for use if required.

A second potential limitation of the IORA technique is the lower systemic concentration when the tourniquet is released. Many surgeons routinely continue antibiotics for 24 hours postoperatively, and further systemic vancomycin doses after IORA would still be required to maintain tissue levels. However, due to the high initial concentrations achieved, vancomycin levels in perioperative tissues are likely to remain elevated for some time. Hoddinott et al<sup>77</sup> demonstrated persistently elevated antibiotic levels in drain fluid on the morning after surgery in patients undergoing TKA and given prophylactic cefazolin via a regional route. Additionally, a distinction should be made between use of an antibiotic for prophylaxis versus its use to treat an established infection. The goal of prophylaxis is to prevent initial bacterial adherence and colonisation during the period the wound is open, when contamination is occurring.<sup>39</sup> Therefore, the critical period when adequate antibiotic concentrations must be present in the tissues is from the time of incision to the time of closure, and was achieved in both IORA groups in this study. Moreover, a number of randomised controlled trials have shown no difference in infection rates between patients who receive a single preoperative antibiotic dose and those in whom antibiotics are continued for 24 hours<sup>162 84</sup>, implying further doses after IORA may be unnecessary.

Timing of prophylactic antibiotics is critical to their effectiveness; maximum benefit is achieved when they are administered in the 60 minutes before skin incision<sup>49</sup>.

Protocols for systemic vancomycin require infusion rates of no greater than 1 g per 60 minutes, so a prophylactic dose of 1 g needs to be started 1–2 hours before surgery<sup>153</sup>.

This is difficult to incorporate into operating room protocols<sup>163</sup>, and clinical studies

show that optimal timing of vancomycin is rarely achieved in practice<sup>87,155</sup>. An advantage of IORA over the systemic route is that it ensures appropriate timing of administration, and very high tissue levels of antibiotic were present immediately after skin incision both in this study and in a previous investigation of IORA<sup>158</sup>. A disadvantage is that injection by IORA occurs after tourniquet inflation, adding 2–4 minutes to overall tourniquet time. The intraosseous needles are also an additional cost.

Regional administration of prophylactic antibiotics in TKA has been investigated previously using teicoplanin, a glycopeptide antibiotic with a spectrum of activity similar to that of vancomycin. de Lalla et al<sup>76</sup> reported that intravenous regional administration (IVRA) of teicoplanin 400 mg via a foot vein provided tissue concentrations 2–10 times higher than teicoplanin 800 mg given via the systemic route. The same authors later prospectively evaluated this IVRA protocol in 250 patients undergoing TKA and reported a 0% deep infection rate<sup>75</sup>.

While this is the first study of IORA vancomycin in humans, the veterinary literature contains a number of reports of regional antibiotic administration via this route for the treatment of limb infections. Rubio-Martínez et al<sup>102</sup> compared IORA vancomycin versus IVRA vancomycin in 12 horses. No complications were reported, and the tissue concentrations achieved by the two routes were similar. That study and a number of other animal studies<sup>104,105,164</sup> have demonstrated that the tissue antibiotic concentrations reached using the IVRA and IORA routes are equivalent. IORA injections also travel directly into the intravascular space, and in TKA surgery the main advantages of this route over IVRA are reliability and speed of access. Cannulation of the proximal tibia using modern intraosseous kits is rapid and

reproducible<sup>94</sup>, and unlike cannulation of a foot vein, does not require any changes to standard sterile draping.

Potential complications of intraosseous infusion include extravasation of fluid with compartment syndrome related to incorrect needle placement in emergency situations<sup>96</sup>. Infection at the needle site is rare and correlates with the length of time the needle is left in situ<sup>96</sup>. Fat embolus is a theoretical concern, and subclinical lung microemboli have been seen histologically following intraosseous infusion in some animal studies<sup>115,116</sup>. However, no measurable effects on ventilation-perfusion performance have been found<sup>116,165</sup>, and other studies report no difference in histological fat embolus rates between intraosseous and intravenous infusions<sup>165</sup>. To date, no cases of clinical fat emboli associated with intraosseous infusion have been reported in humans<sup>96</sup>.

Accepted indications for vancomycin prophylaxis in TKA include beta-lactam allergy and known colonisation with MRSA<sup>153</sup>. High institutional prevalence of MRSA has also been suggested as an indication<sup>154</sup>, but the prevalence at which routine prophylaxis with vancomycin becomes beneficial is controversial<sup>155</sup>. Promotion of further antibiotic resistance with routine vancomycin prophylaxis remains a significant concern, because prolonged exposure to sublethal concentrations may promote the emergence of resistant organisms<sup>166</sup>. In theory, low-dose IORA may exert less selection pressure than systemic administration by maximising tissue levels at the site of action and reducing the overall exposure; however, any advantage is difficult to quantify.

In conclusion, while concerns about the routine use of vancomycin for prophylaxis remain, use of low-dose IORA vancomycin can achieve higher tissue concentrations

than systemic administration without prolonged preoperative infusion times. This may optimise the timing of administration of vancomycin and reduce the risk of systemic side effects, while providing equal or enhanced prophylaxis in TKA.

#### 4.2.7 Acknowledgments

We thank Irene Zeng MSc (Hons) for her assistance with the statistical analysis, the Awhina Trust for its funding support, and Dr Kelly Vince for his advice and guidance on the project. We also thank Vidacare Corp for supplying the intraosseous needles without charge.

### 4.3 Discussion of article

#### 4.3.1 Contribution and significance

The main finding of this study was that the IORA technique provided very high tissue drug concentrations when low-dose vancomycin was used as the prophylactic agent. In comparison with cefazolin, prophylaxis with vancomycin covers cefazolin-resistant Gram-positive organisms that are common causes of PJI, as seen in Chapter 3.

#### 4.3.2 Vancomycin and red man syndrome

This study found that IORA vancomycin could be safely administered as a bolus injection. This ensures that the prophylactic antibiotic is administered with optimal timing, which is known to be important for efficacy.<sup>49</sup> This contrasts with the 1–2-hour infusion required when vancomycin is administered systemically.

Further, no evidence of red man syndrome was seen in the IORA vancomycin groups at either dose. This is likely because of both the lower vancomycin dose and the depot

effect of the high tissue concentration causing antibiotic to be released gradually into the systemic circulation after tourniquet deflation<sup>101</sup>. Red man syndrome is an anaphylactoid reaction caused by degranulation of mast cells resulting in histamine release. It is not an allergic reaction and is independent of preformed immunoglobulin E. It occurs in 30%–90% of healthy volunteers given vancomycin.<sup>159</sup> Symptoms are usually mild and alleviated by use of an antihistamine. The incidence is related to both dosage and rate of infusion; Polk et al<sup>160</sup> observed the reaction during systemic infusion of vancomycin 1 g in 82% of volunteers, but no reaction occurred when a 500 mg dose was used. Healy et al<sup>161</sup> noted symptoms in eight of 10 volunteers (80%) who received vancomycin 1 g over one hour, but in only three of 10 volunteers (30%) who received the same dose over 2 hours. Because development of red man syndrome is related to dosage<sup>159</sup>, we used two different doses of vancomycin to determine if one was more likely to cause red man syndrome. Given that no such effect was seen at either dose, it seems reasonable to recommend IORA vancomycin at the 500 mg dose because this resulted in higher tissue concentrations.

In this study, the tissue concentrations of vancomycin achieved by the IORA route were many times greater than those achieved by systemic administration, despite using a lower dose. In theory, this would reduce both the risk of systemic toxicity (particularly nephrotoxicity) and antibiotic selection pressure with subsequent development of bacterial resistance.

#### 4.3.3 Efficacy of vancomycin administered by IORA

IORA clearly provides higher tissue concentrations, but it is unclear whether this translates into a reduction in deep infection rates following TKA in the clinical

setting. As reported in Chapter 3, the infection rate following TKA due to intraoperative contamination is 1.0% (22 of 2157 TKA procedures).

As a theoretical exercise, we can first assume IORA reduces the rate of PJI by 50% to 0.5%, given that more than 50% of bacteria causing PJI are resistant organisms. A sample power calculation would then proceed using the method described by Woodward<sup>167</sup> as follows:

$$H_0: \pi_1 = \pi_2$$

$$H_1: \pi_1 / \pi_2 = \lambda \quad (\lambda \neq 1)$$

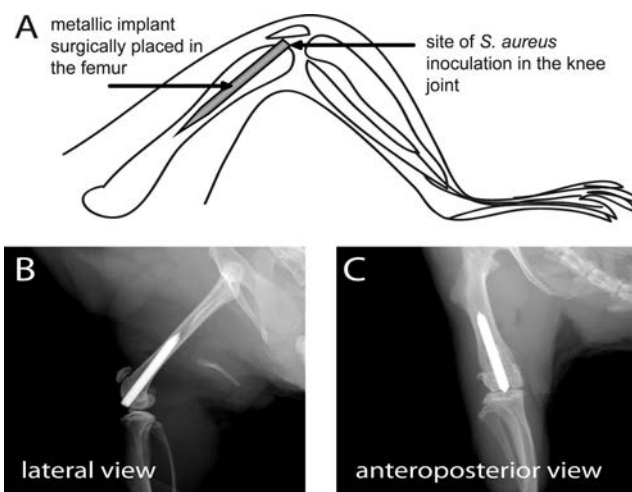
For a two-sided test:

$$n = \frac{r + 1}{r(\lambda - 1)^2 \pi^2} \left[ z_{\alpha/2} \sqrt{(r + 1)p_c(1 - p_c)} + z_{\beta} \sqrt{(\lambda\pi(1 - \lambda\pi) + r\pi(1 - \pi))} \right]^2$$

Where  $r$  is the ratio of sample size between the interosseous group and the control group and  $P_c$  represents the average of the two rates when the ratio ( $r$ ) = 1. If the PJI rate following TKA using systemic vancomycin prophylaxis is 1.0% and that following IORA with vancomycin prophylaxis is 0.5%, then assuming a 5% type I error, 80% power, and using a two-sided test comparing proportions, a study including 9346 patients (4673 in each arm) would then be required to demonstrate a difference. Such a study is unlikely to be feasible in clinical practice.

Therefore, an animal model is required to assess whether IORA provides more effective prophylaxis in an experimental setting. Researchers at University of California Los Angeles have developed a mouse model of TKA infection<sup>60</sup> that is suitable for this purpose. The model involves a medical-grade metal stainless steel K-wire surgically implanted into the intramedullary canal of the femur (Figure 4.5). The wire is cut so that the intra-articular aspect extends 1 mm into the joint space. An

inoculum of *S. aureus* is then placed into the joint to simulate contamination of the wound. This model was used as the basis for the study in the following chapter.



**Figure 4.5** Placement of K-wire implant (A) A medical-grade metal (stainless steel or titanium) K-wire implant was surgically placed in a retrograde fashion in the intramedullary canal of the right femur and cut so that the intra-articular end extended 1 mm into the joint space (left arrow). (B, C) Representative lateral (B) and anteroposterior (C) X-ray images demonstrating placement of the implant in the femoral canal and the cut end within the knee joint.

## **Chapter 5 Regional intraosseous administration of prophylactic antibiotics is more effective than systemic administration in a mouse model of TKA**

### **5.1 Preface**

While the previous chapters demonstrated that IORA provides higher tissue concentrations of antibiotics during TKA surgery, it is unclear whether these higher concentrations provide more effective prophylaxis against infection.

The following section contains a modified version of an article entitled ‘Regional intraosseous administration of prophylactic antibiotics is more effective than systemic administration in a mouse model of TKA’ published in 2015 in *Clinical Orthopaedics and Related Research* (volume 473, pages 3573–3584). *Clinical Orthopaedics and Related Research* has a 2016 impact factor of 3.127. The article was the subject of an editorial published in the same issue by Charalampos G. Zalavras MD, PhD ‘CORR Insights’<sup>168</sup>. The editorial is reproduced in Appendix 2.

### **5.2 Published article**

#### 5.2.1 Title page

Regional intraosseous administration of prophylactic antibiotics is more effective than systemic administration in a mouse model of TKA

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The institutions of three of the authors (SW, JD, SJ) received a grant-in-aid from the New Zealand Orthopaedic Association in relationship to this work. One of the authors (SW) was supported by a Sir Charles Hercus Fellowship from the Health Research Council of New Zealand (09/099).

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.

Each author certifies that his or her institution approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

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### 5.2.2 Abstract

*Background* In human total knee arthroplasty (TKA) studies, intraosseous regional administration (IORA) of prophylactic antibiotics achieves local tissue antibiotic concentrations 10 times higher than those achieved by systemic administration.

However, it is unclear if such high concentrations provide more effective prophylaxis.

*Questions/purposes* We asked: (1) What prophylactic antibiotic dosage and route (intravenous [IV] versus IORA) produces less bacterial burden in vivo when compared with no-antibiotic controls? (2) Compared with controls, what prophylactic antibiotic dosage and route yields fewer colony-forming units (CFU) in an animal model of TKA? (3) Is prophylactic antibiotic therapy given by the IORA route more effective than the same dose administered by the IV route in reducing CFU?

*Methods* Mice (6–9 per group) were randomised in blocks to one of six prophylaxis regimens: control, systemic cefazolin (C100<sub>IV</sub>), IORA cefazolin (C100<sub>IORA</sub>), systemic vancomycin (V110<sub>IV</sub>), low-dose systemic vancomycin (V25<sub>IV</sub>), or low-dose IORA vancomycin (V25<sub>IORA</sub>). Surgery involved placement of an intra-articular knee prosthesis followed by an inoculum of bioluminescent *Staphylococcus aureus* strain Xen36. Bacterial loads were assessed in vivo using biophotonic imaging. After 4 days, the bacterial load was quantified using culture-based techniques and compared between the group allocated to IORA prophylactic antibiotics and the group allocated to the same dose of prophylactic antibiotic by the IV route, and between these groups and the control group.

*Results* Mice treated with high-dose systemic vancomycin, IORA vancomycin, or IORA cefazolin had a lower in vivo *S. aureus* burden (median area under curve: control,  $5.0 \times 10^6$ ; V110<sub>IV</sub>,  $1.5 \times 10^6$  (difference of medians  $3.5 \times 10^6$ ,  $p=0.003$ ); V25<sub>IV</sub>,  $1.94 \times 10^6$  (difference  $3.07 \times 10^6$ ,  $p=0.49$ ); V25<sub>IORA</sub>,  $1.51 \times 10^6$  (difference  $3.5 \times 10^6$ ,  $p=0.0011$ ); C100<sub>IORA</sub>,  $1.55 \times 10^6$  (difference  $3.46 \times 10^6$ ,  $p=0.0016$ ); C100<sub>IV</sub>,  $2.35 \times 10^6$  (difference  $2.66 \times 10^6$ ,  $p=0.23$ ). Findings on recovered implants using culture-based techniques were similar. At the same dose, antibiotic prophylaxis via the IORA route was more effective than when administered IV in reducing the bacterial load on recovered implants ( $<7.0 \times 10^0$  versus  $2.83 \times 10^2$  median CFU,  $p=0.0183$ ).

*Conclusions* Prophylactic cefazolin and vancomycin by IORA was more effective than the same dose of antibiotic given systemically. The effectiveness of prophylactic vancomycin in particular was enhanced when delivered by the IORA route, despite using a lower dose.

*Clinical relevance* These findings are consistent with previous studies of IORA prophylactic antibiotics in humans, and suggest this novel route of administration has the potential to enhance the effectiveness of antibiotic prophylaxis in TKA.

### 5.2.3 Introduction

Prophylactic antibiotics are used to protect against the bacteria most likely to cause contamination during surgery.<sup>39,158</sup> The two most common types of bacteria causing contamination and subsequent deep infection in total knee arthroplasty (TKA) are *Staphylococcus aureus* and coagulase-negative staphylococci.<sup>12,18,21</sup> In the 1960s and

1970s when preoperative prophylactic antibiotics were introduced, up to 98% of coagulase-negative staphylococci and 97% of *S. aureus* isolated in hospital were sensitive to cephalosporins<sup>64,169-171</sup>. Therefore, these agents were commonly recommended for prophylaxis in arthroplasty<sup>41,47,118,172</sup>. However, around 90% of coagulase-negative staphylococci isolated in hospital are now resistant to cephalosporins<sup>12,18,21,61,136</sup> and 30%–56% of *S. aureus* cultured from infected joint arthroplasties are methicillin-resistant (MRSA)<sup>74,125,135,152</sup>. Vancomycin has been suggested as an alternative prophylactic agent because it remains effective against MRSA and coagulase-negative staphylococci resistant to cefazolin.<sup>87,173</sup> However, injudicious use of vancomycin may risk further resistance, and in clinical studies it is a less effective prophylactic agent than cefazolin for methicillin-sensitive *S. aureus* (MSSA) strains.<sup>87,174</sup> This may be because adequate tissue levels are not achieved by typical systemic doses of vancomycin<sup>157,170</sup>, particularly when the timing of prophylactic antibiotic administration is suboptimal<sup>87,147</sup>.

Higher tissue levels of antibiotic can be achieved using alternative methods of administration. Intraosseous regional administration (IORA) of prophylactic antibiotics is a novel form of administration that involves intraosseous injection after inflation of a tourniquet but before skin incision. In a randomised trial comparing cefazolin 1 g given by IORA or the systemic route in patients undergoing TKA, IORA achieved 10 times greater tissue antibiotic concentrations.<sup>158</sup> IORA also achieves high tissue concentrations when lower doses of prophylactic antibiotic are used<sup>175</sup>, which is an advantage when using agents such as vancomycin where systemic toxicity, including red man syndrome, is a concern<sup>146</sup>. However, it is unclear if the high tissue concentrations seen in clinical studies of IORA using vancomycin or cefazolin provide more effective prophylaxis.

The aim of this study is to compare the effectiveness of prophylactic antibiotics via the IORA route with that of the systemic route using an in vivo murine model of TKA<sup>60</sup>. Specifically, we asked: (1) What antibiotic dosage and route of administration (intravenous [IV] versus IORA) produces less bacterial burden in vivo when compared with no-antibiotic controls? (2) Compared with controls, what prophylactic antibiotic dosage and route of administration yields fewer colony-forming units (CFU) in an animal model of TKA? (3) Is prophylactic IORA more effective than same-dose IV antibiotic administration in reducing CFU?

#### 5.2.4 Materials and methods

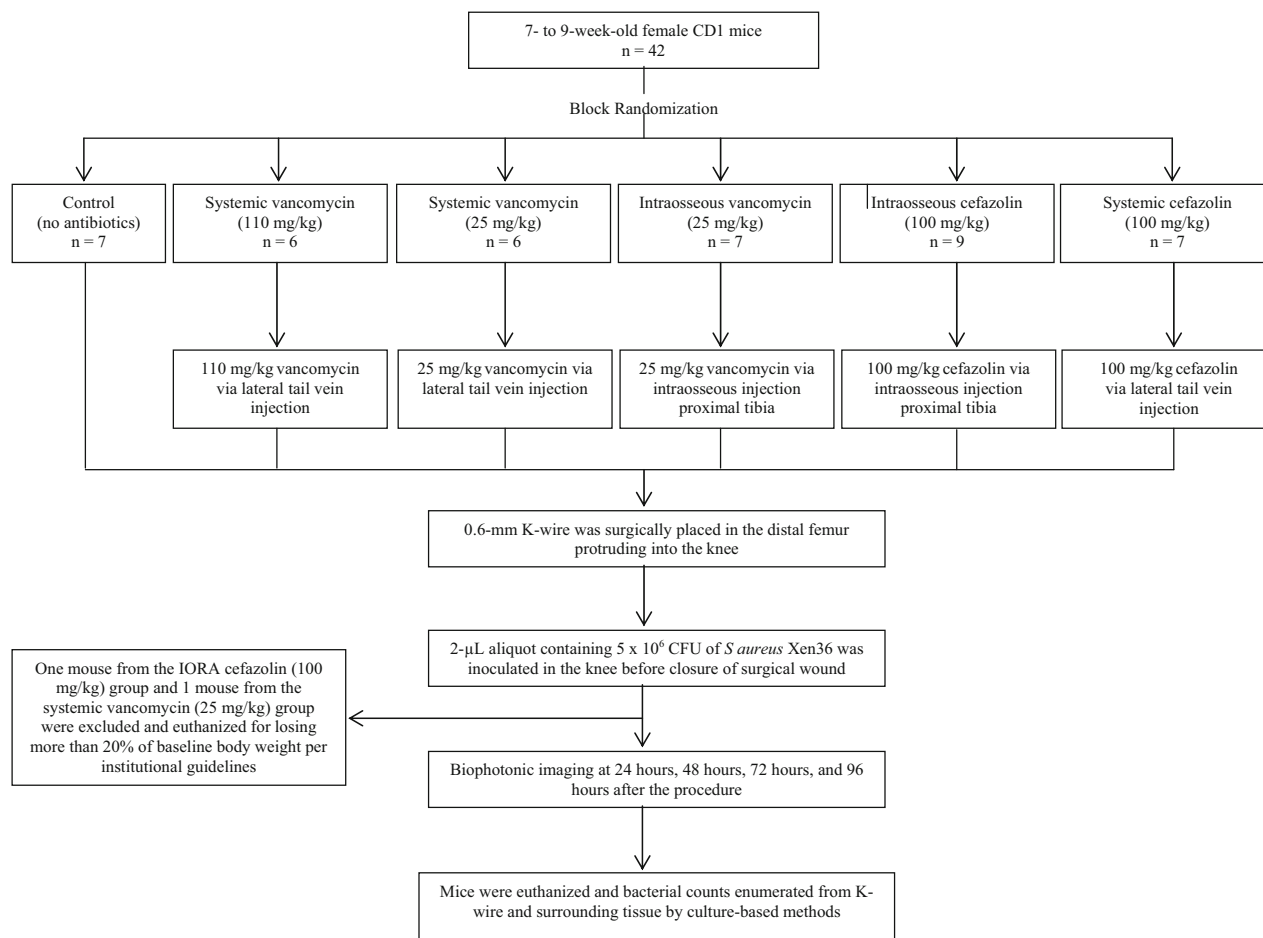
##### *Bioluminescent S. aureus*

Bioluminescent MSSA Xen36 (Perkin Elmer, Waltham, MA, USA) was used in all experiments. Xen36 is a derivative of ATCC 49525 (Wright), an isolate causing clinical bacteraemia that has a modified *lux* operon from *Photobacterium luminescens* stably integrated in a native plasmid<sup>176</sup>.

Bacteria were grown overnight in Tryptic Soy Broth (Fort Richard Laboratories Ltd, Auckland, New Zealand) at 37°C with shaking at 200 rpm, then reinoculated in fresh medium at 1:5 and incubated for a further 90 minutes. The bacteria were then checked for light expression, washed three times in phosphate-buffered saline and resuspended in fresh phosphate-buffered saline to obtain approximately  $5 \times 10^9$  CFU/mL. The concentration of bacteria in solution was verified retrospectively by plating and culture.

##### *Animals*

Female CD1 mice were obtained from the specific pathogen-free breeding facility at the University of Auckland. The mice were 7–9 weeks of age on arrival and had access to food and water ad libitum. The animals were housed and cared for in accordance with the New Zealand Animal Welfare Act<sup>177</sup> and the institutional guidelines provided by the University of Auckland Animal Ethics Committee, which reviewed and approved these experiments under application R1134. Single housing of these animals is discouraged, so all experiments were performed using female mice, which are less aggressive than male mice and so less likely to injure themselves or each other when housed together. The housing conditions and diet were identical for all animals. To minimise the number of animals required, while accounting for any host, bacterial, or surgical variation, one experiment was performed using a block design (Figure 5.1). Surgery was performed on six separate occasions using a different cohort of mice each time and a fresh preparation of bacteria. At each surgery, 6–8 animals were randomised to each of the six experimental groups.



**Figure 5.1** Schematic of the experimental design used in this study.

### Antibiotic prophylaxis

Mice were randomised into six experimental groups: no antibiotic prophylaxis (n=7); systemic vancomycin (110 mg/kg, V110<sub>IV</sub>, n=6); systemic vancomycin (25 mg/kg, V25<sub>IV</sub>, n=6); intraosseous vancomycin (25 mg/kg, V25<sub>IORA</sub>, n=7); intraosseous cefazolin (100 mg/kg, C100<sub>IORA</sub>, n=9); and systemic cefazolin (100 mg/kg, C100<sub>IV</sub>, n=7). These experimental groups represent vancomycin IV at either a high therapeutic dose (110 mg/kg) or a suboptimal dose (25 mg/kg) or by IORA at a low dose (25 mg/kg). We administered cefazolin at a standard therapeutic dose via either the IV or intraosseous route. These reflect the dosages and routes of administration used in two

previous human studies of IORA<sup>158,175</sup>. Two mice (one from the IORA cefazolin 100 mg/kg group and one from the systemic vancomycin 25 mg/kg group) lost more than 20% of their baseline body weight so were euthanised per institutional guidelines and excluded from the analysis (Figure 5.1).

Antibiotics, when used, were administered either systemically via the IV route or regionally (below a tourniquet) via IORA. Systemic antibiotics were introduced by injection into the lateral tail vein 30 minutes before surgery. However, regional intraosseous antibiotics were administered by direct injection into the proximal tibia after tourniquet inflation to the extremity and immediately before surgery. Antibiotics were given by intraosseous injection into the tibia using a 26-gauge needle as previously described.<sup>178-180</sup> The 110 mg/kg dose of vancomycin is an effective dose in mice, approximating an area under the curve (AUC) of 400 mg·hour/L for a typical human dose of vancomycin (1 g every 12 hours).<sup>60,181</sup> Using the body surface normalisation method<sup>182</sup>, this represents a human dose of approximately 10–15 mg/kg. We used an IORA dose of vancomycin that was approximately 25% of this, as reported in a human study where a lower dose was used to protect against systemic effects such as red man syndrome<sup>175</sup>. Because cefazolin has minimal systemic toxicity, we used the same dose for the systemic and IORA routes as in a previous IORA study in humans<sup>158</sup>. The cefazolin dose of 100 mg/kg in mice gives serum concentrations similar to those of a 1–2 g prophylactic dose in humans<sup>81,183,184</sup>.

### *Surgical procedure*

The mice were weighed preoperatively and inhalational isoflurane (3.0%) was administered for anaesthesia. When loss of the toe pinch reflex was confirmed, the right leg was depilated using clippers and an above-knee tourniquet was applied. The



surgical site was prepared using an iodine-povidone swab followed by an alcohol swab and a final iodine-povidone wash.

The knee was accessed using a medial parapatellar approach and the intercondylar region of the distal femur was identified. The femoral medullary canal was reamed manually with sequentially larger-gauge needles for the stainless steel implant, starting with a 26-gauge needle. A sterile 0.6 mm K-wire was then inserted in a retrograde fashion through the intercondylar region into the intramedullary cavity of the distal femur. The K-wire was cut with approximately 1 mm of wire protruding in the joint cavity.

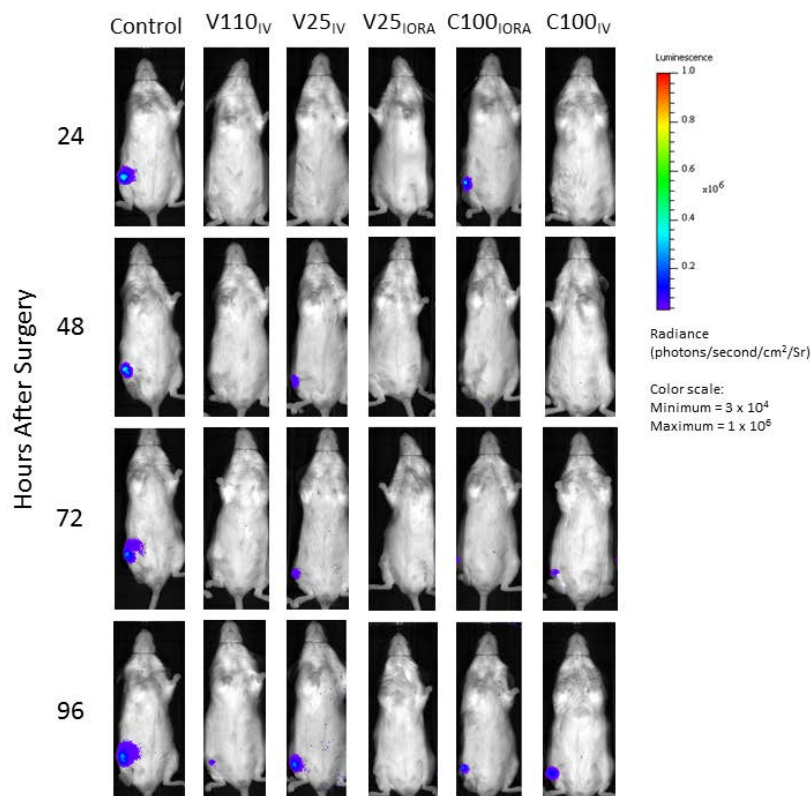
Before closing, a 2  $\mu$ L aliquot containing approximately  $5 \times 10^6$  CFU of *S. aureus* Xen36 was pipetted into the joint. The patella complex was then reduced and the incision closed with 6-0 Monocryl™ sutures (Ethicon, Somerville, NJ, USA). The total tourniquet time for each mouse was 30 minutes.

Postoperatively, acetaminophen (paracetamol; 6 mg/mL) was provided in the drinking water and carprofen 5 mg/kg subcutaneously once daily for pain relief.

### *Biophotonic imaging*

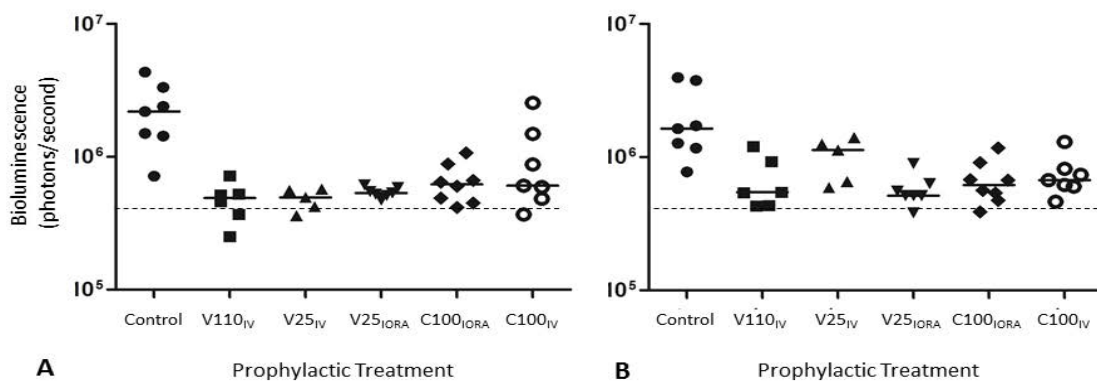
Biophotonic imaging was used to measure the bioluminescent signal emitted by *S. aureus* Xen36 from anaesthetised mice in a noninvasive manner and provide information regarding the localisation of the bacterium (expressed as photons per second per square centimetre per steradian [photons second/cm<sup>2</sup>/sr]; Figure 5.2). We also quantified the bacterial burden in vivo from the biophotonic signal of selected regions of interest (expressed as photons/second) using Living Image software (Perkin Elmer; Figure 5.3). Measurements were obtained daily to determine the AUC for each animal (Figure 5.4).

Assessment of bioluminescence (photons/second/cm<sup>2</sup>/sr) from living animals was measured after gaseous anaesthesia with isoflurane using the IVIS® kinetic camera system (Perkin Elmer). A photograph (reference image) was taken under low illumination before quantification of photons emitted from *S. aureus* Xen36 at a binning of four over 5 minutes using the Living Image software. For anatomical localisation, a pseudocolour image representing light intensity (blue, least intense; red, most intense) was generated using the Living Image software and superimposed over the grey-scale reference image. Bioluminescence in specific regions of individual mice was also quantified using the region of interest tool in the Living Image software program (expressed as photons per second).

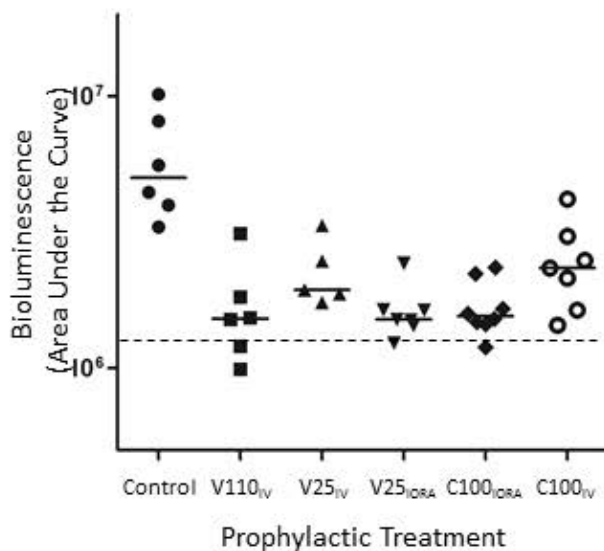


**Figure 5.2** Bioluminescence from *Staphylococcus aureus* Xen36 was assessed after surgery in anaesthetised animals.

The images show peak bioluminescence with variations in colour representing light intensity at a given location. Red represents the most intense light emission, whereas blue corresponds to the weakest signal. The colour bar indicates relative signal intensity (as photons/second/cm<sup>2</sup>/steradian [sr]). Mice were imaged at various times after surgery with an integration time of 5 minutes. One representative animal is shown for each group. Abbreviations: IV, intravenous; IORA, intraosseous regional administration; V110IV, systemic vancomycin 110 mg/kg; V25IV, systemic vancomycin 25 mg/kg; V25IORA, IORA vancomycin 25 mg/kg; C100IORA, IORA cefazolin 100 mg/kg; C100IV, systemic cefazolin 100 mg/kg.



**Figure 5.3** Quantification of bioluminescence from *Staphylococcus aureus* Xen36 in anaesthetised animals after surgery. The bioluminescent signals originating from individual animals at (A) 1 day and (B) 4 days after surgery were obtained using the region of interest tool in the Living Image software program (given as photons per second). The dotted line represents the level of background from uninfected animals. Median values per group are denoted by solid lines. Each symbol represents an individual animal. Data are pooled from six independent repeats with 1–2 animals per group per repeat. Abbreviations: IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg.



**Figure 5.4** Area under the curve values (summation during entire test period) from bioluminescent signals obtained throughout the experiment. The dotted line represents the level of background from uninfected animals. Median values per group are denoted by solid lines. Each symbol represents an individual animal. Data are pooled from six independent repeats with 1–2 animals per group per repeat. Abbreviations: IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg.

#### *Quantification of bacteria in the knee and implant*

The mice were euthanised by cervical dislocation under anaesthesia. The hind limb was surface-sterilised with 70% ethanol and the skin removed. The knee joint (including approximately 5 mm of the proximal end of the tibia and distal end of the femur) and surrounding tissue were excised. The K-wire was extracted from the femur and placed in a 1.5 mL microtube containing 0.5 mL of phosphate-buffered saline. The excised knee joint was placed in a 2 mL sample tube containing ceramic

beads and 1 mL of phosphate-buffered saline, and homogenised ( $3 \times 10$  seconds at 3.55 m/second) using a tissue disruptor (OMNI International, Kennesaw, GA, USA). Serial dilutions were plated on Mannitol salt agar (Fort Richard Laboratories Ltd) and grown overnight at 37°C for enumeration of viable bacteria. The plates were then imaged using the IVIS kinetic camera system to confirm recovery of bioluminescent *S. aureus* Xen36.

#### *Statistical analysis*

The data were analysed using GraphPad Prism version 6 software (GraphPad Software Inc, La Jolla, CA, USA). Briefly, in vivo bacterial burdens (measured as photons per second and calculated AUC values for each animal) were compared between the control group and each treatment group using the Kruskal-Wallis test and Dunn's post hoc analysis. Ex vivo bacterial burdens from tissue samples and implanted K-wires (measured as CFU for each animal) were compared between the control group and each treatment group using the Kruskal-Wallis test and Dunn's post hoc analysis. Ex vivo bacterial burdens from tissue samples and implanted K-wires were also compared between the same-dose IV and IORA treatments using a two-tailed Mann-Whitney test. The numbers of animals with culture-positive or negative K-wires in the same-dose IV and IORA treatment groups were compared using Fisher's exact test.

#### 5.2.5 Results

##### Influence of antibiotic dosage and route on bacterial burden (biophotonic imaging)

Biophotonic imaging showed lower median bioluminescence levels in bacteria from all vancomycin-treated animals as early as day 1 after surgery (Table 5.1: control, 2.2

$\times 10^6$  [range  $7.2 \times 10^5$  to  $4.3 \times 10^6$ ]; V110<sub>IV</sub>,  $4.9 \times 10^5$  [range  $2.5 \times 10^5$  to  $7.2 \times 10^5$ ], difference of medians  $1.7 \times 10^6$ ,  $p=0.0016$ ; V25<sub>IV</sub>:  $4.9 \times 10^5$  [range  $3.6 \times 10^5$  to  $5.7 \times 10^5$ ], difference of medians  $1.7 \times 10^6$ ,  $p=0.0028$ ; V25<sub>IORA</sub>,  $5.3 \times 10^5$  [range  $4.73 \times 10^5$  to  $6.15 \times 10^5$ ], difference of medians  $1.7 \times 10^6$ ,  $p=0.0148$ ; Figure 5.3A). With the numbers available, there was no difference in median bioluminescence between untreated animals and those treated with cefazolin (control,  $2.2 \times 10^6$  [range  $7.15 \times 10^5$  to  $4.34 \times 10^6$ ]; C100<sub>IORA</sub>,  $6.2 \times 10^5$  [range  $4.1 \times 10^5$  to  $1.1 \times 10^6$ ],  $p=0.23$ ; C100<sub>IV</sub>,  $6.1 \times 10^5$  [range  $3.68 \times 10^5$  to  $2.55 \times 10^6$ ],  $p=0.06$ ; Figure 5.3A).

**Table 5.1** Bioluminescence in *Staphylococcus aureus* bacteria exposed or not to antibiotics on postoperative day 1

Treatment	Median (range)	Difference of median versus control	p-value
Control	$2.19 \times 10^6$ ( $7.15 \times 10^5$ to $4.34 \times 10^6$ )		
V110 <sub>IV</sub>	$4.90 \times 10^5$ ( $2.51 \times 10^5$ to $7.20 \times 10^5$ )	$1.70 \times 10^6$	0.0016
V25 <sub>IV</sub>	$4.94 \times 10^5$ ( $3.60 \times 10^5$ to $5.70 \times 10^5$ )	$1.70 \times 10^6$	0.0028
V25 <sub>IORA</sub>	$5.34 \times 10^5$ ( $4.73 \times 10^5$ to $6.15 \times 10^5$ )	$1.66 \times 10^6$	0.0148
C100 <sub>IORA</sub>	$6.21 \times 10^5$ ( $4.14 \times 10^5$ to $1.07 \times 10^6$ )	$1.57 \times 10^6$	0.0606
C100 <sub>IV</sub>	$6.06 \times 10^5$ ( $3.68 \times 10^5$ to $2.55 \times 10^6$ )	$1.58 \times 10^6$	0.2335

The data are expressed as photons/sec. Abbreviations: C, cefazolin; IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg; V, vancomycin

At 4 days after surgery, the bioluminescent signals from animals treated with a suboptimal concentration of vancomycin IV (V25<sub>IV</sub>) returned to near control levels. However, the median bioluminescence signals obtained from animals administered high-dose systemic IV vancomycin (V110<sub>IV</sub>), low-dose regional intraosseous vancomycin (V25<sub>IORA</sub>), or regional intraosseous cefazolin (C100<sub>IORA</sub>) were lower than those from the control animals at this time (Table 5.2: control,  $1.64 \times 10^6$  [range  $7.76 \times 10^5$  to  $3.96 \times 10^6$ ]; V110<sub>IV</sub>,  $5.45 \times 10^5$  [range  $4.30 \times 10^5$  to  $1.20 \times 10^6$ ], difference of medians  $1.10 \times 10^6$ ,  $p=0.013$ ; V25<sub>IV</sub>,  $1.13 \times 10^6$  [range  $5.91 \times 10^5$  to  $1.40 \times 10^6$ ], difference of medians  $5.10 \times 10^6$ ,  $p>0.99$ ; V25<sub>IORA</sub>,  $5.14 \times 10^5$  [range  $3.83 \times 10^5$  to  $8.96 \times 10^5$ ], difference of medians  $1.13 \times 10^6$ ,  $p=0.0012$ ; C100<sub>IORA</sub>,  $6.18 \times 10^5$  [range  $3.88 \times 10^5$  to  $1.17 \times 10^6$ ], difference of medians  $1.02 \times 10^6$ ,  $p=0.0140$ ; C100<sub>IV</sub>,  $6.72 \times$

$10^5$  [range  $4.63 \times 10^5$  to  $1.30 \times 10^6$ ], difference of medians  $9.68 \times 10^5$ ,  $p=0.101$ ; Figure 5.3B). Likewise, AUC values calculated for the bioluminescence signals from treated mice throughout the experiment were approximately one quarter of the value of those calculated for the untreated controls (Table 5.3, median bioluminescence: control,  $5.01 \times 10^6$  [range  $3.30 \times 10^6$  to  $1.02 \times 10^7$ ]; V110<sub>IV</sub>,  $1.52 \times 10^6$  [range  $9.93 \times 10^5$  to  $3.13 \times 10^6$ ], difference of medians  $3.49 \times 10^6$ ,  $p=0.0026$ ; V25<sub>IV</sub>,  $1.94 \times 10^6$  [range  $1.75 \times 10^6$  to  $3.35 \times 10^6$ ], difference of medians  $3.07 \times 10^6$ ,  $p=0.4934$ ; V25<sub>IORA</sub>,  $1.51 \times 10^6$  [range  $1.25 \times 10^6$  to  $2.43 \times 10^6$ ], difference of medians  $3.50 \times 10^6$ ,  $p=0.0011$ ; C100<sub>IORA</sub>,  $1.55 \times 10^6$  [range  $1.19 \times 10^6$  to  $2.35 \times 10^6$ ], difference of medians  $3.46 \times 10^6$ ,  $p=0.0016$ ; C100<sub>IV</sub>,  $2.35 \times 10^6$  [range  $1.44 \times 10^6$  to  $4.16 \times 10^6$ ], difference of medians  $2.66 \times 10^6$ ,  $p=0.2312$ ; Figure 5.4).

**Table 5.2** Bioluminescence in *Staphylococcus aureus* bacteria exposed or not to antibiotics on postoperative day 4

Treatment	Median (range)	Difference of median versus control	p-value
Control	$1.64 \times 10^6$ ( $7.76 \times 10^5$ to $3.96 \times 10^6$ )		
V110 <sub>IV</sub>	$5.45 \times 10^5$ ( $4.30 \times 10^5$ to $1.20 \times 10^6$ )	$1.10 \times 10^6$	0.0126
V25 <sub>IV</sub>	$1.13 \times 10^6$ ( $5.91 \times 10^5$ to $1.40 \times 10^6$ )	$5.10 \times 10^5$	>0.9999
V25 <sub>IORA</sub>	$5.14 \times 10^5$ ( $3.83 \times 10^5$ to $8.96 \times 10^5$ )	$1.13 \times 10^6$	0.0012
C100 <sub>IORA</sub>	$6.18 \times 10^5$ ( $3.88 \times 10^5$ to $1.17 \times 10^6$ )	$1.02 \times 10^6$	0.0140
C100 <sub>IV</sub>	$6.72 \times 10^5$ ( $4.63 \times 10^5$ to $1.30 \times 10^6$ )	$9.68 \times 10^5$	0.1015

The data are expressed as photons/sec. Abbreviations: C, cefazolin; IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg; V, vancomycin



**Table 5.3** Bioluminescence in *Staphylococcus aureus* bacteria exposed or not to antibiotics: area under the curve values over 4 days.

Treatment	Median (range)	Difference of median versus control	p-value
Control	$5.01 \times 10^6$ ( $3.30 \times 10^6$ to $1.02 \times 10^7$ )		
V110 <sub>IV</sub>	$1.52 \times 10^6$ ( $9.93 \times 10^5$ to $3.13 \times 10^6$ )	$3.49 \times 10^6$	0.0026
V25 <sub>IV</sub>	$1.94 \times 10^6$ ( $1.75 \times 10^6$ to $3.35 \times 10^6$ )	$3.07 \times 10^6$	0.4934
V25 <sub>IORA</sub>	$1.51 \times 10^6$ ( $1.25 \times 10^6$ to $2.43 \times 10^6$ )	$3.50 \times 10^6$	0.0011
C100 <sub>IORA</sub>	$1.55 \times 10^6$ ( $1.19 \times 10^6$ to $2.35 \times 10^6$ )	$3.46 \times 10^6$	0.0016
C100 <sub>IV</sub>	$2.35 \times 10^6$ ( $1.44 \times 10^6$ to $4.16 \times 10^6$ )	$2.66 \times 10^6$	0.2312

The data are expressed as photons/sec. Abbreviations: C, cefazolin; IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg; V, vancomycin

#### Influence of antibiotic dosage and route of administration on survival of *S. aureus*

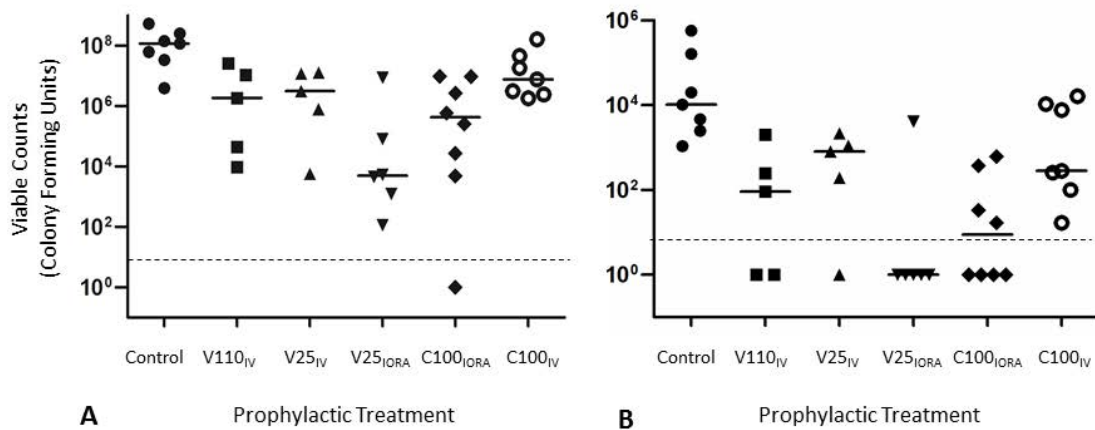
Similar to data from biophotonic imaging, the median CFU obtained from the implanted K-wire were lower in the high-dose systemic IV vancomycin, low-dose regional intraosseous vancomycin, and regional intraosseous cefazolin groups than in controls (Table 5.4: control,  $1.03 \times 10^4$  [range  $1.08 \times 10^3$  to  $5.75 \times 10^5$ ]; V110<sub>IV</sub>,  $9.17 \times 10^1$  [range  $<7.0 \times 10^0$  to  $2.00 \times 10^3$ ], difference of medians  $1.02 \times 10^4$ ,  $p=0.0313$ ; V25<sub>IV</sub>,  $4.96 \times 10^2$  [range  $<7.0 \times 10^0$  to  $2.13 \times 10^3$ ], difference of medians  $9.80 \times 10^3$ ,  $p=0.0905$ ; V25<sub>IORA</sub>,  $<7.0 \times 10^0$  [range  $<7.0 \times 10^0$  to  $4.08 \times 10^3$ ], difference of medians  $1.03 \times 10^4$ ,  $p=0.0013$ ; C100<sub>IORA</sub>,  $8.85 \times 10^0$  [range  $<7.0 \times 10^0$  to  $6.17 \times 10^2$ ],

difference of medians  $1.03 \times 10^4$ ,  $p=0.0020$ ; C100<sub>IV</sub>,  $2.83 \times 10^2$  [range  $1.67 \times 10^1$  to  $1.62 \times 10^4$ ], difference of medians  $1.00 \times 10^4$ ,  $p=0.8858$ ; Figure 5.5).

**Table 5.4** Colony-forming units of *Staphylococcus aureus* bacteria exposed or not to antibiotics recovered from implant 4 days postoperatively

Treatment	Median (range)	Difference of median versus control	p-value
Control	$1.03 \times 10^4$ ( $1.08 \times 10^3$ to $5.75 \times 10^5$ )		
V110 <sub>IV</sub>	$9.17 \times 10^1$ ( $<7.0 \times 10^0$ to $2.00 \times 10^3$ )	$1.02 \times 10^4$	0.0313
V25 <sub>IV</sub>	$4.96 \times 10^2$ ( $<7.0 \times 10^0$ to $2.13 \times 10^3$ )	$9.80 \times 10^3$	0.0905
V25 <sub>IORA</sub>	$<7.0 \times 10^0$ ( $<7.0 \times 10^0$ to $4.08 \times 10^3$ )	$1.03 \times 10^4$	0.0013
C100 <sub>IORA</sub>	$8.85 \times 10^0$ ( $<7.0 \times 10^0$ to $6.17 \times 10^2$ )	$1.03 \times 10^4$	0.0020
C100 <sub>IV</sub>	$2.83 \times 10^2$ ( $1.67 \times 10^1$ to $1.62 \times 10^4$ )	$1.00 \times 10^4$	0.8858

Abbreviations: C, cefazolin; IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg; V, vancomycin



**Figure 5.5** Quantification of viable *Staphylococcus aureus* Xen36 after surgery. The mice were euthanised 96 hours after surgery for quantification of bacteria remaining in the (A) knee and surrounding tissue and (B) implanted K-wire. The dotted line represents the limits of detection. Median values per group are denoted by solid lines. Each symbol represents an individual animal. Data are pooled from six independent repeats with 1–2 animals per group per repeat. Abbreviations: IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg.

Although bacteria were recovered from the tissues surrounding the implant site for all but one animal, mice treated with intraosseous vancomycin or cefazolin had lower median CFU (Table 5.5: control,  $1.17 \times 10^8$  [range  $3.94 \times 10^6$  to  $5.37 \times 10^8$ ]; V110<sub>IV</sub>,  $1.86 \times 10^6$  [range  $9.59 \times 10^3$  to  $2.60 \times 10^7$ ], difference of medians  $1.15 \times 10^8$ ,  $p=0.1376$ ; V25<sub>IV</sub>,  $1.95 \times 10^6$  [range  $6.64 \times 10^2$  to  $1.27 \times 10^7$ ], difference of medians  $1.15 \times 10^8$ ,  $p=0.0454$ ; V25<sub>IORA</sub>,  $4.92 \times 10^3$  [range  $1.16 \times 10^2$  to  $8.69 \times 10^6$ ], difference of medians  $1.17 \times 10^8$ ,  $p=0.0005$ ; C100<sub>IORA</sub>,  $4.23 \times 10^5$  [range  $<1.30 \times 10^1$  to  $9.69 \times 10^6$ ] difference of medians  $1.17 \times 10^8$ ,  $p=0.0049$ ; C100<sub>IV</sub>,  $7.67 \times 10^6$  [range  $1.82 \times 10^6$  to  $1.63 \times 10^8$ ], difference of medians  $1.09 \times 10^8$ ,  $p=0.8699$ ; Figure 5.5).

**Table 5.5** Colony-forming units of *Staphylococcus aureus* bacteria exposed or not to antibiotics recovered from periprosthetic tissue 4 days postoperatively

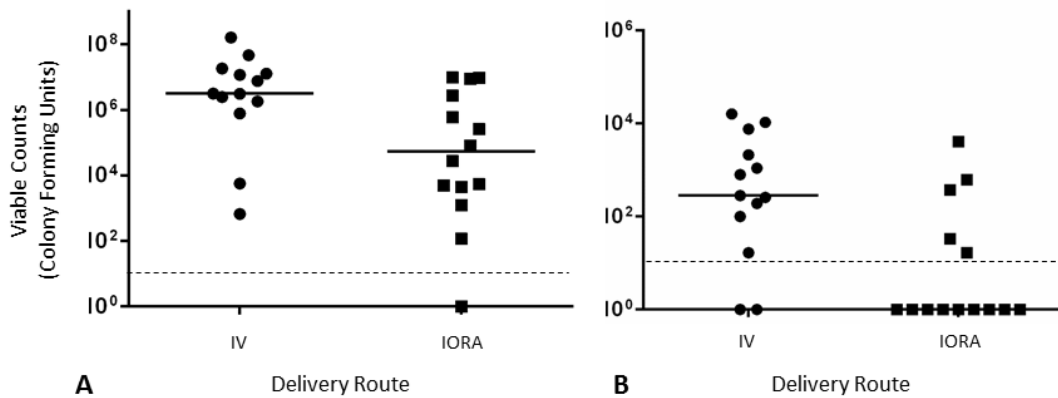
Treatment	Median (range)	Difference of median versus control	p-value
Control	$1.17 \times 10^8$ ( $3.94 \times 10^6$ to $5.37 \times 10^8$ )		
V110 <sub>IV</sub>	$1.86 \times 10^6$ ( $9.59 \times 10^3$ to $2.60 \times 10^7$ )	$1.15 \times 10^8$	0.1376
V25 <sub>IV</sub>	$1.95 \times 10^6$ ( $6.64 \times 10^2$ to $1.27 \times 10^7$ )	$1.15 \times 10^8$	0.0454
V25 <sub>IORA</sub>	$4.92 \times 10^3$ ( $1.16 \times 10^2$ to $8.69 \times 10^6$ )	$1.17 \times 10^8$	0.0005
C100 <sub>IORA</sub>	$4.23 \times 10^5$ ( $<1.30 \times 10^1$ to $9.69 \times 10^6$ )	$1.17 \times 10^8$	0.0049
C100 <sub>IV</sub>	$7.67 \times 10^6$ ( $1.82 \times 10^6$ to $1.63 \times 10^8$ )	$1.09 \times 10^8$	0.8699

Abbreviations: C, cefazolin; IV, intravenous; IORA, intraosseous regional administration; V110<sub>IV</sub>, systemic vancomycin 110 mg/kg; V25<sub>IV</sub>, systemic vancomycin 25 mg/kg; V25<sub>IORA</sub>, IORA vancomycin 25 mg/kg; C100<sub>IORA</sub>, IORA cefazolin 100 mg/kg; C100<sub>IV</sub>, systemic cefazolin 100 mg/kg; V, vancomycin

#### IORA versus IV administration of same-dose antibiotic prophylaxis

Overall, intraosseous antibiotic administration was more effective at reducing the burden of contaminating bacteria in tissue than the same dose of antibiotic administered IV (median CFU: IV,  $3.16 \times 10^6$  [range  $6.64 \times 10^2$  to  $1.63 \times 10^8$ ]; IORA,  $5.43 \times 10^4$  [range  $<1.30 \times 10^1$  to  $9.69 \times 10^6$ ], difference of medians  $3.11 \times 10^6$ ,  $p=0.0163$ ; Figure 5.6A). Bacteria were recovered from the K-wires implanted in only five of 14 IORA-treated animals compared with 11 of 13 animals treated intravenously with the same dose of antibiotic; the difference was statistically significant ( $p=0.0183$  [Fisher's exact test], median CFU: IV,  $2.83 \times 10^2$  [range  $<7.0 \times$

$10^0$  to  $1.62 \times 10^4$ ]; IORA,  $<7.0 \times 10^0$  [range  $<7.0 \times 10^0$  to  $4.08 \times 10^3$ ] difference of medians  $2.76 \times 10^2$ ,  $p=0.0073$ ; Figure 5.6B).



**Figure 5.6** Effect of route used to deliver antibiotic prophylaxis on survival of *Staphylococcus aureus* Xen36. Mice treated with either 25 mg/kg vancomycin or 100 mg/kg cefazolin were euthanised 96 hours after surgery for quantification of bacteria remaining in the (A) knee and surrounding tissue and (B) implanted K-wire. The dotted line represents the limits of detection. Median values are denoted by solid lines. Each symbol represents an individual animal. Data are pooled from six independent repeats. Abbreviations: IV, systemic administration; IORA, intraosseous regional administration.

### 5.2.6 Discussion

Prophylactic antibiotics reduce deep infection rates in arthroplasty.<sup>41,151</sup> To be effective, prophylactic antibiotics must be present in adequate tissue concentrations at the operative site from the time of incision until the time of closure.<sup>39</sup> As antibiotic resistance increases, systemic administration of cephalosporins may no longer provide tissue concentrations that are effective against coagulase-negative staphylococci and MRSA. IORA allows much higher tissue concentrations to be achieved.<sup>158,175</sup> In the present study, cefazolin and vancomycin by the IORA route provided more effective

prophylaxis than the same dose of antibiotic given systemically in a murine model of TKA.

There are several limitations to this study. Firstly, although we attempted to use the equivalent antibiotic doses and replicate the clinical situation of an intra-articular implant, it is unclear how well this model approximates the clinical situation of TKA in humans. However, because clinical TKA infection rates in the range of 0.86%–2.5% have been reported<sup>10-13</sup>, animal models such as this remain the only practical way of providing adequate power to compare prophylaxis regimens. Secondly, we chose to investigate only MSSA, because vancomycin is likely to be more effective than cefazolin against coagulase-negative staphylococci and MRSA strains resistant to cefazolin. As in previous studies<sup>60,185</sup>, we used a relatively high inoculum of bacteria to discriminate better between the effectiveness of prophylactic regimens for the three endpoints used (in vivo bioluminescence, ex vivo implant, and periarticular tissue counts). This may differ from the clinical situation in TKA, because although contamination occurs in most if not all TKAs<sup>20</sup>, the overall bacterial inoculum is likely to be lower than that used in this model. In addition, vancomycin has a longer half-life than cefazolin, and this may have affected the comparison between groups because we used only one preoperative dose. However, clinical data suggest that the preoperative dose is the most important determinant of effective prophylaxis<sup>50,84,162,186</sup>, and the faster rate of drug metabolism in the mouse means the effect of differing half-lives is reduced<sup>182</sup>. Further, we used only female mice because male mice are more likely to fight and injure themselves or other mice when housed in a group. In murine models, female mice are generally more resistant to bacterial infection<sup>187</sup>; however, previous studies using this TKA model have also been single-sex studies<sup>60,188</sup>, so any sex-related differences in this regard are not known.

We found the *in vivo* bacterial burden 4 days after simulated TKA to be lower than that of controls in both IORA groups (low-dose vancomycin and standard-dose cefazolin) and in the group given high-dose systemic vancomycin. The rationale for using a low dose of vancomycin for IORA relates to the multiple disadvantages of systemic vancomycin prophylaxis. Systemic vancomycin requires a prolonged administration time to prevent red man syndrome, a pruritic, erythematous rash related to histamine release that can occur with rapid infusion<sup>156,159</sup>. A prophylactic dose of 1 g requires the infusion to be started a minimum of 1 hour before surgery, which is difficult to achieve in an arthroplasty practice<sup>87</sup>. Vancomycin can also cause renal and other systemic toxicities.<sup>146,156</sup> Use of a lower targeted vancomycin dose via IORA optimises the timing of administration and reduces the risk of such systemic toxic effects.

Similar to the data from biophotonic imaging, bacterial CFU counts from the implanted K-wire were lower than those of controls in both IORA groups and in the group that received high-dose systemic vancomycin. This suggests that high tissue concentrations are important in the efficacy of vancomycin as a prophylactic agent. The killing power of vancomycin is proportional to the area under the concentration-time curve over the minimum inhibitory concentration for the bacterium (AUC/MIC)<sup>141,189</sup>; thus, higher concentrations are likely to enhance efficacy, as seen in our study. Inadequate tissue concentrations have been implicated as the reason why systemic vancomycin is less effective than cephalosporins against MSSA.<sup>87,155,170</sup> Niska et al<sup>60</sup> used a murine model to investigate the efficacy of varying doses of antibiotic on prophylaxis against implant infection. They found vancomycin to have a narrower effective dose range than daptomycin or tigecycline, with a 110 mg/kg dose being markedly more effective than a 10 mg/kg dose. Although estimation of

equivalent human and mouse dosages is imperfect, our study supports the finding that the efficacy of vancomycin as a prophylactic agent depends on achieving high tissue concentrations. Vancomycin by IORA, which achieves high concentrations despite the lower dose, resulted in lower bacterial counts and appeared at least as effective as cefazolin for prophylaxis against MSSA in our model. Vancomycin performs less well than cephalosporins against MSSA in clinical studies of prophylaxis in arthroplasty.<sup>87,174</sup> It seems likely that the clinical efficacy of vancomycin for prophylaxis against MSSA would be enhanced if higher tissue concentrations can be achieved.

We found that the same doses of vancomycin and cefazolin were more effective via IORA than via IV administration. The bactericidal activity of cefazolin is normally considered to be time-dependent (i.e., concentration-independent), and once tissue levels are 4–5 times the MIC, further increases do not increase efficacy<sup>59</sup>. Therefore, while high tissue concentrations of cefazolin by IORA may provide benefit against organisms with high MICs against cefazolin, such as coagulase-negative staphylococci<sup>61</sup>, they would be expected to have less effect on more sensitive strains such as the MSSA used in our study. However, these data are based on animal models of treatment of established infections<sup>125,126</sup>, rather than models of prophylaxis such as ours, in which prevention of infection is the goal. Initiation of bacterial killing is known to occur earlier with increasing cefazolin concentrations<sup>59</sup>, a factor likely to be more important in prophylaxis where preventing initial bacterial adherence and subsequent formation of biofilm is required. This may explain our finding that cefazolin provided more effective prophylaxis via the IORA route than the same cefazolin dose administered via the systemic route.



Cefazolin and vancomycin provided more effective prophylaxis via the IORA route than the same doses of these antibiotics given systemically. The effectiveness of vancomycin in particular was enhanced by IORA despite a lower dose, suggesting vancomycin is more effective against MSSA when high tissue concentrations are achieved, such as with IORA. Further clinical studies are needed to identify any unforeseen complications with IORA, particularly for vancomycin. Use of a lower dose and the depot effect may reduce the risk of red man syndrome after deflation of the tourniquet; this complication has not yet been seen in human studies of vancomycin via the IORA route. Concerns remain regarding antibiotic stewardship, and routine use of vancomycin by any route may not be justified. Vancomycin by IORA may be more appropriately limited to patients at higher risk of infection, such as those undergoing revision procedures and those with a high body mass index<sup>26</sup>. Future clinical studies will focus on these areas.

### 5.2.7 Acknowledgements

We thank Thomas Lumley PhD, Department of Statistics, University of Auckland, for helpful advice regarding the statistical analysis.

## 5.3 Discussion of article

### 5.3.1 Contribution and significance

This study found that the IORA technique provided more effective antibiotic prophylaxis than systemic administration in a mouse model of TKA. The effectiveness of vancomycin was particularly enhanced by IORA, despite the lower dose used. However, there are significant limitations to this study, the most important of which is how accurately the model simulates the clinical situation of TKA.

However, it is certainly encouraging that the theoretical advantages of IORA in terms of providing more effective prophylaxis were demonstrated in this experimental study.

Vancomycin appeared to provide the most effective antibiotic prophylaxis, even though an MSSA strain was used. In the setting of contamination by a cefazolin-resistant organism such as MRSA or CoNS, vancomycin is likely to be even more effective than cefazolin. This would support the choice of vancomycin over cefazolin for IORA in clinical practice.

### 5.3.2 Relationship to clinical practice and potential negatives of IORA antibiotic prophylaxis in TKA

The preceding studies in this thesis have demonstrated that IORA provides markedly higher tissue concentrations of antibiotics, appears to do so reliably and safely in TKA, and provides enhanced prophylaxis against infection in an animal model. However, there are some potential negatives of IORA prophylaxis in clinical practice, including needle cost, additional tourniquet time, possible complications, and issues concerning antibiotic stewardship and vancomycin resistance.

#### 5.3.2.1 Needle cost

The intraosseous needle system (Vidacare) used in the clinical studies costs approximately \$NZ100 per needle, representing a significant additional cost for each TKA procedure, particularly when the clinical benefit is unproven and the complication (PJI) it aims to prevent is rare.

There are cheaper intraosseous systems available, and even using the current needles the additional expense is small in the context of the total cost of a TKA procedure. In

New Zealand, a private TKA procedure including a prosthesis costs \$20,000–\$30,000, meaning a \$100 needle adds an additional 0.3%–0.5% to the total cost.

As a thought experiment, if IORA can reduce the deep infection rate following TKA from 1.0% to 0.5%, then the number-needed-to-treat to prevent one PJI is 200 patients. This represents a \$20,000 cost if needles are priced at \$100. This compares favourably with the total cost of treating one knee PJI, which is estimated to be at least \$NZ130,000<sup>6,7</sup>. This cost does not take into account the fact that even after successful treatment, patients with PJI are often left with residual pain and compromised function.

#### *5.3.2.2 Additional tourniquet time*

Inserting the intraosseous needle and performing the injection adds 2–4 minutes to the tourniquet time. Tourniquets cause tissue ischaemia while inflated, and it is generally recommended that the total tourniquet time should not exceed 120 minutes.<sup>190</sup>

Therefore, the additional 2–4 minutes required for the IORA injection leaves less time to perform the procedure. However, given that the average tourniquet time in our clinical study was 83 minutes, this should have minimal impact.<sup>158</sup>

Some surgeons also prefer to perform TKA without a tourniquet, in which case IORA could not be used. However, a recent survey of members of the American Association of Hip and Knee Surgeons reported that 96% of surgeons routinely use a tourniquet during TKA.<sup>191</sup>

#### *5.3.2.3 Potential complications*

Potential complications of the intraosseous route, such as extravasation of fluid (due to incorrect needle placement) and subsequent compartment syndrome, fracture, and

fat embolus were discussed in Section 1.6.5. Use of vancomycin also involves a risk of red man syndrome and the IORA technique may be associated with other unforeseen complications. Such complications have not been seen so far in either of the two studies presented in this thesis. However, the total number of patients receiving IORA antibiotics was only 31. When IORA antibiotic prophylaxis becomes more established in clinical practice, it will be possible to monitor the incidence of rare complications in larger prospective cohort studies.

#### 5.3.2.4 *Vancomycin resistance and antibiotic stewardship*

Vancomycin (or other glycopeptide antibiotics such as teicoplanin) may be the only available treatment option for infections caused by beta-lactam-resistant organisms such as MRSA and CoNS. Therefore, there is concern that using vancomycin for routine antibiotic prophylaxis may eventually lead to resistance to this agent as well.

Despite widespread use, it was not until 40 years after the introduction of vancomycin that clinical isolates with a high level of resistance to this drug emerged.<sup>192</sup> Resistance to vancomycin is more common in *Enterococcus* species (vancomycin-resistant enterococci [VRE]). Among the staphylococci, reduced susceptibility to vancomycin is categorised by two distinct phenotypes: intermediate susceptibility to vancomycin (vancomycin-intermediate *S. aureus*, MIC 4–8 µg/mL) and (rarely) high-level resistance to vancomycin (vancomycin-resistant *S. aureus*, MIC ≥16 µg/mL).<sup>193</sup> In contrast with VRE, vancomycin-resistant *S. aureus* remains rare, with only 16 isolates described in the USA as of 2015.<sup>192</sup> This may be because the typical genetic mechanism of vancomycin resistance in staphylococci (which differs from the mechanism in VRE) imposes a ‘fitness burden’. Therefore, this trait is selected against *in vivo* once the vancomycin exposure is removed, with restoration of fitness

incurring the price of loss of resistance.<sup>194</sup> However, intermediate-susceptible vancomycin-intermediate *S. aureus* strains are seen with increasing frequency, and many laboratories are reporting that the MICs of vancomycin for staphylococcal species are increasing over time, a phenomenon known as ‘MIC creep’.<sup>195</sup>

Data concerning how systemic prophylactic antibiotics may affect development of resistance is mixed. Stefánsdóttir et al reported an increase in methicillin-resistant CoNS on groin swabs from 20% preoperatively to 50% postoperatively following TKA in patients who received three doses of systemic cefazolin prophylaxis.<sup>196</sup> However, Pfundstein et al found no difference in the rate of VRE colonisation in organ transplant patients randomised to receive vancomycin or cefazolin prophylaxis.<sup>197</sup>

Whether IORA prophylaxis will have more or less impact than systemic prophylaxis on development of resistance is unclear. In vitro, antibiotic resistance is promoted by prolonged exposure of a bacterial inoculum to drug concentrations at or around the MIC.<sup>124</sup> The concept of the ‘mutant selection window’ describes the range of antibiotic concentrations in which resistant mutants may be selected for. At concentrations below the MIC, there is no selective pressure and therefore growth of resistant mutants is not favoured. At very high concentrations, no mutants will be selected because it is thought that a two-stage mutation is necessary for growth. This concept has been shown to apply in vitro to the development of vancomycin resistance in staphylococci<sup>198</sup>; therefore, to prevent resistance in clinical practice, the recommendation is to achieve the highest tolerated concentration of antibiotic.<sup>124</sup>

Low-dose prophylaxis with IORA vancomycin achieves this aim by limiting administration to the limb, which results in very high tissue concentrations but limited

overall exposure. Therefore, IORA may offer a theoretical advantage over systemic administration of vancomycin in reducing the development of resistance. However, because of antibiotic stewardship concerns, some doctors will remain uncomfortable about using vancomycin as prophylaxis in any form.

### 5.3.3 Selective use of IORA

In view of the negative aspects of IORA outlined above, it may be that selective use of IORA prophylaxis in patients at highest risk of infection is an appropriate strategy. Revision TKA procedures, performed after a previous TKA procedure has failed, are more complex, take longer to perform, and have a higher reported infection rate than primary TKA. The following chapter describes a study that identified the causes of TKA failure leading to revision surgery, and the subsequent chapter describes an investigation of whether IORA prophylaxis can be successfully performed prior to revision TKA.

## **Chapter 6 Importance of periprosthetic joint infection as a failure mechanism in modern knee arthroplasty**

### **6.1 Introduction**

Total knee arthroplasty (TKA) is considered to be one of the most successful procedures in modern medicine. However, complications such as prosthetic joint infection (PJI) do occur. The outcome of TKA is recorded in many countries using national registries, including in New Zealand. In such registries, 'failure' is defined as the need for revision surgery. PJI is a common cause of failure, but its relative importance in the context of other mechanisms of failure is unclear.

Although registry data often record the reason for revision TKA, interpretation is limited by the lack of standardised definitions for mechanisms of failure or objective assessment of radiological and laboratory parameters<sup>199</sup>. Further, studies have shown that the reasons for revision recorded in registries are often inaccurate, particularly in regard to PJI<sup>200</sup>. Alternatively, large series of revision TKAs from tertiary referral centres are able to provide a more accurate assessment of failure mechanisms because standardised definitions can be applied. However, studies from referral centres lack information on the original primary TKA population, and as a consequence, the true incidence and relative importance of each failure mechanism remains unknown. Moreover, the original primary TKAs in such studies were often performed in the distant past, and may not reflect modes of failure in contemporary primary TKA<sup>201,202</sup>.

The aims of this study were to identify the most common failure mechanisms in contemporary primary TKA, to assess the relative importance of PJI as a failure mechanism, and to analyse when TKA failures occur.

Aspects of this chapter were published in an article entitled 'Periprosthetic Joint Infection Is the Main Cause of Failure for Modern Knee Arthroplasty: An Analysis of 11,134 Knees' by Chuan Kong Koh, Irene Zeng, Saiprasad Ravi, Mark Zhu, Kelly G. Vince & Simon W. Young, published in *Clinical Orthopaedics and Related Research*, in June 2017. The article received an attention score in the top 5% of all research outputs scored by altmetric (<https://springerlink.altmetric.com/details/20782913>). The data in this chapter were also presented at the American Association of Hip and Knee Surgeons Annual Meeting in Dallas in November 2016, being one of 52 papers selected for a podium presentation from 1600 submissions.

## **6.2 Methods**

We performed a multicentre retrospective review of all primary TKA procedures (n=11,134) performed at three tertiary hospitals, i.e., Middlemore Hospital, North Shore Hospital, and Auckland Hospital, between January 1, 2000 and December 31, 2015. Exclusion criteria were unicompartmental knee arthroplasty, constrained TKA including hinged and non-hinged designs, and any tumour prosthesis.

Three hundred and fifty-seven patients who underwent subsequent revision surgery were identified by an individual search of patient records and supplemented with New Zealand Joint Registry (NZJR) data to identify revision TKAs performed at other hospitals. The most recent compliance audit of the NZJR in 2015 reported a capture rate of over 95%<sup>1</sup>. All patients were identified by the unique patient identifier



(National Health Index number) used by the New Zealand health system. Approval to conduct the study was obtained from all three district health boards and Health and Disability ethics committees. Local hospital data were used firstly to confirm the NZJR data and to identify patients who underwent revision surgery but were not captured by the registry. If the revision surgery was performed at an institution other than one of the three primary study hospitals, consent was obtained to collect clinical and radiographic data to ensure complete data capture.

Clinical, laboratory, and radiographic data for all patients with revision TKA were analysed using a standardised written protocol, and the primary mode of failure was determined independently by two authors, with disagreements resolved by consensus in conjunction with a third author. Where more than one mode of failure was thought to contribute, the cause that was most significant in the decision for the first revision procedure was listed as the primary reason.

‘Failure’ was defined as revision surgery in which one or more components was exchanged, removed, manipulated, or added surgically, or any reoperation for PJI as defined by the Musculoskeletal Infection Society criteria<sup>203</sup>.

The mechanism of failure was divided into nine categories as defined by Vince: PJI, aseptic loosening, patellofemoral arthrosis, arthrofibrosis or stiffness, tibiofemoral instability, periprosthetic fracture, patellar maltracking, polyethylene wear, or extensor mechanism deficiency.<sup>204</sup> PJI was defined according to the Musculoskeletal Infection Society definition.<sup>203</sup>

Aseptic loosening was defined as documented radiographic migration of components by more than 2 mm, progressive radiolucent lines of more than 2 mm, or

intraoperative finding of loose components<sup>205</sup>. If component loosening was present, aseptic loosening was recorded as the primary cause of failure, and if present, polyethylene wear, osteolysis, failure of ingrowth of uncemented implants, and bone collapse causing malalignment were considered secondary or predisposing factors<sup>206</sup>. Technetium bone and computed tomography scans were also reviewed to confirm periprosthetic lucency or increased tracer uptake.

Patients with patellofemoral arthrosis underwent clinical and radiographic examination (Merchant or Skyline patella view) to confirm chondral loss and osteophyte formation before secondary patellar resurfacing.

Arthrofibrosis was defined as a flexion contracture of 15° and/or less than 75° of flexion, and was considered the primary cause of failure in patients with a stiff but otherwise functional TKA when all other mechanisms were excluded.<sup>207</sup>

Primary tibiofemoral instability was considered if investigations excluded aseptic loosening, disruption of the extensor mechanism, PJI, and fracture. Clinical documentation of symptomatic instability and presence of varus or valgus laxity when the knee was assessed at 0° and 30° of flexion and/or instability in flexion were considered to assist diagnosis<sup>208,209</sup>.

Extensor mechanism deficiency included discontinuity of the patella and/or quadriceps tendon and transverse patella fracture. Patellar maltracking or dislocation was defined as symptomatic subluxation and/or dislocation of the patella from the trochlear groove. Polyethylene wear was described as macroscopic evidence of wear particles and delamination on the surface of the polyethylene liner without signs of aseptic loosening. Malalignment in the coronal, sagittal, or rotational plane was

recorded when present, but was considered a secondary cause of failure rather than one of the nine primary failure mechanisms.<sup>204</sup>

### *Statistical analysis*

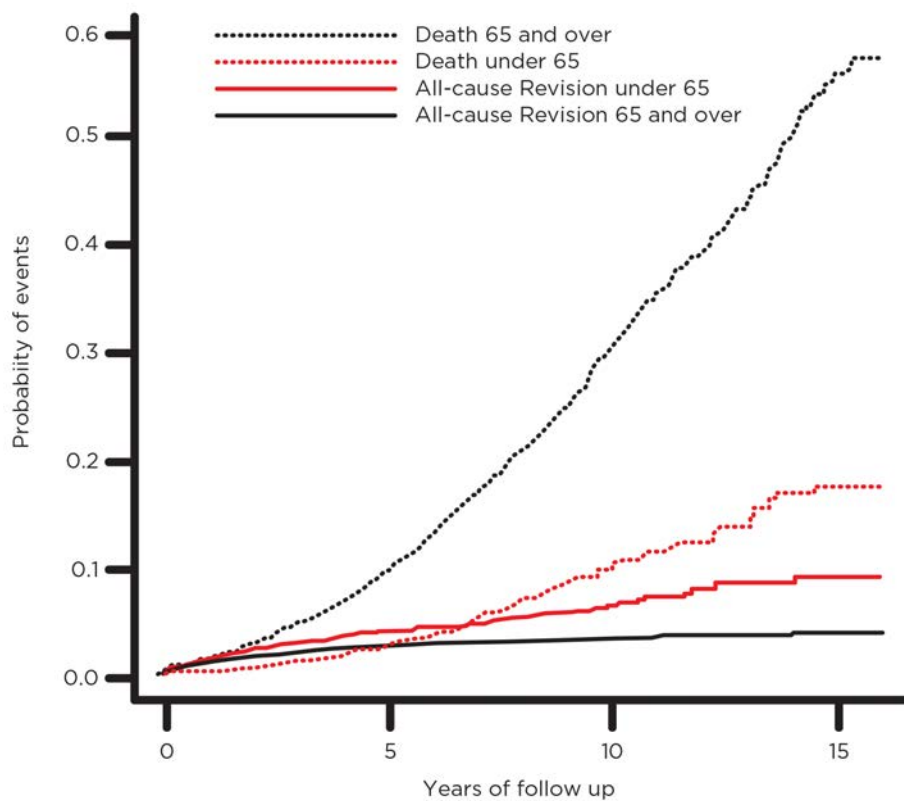
The Kaplan-Meier method was used to estimate the unadjusted mortality, revision rate, and standard error from postoperative years 1 to 15. The competing risk method, which copes with the simultaneous risk of different types of events including mortality, was used for the 15 years of follow-up of the primary TKA. The competing risk method was devised by Fine and Gray to cope with censoring subjects who failed because of causes other than those of interest<sup>210</sup>. These other causes, which are referred to as competing events, and the event of interest are considered to be mutually exclusive. The competing risk method estimates the probability of the event adjusted for other causes and has been reported to have a higher accuracy in assessing cumulative incidence when compared with the traditional Kaplan-Meier method<sup>211</sup>.

Outputs from the competing risk method sum to 100% when all competing events and all event-free probability are included. The standard output from cumulative function calculated the probability of each event at different defined time points. The ‘cmprsk’ and ‘cuminc’ functions of the R package (R Foundation for Statistical Computing, Vienna, Austria) were applied to estimate the cumulative incidences of the different revisions and mortality for each year of follow-up, along with their 95% confidence intervals<sup>212</sup>.

## **6.3 Results**

A total of 11,134 primary TKAs in 8830 patients met the study inclusion criteria (Table 6.1). The mean patient age was 68.8 (range 18–98) years. The most common

indication for primary TKA was osteoarthritis (95.6%). The median follow-up duration was 5 years (range 1–16) years. There were 1368 patient deaths (1653 TKAs) during the study period. The mortality rate was 8.62% at 5 years, 28.0% at 10 years, and 52.1% at 15 years from the index operation (Table 6.2). The mortality rate was 10.0% at 5 years, 31.1% at 10 years, and 56.3% at 15 years from the index surgery in patients aged 65 years or older and 8.6% at 10 years and 17.9% at 15 years in patients younger than 65 years (Figure 6.1).



**Figure 6.1** Cumulative incidence of death and revision total knee arthroplasty.

**Table 6.1** Demographic and clinical data for primary TKA patients and those who underwent subsequent revision TKA

	<b>Primary TKA (n=11,134)</b>	<b>Revision TKA (n=357)</b>
<b>Demographic data</b>		
Mean (SD) age at surgery, years	68.8 (9.7)	65.2* (9.7)
Mean BMI (SD, total recorded)	32.6 (6.88, 3040)	33.2 (7.23, 75)
Male sex (%)	4792 (43)	163 (46)
<b>Indication for primary TKA</b>		
Osteoarthritis	10,648	331
Rheumatoid arthritis	329	20
Other inflammatory arthritis	48	4
Fracture	50	2
Other	59	0
<b>Mean skin to skin time, minutes (range, total recorded)</b>	92.7 (25–402, 10,402)	117.3 (26–511, 287)
<b>ASA score</b>		
1	529 (6%)	23 (8%)
2	5232 (60%)	139 (49%)
3	2862 (33%)	115 (40%)
4	53 (0.6%)	9 (3%)
Total ASA score recorded (n)	8676	286
<b>Details of primary surgery</b>		
Patella resurfaced	4858 (43.6%)	
Cemented TKA	10,624 (95.4%)	
Hybrid TKA	499 (4.5%)	
Uncemented TKA	11 (0.1%)	
Cruciate-retaining knee	7880	
Posterior-substituting knee	2278	
<b>Hospital</b>		
A	4527 (40.7%)	118 (2.6%**)
B	4897 (44.0%)	183 (3.7%**)
C	1710 (15.3%)	50 (2.9%**)

\*Age at time of revision; \*\*revision rate per hospital. Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index; SD, standard deviation; TKA, total knee arthroplasty

**Table 6.2** Incidence of revision total knee arthroplasty and mortality rate.

	Follow-up (years)															
	0.5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<b>Revision TKA</b>																
Cumulative incidence (%)	0.76	1.17	1.89	2.29	2.79	3.11	3.36	3.53	3.88	4.18	4.44	4.66	5.38	5.63	5.86	6.09
Annual incidence (%)	-	0.41	0.71	0.48	0.30	0.24	0.14	0.29	0.24	0.20	0.35	0.31	0.16	0.13	0.13	0.23
Standard error	0.08	0.10	0.13	0.15	0.17	0.20	0.20	0.20	0.23	0.25	0.27	0.29	0.38	0.41	0.47	0.53
<b>Mortality</b>																
Kaplan-Meier estimate (%)	0.58	1.10	2.38	4.24	5.87	8.62	11.65	14.98	18.62	22.69	28.04	32.39	35.95	40.3	46.5	52.1
Standard error	0.0008	0.001	0.002	0.002	0.003	0.004	0.004	0.005	0.006	0.007	0.008	0.009	0.01	0.01	0.01	0.02
Patients at risk (n)	8701	8208	6992	6031	5052	4255	3564	2946	2417	1849	1351	925	620	464	300	166

Abbreviation: TKA, total knee arthroplasty

Of the 11,134 primary TKAs performed, 357 underwent revision surgery, giving a 15-year incidence of revision of 6.1%. Clinical information was also obtained for 28 patients who underwent revision TKA outside the three study hospitals, resulting in 100% data capture for all recorded failures. The NZJR captured 302 revisions from the primary TKA cohort and local hospital records added 55 further revisions. Forty of the additional 55 revisions identified were secondary to PJI. The overall sensitivity of the NZJR in capturing revision TKA was 85% when cross-matched with data obtained from local hospital records, 76% for revisions due to PJI, and 92% for aseptic revisions.

The cumulative incidence of revision TKA was 1.2% at 1 year, 1.9% at 2 years, 3.1% at 5 years, 4.4% at 10 years, and 6.1% at 15 years from the index TKA (Table 6.2).

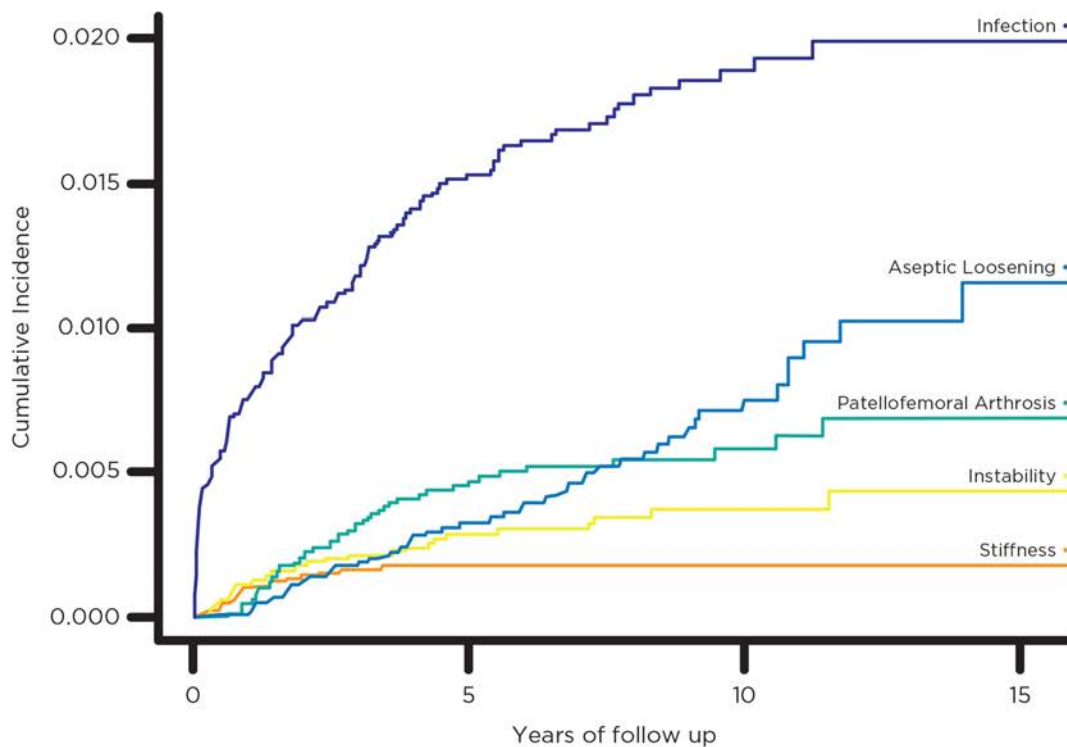
The annual incidence of revision was highest within the first 3 years, and after 4 years the annual risk of revision ranged from 0.1% to 0.3%. The five most common reasons for revision were PJI, aseptic loosening, patellofemoral arthrosis, tibiofemoral instability, and stiffness/arthrofibrosis (Table 6.3, Figure 6.2). The cumulative incidence of aseptic loosening and polyethylene wear was higher in patients under the age of 65 years (Figures 6.3 and 6.4).

**Table 6.3** Adjusted cumulative incidence (%) of reasons for revision total knee arthroplasty during 15 years of follow-up.

Revision Reason	Follow Up (Years)															
	0.5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<b>Infection</b> (95% CI)	0.56 (0.42-0.7)	0.76 (0.6-0.92)	1.03 (0.84-1.22)	1.18 (0.97-1.38)	1.41 (1.17-1.64)	1.52 (1.28-1.77)	1.65 (1.38-1.91)	1.68 (1.42-1.95)	1.80 (1.51-2.08)	1.86 (1.56-2.15)	1.89 (1.59-2.19)	1.93 (1.62-2.24)	1.99 (1.66-2.32)	1.99 (1.66-2.32)	1.99 (1.66-2.32)	1.99 (1.66-2.32)
<b>Aseptic Loosening</b> (95% CI)	0.01 (0-0.03)	0.02 (0-0.04)	0.13 (0.06-0.20)	0.19 (0.1-0.27)	0.28 (0.17-0.39)	0.32 (0.2-0.45)	0.38 (0.24-0.51)	0.45 (0.30-0.61)	0.54 (0.36-0.72)	0.65 (0.44-0.86)	0.71 (0.5-0.94)	0.91 (0.51-1.18)	1.02 (0.69-1.35)	1.02 (0.69-1.35)	1.15 (0.73-1.57)	1.15 (0.73-1.57)
<b>Patellofemoral Arthrosis</b> (95% CI)	0	0.05 (0.01-0.09)	0.20 (0.12-0.29)	0.32 (0.21-0.43)	0.41 (0.28-0.54)	0.47 (0.32-0.51)	0.50 (0.35-0.65)	0.52 (0.36-0.67)	0.54 (0.38-0.70)	0.54 (0.38-0.70)	0.58 (0.40-0.75)	0.62 (0.42-0.82)	0.68 (0.45-0.91)	0.68 (0.45-0.91)	0.68 (0.45-0.91)	0.68 (0.45-0.91)
<b>Instability</b> (95% CI)	0.05 (0.01-0.10)	0.11 (0.05-0.17)	0.18 (0.10-0.26)	0.21 (0.12-0.30)	0.24 (0.14-0.33)	0.28 (0.17-0.39)	0.30 (0.19-0.41)	0.30 (0.19-0.41)	0.34 (0.21-0.47)	0.37 (0.23-0.51)	0.37 (0.23-0.51)	0.37 (0.23-0.51)	0.43 (0.25-0.62)	0.43 (0.25-0.62)	0.43 (0.25-0.62)	0.43 (0.25-0.62)
<b>Stiffness</b> (95% CI)	0.36 (0-0.07)	0.10 (0.04-0.16)	0.14 (0.07-0.21)	0.16 (0.09-0.24)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)	0.18 (0.09-0.26)
<b>Polyethylene Wear</b> (95% CI)	0	0	0	0	0	0.01 (0-0.04)	0.01 (0-0.04)	0.01 (0-0.04)	0.01 (0-0.04)	0.07 (0-0.15)	0.14 (0.01-0.26)	0.18 (0.03-0.32)	0.18 (0.03-0.32)	0.34 (0.07-0.61)	0.34 (0.07-0.61)	0.47 (0.10-0.84)
<b>Periprosthetic Fracture</b> (95% CI)	0.03 (0-0.06)	0.03 (0-0.06)	0.04 (0-0.07)	0.04 (0-0.07)	0.05 (0-0.09)	0.05 (0-0.09)	0.07 (0.01-0.12)	0.07 (0.01-0.12)	0.09 (0.02-0.16)	0.09 (0.02-0.16)	0.09 (0.02-0.16)	0.13 (0.12-0.25)	0.13 (0.12-0.25)	0.13 (0.12-0.25)	0.13 (0.12-0.25)	0.13 (0.12-0.25)
<b>Patella Maltracking</b> (95% CI)	0.02 (0-0.04)	0.04 (0-0.07)	0.06 (0.01-0.1)	0.06 (0.01-0.1)	0.06 (0.01-0.1)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)	0.07 (0.32-0.51)
<b>Extensor Mechanism Dysfunction</b> (95% CI)	0.01 (0-0.03)	0.02 (0-0.43)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)	0.03 (0-0.06)
<b>Other</b> (95% CI)	0.05 (0.01-0.08)	0.05 (0.01-0.10)	0.07 (0.02-0.13)	0.09 (0.03-0.14)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)	0.10 (0.04-0.16)
<b>TKAs at risk (n)</b>	10989	10423	8946	7766	6603	5575	4775	4077	3232	2526	1909	1519	919	756	405	399

Abbreviations: CI, confidence interval; TKA, total knee arthroplasty



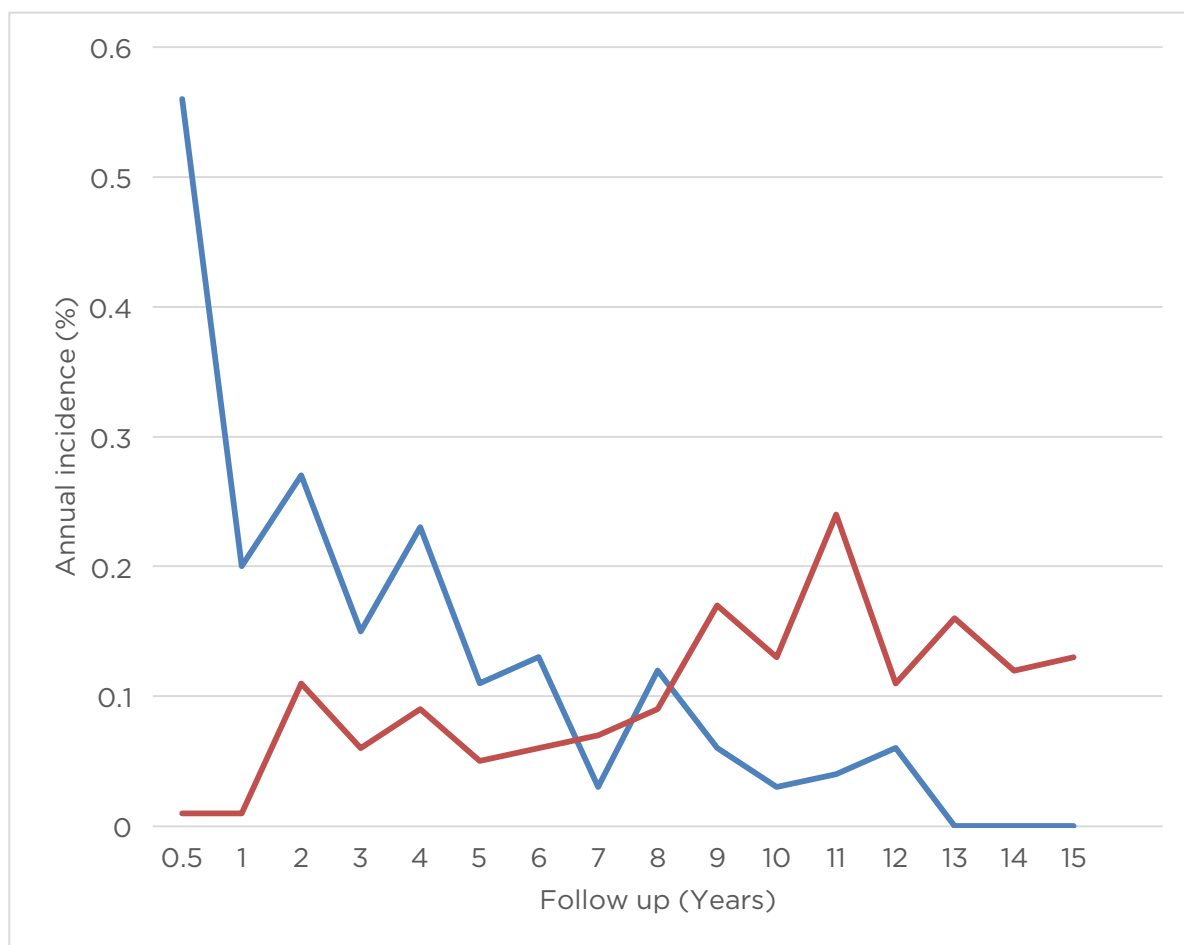


**Figure 6.2** Cumulative incidence of the five most common reasons for revision total knee arthroplasty.

There were 169 revision TKAs performed due to PJI. Eighteen had culture-negative PJI and three revision TKA procedures did not have component exchange (two had arthroscopic lavage and one patient with a monoblock tibial component underwent open debridement and lavage without component exchange). The cumulative incidence of PJI was 0.8% at 1 year, 1% at 2 years, 1.5% at 5 years, and 2% at 15 years after the index operation (Table 6.3, Figure 6.2).

Fifty-two revision TKA procedures were performed for aseptic loosening. Aseptic loosening and polyethylene wear combined were the primary causes for revision after 8 years from index surgery. The annual incidence of PJI was highest in the first 4 years post primary TKA, with aseptic loosening and polyethylene wear becoming the

most important after 8 years (Figure 6.3).



**Figure 6.3** Annual incidence of periprosthetic joint infection against aseptic loosening and polyethylene wear (blue line indicates periprosthetic joint infection, orange line indicates aseptic loosening and polyethylene wear).

The original cohort of 11,134 primary TKAs included 6276 knees in which the patella was not resurfaced. Forty-nine of these TKAs underwent secondary patella resurfacing for patellofemoral arthrosis. The cumulative incidence of secondary patella resurfacing was highest (0.5%) during the first 5 years after primary TKA (Figure 6.2).

Thirty-two revisions were performed for instability and 18 for stiffness/arthrofibrosis, with an incidence of 0.4% and 0.2%, respectively (Figure 6.2). Revision secondary to

stiffness tended to present early, with all 18 revisions performed within 4 years of the index surgery.

Ten revision TKAs were performed for reasons other than the nine modes of failure. There were two cases of inappropriate seating of the tibial component causing overhang and symptomatic soft tissue irritation, requiring revision of the tibial component. There were two cases of wound dehiscence, five of haematoma, and one of acute inflammatory synovitis that did not fulfil the diagnostic criteria for PJI.

For the remaining four revision TKA categories, there were nine cases of polyethylene wear, eight of periprosthetic fracture, seven of patellar maltracking, and three of disruption of the extensor mechanism (Table 6.3).

## **6.4 Discussion**

### **6.4.1 Contribution and significance**

Revision surgery following TKA has long been used as a marker of failure, and is the primary outcome used by joint registries worldwide<sup>1,213,214</sup>. However, such registries lack clinical and radiographic data, making accurate analysis of failure mechanisms difficult. Further, multiple studies show that joint registries have a poor ability to capture certain mechanisms of failure. The present study is unique in the literature in that it combined local hospital and national joint registry data to allow long-term follow-up in a large group of TKA patients and accurate clinical and radiographic analysis of failure mechanisms. Such a study is perhaps only possible in a small country like New Zealand, where each patient has a national health identifier to enable tracking when they move out of an area and surgeons were willing to collaborate and share clinical and radiographic data to allow accurate identification of the reasons for revision. In contrast with registry findings and previous reports from

tertiary revision centres<sup>1,202,215,216</sup>, we found PJI to be the dominant mechanism of failure in the first 15 years following primary TKA.

#### 6.4.2 Limitations

There are a number of limitations to this study. Firstly, while the national registry allowed capture of patients who had moved to other cities or had revision surgery at other institutions in New Zealand, any patients receiving revision surgery overseas would not have been captured. However, the average patient age at the time of primary TKA was 69 years in this study, and the likelihood of emigration in this age group would be low. Secondly, while we defined ‘failure’ as revision surgery involving addition or exchange of one or more components, we also included any reoperation due to confirmed PJI. While this would tend to increase the relative importance of PJI as a failure mechanism, only three of 169 patients with PJI did not undergo some form of component exchange (i.e., ‘revision’), and most surgeons would consider PJI a ‘failure’ regardless of how it was treated. Thirdly, there are no universally accepted criteria to define modes of failure. Registries in particular vary widely in this regard, with the number of recorded modes ranging from 8 to 33 across national registries<sup>217</sup>. For this reason, we used the method devised by Vince<sup>204</sup>, applying standardised criteria to define each mode of failure as objectively as possible. Further, registries have no defined criteria for PJI. Using the consensus criteria established by the Musculoskeletal Infection Society, we identified 18 culture-negative PJIs and three patients who had a PJI but were managed operatively without component exchange. Failure mechanisms in such patients would almost certainly be incorrectly identified by registry data capture mechanisms.

### 6.4.3 Importance of PJI as a failure mechanism

PJI was the dominant mechanism of failure, accounting for 47% of revision TKAs, and had an incidence of 1.0% at 2 years and 2.0% at 15 years. The second most common mechanism was aseptic loosening, with a 15-year incidence of 1.2%, accounting overall for 15% of all revision TKAs. This contrasts with previous studies from tertiary referral centres that report aseptic loosening to be the most common cause. Thiele et al and Sharkey et al reported that aseptic loosening contributed to 39.9% and 21.8% of their revisions<sup>216,218</sup>. Similarly, Fehring et al and Dalury et al found that 27% and 23.1% of their revisions were secondary to aseptic loosening<sup>201,219</sup>. Such reports are likely to underestimate the importance of PJI as a failure mechanism because they are based on data from referral centres. Treatment of PJI is often performed acutely, without time for transfer from a primary institution. More complex cases, such as those involving massive osteolysis or disruption of the extensor mechanism, are also more likely to be referred, potentially increasing their relative prevalence at referral centres. Such reports also include revisions of implants placed more than 15 years earlier, so may not reflect the mechanisms of failure of more modern prostheses, and would emphasise failure mechanisms that become more common with longer-term follow-up, such as aseptic loosening. For example, Sharkey et al reported that 41% of their patients were referred from outside institutions and time to revision ranged from 1 day to 30 years<sup>202</sup>. Thiele et al reported that a ‘substantial’ number of revision TKAs in their study were referrals from outer regions with incomplete baseline information and that the index surgery was performed before the year 2000 in 16% of their patients<sup>218</sup>. Similarly, in the report by Fehring et al, all revision TKAs were performed between 1986 and 1999, and most of these revisions were from primary cases performed in outlying areas<sup>201</sup>. In our study, the

availability of a known denominator of patients who had undergone primary TKA enabled us to calculate the incidence of each reason for revision, allowing more accurate analysis of the relative importance of each mechanism.

National joint registries in Australia, the UK, Sweden, and New Zealand report aseptic loosening to be the most common failure mechanism following primary TKA<sup>1,214,220</sup>. However, capture of revisions due to infection by registries is often poor. Lindgren et al reported that the Swedish Hip Arthroplasty Register had a capture rate of 67% for reoperation due to PJI following total hip arthroplasty<sup>221</sup>. Similarly, Zhu et al reported that the NZJR was 63% accurate in detecting reoperations for PJI (in both hip and knee arthroplasties) when compared with data from ICD-9/10 codes<sup>200</sup>. This is consistent with the findings of our study, which showed a 76% capture rate for revision TKA due to PJI. There are several potential reasons for this. Firstly, in our study, many revisions for PJI were performed in an acute setting outside normal hours, where different staffing levels may compromise protocols for reporting to national registries<sup>221</sup>. Secondly, registry data sheets are typically collected at the time of revision surgery before culture results are available. Thirdly, such data sheets do not apply standardised definitions of PJI, such as the Musculoskeletal Infection Society criteria used in this study. The importance given to aseptic loosening as a failure mechanism in both national registries and revision TKA series is reflected in technological efforts to improve the outcome of primary TKA, i.e., computer navigation, patient-specific instrumentation, use of cross-linked polyethylene, and uncemented fixation, all of which aim to reduce failure caused by aseptic loosening. This study suggests that future efforts should also place similar emphasis on reducing the risk of PJI.

#### 6.4.4 Importance of prophylaxis

We found that the highest incidence of PJI occurred within the first 2 years following primary TKA (1.0%) and that the annual incidence decreased to less than 0.2% after 5 years. Many studies have reported infection as the primary cause of early failure, contributing to 18%–27% of early TKA revisions<sup>201,215,216</sup>.

Preventing infection should be a key goal in efforts to improve patient outcomes following TKA. As highlighted in Chapter 3, the majority of PJIs occurring in the first two years following primary TKA are due to intra-operative contamination. Therefore, enhancing the effectiveness of prophylaxis has the potential to reduce the risk of PJI and have a significant impact on the overall failure rate of TKA.

#### 6.4.5 Conclusion

We found PJI to be the dominant failure mechanism in the first 15 years following modern TKA. Aseptic loosening remains an important cause of failure, particularly in younger patients. Therefore, efforts to improve the outcome following primary TKA should focus on these areas, particularly prevention of PJI.

This study was limited by its follow-up duration of only 15 years, and we found that the incidence of aseptic loosening and polyethylene wear increased significantly after 8 years. As the number of patients living with TKA implants increases, the proportion of those requiring revision TKA for any reason will also increase. Using national health care and demographic data, Kurtz et al projected that the demand for revision TKA will increase by 601% in the USA by 2030.<sup>2</sup> Revision TKA tends to have a higher risk of complications, including PJI, and the following chapter investigates the use of IORA in revision TKA procedures.

## **Chapter 7 Higher tissue concentrations of vancomycin with intraosseous regional prophylaxis in revision TKA: a randomised trial**

### **7.1 Preface**

Revision total knee arthroplasty (TKA) has a higher reported infection rate than primary TKA, so strategies aiming to prevent prosthetic joint infection (PJI), such as intraosseous regional administration (IORA) of antibiotic prophylaxis, are particularly relevant in this patient population. However, it is unclear whether intraosseous injection can be successfully performed in revision TKA, given that a metal component is already present in the tibia and may compromise the injection. Moreover, because revision TKA procedures take longer to perform, the tourniquet is often released and then reinflated, which may affect antibiotic tissue concentrations when using IORA.

The following section contains a reproduction of an article entitled ‘The John Insall Award: Higher tissue concentrations of vancomycin with intraosseous regional prophylaxis in revision TKA - a randomized trial’, accepted for publication in *Clinical Orthopaedics and Related Research* in June 2017.

The paper received the 2017 Knee Society John Insall award, presented in San Diego, USA. This is the first time this award has been received by a New Zealand researcher. It was presented at the 2014 open meeting of the Knee Society in Chicago and published as part of the Knee Society Symposium. The article was also presented at the American Association of Hip and Knee Surgeons Annual Meeting in Dallas, November 2016, and was one of 52 papers selected for a podium presentation out of 1600 submissions. *Clinical Orthopaedics and Related Research* has a 2016 impact factor of 3.127.



## 7.2 Manuscript in Press

### 7.2.1 Title page

The John Insall Award: Higher tissue concentrations of vancomycin with intraosseous regional prophylaxis in revision TKA: a randomized trial

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Clinical Trial Registration: NCT02020031

The institution of two of the authors (MS, HC) received funding from Vidacare, the manufacturer of the intraosseous needles used in this study.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.

Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

Procedures and sample collection were performed at the Mayo Clinic, AZ, USA. Samples were analysed at Canterbury Health Laboratories, Christchurch, New Zealand.

### 7.2.2 Abstract

*Introduction* In primary TKA, prophylaxis with low-dose vancomycin via intraosseous regional administration (IORA) achieves tissue concentrations 6–10 times higher than those achieved by systemic administration, and has been shown to provide more effective prophylaxis in an animal model. However, in revision TKA, the presence of a tibial implant may compromise injection via IORA and tourniquet deflation during a prolonged procedure may lower tissue concentrations. This study compared tissue concentrations of vancomycin administered intravenously (IV) with those achieved by IORA in revision TKA.

*Methods* Twenty patients undergoing aseptic revision TKA were randomized to receive 1 g of systemic IV prophylactic vancomycin (IV group) or 500 mg of vancomycin as a bolus injection into a tibial intraosseous cannula below an inflated thigh tourniquet (IORA group) before skin incision. Subcutaneous fat and bone samples were taken at regular intervals during the procedure. Tissue vancomycin concentrations were measured using high-performance liquid chromatography.

*Results* Tibial intraosseous injection was unaffected by the tibial implant in all patients in the IORA group. The mean operating time was 3.5 hours in both groups. The mean initial tourniquet inflation time was 1.5 hours in the IORA group, with a second inflation for a mean of 35 minutes during cementation. Overall, the mean concentration of vancomycin in fat samples was 4.1  $\mu\text{g/g}$  in the IV group versus 115  $\mu\text{g/g}$  in the IORA group ( $p<0.001$ ); tissue concentrations in femoral bone were 7.2  $\mu\text{g/g}$  in the IV group and 101  $\mu\text{g/g}$  in the IORA group. The vancomycin concentration in the final subcutaneous fat sample taken before closure was 5.3 times higher in the IORA group when compared with the IV group ( $p<0.001$ ). The intra-articular concentration of vancomycin in drain fluid samples on postoperative day 1 was similar between the two groups (mean 4.6  $\mu\text{g/g}$  in the IV group and 6.6  $\mu\text{g/g}$  in the IORA group;  $p=0.08$ ).

*Conclusion* Vancomycin via the IORA route is effective in revision TKA, resulting in tissue concentrations of vancomycin that are 5–20 times higher despite the lower dose than those achieved by systemic IV administration. High tissue concentrations were maintained throughout the procedure, despite a period of tourniquet deflation. IORA may be more clinically important in revision TKA, where the risk of infection is higher.

### 7.2.3 Introduction

Periprosthetic joint infection (PJI) is more common following revision total knee arthroplasty (TKA) than after primary surgery, with rates reported to be as high as 9%<sup>222</sup>. Such PJIs are more challenging to treat because revision implants often involve use of stems, cones, and augments that make thorough debridement or removal difficult. Prophylactic antibiotics reduce the risk of developing PJI<sup>41,151</sup>; however, bacterial resistance to common prophylactic antibiotics such as cephalosporins is increasing<sup>12,61,136</sup>. Vancomycin has been proposed as an alternative prophylactic antibiotic<sup>173</sup>, but requires a prolonged administration time, carries a risk of systemic toxicity, and risks promoting further antibiotic resistance. Low-dose prophylactic vancomycin via intraosseous regional administration (IORA) may mitigate these issues, and in primary TKA achieves tissue concentrations 6–10 times higher than those achieved by systemic administration<sup>175</sup>. In an animal model of TKA, IORA was also shown to provide more effective prophylaxis against PJI than systemic administration<sup>223</sup>.

In TKA, IORA involves intraosseous injection of prophylactic antibiotics into the proximal tibia after tourniquet inflation and before skin incision. In adults and in children, the distribution of an intraosseous injection is equivalent to that of an intravenous injection<sup>96</sup>, and is a reliable route for antibiotic administration in primary TKA<sup>158,175</sup>. However, in revision TKA, it is unclear if the presence of a tibial implant can alter the effectiveness of intraosseous injection. Further, revision TKA surgery is often prolonged and the tourniquet may be deflated during the procedure. This removes the restriction of the antibiotic to the circulation in the affected limb, potentially lowering tissue concentrations at the surgical site following deflation. Given that the goal of prophylaxis is to provide adequate concentrations of antibiotic

‘from the time of incision to the time of closure’<sup>39</sup>, any lowering of tissue concentrations may lessen the effectiveness of IORA in revision TKA.

This study was performed to compare tissue concentrations of vancomycin administered via the systemic intravenous (IV) route versus IORA in revision TKA, where the risk of PJI is higher. We aimed to answer these questions:

- 1) Does low-dose IORA consistently provide equal or higher tissue concentrations of vancomycin when compared with systemic IV administration in revision TKA?
- 2) Are tissue concentrations of vancomycin following IORA maintained for the duration of the revision TKA procedure, despite a period of tourniquet deflation?
- 3) Is there any difference in short-term complications between IORA and systemic IV administration of vancomycin in revision TKA?

#### 7.2.4 Methods

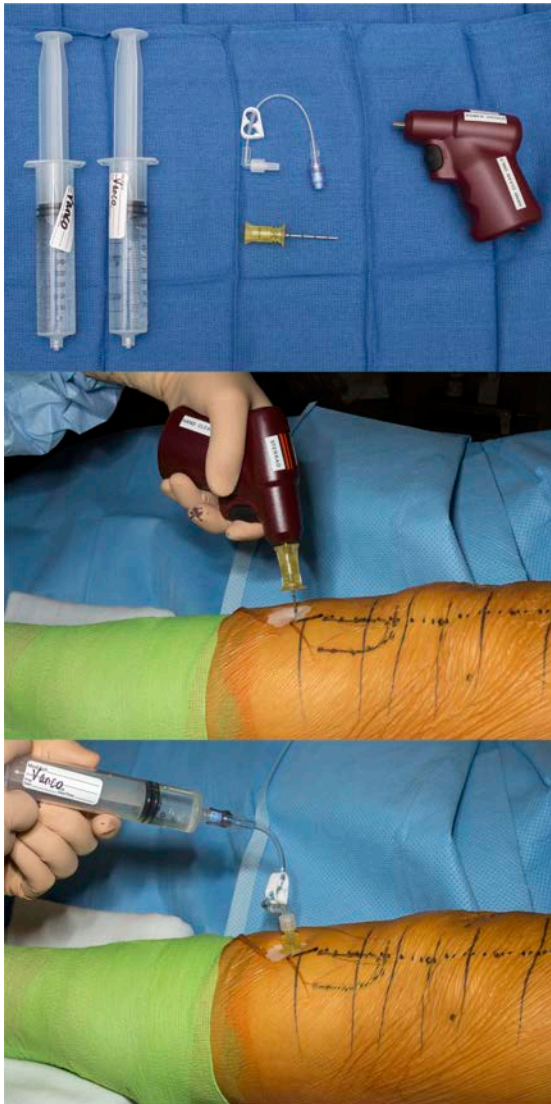
Patients undergoing unilateral revision TKA at a single tertiary institution were eligible for enrolment in this prospective, randomized controlled trial. Ethical approval was obtained from the institutional review board, and the trial and protocol were registered with ClinicalTrials.gov (Identifier: NCT02020031). The inclusion criterion was single-stage aseptic revision TKA with change of both tibial and femoral components. Exclusion criteria were previous or current PJI, known hypersensitivity to vancomycin, and significant cardiac or respiratory disease. All procedures were performed by either of two fellowship-trained arthroplasty surgeons (HDC, MJS).

Between January 2014 and April 2015, 22 patients were enrolled by a trained research nurse (DLR) in an outpatient setting. Patients were randomized to an IV group or an

IORA group using computer-generated random allocations placed in numbered, opaque, sealed envelopes. The IV group received 1 g of systemic IV prophylactic vancomycin as a one-hour infusion into an arm vein, timed to finish immediately prior to tourniquet inflation. The IORA group received 500 mg vancomycin in 150 mL of saline as a bolus injection via a tibial intraosseous cannula below an inflated thigh tourniquet immediately (<2 minutes) before skin incision (Figure 7.1).

Both groups received 2 g of systemic cefazolin 15 minutes prior to tourniquet inflation to ensure that all patients received effective antibiotic prophylaxis regardless of treatment allocation. Two patients were withdrawn from the study following an intraoperative decision not to proceed with a full revision of both components, leaving 20 patients for analysis (10 in the IV group and 10 in the IORA group, Table 7.1). All patients received a general anaesthetic combined with periarticular injection of local anaesthetic; 8 patients (4 in each group) also received a peripheral nerve block.

Revision TKA was performed using components from either of two suppliers (Stryker Inc, Mahwah, NJ, USA; Zimmer, Warsaw, IN, USA).



**Figure 7.1** Intraosseous injection performed after tourniquet inflation. Following injection, the needle is removed and the injection site is covered with Ioban before proceeding with skin incision and surgery.

**Table 7.1** Patient demographic and clinical data.

	<b>Systemic vancomycin 1 g (n=10)</b>	<b>IORA vancomycin 500 mg (n=10)</b>
<b>Sex</b>		
Male	5	3
Female	5	7
<b>Age (years)</b>	67.4 (54–82)	69.3 (43–83)
<b>Body mass index</b>	32.6 (22–42)	32.3 (26–42)
<b>First tourniquet time (min)*</b>	94 (85–108)	91 (89–96)
<b>Tourniquet deflation time (min)*</b>	37 (15–88)	61 (18–109)
<b>Second tourniquet time (cementation, min)*</b>	35 (25–48)	35 (21–52)
<b>Total procedure time (min, skin to skin)</b>	212 (177–282)	219 (167–263)
<b>ASA score</b>	2.4 (2–3)	2.7 (2–3)

Values are shown as the mean with range in parentheses. \*Excludes three patients (one in the intravenous group and 2 in the IORA group) in whom the tourniquet was used for 120 minutes and not reinflated. Abbreviations: ASA, American Society of Anesthesiologists; IORA, intraosseous regional administration

The revision TKA procedure was performed with the tourniquet initially inflated for exposure and implant removal, followed by deflation and then reinflation for cementation of the implant. Patients were monitored for clinical signs of red man syndrome, particularly after tourniquet deflation. An antihistamine was available for use if required. During the procedure, subcutaneous fat and bone samples (approximately 0.5 cm<sup>3</sup>) were taken at regular intervals until skin closure; a total of six fat samples and four bone samples were taken for each patient (Table 7.2). All bone samples were taken from the femur, distant from the tibial intraosseous injection



site. Tissue samples were stored at  $-90^{\circ}\text{C}$  until analysed. Vancomycin concentrations were determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using a technique that has been previously described and validated<sup>175,224</sup>. On postoperative day 1, intra-articular vancomycin concentrations were determined from a drain fluid sample. All patient samples were analysed in duplicate, and the laboratory analysis was carried out blinded as to group allocation.

#### *Power calculation*

Data from a previous randomised trial comparing vancomycin 250 mg via the IORA route versus vancomycin 1 g administered via a systemic route<sup>175</sup> showed mean ( $\pm$  standard deviation) vancomycin concentrations in subcutaneous fat at different collection points ranging from  $8.1 \pm 5.6 \mu\text{g/g}$  to  $19.4 \pm 11.7 \mu\text{g/g}$  in the IORA group and from  $2.4 \pm 1.5 \mu\text{g/g}$  to  $4.4 \pm 2.0 \mu\text{g/g}$  in the IV systemic group. Therefore, the concentration of vancomycin was approximately 3.3 times higher in the IORA group. In bone samples, the difference in vancomycin concentration was 4.5-fold. Using these data, an a priori power analysis calculated 10 patients in each arm would provide  $>90\%$  statistical power to detect the expected fold difference in subcutaneous fat and bone concentrations at a 5% significance level using IORA doses that were 25% of the systemic dose (250 mg vs 1 g). Due to the more prolonged nature of revision surgery, we chose to use a higher IORA dose of 500 mg, so this power analysis represents a conservative estimate and likely overestimated the number of patients required.

**Table 7.2** Mean tissue concentrations of vancomycin at each sample point.

Sampling time	Systemic vancomycin 1 g		IORA vancomycin 500 mg		p-value
	Time (min)	Concentration ( $\mu\text{g/g}$ )	Time (min)	Concentration ( $\mu\text{g/g}$ )	
Subcutaneous fat 1 (S1)	2 (1)	3.2 (1.8)	3 (4)	94.1 (69)	<0.0001
Subcutaneous fat 2 (S2)	34 (7)	5.0 (2.9)	30 (6)	88.3 (131)	<0.0001
Subcutaneous fat 3 (S4)	50 (10)	4.2 (2.5)	48 (12)	69.4 (50)	<0.0001
Subcutaneous fat 4 (S6)	115 (30)	4.7 (2.6)	119 (30)	173 (445)	<0.0001
Subcutaneous fat 5 (S8)	145 (34)	4.0 (2.2)	151 (36)	249 (639)	<0.0001
Subcutaneous fat 6 (S10)	180 (64)	3.6 (2.5)	193 (82)	18.2 (11.6)	<0.0001
Bone 1 (S3)	34 (7)	7.9 (5.7)	30 (6)	90.7 (77)	<0.0001
Bone 2 (S5)	50 (10)	8.6 (5.9)	48 (12)	193 (191)	<0.0001
Bone 3 (S7)	115 (30)	5.0 (2.3)	119 (30)	59.8 (63)	<0.0001
Bone 4 (S9)	145 (34)	7.1 (4.4)	151 (36)	62.9 (62)	<0.0001

Times are given as minutes after surgical incision. Differences in mean tissue concentrations between the two groups were statistically significant ( $p < 0.0001$ ) for all comparison points after adjustment by sex, age, body mass index, American Society of Anesthesiologists score, interaction between groups, and time from incision.

There are limited data on the pharmacodynamics of vancomycin when used for surgical prophylaxis; however, in models of treatment of infection, the pharmacokinetic-pharmacodynamic parameter most predictive of efficacy is the area under the concentration-time curve (AUC) divided by the minimum inhibitory concentration (MIC)<sup>59</sup>. Therefore, increased tissue concentrations can be expected to enhance the effectiveness of antibiotic prophylaxis with vancomycin, especially when the MIC is  $\geq 1$  mg/mL, as for MRSA and CoNS<sup>153</sup>. An animal study of vancomycin via the IORA route supports the expectation that higher concentrations will enhance effectiveness<sup>223</sup>, so the differences used in our power analysis are likely to be clinically relevant.

#### *Statistical analysis*

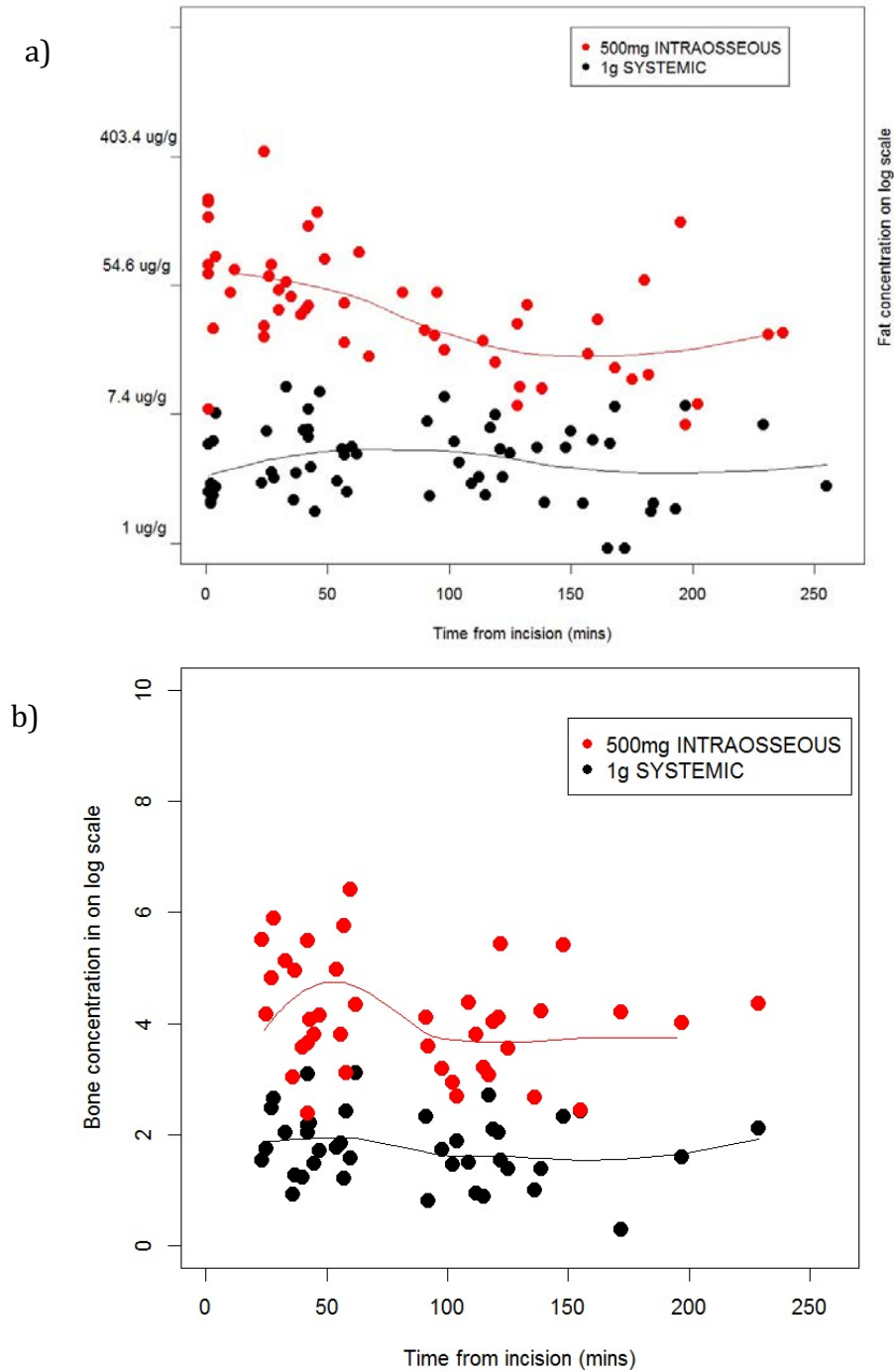
Means, standard deviations, and 95% confidence limits were calculated for the concentrations in the different samples. Coefficients of variation (CVs) in concentration level were summarised at each surgical step for comparison between the two groups. Repeated measures analysis of variance was used to compare the average concentration levels across time between groups adjusted for body mass index, age, and duration of the surgical procedure. The Shapiro-Wilk test was used to assess the normality of the residuals. Adverse events were recorded by contingency table.

#### 7.2.5 Results

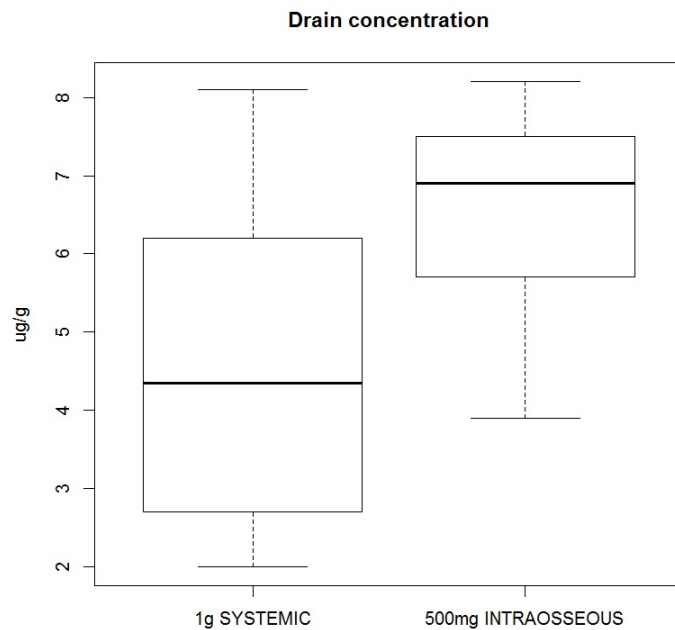
Tibial intraosseous injection was successful in all patients in the IORA group and was unaffected by the presence of a tibial implant. The mean tissue concentration of vancomycin in fat samples was 4.1  $\mu\text{g/g}$  in the IV group and 115  $\mu\text{g/g}$  in the IORA group ( $p < 0.001$ ; (Table 7.2). The overall mean tissue concentration of vancomycin in

femoral bone was  $7.2 \mu\text{g/g}$  in the IV group and  $101 \mu\text{g/g}$  in the IORA group ( $p < 0.001$ ; Figure 7.2).

The mean procedure duration was 3.5 hours in both groups. The mean duration of initial tourniquet inflation was 1.5 hours, with a second inflation for a mean 35 minutes during cementation of the implant. In three patients (one in the IV group and two in the IORA group), the tourniquet was used for 120 minutes and not reinflated. The mean ( $\pm$  standard deviation) vancomycin concentration in the final subcutaneous fat samples taken prior to closure was 5.3 times higher in the IORA group than in the IV group ( $18.2 \pm 11.6 \mu\text{g/g}$  versus  $3.6 \pm 2.5 \mu\text{g/g}$ ;  $p < 0.001$ ). The mean intra-articular concentration of vancomycin on postoperative day 1 was similar in both groups ( $4.6 \pm 2.1$  [range 2.0–8.1]  $\mu\text{g/mL}$  in the IV group and  $6.6 \pm 1.4$  [range 3.9–8.2]  $\mu\text{g/mL}$  in the IORA group;  $p = 0.07$ , Figure 7.3).



**Figure 7.2** Scatterplots showing concentration of vancomycin (A) Scatterplot showing tissue concentration of vancomycin in subcutaneous fat at various time points following incision. (B) Scatterplot showing tissue concentration of vancomycin in bone at various time points following incision. Note the scales are logarithmic.



**Figure 7.3** Graph showing the intra-articular concentration of vancomycin in drain fluid drawn the morning following surgery. The central line represents the median; the box represents the 25% and 75% quartiles; the whiskers represent the range.

No patient in either group developed symptoms of red man syndrome. There were no thromboembolic complications or deep or superficial infections in either group. One patient in the IORA group developed a foot drop postoperatively, and underwent exploration and decompression of the common peroneal nerve 3.5 months postoperatively. There were no other reoperations in either group.

#### 7.2.6 Discussion

PJI is more common after revision surgery than after primary TKA<sup>222,225</sup>, and has been reported to account for over 44% of failures following revision TKAs<sup>226</sup>. This study found that low-dose vancomycin via the IORA route was effective in the setting of revision TKA, consistently providing tissue concentrations that were 5–20 times

higher than those achieved by systemic IV administration for the duration of the procedure. Measures aiming to reduce PJI are particularly relevant in revision TKA, given both the higher incidence of PJI and the associated treatment difficulties in the presence of revision implants.

This study has several limitations. Firstly, the power analysis was based on the tissue concentration of vancomycin and not the subsequent development of PJI. The reason for this was that the numbers required to detect a difference in incidence of PJI between the two techniques would be prohibitive. However, the pharmacokinetic-pharmacodynamic parameter most predictive of the efficacy of vancomycin is the area under the concentration-time curve divided by the minimum inhibitory concentration (AUC/MIC)<sup>59</sup>. Therefore, higher tissue concentrations are likely to enhance the effectiveness of vancomycin. This observation is supported by a recent study in a murine model of TKA that found low-dose vancomycin via the IORA route to be significantly more effective than standard-dose systemic vancomycin for prevention of PJI<sup>223</sup>. Secondly, we used a standard 1 g dose of vancomycin, whereas some authors have advocated weight-based dosing (e.g., 15 mg/L) to ensure adequate tissue concentrations are achieved<sup>149</sup>. However, a 1 g dose for orthopaedic prophylaxis is commonly reported<sup>153,154,173</sup>, and given the magnitude of the difference seen, a weight-based systemic vancomycin dose would have been unlikely to alter our findings. Finally, while we found no difference in complication rates between the two groups, the number of patients was relatively small. Two previous studies of IORA in primary TKA also found no increase in complications<sup>158,175</sup>. Further, administration of vancomycin and other antibiotics via the IORA route is well described in the treatment of equine septic arthritis in the veterinary literature, again without reported complications<sup>101,104</sup>.

We found the intraosseous injection to be successful in all patients despite the presence of a tibial implant. Rapid distribution through the circulation of the limb following injection was evident from the very high vancomycin concentrations seen in the first tissue sample, which was taken within minutes of the IORA injection. Regional administration of prophylactic antibiotics in TKA has been previously investigated by de Lalla et al, who compared intravenous regional administration (IVRA) of teicoplanin 400 mg via a foot vein with teicoplanin 800 mg administered systemically<sup>76</sup>. They reported tissue concentrations 2–10 times higher in the IVRA group. They evaluated the IVRA protocol in 250 patients who underwent TKA and reported a 0% PJI rate<sup>75</sup>. In the present study, we used the intraosseous route for regional administration of antibiotic prophylaxis, and the main advantages of IORA over IVRA were reliability and speed. Cannulation of a foot vein can be difficult in obese patients, and involves exposing an area typically covered in sterile drapes. In contrast, intraosseous injection using modern equipment is rapid and reproducible<sup>90</sup>, and the fluid and medication injected travels directly into the intravascular space in a manner equivalent to intravenous injection in both adults and children<sup>96</sup>. A small area of cancellous bone is all that is required, and the presence of tibial implants in the present study did not alter the effectiveness of IORA. In the setting of severe proximal tibial bone loss, the IORA technique may not be feasible, although intraosseous injection can also be performed via the distal tibia, distal femur, or calcaneus<sup>227-229</sup>.

We found higher tissue concentrations of vancomycin throughout the duration of the surgical procedure in the IORA group, despite intraoperative release of the tourniquet. Revision TKA procedures are often prolonged, and inflation of the tourniquet for the entire procedure risks nerve or ischaemic injury. Once the tourniquet is released, vancomycin levels at the operative site can be expected to decrease, although prior to



this study the rate at which they did so was unclear. We found that intra-articular vancomycin levels on postoperative day 1 remained above the typical MIC reported for methicillin-resistant *Staphylococcus aureus* ( $1.0 \mu\text{g/mL}$ ) and coagulase-negative staphylococci ( $2.0 \mu\text{g/mL}$ )<sup>230</sup>. There is likely to be a depot effect of the initial high tissue concentrations, causing the antibiotic to be released gradually into the systemic circulation after tourniquet deflation<sup>101</sup>. A potential weakness of the IORA technique is that many surgeons routinely continue antibiotics for 24 hours postoperatively, so further systemic vancomycin doses would still be required after IORA. However, randomised trials have shown no difference in infection rates between a single preoperative antibiotic dose and continuing antibiotics for 24 hours postoperatively<sup>84,162</sup>. This supports Burke's original theory of antibiotic prophylaxis, which states that adequate antibiotic tissue concentrations must be maintained from the time of incision to the time of closure, when contamination is occurring<sup>39</sup>. This outcome was clearly achieved in the IORA group in this study, despite the use of a lower vancomycin dose. The lower dose allows bolus administration instead of a prolonged systemic infusion, and minimises the risk of systemic complications such as red man syndrome<sup>160</sup> and nephrotoxicity<sup>146</sup>. The use of IORA also avoids the need for preoperative coordination to ensure appropriate timing of administration of prophylaxis, considering that most hospital protocols require infusion of 1 g of vancomycin when given systemically over 1–2 hours, to avoid red man syndrome.

The very high antibiotic concentrations seen with IORA raises the question of the potential for local toxicity. The effect of high antibiotic concentrations on musculoskeletal cells has been investigated in vitro in the context of local delivery of antibiotic-impregnated cement for the treatment of bone or periprosthetic infection. Antoci et al reported minimal toxicity to osteoblastic and chondroblastic cell lines at

vancomycin concentrations of 250  $\mu\text{g/mL}$ , with a significant reduction in cellular proliferation becoming apparent at concentrations above 2000  $\mu\text{g/mL}$ <sup>231</sup>. Similarly, Rathbone et al reported vancomycin to be the least toxic of 21 antibiotics tested, with no effect on survival of osteoblasts or metabolic function until exposure to concentrations in excess of 2000  $\mu\text{g/mL}$  for 10–14 days<sup>80</sup>. The IORA tissue concentrations in our study were well below these levels, and the duration of exposure shorter, suggesting local toxicity is unlikely to occur.

In conclusion, this study found low-dose IORA vancomycin to be effective in revision TKA, resulting in tissue concentrations of vancomycin 5–20 times higher than those achieved by systemic IV administration. The high tissue concentrations of vancomycin following IORA were maintained throughout the procedure and on the first postoperative day, despite a period of tourniquet deflation during surgery. Use of IORA may be more clinically important in revision TKA, when the risk of infection is higher than for primary TKA.

#### Acknowledgements

The authors would like to thank Debra L Ryan, research assistant, for her help in patient recruitment, sample management, and data

## 7.3 Discussion

### 7.3.1 Contribution and significance

This study found that IORA provided very high tissue concentrations of antibiotics in the more complex setting of revision TKA. This is relevant to clinical practice, considering that the infection rate following revision TKA is higher than primary procedures. The consequences of infection are also more serious, as treatment often requires removal of implants. Revision implants typically include stems and augments, and removal is extremely challenging and may cause significant tissue damage and bone loss.

Therefore, revision TKA is likely to be a key indication for surgeons preferring to use IORA selectively because of concerns regarding, e.g., the additional tourniquet time and needle cost associated with intraosseous injection. Until this study, it was unclear if IORA would be possible in revision procedures. The presence of a tibial implant could compromise intraosseous injection and the prolonged nature of the procedure with a period of tourniquet deflation may cause antibiotic concentrations to fall to low levels. The findings of this study indicate that such concerns are unwarranted: intraosseous injection was successful in every case, and tissue levels were very high throughout the procedure.

A limitation of this study is that there were only 10 patients in the IORA group. Revision TKA cases are extremely heterogeneous, and can differ significantly in indications and complexity. It is likely that intraosseous injection would not be possible in cases with severe proximal tibial bone loss. In this situation, the distal tibia, distal femur, or calcaneus may offer alternative access points <sup>227-229</sup> ..

### 7.3.2 Further antibiotic doses

This study also found that intra-articular concentrations of vancomycin, as measured by drain fluid samples, remained above the typical MIC for *S. aureus* on the morning following surgery. This is likely attributable to the depot effect of the high concentrations in the limb achieved at the time of surgery, and would support the use of a single preoperative IORA dose. Whenever this issue has been examined, the preoperative dose has been found to be the most crucial, and further doses of antibiotics beyond the duration of surgery do not alter the rate of infection.<sup>56,57,84</sup> The American Academy of Orthopaedics recommends that antibiotics should be discontinued within 24 hours of the end of surgery<sup>45</sup>. Multiple studies have showed that extended prophylaxis after surgery increases the risk of development of resistant organisms<sup>52,57</sup>, so a single preoperative dose is most appropriate when using the IORA technique.

There is evidence that additional intraoperative antibiotic doses may be beneficial for reducing the risk of infection during very prolonged procedures<sup>232</sup>. Steinberg et al investigated 4472 cardiac and orthopaedic procedures and found that, in cases lasting over 4 hours, 2 (1.8%) of 112 patients who were re-dosed intraoperatively developed infection compared with 22 (5.5%) of 400 who were not re-dosed (odds ratio 3.1;  $p=0.06$ ).<sup>50</sup> In a randomised trial of 801 patients undergoing clean-contaminated operations, Scher et al compared 1 g of cefazolin preoperatively versus 1 g of cefazolin preoperatively with another dose 3 hours later. Infection rates were no different for surgeries lasting up to 3 hours; however, for those lasting more than 3 hours, the group that received only the single preoperative dose had a higher infection rate than those who also received the second cefazolin dose (6.1% versus 1.3%;

$p < 0.01$ ).<sup>233</sup> Such additional intraoperative doses will maintain adequate tissue antibiotic concentrations while the wound remains open and contamination is occurring. In our study, we found tissue concentrations remained elevated in the IORA group even during the prolonged revision procedures. However, because the number of cases was limited, in the case of extremely long procedures (more than 3–4 hours) a further systemic dose of either vancomycin or cefazolin would be prudent.

## **Chapter 8 Conclusion**

### **8.1 Summary**

This thesis has shown that infection is the main cause of failure following modern total knee arthroplasty (TKA) and that the bacteria causing infection are usually resistant organisms. It has introduced a novel form of prophylaxis using intraosseous regional administration (IORA), and showed that this technique provides tissue concentrations of cefazolin and vancomycin that are 5–10 times higher than those achieved by standard systemic administration. This research has shown that the IORA technique provides more effective prophylaxis against infection in a murine model of TKA, and that it can also be applied successfully in the more complex setting of revision TKA.

### **8.2 Contribution of this thesis**

While the techniques of intraosseous injection and regional administration are not new, this is the first time the two have been combined to provide antibiotic prophylaxis prior to TKA. Intraosseous administration is reliable and feasible, allowing simple translation into clinical practice. Many surgeons worldwide now use the IORA technique.

### **8.3 Recognition of the work in this thesis**

John Insall Award 2017

*Awarded annually by the North American Knee Society to the best work on a clinical subject or outcomes report in knee surgery. Presented at the Knee Society Open Meeting, American Academy of Orthopaedic Surgeons, San Diego, CA, USA, 2017*

For paper entitled “Higher tissue concentrations of vancomycin with intraosseous regional prophylaxis in revision TKA: a randomized trial”

Top 5 Paper Award - ICJR East, New York 2013

*Awarded at the International Congress for Joint Reconstruction Meeting, New York*

For paper entitled “Higher concentrations of vancomycin with low-dose intraosseous regional prophylaxis in TKA: a randomised trial”

Mark Coventry Award 2013

*Awarded annually by the North American Knee Society to the best work on a surgical technique in knee surgery. Presented at the Knee Society Open Meeting, American Academy of Orthopaedic Surgeons, Chicago, IL, USA, 2013*

For paper entitled “Higher concentrations of vancomycin with low-dose intraosseous regional prophylaxis in TKA: a randomised trial”

Louis Barnett Prize 2011

*From College website: ‘This Prize was established by the New Zealand Committee of the College in 1962 and has been awarded over the years to many prestigious New Zealand surgeons. It is awarded annually and commemorates Sir Louis Barnett CMG, the first New Zealander to become President of this College.’*

For paper entitled “Intraosseous administration of prophylactic antibiotics: a randomised controlled trial”

Auckland Orthopaedic Society Academic Meeting, 2010

*Registrar Winner - Best Presentation*

For paper entitled “Intraosseous administration of prophylactic antibiotics”

#### **8.4 Future directions**

While the results of the animal study in this thesis were promising, it is unclear whether IORA will result in a reduction in infection rates in clinical practice.

Additionally, while no complications were seen with use of IORA in this thesis, the total number of patients receiving IORA was small. Therefore, larger cohort studies are required to identify any rare complications that may be associated with IORA prophylaxis and to provide an indication of the deep infection rate, which can then be compared with that in historical controls. Currently, a multicentre prospective cohort study is underway combining data on the incidence of PJI following TKA with use of IORA prophylaxis from North Shore Hospital in Auckland and the Mayo Clinic in Scottsdale, AZ, USA.

Further research into the use of IORA in patients at higher risk of infection may also be beneficial. This thesis identified patients undergoing revision TKA as an at-risk subgroup; however, surgeons interested in using IORA prophylaxis selectively in primary TKA may target other risk factors for PJI. For example, obesity is a known risk factor for infection, and multiple studies have shown an association between an elevated body mass index and increased risk of PJI in TKA<sup>26,234,235</sup>. This may at least in part be because systemic prophylactic antibiotics may not reach adequate tissue concentrations in obese patients, particularly when vancomycin is used.<sup>149,236</sup>

Therefore, IORA may be ideal for use in this patient subgroup, but the pharmacokinetics of IORA in obese patients is unknown. A randomised controlled trial is currently underway at North Shore Hospital in Auckland comparing tissue



concentrations of vancomycin in obese patients (body mass index >35) undergoing TKA.

In conclusion, IORA shows promise as a means of enhancing the effectiveness of antibiotic prophylaxis in TKA. It is hoped that the information presented in this thesis will provide a basis for further clinical research into this technique, with the goal of preventing infection and enhancing outcomes for patients undergoing TKA.

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**Appendix 1 ICD-9 and ICD-10 procedure codes searched in Chapter 2**

4930300	Arthrotomy of hip
4931200	Excision arthroplasty of hip
4932400	Revision of total arthroplasty of hip
4934600	Revision of partial arthroplasty of hip
4936000	Arthroscopy of hip
4950001	Arthrotomy of knee
4951500	Removal of knee prosthesis
4952700	Revision of total arthroplasty of knee
4953000	Revision of total arthroplasty of knee with bone graft to femur
4953001	Revision of total arthroplasty of knee with bone graft to tibia
4953300	Revision of total arthroplasty of knee with bone graft to femur and tibia
4955400	Revision of total arthroplasty of knee with anatomic specific allograft
4955700	Arthroscopy of knee
4955800	Arthroscopic debridement of knee
9056200	Patella resurfacing



## Appendix 2 Editorial by Charalampos G. Zalavras MD, PhD ‘CORR Insights’<sup>168</sup>.

Clin Orthop Relat Res (2015) 473:3585–3587 / DOI 10.1007/s11999-015-4521-5

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Published online: 21 August 2015  
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# CORR Insights

## CORR Insights®: Regional Intraosseous Administration of Prophylactic Antibiotics is More Effective Than Systemic Administration in a Mouse Model of TKA

Charalampos G. Zalavras MD, PhD

### Where Are We Now?

Despite the best preoperative and intraoperative practices, contamination of the surgical

*This CORR Insights® is a commentary on the article “Regional Intraosseous Administration of Prophylactic Antibiotics is More Effective Than Systemic Administration in a Mouse Model of TKA” by Young and colleagues available at: DOI: 10.1007/s11999-015-4464-x.*

The author certifies that he, or a member of his immediate family, has no funding or commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

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This *CORR Insights*® comment refers to the article available at doi:10.1007/s11999-015-4464-x.

site may be unavoidable due to airborne or skin bacteria. Prophylactic antibiotics can help inhibit or kill contaminating bacteria, thereby preventing bacterial adherence to the arthroplasty implants, biofilm formation, and infection [7]. Additionally, systemic antibiotic prophylaxis has been shown to reduce infections in orthopaedic procedures [5, 6] and is part of well-established guidelines [2]. However, optimal antibiotic selection remains unclear. Cefazolin and cefuroxime are the recommended antibiotics, unless the patient has  $\beta$ -lactam allergy, in which case clindamycin or vancomycin should be used [2]. The increasing prevalence of infections by resistant microorganisms, such as methicillin resistant *Staphylococcus aureus* (MRSA), has raised the

question whether prophylaxis with vancomycin is necessary. No definite criteria exist, but vancomycin should be considered for patients at high risk for infection, such as patients colonized with MRSA or treated in institutions with recent MRSA outbreaks [7].

The protective effect of antibiotics depends on achieving adequate local tissue levels, but systemic administration of high doses of antibiotics may be limited by associated adverse effects. For this reason, local antibiotic delivery becomes an attractive option that could achieve high local levels while avoiding systemic toxicity. Antibiotic-impregnated cement has been widely employed in arthroplasty procedures and has been shown to reduce revisions due to infection in total hip arthroplasty [4, 5]. On the other hand, the benefit of adding antibiotics to the cement in TKA has not been conclusively demonstrated [1].

Intraosseous regional administration (IORA) of antibiotics was recently

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### Appendix 3. Revision IORA study outline (Chapter 7)

Event (approximate time)	IORA group	IV group
-90 minutes		<b>1 g systemic IV vancomycin infusion</b>
-10 minutes	<b>1 g systemic IV cefazolin injected</b>	<b>1 g systemic IV cefazolin injected</b>
-1 minute	Routine preparation and draping	Routine preparation and draping
<b>Tourniquet inflation</b> 0 minutes	Exsanguination and tourniquet inflation	Exsanguination and tourniquet inflation
1 minute	<b>500 mg intraosseous vancomycin</b> (injected into tibial cannula)	--
<b>Surgery commences</b> 2 minutes	Skin incision	Skin incision
<b>Post incision</b> 2 min	First sample – subcutaneous fat	First sample – subcutaneous fat
<b>Femoral component removed</b> 15–25 min	Second and third samples – fat and bone	Second and third samples – fat and bone
<b>Tibial component removed</b> 40–60 min	Fourth and fifth samples – fat and bone	Fourth and fifth samples – fat and bone
<b>Trialling of implant</b> 70–100 min	Sixth and seventh samples – fat and bone	Sixth and seventh samples – fat and bone
<b>Cementing of implant</b> 120 min	Eighth and ninth samples – fat and bone	Eighth and ninth samples – fat and bone
<b>Prior to closure</b> 150+ min	Tenth sample - fat	Tenth sample - fat
<b>Surgery complete</b>	Tourniquet deflation	Tourniquet deflation
<b>Next morning</b>	Drain sample	Drain sample