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Microorganisms in Presumed Aseptic Revision Hip and Knee Arthroplasty

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Abstract

The role and clinical significance of microorganisms in presumed aseptic revision total hip (THA) and knee arthroplasty (TKA) is unclear. The primary aim of this thesis was to determine the prevalence and infection-free survival of presumed aseptic revisions with unexpected positive intraoperative cultures (UPC) by analyzing the largest cohorts of UPC in the literature. Secondarily, a prospective pilot study using modern molecular techniques with an emphasis on stringent control of contamination was designed to determine how frequently microorganisms are present on implants of presumed aseptic revisions, as well as their location and association with reason for revision.

The prevalence of UPC was approximately 10%, the infection-free survival is encouraging, and the infection-free survival from the same UPC microorganism is outstanding. Patients with ≥2 UPC or a single UPC treated with antibiotics were more likely to have recurrent infection caused by the UPC microorganism. Patients with a single UPC and no other signs of infection do not require antibiotic treatment.

The rate of UPC in the prospective molecular pilot study was also approximately 10% and we hypothesize that microorganisms will frequently be found on implants of ‘aseptic’ failures and associated with location and reason for revision.

Keywords

Total hip arthroplasty, total knee arthroplasty, revision, revision hip arthroplasty, revision knee arthroplasty, aseptic, aseptic revision, unexpected positive cultures, microorganisms, polymerase chain reaction, PCR, periprosthetic infection, prevalence, survival, outcomes

Lay Summary

Over 1-million total hip (THA) and knee replacements (TKA) are performed in North America every year. Unfortunately, about 12% of these fail by the 10-year mark and require revision surgery to treat. Infection is a common reason for failure, however, there is no perfect test to diagnose infection. This can lead to the problem of unexpected positive bacterial cultures (UPC) in revisions done for non-infected reasons (loosening, instability, others). This is a problem because the surgical treatment of infected versus non-infected failure differs greatly. How often UPC occurs in presumed non-infected revisions is unclear. The optimal treatment and outcomes for these patients is also unclear.

The first goal of this thesis was to study UPC in the largest group of presumed non-infected revision THA and TKA patients to date. We found that about 10% of presumed 'non-infected' revisions have UPC. Most patients with UPCs did well and only a small number required more surgery for infection-related failure. Patients with a higher number of UPCs and those that were deemed to require antibiotic treatment were more likely to have infection-related failure caused by the same UPC bacterial microorganism. Lastly, patients with only one UPC and no other signs of infection do not require antibiotic treatment.

Furthermore, it is now suspected that a large number of 'non-infected' loose or failed implants are actually undiagnosed infection because modern molecular methods have identified bacterial microorganisms on some failed implants and a proportion of known infections never grow a bacterial microorganism.

Therefore, the second goal of this thesis was to design a modern molecular gene sequencing study to identify how often and what type of bacterial microorganisms are on THA/TKA implants that required revision surgery for 'non-infection' causes. We expect to show that implants are frequently contaminated with bacterial microorganisms, and that bacterial microorganisms are associated with certain locations of failed joint implants and the reason for failure. These results will provide an important steppingstone to develop future studies that can determine the role and significance of microorganisms in presumed 'non-infected' THA and TKA failure, which we think may be greatly underestimated.

Co-Authorship Statement

Chapter 1	Michael Neufeld – Sole author
Chapter 2	Michael Neufeld – Sole author
Chapter 3	Michael Neufeld – Study conceptualization, study design, data collection, statistical analysis, manuscript preparation Brent Lanting – Study design, manuscript preparation Edward Vasarhelyi – Study design, manuscript preparation
Chapter 4	Michael Neufeld – Study conceptualization, study design, data collection, statistical analysis, manuscript preparation Brent Lanting – Study design, manuscript preparation Edward Vasarhelyi – Study design, manuscript preparation
Chapter 5	Michael Neufeld – Study design, patient recruitment, sample/data collection, chapter preparation Mathew Teeter – Study conceptualization, study design, chapter edits Brent Lanting – Study design, chapter edits Edward Vasarhelyi – Study design, chapter edits Jeremy Burton – Study design, chapter preparation and edits
Chapter 6	Michael Neufeld – Sole author

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List of Abbreviations

16S rRNA	16S ribosomal ribonucleic acid
AF	Aseptic failures
AL	Aseptic loosening
BMI	Body mass index
ICM	International consensus meeting
IDSA	Infectious Disease Society of America criteria
CNS	Coagulase-negative *Staphylococcus* species
CRP	C-reactive protein
C. acnes	*Cutibacterium acnes*
DAIR	Debridement, antibiotics, and implant retention
DDH	Developmental dysplasia of the hip
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EMR	Electronic medical records
ESR	Erythrocyte sedimentation rate
ISF	Implant sonication fluid
IV	Intravenous
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
MSIS	Musculoskeletal Infection Society
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MSSE	Methicillin-sensitive *Staphylococcus epidermidis*

OA	Osteoarthritis
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PJI	Periprosthetic joint infection
PO	Oral route
RDP	Ribosomal Database Project
REB	Research ethics board
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
SONK	Spontaneous osteonecrosis of the knee
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
UPC	Unexpected positive intraoperative cultures
USA	United States of America

Chapter 1

1. Introduction and Literature Review

1.1 Total Hip and Total Knee Arthroplasty

Arthritis of the hip and knee are among the most symptomatic and disabling of all joints(1). Hip and knee arthritis is characterized by pain and stiffness that results in substantial loss of function and quality of life(2). Total hip arthroplasty (THA) and total knee arthroplasty (TKA) are the modern treatment for symptomatic, end-stage arthritis of these joints when conservative measures fail. In this patient population, THA and TKA are highly successful at relieving pain, restoring function, and improving quality of life(3,4). In fact, total joint replacement is amongst the most successful and cost-effective treatments in all of medicine, and THA has been labelled as “the operation of the century”(5).

1.1.1 Indications for Total Hip and Knee Arthroplasty

The most common etiology for both THA and TKA is primary osteoarthritis (OA), accounting for more than 90-98% of patients(3,4,6). Other less frequent aetiologies include post-traumatic osteoarthritis, inflammatory arthritis, osteonecrosis, fracture, congenital abnormalities such as development dysplasia, childhood conditions such as a slipped capital femoral epiphysis or Perthes disease, or sequelae from a remote septic native joint arthritis(3,4,6).

Diagnosis is based on a detailed history, physical examination, x-rays, and occasionally additional tests such as laboratory studies or advanced imaging modalities. Initial management for OA, the most common etiology, should be conservative and include patient education, activity modification, weight loss, targeted physiotherapy, non-narcotic pain medication, injections, and the use of mobility aids when applicable(7,8).

There are no treatments currently available that can prevent or reverse OA. When hip or knee arthritis leads to unacceptable pain, loss of function, and reduced quality of life despite conservative or less invasive surgical treatments, the patient is a candidate for THA/TKA. Traditional indications were limited to very elderly patients with severe-end stage OA, pain, and

disability because of concerns of implant longevity. However modern studies have supported the expansion of these indications to include younger patients and those with less than end-stage OA or disability(3,4,9).

1.1.2 Epidemiology and Economic Impact of Arthritis

Arthritis is a leading cause of disability globally that causes significant societal burden, as well as physical and psychological suffering to affected individuals(2,10,11). OA is the leading cause of physical disability in Canada affecting over 4.6 million people and costing over $33 billion annually in healthcare costs and lost productivity(12,13). The prevalence of hip and knee OA is projected to increase substantially in North America and globally, in part due to an ageing population and increasing obesity(11,14). This has important economic implications, with the economic burden of OA in Canada projected to exceed $405 billion by the year 2020(12).

1.1.3 Epidemiology of Total Hip and Knee Arthroplasty

THA and TKA are very common procedures globally and the demand is increasing rapidly(3,4). In Canada more than 130,000 hip and knee replacements were performed between 2017 to 2018(6). In the United States of America (USA) approximately 400,000 THA and 700,000 TKA are performed annually(15,16). It has been projected that by the year 2030 in the USA the demand for THA and TKA will increase by 174% and 673%, respectively(14). Recent data suggests that the demand for THA and TKA by 2030 will likely exceed these estimates(16). Meeting this demand has important implications for patients and society. Enhancing access to THA/TKA would result in an estimated cost savings of over $17 billion to the Canadian economy by 2040(12). Conversely, failing to meet these demands would result in substantial physical and psychological disability to patients and cost to society.

1.2 Revision Total Hip and Knee Arthroplasty

Despite advances in implant technology, perioperative care, and surgical technique, THA and TKA do not always last the patient’s entire life and can require revision surgery to replace the failed components. A systematic review of national joint registers and clinical studies demonstrated that revision rates are about 6% at 5 years and 12% at 10 years for both primary

THA and TKA(17). A recent systematic review and meta-analysis looking of case series, cohort studies, and registry data with a minimum of 15-year follow-up showed that the approximate 25-year survival of TKA is 82%(18). In a separate publication the same authors found that the 25-year survivorship of THA is 77.6% from case series and 57.9% from joint replacement registries(19). However, the introduction of modern highly cross-linked polyethylene acetabular component liners may improve the survival of THA(20,21). Furthermore, there are long-term reports of modern uncemented femoral implants with 15-year survival of 99.5% with revision for aseptic loosening as the end-point(22).

1.2.1 Epidemiology and Outcomes

In Canada from 2017 to 2018 more than 9,700 THA and TKA revision surgeries were performed, representing 8.2% and 6.9% of all THA and TKA performed(6). Revisions cost nearly 80% more than primary replacements when considering inpatient costs, amounting to $163 million during the same time period(6). As the demand for primary THA and TKA continues to increase, so too does the number of revision surgeries(3,4,6). It is estimated that between 2005 and 2030, THA and TKA revisions in the USA will increase by 137% and 601%, respectively(14). In the USA, the mean total charge for revision THA and TKA is $77,851 and $75,028 respectively, costing the healthcare system well over 1 billion dollars annually(23,24).

There are many documented risk factors for revision of primary THA and TKA. These include but are not limited to diagnoses other than primary OA as the indication for primary joint replacement, implant design/material, male sex, younger age, obesity, increased patient medical comorbidity, excessive alcohol use, active drug use, coagulopathy, mental health disorders, and low hospital/surgeon volume(25–28).

Although revision surgery can restore function and quality of life, revisions are associated with increased risk of morbidity, increased costs, lower patient satisfaction, and reduced patient reported outcomes when compared to primary THA/TKA(29,30).

1.2.2 Etiology for Revision

Canadian registry data from 2017 to 2018 shows that the most common causes for revision THA are instability, aseptic loosening (AL), and infection, and for revision TKA are AL

(26.7%), infection (21.2%), and instability (15.8%)(6). The Australian Orthopaedic Association National Joint Replacement Registry, considered the gold standard for joint registries, showed similar findings with a revision burden of 8.4% for THA and 8.7% for TKA(21). The most common reasons for revision THA were infection, AL, dislocation, fracture, and metal reaction. The most common reasons for revision TKA were infection, loosening, instability, and pain(21).

A 2009 USA database study found that the most common reasons for revision THA were instability (22.5%), AL (19.7%), and infection (14.8%), followed by implant failure, osteolysis, and periprosthetic fracture(31). More recent USA data shows that instability and AL remain the most common reasons for revision THA, followed closely by infection(23). However, infection has been reported as the most common cause of revision THA failure (30%), followed by instability (25%) and loosening (19%)(32). Current USA data shows that the most common cause for revision TKA is infection (20.4%), followed very closely by AL (20.3%)(24). Another large single center study listed causes for revision TKA in decreasing order as AL, infection, instability, periprosthetic fracture, and arthrofibrosis(33). When looking at early versus late failures, the authors found that infection was the most common cause for early revision and AL was the most common cause for late revision.

In summary, the current evidence shows that infection and AL are the most common causes for revision of primary THA and TKA, with instability being another leading cause. Additionally, infection is a leading cause of failure for revision THA and TKA with rates as high as 30%.

1.3 Periprosthetic Joint Infection in Total Hip and Knee Arthroplasty

The definition of periprosthetic joint infection (PJI) is an infection involving the prosthesis of the THA/TKA and the contiguous tissue. Despite considerable efforts, PJI remains one of the most common causes of revision in primary THA/TKA, and a leading cause of failure in revision THA/TKA.

Several classification systems have been developed for PJI. The simplest classification scheme devised is based solely on time from joint replacement to infection. Although controversy exists regarding the exact time cut-offs, PJI can be classified as early, delayed, or

late-onset(34). Early-onset PJI occurs less than 3 months from the time of surgery. Early-onset PJI is often caused by more virulent microorganisms through intraoperative contamination. The time period of delayed-onset PJI is between 3 and 12-24 months. Delayed-onset PJI is also thought to originate at the time of surgery but by less virulent organisms, leading to the delayed onset of symptoms and diagnosis(34). Late-onset PJI occurs after the 12-24 month time period, and is thought to be caused most commonly by hematogenous infection or at the time of surgery by extremely low virulent microorganisms(34).

Another popular and commonly used PJI classification scheme categorizes PJI by presumed mode of infection in addition to time since surgery(35,36). The first category is patients undergoing presumed aseptic revision surgery with unexpected positive intraoperative cultures (UPC). This is an area of considerable debate and not all patients with an UPC have a diagnosis of PJI. The second category is early postoperative infection, defined as occurring within 1 month from surgery in this classification scheme. PJI that occurs later than 1 month after the index procedure is classified as late chronic PJI, and like the delayed and late-onset PJI of the previous classification scheme, is usually characterized by indolent microorganisms and symptoms. The fourth and final category of PJI is acute hematogenous spread. This classification system has gained popularity because it can help guide the medical and surgical management of PJI. Lastly, authors have modified these classification schemes to include the status of the host patient in terms of comorbidity and immune function(37).

In response to the burden of PJI, leading experts from many subspecialty fields across the globe met to evaluate the evidence and reach a consensus on the prevention, diagnosis, and management of PJI. This culminated in the first International Consensus Meeting (ICM) on Periprosthetic Joint Infection in 2013 and the second ICM on Musculoskeletal Infection in 2018(38,39). Despite these considerable efforts, PJI remains a devastating complication of total joint replacement surgery and poses an incredible burden on patients, healthcare systems, and society.

1.3.1 Epidemiology of PJI

The risk of PJI after primary THA or TKA is approximately 2% per year(40–43). Nearly 70-80% of PJI is diagnosed within the first 2 years, however, one-fourth to one-third are

diagnosed after the 2-year mark(44,45). Despite all efforts toward the prevention and treatment of PJI, the incidence is not decreasing and may actually be increasing(40–42). Additionally, PJI remains the leading cause of failure of TKA and amongst the most commons causes for THA. Since the incidence of PJI is not decreasing, the number of PJI cases are expected to increase proportionately with the projected increases in the numbers THA and TKA performed(14,16,41).

The economic cost of PJI is enormous. The mean annual cost to treat chronic PJI case in a tertiary center in the USA was $88,623 for THA and $116,383 for TKA(46,47). The same authors showed that the cost of treating septic revisions was 3 to 4 times higher compared to aseptic revisions. Other authors have reported that PJI costs 5 to 6 times that of primary total joint replacement(48). Similar high costs for the treatment of PJI in THA/TKA are reported in the literature(40,49). The cost to the USA healthcare system of treating PJI is projected at $1.6 billion by 2020(40). However, this figure is a gross underestimation of the overall costs of PJI because authors only considered the direct hospital costs, disregarding many other substantial direct and indirect costs(34). The economic burden of the projected increased numbers of PJI in the future has the potential to overwhelm healthcare systems worldwide.

The identification of risk factors for PJI in THA/TKA has been an area of great interest. The goal is to detect high risk patients and develop strategies to prevent PJI by reducing nonmodifiable risk factors and optimizing modifiable risk factors. Several nonmodifiable and modifiable patient, surgical, and healthcare risk factors for PJI have been identified. A prospective, observational cohort study of over 620,000 primary TKA patients with a median follow-up of 4.6 years identified male sex, younger age, elevated body mass index (BMI), diabetes, dementia, previous septic arthritis, a diagnosis of fractured femoral neck, the use of metal versus ceramic bearings, and the surgical approach (controversial) as significant risk factors for PJI(50). Three recently published reviews on PJI identified male gender, obesity, increased BMI, low BMI/malnutrition (Albumin <34g/L or total lymphocyte count less than 1200 cells per μL), diabetes, increased hemoglobin A1c, chronic kidney disease, rheumatoid arthritis, malignancy, immunosuppressive medications (steroids, biologic disease-modifying antirheumatic drugs, chemotherapy) immunosuppressive disorders (human immunodeficiency virus/AIDS, Hepatitis C), smoking, nasal *Staphylococcus aureus* colonization, substance abuse, prolonged surgical time, post-traumatic arthritis (particularly requiring previous surgery/hardware), revision joint replacement surgery as compared to primary surgery, previous

septic arthritis, peri-operative allogeneic blood transfusion, active infection at a distant site, and prolonged drainage of the surgical wound as risk factors for PJI in THA/TKA(34,43,51).

1.3.2 Pathogenesis

The most common cause of PJI is the introduction of microorganisms to the prosthetic joint or surrounding periprosthetic tissue during the index surgery(34,52). Approximately two-thirds of PJI are originated from this mechanism, as are the vast majority of early PJI and those during the first year. The contamination of microorganisms during index procedure occurs through aerosol contamination or direct contact(34). High virulence microorganisms are more likely to cause early PJI with clear signs of aggressive infection, whereas lower virulence microorganisms are more likely to cause late or chronic PJI with indolent signs of infection such as progressive pain or component loosening(51,52).

The second mechanism is contiguous spread of infection from an external or adjacent site(34,43,51). This could include direct spread of microorganisms from a superficial surgical site infection in the setting of prolonged drainage, adjacent soft tissue or bone infection (osteomyelitis), or an injury that exposes the prosthetic implants to the external environment (open periprosthetic fracture). Lastly, all prosthetic joints are at risk of hematogenous seeding of microorganisms from a distant primary focus of infection(34,51,53). Although these infections can occur during any period of life of the prosthesis, acute hematogenous PJI occurs most commonly in the first year and presents as an aggressive acute PJI after a pain-free interval(53). Common foci of infection include other prosthetic devices, and skin, oral cavitary, genitourinary, gastrointestinal, and cardiovascular sources(53). Bacteremia is commonly associated with acute hematogenous PJI, commonly caused by being *Staphylococcus aureus*, *Streptococci*, and *Enterococci* (34,53). Other gram-negative microorganisms, coagulase-negative *Staphylococci,* and rare microorganisms are encountered as well(34,53).

A major factor in the pathogenesis of PJI in THA/TKA is the presence of biofilms. In the presence of a foreign body such as a THA/TKA prosthetic implant, the concentration of bacteria required to induce a infection is decreased by greater than 100,00 times(54). Microorganisms have developed the basic survival mechanism of adhering to surfaces of foreign bodies and creating microcolonies by multiplying while encasing themselves within a glycocalyx biofilm to

resist environmental factors(43,55). The two most common microorganisms involved in PJI, *Staphylococcus aureus* and coagulase-negative *Staphylococci*, are recognized as biofilm forming microorganisms. It is through the formation of biofilm that normal microbial flora (considered innocuous) become pathogens in the presence of THA/TKA prostheses(34). Biofilm formation takes up to 4 weeks to mature and occurs in 4 general stages: attachment, initial growth, maturation, and detachment(34,55). Microorganisms in the biofilm state are difficult to isolate and are protected from the host immune system and antimicrobials, hence why surgical management is often required for eradication(34,55,56). Microorganisms in biofilms are resistant to antimicrobial agents because of their slow rate of growth, subpopulations of resistant bacteria, and the biofilm microenvironment that impedes microbial activity(51,57,58). A majority of the microorganisms in biofilms remain adherent to the surface of prosthesis limiting the sensitivity of traditional synovial fluid and tissue cultures, particularly in late or chronic PJI(34). Furthermore, microorganisms in chronic PJI biofilms can remain in dormant states resistant to traditional culture techniques(34). Thus, the presence of biofilms can impede the diagnosis of PJI in THA/TKA.

1.3.3 Microbiology and Microorganisms

Nearly all PJI in THA and TKA is caused by bacterial microorganisms. Methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), and methicillin-sensitive *Staphylococcus epidermidis* (MSSE) are the most common PJI microorganisms in the USA(59). Conversely, in Europe the most common microorganisms in order of descending frequency are coagulase-negative *Staphylococcus* species (CNS), *Staphylococcus aureus* (*S. aureus*), *Streptococci*, and *Enterococci*(60). The incidence of MRSA infection in THA/TKA is estimated at 12-23% in the USA, and the increasing incidence of antimicrobial resistant microorganisms is associated with increased costs of treatment and poorer outcomes(43,49).

A comprehensive and well-written microbiological review of PJI synthesized the findings of 14 large studies on PJI including over 2400 patients from different geographic locations and time periods(34). The majority of THA/TKA PJI involve gram-positive cocci, with over 50 to 60% of PJI caused by *S. aureus* and CNS. In all infections *S. aureus* and CNS contributed equal

amounts, however CNS tended to be more common in THA with equal contributions in early and delayed/late infections, whereas *S. aureus* was more common in early-onset infections and TKA(34). *S. aureus* is also the most common causative microorganism in hematogenous PJI in the delayed or late time period. The gram-positive *Streptococci* and *Enterococci* species accounted for less than 10% of PJI cases. *Streptococcus* species PJI most commonly present in the delayed or late onset time-period and are often presumed to be caused by acute hematogenous seeding from gastrointestinal, genitourinary, and skin sources(34,61). Patients with *Streptococci* species PJI tend to present acutely, are often systemically ill with associated bacteremia (as many hematogenous PJI are), and can be difficult to treat(62,63). *Enterococci* species PJI are rare but contributes to 12-15% of early PJI, can be associated with lower virulence delayed PJI, and is associated with polymicrobial infections(34,64). Aerobic gram-negative bacilli constitute less than 10% of all PJI (most commonly *Escherichia coli* & *Pseudomonas aeruginosa*), but account for one-quarter of early-onset PJI(34). *S. aureus* and gram-negative bacilli account for more than 60% of early infections due to the high virulence nature of these microorganisms, and tend to present as acute infections(34).

Delayed or late PJI tends to be caused by lower virulence microorganisms such as CNS (normal human microbiome on skin) or *Enterococcus* species with an insidious onset of nonspecific symptoms such as pain and swelling(34,43,51). The most common CNS microorganism in PJI is *Staphylococcus epidermidis* (*S. epidermidis*), a known former of biofilm(34,43,55). *S. epidermidis* is the second most common causative microorganism in early PJI, but also a player in delayed or late PJI due to its low virulence(34,43,55). Another low virulence microorganism increasingly being recognized as a contributor to late onset PJI is the anaerobic gram-positive bacilli *Cutibacterium acnes* (*C. acnes*), formerly known as *Propionibacterium acnes*(34,43,51). *C. acnes* is part of the normal human microbiome found on skin and sebaceous glands, has been implicated more in THA versus TKA PJI, and is most commonly introduced into the joint from contamination during the index surgery(34,65). *C. acnes* represents a diagnostic challenge because it is difficult to grow with standard laboratory techniques (requiring extended anaerobic cultures), presents with indolent symptoms, often does not induce increased or abnormal standard diagnostic inflammatory markers, and may be considered a contaminant(43,66).

Only 15% of PJI in THA/TKA is polymicrobial, however these infections account for nearly 31% of early onset PJI(67). Anerobic bacterial microorganisms are a less common cause of PJI, but when present are often associated with polymicrobial infections(67). Mycobacteria are an extremely rare cause of PJI, but can occur in developing countries(68). Many other bacterial microorganisms have been associated with PJI, although much less common and outside of the scope of this review(34,43,51). Less than 1% of PJI are caused by fungal microorganisms, however fungal infections are more common in multiply revised cases and Candida species are the causative microorganisms in over 80%(69).

Culture-negative PJI is increasingly recognized and presents multiple diagnostic and treatment challenges, however, this will be discussed in the next section.

1.3.4 The Diagnosis of PJI in THA and TKA

The diagnosis of PJI is based on a combination of clinical, serum, synovial fluid, microbiologic culture, histopathology, and intraoperative findings. Despite enormous focus and scientific efforts, there is no gold standard or perfect test to diagnose PJI.

Early PJI often presents with wound drainage, erythema, swelling, severe pain, and aggressive clinical signs secondary to the high virulence microorganisms often involved. Delayed and certainly late-onset PJI often present with indolent local non-specific signs of infection such as pain, swelling, or implant loosening due to the lower virulence of causative microorganisms. However, a draining sinus can still occur in the late time period. Additionally, if secondary to acute hematogenous seeding the patient can present with acute, aggressive local and systemic signs of infection. Plain radiographs are obtained routinely in the evaluation of any THA or TKA suspected of PJI, pain, or dysfunction. Radiographic signs of loosening can suggest an infectious process, but no radiographic signs are sensitive or specific for the diagnosis of PJI(70).

Screening bloodwork and joint synovial fluid aspiration (when infection is suspected) are the next diagnostic steps and critical to the evaluation of PJI. Advanced nuclear imaging modalities have been investigated, however are not routinely used to make the diagnosis of PJI due to inadequate specificity and increased costs(51,71,72).

In 2011 a workgroup created by the Musculoskeletal Infection Society (MSIS) analyzed the best available evidence to produce a landmark consensus statement on the definition of PJI in THA/TKA(73). Prior to this there was no widely accepted standardized criteria to diagnosis PJI. This was an enormous step forward in terms of diagnosing PJI and aiding further scientific research in the area. During the inaugural 2013 ICM of PJI delegates across the globe made a slight alteration to the minor criteria of the 2011 MSIS definition and published the updated document(74). This definition of PJI was widely adopted in both clinical and scientific settings. There are 2 major and 5 minor criteria for the diagnosis of PJI. One of two major criterion is required (I) 2 positive periprosthetic cultures with the same microorganism or (II) a sinus tract communicating directly with the prosthetic joint. If major criteria are not met, there are 5 minor criteria in the 2014 definition, with 3 of 5 required for a diagnosis of PJI(74). Minor criteria are (i) elevated serum c-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), (ii) elevated synovial fluid white count (or + + change of leukocyte esterase test strip), (iii) elevated synovial polymorphonuclear neutrophil (PMN) percentage, (iv) a single positive microorganism culture, and (v) positive histologic analysis of operative periprosthetic tissue with microscopy. Specific numeric cut-offs for acute and chronic PJI for these criteria were published(74). The sensitivity and specificity of these criteria have been validated. Cultures can be obtained preoperatively from synovial fluid aspiration of the prosthetic joint or intraoperatively at the time of revision surgery. Ideally, a diagnosis prior to revision surgery is the goal. There has been extensive study on cultures in the setting of PJI(34,51). Culture isolation of causative microorganisms from periprosthetic tissue or fluid samples remains the gold standard in diagnosing PJI. Numerous periprosthetic tissue cultures should be taken intraoperatively to identify the causative microorganism(75). The practice of sending intraoperative swabs for cultures should be avoided, as it has been shown that tissue samples are both more sensitive and specific(76). It has also been shown that interface membrane periprosthetic tissue samples have the highest diagnostic yield compared to pseudocapsule, other surrounding tissues, or synovial fluid(77,78). Although there has been little and conflicting study on the subject matter, culturing scrapings from the removed implants may be more sensitive than tissue cultures(78,79). Additionally, the advent of extended aerobic and anaerobic cultures (10-14 days) has increased the diagnostic yield of cultures particularly for low virulence or slow growing organisms(51,80).

Indirect biomarkers of infection need to be included in the modern definition of PJI because standard culture techniques are not 100% sensitive in identifying causative microorganisms. Several indirect markers of infection were investigated in recent years, resulting in the need for an updated definition of PJI. An evidence based and weighted scoring system for PJI using the 2014 definition and modern biomarkers from the literature was published in 2018(81). In the same paper the new (albeit more complex) weight adjusted scoring system for PJI was validated against an external cohort and compared to the previous 2014 and MSIS 2011 definitions of PJI. The major criteria are unchanged; however, the minor criteria are now separated into preoperative and intraoperative diagnostic criteria. The minor intraoperative criteria are used only if the diagnosis of PJI is inconclusive after preoperative criteria have been evaluated(81). In the minor preoperative diagnosis criteria section, there has been the addition of serum d-dimer (to some criticism), synovial leukocyte esterase, alpha-defensin (antimicrobial peptide release by neutrophils in response to bacterial infection), and synovial CRP. The minor intraoperative diagnostic criteria for inconclusive cases now include positive purulence in addition to a single positive culture and positive histology. In this seminal paper, the reported sensitivity of this new scoring system is improved over previous definitions (97.7%), with equal specificity (99.5%)(81). This new diagnosis has met some criticism however, with only a 68% agree delegate vote at the most recent 2018 ICM on Prosthetic Joint Infection(82). This definition is more complicated to implement into clinical practice, not all tests are routinely available in healthcare systems (for example alpha-defensin), and there is some controversy over the criteria (for example d-dimer). Thus, its routine use in clinical practice has not yet been widely adopted. There is an ongoing quest to discover other biomarkers to improve the diagnosis of PJI, (for example interleukin-6) however, many remain academic at the present time(34).

Even the most updated definitions of PJI have limitations and there is no perfect test to diagnose PJI(81,83). All definitions of PJI, including the updated 2018 definition, warn that the diagnosis is not 100% specific and there are patient populations in whom the criteria may be inaccurate(81). In the 2018 publication by Parvizi et al(81), the authors warn that patients with slow growing organisms (such as *C. acnes*, CNS, and others), metal on metal reactions, or inflammatory arthropathy are at risk of being misdiagnosed. Furthermore, despite a careful preoperative evaluation, patients who undergo presumed aseptic revision surgery can have

unexpected positive intraoperative cultures (UPC) that are discovered postoperatively(84,85). This forms a major basis of the current thesis and will be discussed in a separate section below.

Culture-negative PJI continues to be problematic towards the diagnosis and management of PJI. It is entirely possible to be diagnosed with PJI by indirect markers of infection (CRP, ESR, PMN number and %, alpha-defensin, leukocyte esterase, purulence, histology) without ever identifying a causative microorganism. Identifying the causative microorganism is a critical aspect of the diagnosis of PJI, as it can guide treatment and antimicrobial therapy. Culture-negative PJI also has a higher rate of treatment failure compared to when a causative microorganism is identified(86). Historically rates of culture-negative PJI in THA/TKA were thought to be around 6%, however recent literature suggests that it is higher with a range of 0-42% and a likely true proportion of 20%(34,87). These infections are typically classified as delayed or late-onset PJI, with a smaller percentage being early onset or hematogenous(88). There are multiple postulated reasons for the proportion and variation of culture-negative PJI in the literature. These include (I) biofilm formation (as discussed previously), (II) the use of preoperative antibiotics, (III) consideration of a positive culture as contamination, (IV) inadequate sampling or use of available microbiologic methods, (V) inability to detect known or previously unknown causative microorganisms for PJI, and (VI) current diagnostic definitions incorrectly identifying an aseptic case as infected (false positive)(34,87,88).

Modalities to overcome biofilm formation on implants and culture independent methods of identifying microorganisms, such as molecular techniques, are being pursued in response to UPC in presumed aseptic revisions and culture-negative PJI. The use of molecular methods to investigate whether microorganisms play a role in presumed aseptic revisions will be discussed in a subsequent section and is another major aim of the current thesis.

Sonication of implants removed in revision surgery is a technique that has been developed to increase the diagnostic yield of cultures in revision THA/TKA. The concept is that removed implants can be subjected to ultrasound in order to dislodge bacteria from mature biofilms that have formed on the prosthetic implants. Early studies utilizing sonication for the culture positive diagnosis of PJI suffered from contamination issues and limited specificity(34). In a landmark study that used improved technique and sealable sterile plastic containers (not plastic bags that leak), sonication of revised THA/TKA implants had improved sensitivity with preserved specificity versus traditional methods to diagnose PJI(89). Recent literature using

modern PJI diagnostic criteria supports sonication of failed implants in the diagnosis of PJI and a meta-analysis of molecular methods using implant sonication fluid concluded that sonication may improve sensitivity and specificity over conventional methods(90,91). However, these findings are not universal and have been challenged by several authors. Modern, well designed, large studies have shown that sonication of removed implants does not improve the diagnostic yield of PJI (false-positives and false-negatives) in presumed aseptic or infected revisions(92–94).

1.3.5 Treatment and Outcomes of PJI

The goals of treatment are eradication of infection, a pain free, high functioning joint, and minimizing the morbidity and mortality associated with PJI. Treatment strategies for PJI are based on the classification of PJI (timing), patient comorbidity profile, surgeon preference, and the implant, bone, or soft tissue status. The treatment strategies are beyond the scope of this thesis, however, often necessitate surgical and medical treatment in combination. Treatment options include debridement, irrigation, and exchange of modular components but retention of well-fixed components (DAIR), a single-stage revision of all prosthetic components, a two-stage revision, or in refractory cases, antimicrobial suppression, resection arthroplasty, or amputation(34,43,51). For a comprehensive review of these strategies I would suggest three excellent review articles on PJI(34,43,51). All strategies are used in combination with antimicrobial treatment. Culture identification of causative microorganisms and antimicrobial sensitivity profiles are the cornerstone of antimicrobial treatment. Regardless of treatment strategy employed, failure to identify the causative microorganism is associated with worse outcomes(86).

Both acute hematogenous and early-onset PJI are often treated with DAIR when specific criteria are met(95). The success rate in terms of infection free survival ranges from 11-100% in the literature, with an estimated pooled success rate of 61%(95). Many factors influence the success of DAIR, including time from onset of symptoms, joint involved, and virulence of the microorganism(s). The standard of care for the treatment of delayed or chronic PJI in North America is a two-stage exchange (51,96). In the two-stage exchange all prosthetic implants are removed in order to treat the infection and biofilm, a temporary local antibiotic cement spacer is

implanted into the joint to allow elution of local antibiotics, systemic antibiotic therapy directed at the causative microorganism(s) is administered, and weeks to months later, a revision joint is implanted if infection is thought to be eradicated. In Europe, it has been popular to do this as a single-stage procedure with an aggressive debridement of infected tissue and antibiotic cement, but the infected prosthetic joint still has to be removed in order to eliminate the microorganisms and biofilm(97).

Success rates (cure of infection) for two-stage exchange ranges from 65% to 100% in the literature, with a success rate of over 80% reported for both one-stage and two-stage exchanges in a recent systematic review(43,98). Failures in treatment can result in repeat surgeries, chronic antimicrobial suppression, amputation, or even death. Chronic PJI and its treatment is associated with considerable patient morbidity and even mortality, as well as poorer patient reported outcomes compared to primary joint replacement(41,62,67,96,98,99).

It is clear that PJI is a tremendous burden to patients, healthcare systems, and society. It is also clear that PJI will be more prevalent in the future. In response to this, there has been considerable funding and scientific efforts aimed at the prevention of PJI. These prevention strategies can be categorized into preoperative, perioperative, intraoperative, and postoperative measures(43,100). Again, the details of these prevention strategies are beyond the scope of this thesis, however readers are directed to two excellent published reviews if interested(43,100). Further study of PJI prevention is mandatory because despite these measures the incidence of PJI is not decreasing and may actually be increasing(40–42).

1.4 Unexpected Positive Intraoperative Cultures in Presumed Aseptic Revision THA and TKA

As described, there remains no perfect method to diagnose PJI in THA/TKA. The preoperative diagnosis of PJI is important because the surgical treatment of aseptic and septic failures differ substantially. Unexpected positive intraoperative cultures (UPC) can occur in presumed aseptic revisions(84,85,101). An UPC is by definition a positive culture(s) obtained during a revision THA/TKA surgery that was presumed to be aseptic. The surgeon becomes aware these single or multiple UPC postoperatively. The current definition of PJI considers 2 cultures of the same microorganism as diagnostic for PJI(38,81). UPCs are likely to remain a

problem since it has been postulated that a proportion of presumed aseptic failures are actually undiagnosed PJI(102–104). However, the true incidence, clinical significance, and optimal treatment of UPC in presumed aseptic revisions is not clear in the literature(105).

1.4.1 Prevalence

There are a small number of studies reporting on the prevalence of UPC using conventional cultures methods (culture of joint fluid aspirate, swabs, periprosthetic tissue) and the true incidence remains unclear. A recently published review on UPC in presumed aseptic revision THA/TKA demonstrated a mean prevalence of 10.5% (379 UPC in 3605 presumed aseptic revision cases), however this varied considerably (4-38%)(105). The review included 10 studies published after the year 2000 that reported on the prevalence of UPC in presumed aseptic THA/TKA failures, as well as their treatment and outcomes. The mean follow-up was 26 months and the prevalence of UPC in hips was nearly two times more common than knees. The majority of the UPC were caused by low virulence microorganisms, such as CNS and *C. acnes*(105). A summary of study characteristics and outcomes can be found in tabular form in the published review(105).

An early retrospective study by Padget et al(106) reported an UPC prevalence of 30.4% in 138 presumed aseptic THA. They used a minimum of 6 intraoperative culture specimens (swab and tissue) and found that the majority (35 hips) of these were a single UPC. Unlike many studies, these authors did report on the outcomes of a single positive UPC. Conversely, a subsequent retrospective study reported a 11% rate of UPC in presumed aseptic revision (31/275), however this study only considered PJI diagnosed by UPC (≥ 2 UPC with the same microorganism growing on solid medium)(35). In one of the very few prospective studies Atkins et al(75) evaluated 297 presumed aseptic THA and TKA and found a that 27.9% of cases had UPC (47 had 1 UPC, 8 had 2 UPC, 28 had 3 UPC). However, the authors considered the vast majority of 1UPC and 2 UPC cases to be false positives based on histology and recommended that histology and multiple tissue samples be obtained based on statistical modelling.

In contrast, another early retrospective study looking at revision TKA found that only 4% (5/133) were diagnosed with PJI based on UPC(107). However, the prevalence UPC in this study may have been lower due to the definition used (only ≥ 2 UPC with the same microorganism

grown on solid medium). In 2007 Berend et al.(108) reported on 106 presumed aseptic failed THA cases and reported an UPC prevalence of only 6.8% (2/7 of these were gram stain positive only). The authors accounted for the low prevalence with a throughout preoperative evaluation and use of swabs in addition to tissue cultures. Similarly, a large retrospective, 3 centre study published in the same year evaluated 692 presumed aseptic failed TKA revisions and found a 5.9% (41/492) rate of UPC(101). The authors reported that 29/41 were single UPC with no other signs of infection and concluded that these were false positives. Of the 41 UPC, 8 had 2 UPC with the same microorganism and 4 had 1 UPC with other signs of infection (elevated ESR/CRP). Parvizi et al.(102) published a retrospective study in 2011 looking at 314 failed THA with the diagnosis of AL to evaluate serum ESR and CRP parameters. Intraoperative cultures (minimum 3 fluid/and or tissue) were obtained in 169 of these cases and 8.3% (14/169) had at least a single UPC grown on solid medium (excluded broth).

However, Ribera et al.(109) performed a prospective evaluation of 89 presumed AL cases (60THA/29TKA) comparing periprosthetic tissue versus implant sonication cultures in the diagnosis of PJI. The authors took a minimum of 5 tissue cultures and considered ≥ 2 UPC with the same microorganism growing on solid medium to be diagnostic of PJI, but reported on cases with a single UPC as well. In contrast to the previous 2 retrospective studies, the incidence of UPC in tissue samples was 38% (34/89); 12 of these were ≥ 2 UPC and 22 of these were single UPC(109). Two recent prospective studies compared the ability of conventional operative cultures versus sonication fluid cultures to diagnose PJI in presumed aseptic failures (AF)(92,103). In 198 cases of presumed AL an incidence of PJI diagnosed by UPCs was 5.4% using conventional culture methods(103). However, only ≥ 2 UPC with the same microorganism growing on solid medium were considered with the exception of two specific virulent microorganisms (11 additional single UPC excluded). Kempthorne et al.(92) reported on 56 AL cases versus 53 other AF controls and found an incidence of UPC (including singe) of 15% in the AL cohort and 2% in the control cohort.

Finally, two retrospective studies were published in 2017(84,94). Jacobs et al.(84) reviewed 679 presumed AF THA/TKA cases with a minimum of 3 tissue samples and found that 10% (12.1% THA and 7.9% TKA) of cases had a UPC diagnosis of PJI with ≥ 2 UPC of the same MO on solid medium (excluded and did not report on single UPC). Van Diek et al.(94) reported a prevalence of PJI diagnosed by UPC (≥ 2 UPC with the same MO on solid medium,

with the exception of a single *S. aureus* UPC) of 18.6% (33/177) using a minimum of 6 tissue cultures in presumed aseptic revisions. Again, this study did not consider a single UPC (with exception of *S. aureus*) to be significant and did not report on this cohort of patients.

The reported prevalence of UPC in presumed aseptic THA/TKA failures in the literature varies considerably. This variability is due to differences in preoperative evaluation, definitions of PJI (many studies pre-dated or did not use modern MSIS criteria), diagnoses for the AF cohort (AL seems to have higher rate of UPC), definitions of UPC used in each study (≥ 2 UPC with the same MO on solid medium versus single UPC versus solid medium or broth), type and number or cultures taken, study design (retrospective versus prospective), and often small sample sizes, differing microbiological laboratory protocols, and potential contamination(105).

1.4.2 Treatment and Outcomes

Far fewer studies report on treatment and outcomes of UPC compared to the number reporting on prevalence. There is no consensus on when and how UPC should treated. The majority of treatment and outcome studies in this patient population are retrospective in nature and have limited sample sizes. Treatment protocols included standard prophylactic antibiotics, 4-6 weeks of intravenous (IV) and/or oral (PO) antibiotics, months of antibiotic treatment, or chronic antibiotic suppression(105). Although controversial, most authors would agree that ≥ 2 UPC with the same microorganism should be treated with 4-6 weeks of antibiotics and can expect to have a satisfactory outcome with 80-100% survival at short term follow-up. Low virulence microorganisms common to UPC and short-term follow-up (mean 26.1 months) are thought to contribute to this reported infection-free survival(105). The clinical significance and treatment of single UPC are a topic of debate with no clear consensus to date.

A successful outcome or survival in these studies was often defined as absence of recurrent or new PJI and/or no revision surgery for infection-related failure. Padgett et al.(106) treated only 11/41 of UPC in hips with 6 weeks of IV and/or oral antibiotics. At mean follow-up of 48 months only 1 case required revision for PJI, leading the authors to question the significance of a single UPC with a low virulence microorganism and to rely on histology in these cases. Tsukayama et al.(35) reported on 31 presumed AL THA cases diagnosed with PJI based on ≥ 2 UPC with the same microorganism on solid medium. All were treated with 6 weeks

of IV antibiotics and at a mean of 3.5 years reported a 90% success rate (3/31 required revision for PJI with same microorganism as UPC and 3 additional cases showed evidence of loosening of x-rays). Segawa et al.(107) reported on 5 TKA revised for presumed AL diagnosed with PJI based on ≥ 2 UPC with the same microorganism on solid medium (all CNS). All 5 cases were treated with 6 weeks of IV antibiotics and at a mean of 4 years survival was 100% (one x-ray consistent with tibial baseplate loosening).

In 2005 Marculescu et al.(110) published a retrospective review of the 16 of 509 PJI cases preformed at their institution that were diagnosed by ≥ 2 UPC (same MO on solid medium) in presumed aseptic TKA revisions. To be included in the study, these 16 patients had to be treated with IV antibiotic strategies that lasted < 6 weeks. Treatment in the study varied; 8 cases were treated with IV antibiotics followed by chronic oral suppression, 4 cases were treated with IV antibiotics alone, 1 case was treated with oral suppression alone, and 3 cases received no antibiotic treatment(110). The treatment mean duration in the 12 patients that received IV antibiotics as part of their regimen was 28 days (range 2-42) and the mean duration in the 4 patients treated with IV antibiotics alone was 15 days (2-28 days). At a mean follow-up of 1057 days (731-1969 days) the 5-year infection free survival was 89% (95% CI 47-98%). The authors concluded that PJI diagnosed by ≥ 2 UPC with the same microorganism of low virulence have favorable outcomes with component retention and IV antibiotic treatment strategies of < 6 weeks.

Berend et al.(108) considered all 7 UPC in revision THA as significant treating with 6 weeks of IV antibiotics (PO antibiotics for 2 that were gram stain positive only), and reported no failures for infection at a mean of 31.6 months. They concluded that all UPC should be treated as significant but advised that more tissue cultures should be taken to allow false positives to be identified (may not need treatment). In the large retrospective 3 center study by Barrack et al.(101), the 41 presumed aseptic TKA that had at least one UPC were followed for a mean 45 months post revision. Twenty-nine of these cases had a single UPC with no other signs of infection and were considered probable false positives; of these only 5 were treated with 4-6 weeks of antibiotics. Twelve UPC cases had signs of infection; 8 with 2 UPC of the same microorganism and 4 with a single UPC on solid media with elevated ESR or CRP elevated. Of these, 11 were treated with 4-6 weeks of antibiotics. No failures (revision or PJI) were encountered for the 29 cases considered false positives (single UPC with no signs infection), but

3 of 12 cases with signs of infection required revision (2 for PJI and 1 for AL). The authors concluded that cases with a single UPC and no other signs of infection can safely be regarded as false positives and don't require treatment. Similarly, Dramis et al.(111) retrospectively reviewed 56 revisions (majority hip and knee) that had at least a single UPC of *C. acnes*. Only 12 of these received 6 weeks of antibiotic therapy as advised by as infectious disease specialist (all sensitive to penicillin) and patients were followed for a mean of 20.5 months. Forty cases had isolated UPC of C. acnes only (8 had ≥ 2 UPC) and 16 cases were mixed UPC. Only 1 required revision due to PJI leading the authors to question the significance of an UPC with *C. acnes*.

Saleh et al.(85) grouped THA/TKA UPC patients into those with a single UPC considered contaminants and not treated with antibiotics (44 cases) versus those with UPC(s) that were treated with antibiotics. The antibiotic cohort was further subdivided into those that did (14 PJI-positive) and did not (45 PJI-negative) meet MSIS criteria for infection. The minimum follow-up was 1 year with a mean of 51 months. Sixty-six percent of the antibiotic cohort received IV antibiotics, 17% PO antibiotics, and 17% both IV and PO antibiotics for 4-6 weeks(85). Patients were treated with antibiotics as per advice of an infectious disease specialist based on institutional criteria. Nine percent of the 'false-positive' no antibiotic treatment cohort developed PJI vs 20% in the antibiotic treatment cohorts (difference not statistically significant). Subsequent PJI in the false-positive no antibiotic treatment cohort were all caused by different microorganisms versus the UPC. In the antibiotic treatment cohort 12/14 of subsequent PJI was caused by the same microorganism as the UPC. Additionally, there was a higher revision rate in the 1 UPC PJI-negative antibiotic group versus the PJI-positive antibiotic group (22 v 14%), but again this difference was not significant. The authors concluded that the 1 UPC cohort not treated with antibiotics seemed to be false positives. However, they cautioned that a single UPC may indicate infection (even MSIS PJI-negative) when associated with high virulent microorganisms or other signs of infection, and that these should be treated.

Kempthorne et al.(92) prospectively evaluated 106 failed hips and knees (56 for AL and 52 for other presumed aseptic causes) for a mean follow-up of 9.7 months. They treated 4/9 of the UPC with antibiotics for 6 weeks and had no reoperations due to infection at short term follow-up. It should be noted that a single UPC was included in the study. In contrast, the prospective study by Fernandez-Sampedro et al.(103) reported a much higher failure rate, however the UPC PJI cohort in this study had to have ≧2 tissue UPC of the same microorganism

(exception is *S. aureus* or *Staphylococcus lugdunensis* because of high virulence). Eleven of the 24 with ≧2 tissue UPC of the same microorganism were treated with antibiotic therapy and minimum follow-up was 2 years (mean 36 months). They found that 37.5% of the ≧2 tissue UPC cohort failed by 2 years versus only 1.1% of the AL cohort(103). Interestingly, the authors found that antibiotic treatment did not improve survival in the ≧2 UPC cohort. Additionally, 11 patients with a single UPC (not diagnosed with PJI) did not fail.

The last study to look at survival in UPC was a large retrospective study of presumed AF hips and knees with a minimum follow-up of 2 years(84). Again, only patients with ≧2 tissue UPC of the same microorganism were included in the UPC cohort. Sixty-five percent of the hips and 53% of the knees were treated with antibiotics based on the microorganism and infectious disease specialist (no standard protocol). The authors reported survival at 2 years for the UPC PJI cohort versus the aseptic cohort (aseptic included those with a single UPC). For THA the difference was non-significant (92% versus 94%) but for TKA the UPC cohort had worse survival (88% versus 98%).

The only study to look at any type of functional outcome in this patient population was Tsukayama et al. in 1996(35). At a mean follow-up of 3.5 years the 31 UPC PJI (≧2 tissue UPC of the same MO) the mean Harris Hip Scores were 79 postoperatively compared to 45 preoperatively(35).

1.4.3 Current state of the literature

The current literature is inadequate to inform surgeons or clinicians in a meaningful way on the prevalence, clinical significance, preferred treatment, or outcomes of UPC in presumed aseptic revision THA/TKA(35,75,106–111,84,85,92,94,101–103,105). Reasons for this include differences in patient population, diagnoses for failure (AL seems to have higher rate of UPC), preoperative evaluation, preoperative definitions of PJI (many studies pre-dated or did not use modern MSIS criteria), definitions of UPC used in each study (≥ 2 UPC with the same MO on solid medium versus single UPC versus solid medium or broth), UPC patients excluded, type and number or cultures taken, microbiologic laboratory protocols, study design (retrospective versus prospective), and the often short follow-up times, small sample sizes in the majority of studies, and potential contamination(105).

The significance of a single UPC is even more unclear, with contradictory findings in the literature. However, two recent retrospective, but well-designed studies would suggest that even a single UPC may be important, may warrant treatment, and has the potential to impact outcomes(85,112). Furthermore, since many studies excluded a single UPC or those grown in broth only, data on this patient population is lacking.

Clearly more data is needed to better understand the clinical significance, expected outcomes, and optimal treatment for patients with UPC in presumed aseptic revision THA/TKA.

1.5 Molecular Methods in Presumed Aseptic Revision THA and TKA

The identification of bacterial microorganisms by conventional culture techniques remains the gold standard in the diagnosis of PJI and has important treatment implications. However, cultures lack sensitivity and fail to identify causative microorganisms in a meaningful proportion of cases(86,87,113).

Thus, the use of culture-independent molecular methods to detect bacterial DNA of microorganisms in PJI has be a topic of interest in the last 1-2 decades. Polymerase chain reaction (PCR) based molecular methods have the potential advantages over traditional cultures of being more sensitive, faster, having the potential to identify non-culturable microorganisms, and not being influenced by prior treatment such as antibiotics(104,114–116). Most molecular studies have looked at PCR and the diagnosis of PJI, however, molecular studies have also identified microorganisms on implants of presumed aseptic failures(116–120).

Furthermore, it has been postulated that presumed aseptic THA/TKA failures may be undiagnosed PJI caused by low virulent, difficult to culture bacterial microorganisms or a pro-inflammatory response to colonization of these microorganisms on prosthetic implants(102–104,109). Proposed mechanisms for this hypothesis include formation of biofilms of prosthetic implants and the implications of this, use of prophylactic antibiotics, and difficult to isolate or rare pathogens that traditional microbiologic laboratory techniques may fail to identify(104).

1.5.1 Molecular Methods in the Diagnosis of PJI

The results of molecular methods, largely PCR based, in the diagnosis of PJI have been contradictory. Molecular PCR analysis of a variety of sample types (tissue, aspirate fluid, sonication fluid of implants) has shown a wide range of sensitivity (50-92%) and specificity (65-94%) in the diagnosis of PJI(115,121). A systematic review and meta-analysis of the literature in 2013 including 14 studies showed that the pooled sensitivity and specificity for PCR assays in the diagnosis of PJI were 0.86 and 0.91, with tissue samples having the highest sensitivity and sonication implant fluid having the highest specificity(121). An updated systematic review and meta-analysis including studies from 2013 to 2017 showed a decreased pooled sensitivity (0.76) and an increased specificity (0.94) compared to the 2013 results(115). Interestingly, the authors of this 2018 meta-analysis did not find that the use of implant sonication fluid samples improved the diagnostic yield with molecular methods. In a large number of studies sonication of implants designed to dislodge bacteria from the biofilm has been shown to increase the sensitivity of PCR based methods(91,118,122,123). However, this is not universal and well-designed molecular studies have shown no difference versus traditional cultures of sonication fluid and even worse performance than standard tissue cultures in some instances(78,113,115,124). Reasons for the reduced sensitivity but increased specificity reported by the authors of the 2018 meta-analysis include the increased use of multiplex PCR techniques as opposed to universal PCR (see below), differences in sequencing techniques used, modern definitions of PJI, stricter definitions of PCR based PJI and lab based techniques to reduce false positives, different types of samples, and differences in the number of samples used(115).

Early PCR based techniques identified bacterial DNA but was not able to identify specific species of bacteria to be meaningfully compared to culture results(120). The use of so called broad-range or universal PCR targets the 16S ribosomal RNA gene (16S rRNA), that is highly preserved and universally present in bacterial microorganisms. Most molecular PJI studies using modern 16S rRNA PCR techniques amplify the 16S rRNA gene and then identify the micoorganism by sequencing the amplified DNA. In contrast to the proposed advantages of molecular techniques, disadvantages of 16S rRNA PCR include the cost/need for sequencing, issues of contamination (false positives) and false negatives (difficulty identifying anaerobes), and difficulty identifying multiple organisms in polymicrobial infections(34,115,125). Promising rapid 16S rRNA PCR based methods have been developed, as have methods to improve

sensitivity and specificity(113–115,125). In an attempt to improve on the limitations of 16S rRNA PCR, “multiplex PCR” has been developed and studied in THA/TKA PJI(124,126,127). Multiplex PCR uses multiple primer assays that are designed to target DNA sequences of specific bacterial microorganisms thought responsible for PJI in THA/TKA. Assays for multiple bacterial microorganisms have been developed, recently including anaerobes such as *C. acnes* and other microorganisms thought to be missed (lower sensitivity) by 16S rRNA PCR(126,127). Polymicrobial infections are also less of a problem for multiplex PCR. However, a limitation is that multiplex PCR techniques can be less sensitive for all microorganisms present and miss microorganisms they do not target or that were not thought to be involved in PJI. A recent meta-analysis shows that specificity may have improved with multiplex PCR, but sensitivity is decreased and reports of 16S rRNA PCR methodology with similar or better specificities do exist(115).

Given that modern definitions of PJI and novel biomarkers (such as alpha-defensin) have a greater diagnostic ability to that of the pooled results of recent molecular studies, the role of molecular methods in PJI is not yet clear. It appears that molecular methods have a clear value in culture-negative PJI and in patients who have been on antibiotics and may potentially improve our ability to diagnose PJI in the future(78,115,116,128). However, the current meaning of these results, contradictory findings, evolving techniques, and potential for contamination continues to be an issue(78,115,116,128).

1.5.2 Molecular Methods and Identification of MO in Presumed Aseptic Revisions

UPC in presumed aseptic revisions, the large proportion of culture-negative PJI, and several molecular studies support the theory that a significant proportion of AF in THA/TKA may be associated with microorganisms and/or unrecognized PJI. In comparison to the evaluation of molecular methods in the diagnosis of PJI, there has been less literature evaluating the use of molecular methods to detect microorganisms in cases of presumed AF or AL. The majority of the data on molecular methods in AF come from studies that evaluate PJI in revision THA/TKA, however molecular studies evaluating presumed AF cases do exist(78,113,125–132,116–120,122–124). However, there is considerable debate on the topic with conflicting

findings. Molecular literature supporting the detection of bacterial microorganisms and the potential role of undiagnosed PJI in presumed aseptic revision exists and continues to be published(116–120,122,123,129). However, several authors question the relevance and meaning of PCR based detection of microorganisms and others do not support detection of true positive microorganisms or undiagnosed PJI in presumed AF with the use of molecular methods(78,113,124–128,130–132).

In 1999 Tunney et al.(120) prospectively evaluated 120 THA septic and aseptic revisions using 16S rRNA PCR (no sequencing) and found tissue and implant sonication fluid (ISF) cultures diagnosed infection in only 22% of cases, whereas PCR of ISF identified bacterial DNA in 73% of cases. They concluded that the incidence of infection is grossly underestimated in previously presumed AF in THA but did not use sequencing and were unable to correlate molecular data to tissue cultures. Similarly, Clarke et al.(119) reported on 31 presumed aseptic revision THA cases that were all tissue culture negative and showed that 46% of these patients were positive for microorganisms using 16S rRNA PCR. However, the threshold for a positive PCR result was lower than used in modern studies, and the authors demonstrated a 29% false-positive contamination rate concluding that PCR was not specific enough to be used in the diagnosis of PJI. In contrast, a similar study prospectively evaluating presumed AL cup failures in 24 THA cases using 16S rRNA PCR methods showed that both tissue cultures and PCR were negative in all patients except 1 and that low virulence infections are likely not the usual cause of AL in THA cups(132). However, in 2008 Kobayashi et al.(122) prospectively evaluated 52 THA/TKA patients with dual assay PCR (both 16S rRNA PCR and *Staphylococcus* species specific primers), 85% of which were presumed AL preoperatively. They showed that 12% of culture-negative AL patients were PCR positive and that PCR was more sensitive than tissue or ISF cultures at detecting microorganisms. The authors reported that perhaps the lower incidence of 12% in their study was due to stringent criteria for a positive PCR result and control of contamination(122). Though soon after Moojen et al.(131) prospectively reported on another cohort of 176 presumed AL THA failures in a multicenter study using 16S rRNA PCR and multiple tissue and fluid samples. Using a very conservative criteria of ≥ 3 separate microorganism specimens as true positive for infection, they found that in the uninfected group (<2 specimens positive for microorganisms) tissue culture was falsely positive in 26% of cases and PCR was false positive in 20% of the cases. Additionally, they reported that 4% to 13% of

presumed aseptic revision cases are suspected or true infections (PCR more sensitive in true infections), although of seemingly little clinical effect at 1-year follow-up.

In one of the many studies on the subject matter out of the Mayo Clinic, Gomez et al.(113) evaluated 366 septic and aseptic THA/TKA revisions and showed that 16S rRNA PCR analysis of ISF was statistically equivalent to traditional cultures of ISF, tissue, and synovial fluid. Using strict criteria and careful methodology (use of real-time PCR threshold to decrease false positives and software to detect microorganisms in polymicrobial infections) to maintain the specificity of PCR, the authors also showed that only 5/231 AF were positive by PCR (similar to cultures), and did not support the hypothesis that AF are often undiagnosed PJI. A follow-up study out of the Mayo Clinic again retrospectively evaluated a large cohort of both aseptic and septic revision THA/TKA (434, 290 diagnosed as AF) with a rapid, multiplex genus/group specific, real-time PCR assay panel targeting bacterial microorganisms that are typically associated with PJI, including anaerobes (new addition versus previous)(127). The authors hypothesized that the new multiplex PCR of ISF would improve sensitively and rapidly diagnose PJI and compared results of PCR to those of cultures (ISF, tissue, and aspirate). The authors concluded that the multiplex PCR of ISF was more rapid and sensitive with similar specificity compared to cultures in diagnosis of PJI, and the high specificity of the assay suggested that typical bacteria may not be the cause of aseptic implant failure(127). It should be noted that multiplex PCR can only detect bacteria that the primers target, so they were at risk of failing to detect non-traditional PJI microorganisms (trade-off between sensitivity and specificity). Portillo et al.(124,130) published 2 prospective studies using multiplex PCR in both septic and aseptic revisions. The AF cohort in both studies had no false positive microorganisms identified using PCR, with equivalent and improved sensitivity compared to ISF culture methods. Multiplex PCR in these studies did not support microorganisms common to PJI as a causative factor in aseptic failures. Importantly though, the assay used did not detect anaerobes such as *C. acnes* or *Corynebacterium* (common to UPC), and the authors had very stringent criteria for what they considered a “contaminant”(124,130).

Bjerkan et al.(78) performed a prospective study using 16S rRNA PCR in 54 loose THA/TKA implants to diagnose PJI (21 preoperative diagnosis of infection) with multiple standardized specimens collected and a special emphasis on minimizing the risk of contaminants and false-positive results. Using real-time 16S rRNA PCR methods to establish stringent “true-

positive" thresholds, surprisingly the authors reported that tissue cultures (especially interface membrane) were more sensitive than PCR methods. Furthermore, the specificity of PCR was able to be maintained with only 2/216 samples in 2/36 AF patients positive and defined these as false positive contaminants as per study criterion(78) . However, other authors that employed careful methodology using both 16S rRNA PCR and multiplex PCR methods when prospectively evaluating larger cohorts of septic and aseptic THA/TKA revisions have supported PCR molecular methods as more sensitive in PJI and better able to detect MO in previously presumed AF cases with ISF and scraping of implants(123,129). Esteban et al.(123) and other authors have warned that pathogens detected in presumed AL and AF patients should not all be confidently disregarded as contaminants (as was done by Bjerke at al. 2012), and assays used is many of these studies had low sensitivities for detecting anaerobes and *C. acnes*.

More recently, Bereza et al.(117) showed using 16S rRNA PCR in a prospective study of 37 THA/TKA revisions that PCR identified a variety of bacterial microorganisms in patients with negative cultures, and that histology supported infection in 41% of these. Likewise, authors applying modern MSIS criteria and 16S rRNA PCR analysis of ISF versus standard cultures in preoperatively assumed aseptic revisions demonstrated that microorganisms were identified by PCR in 13/58 AF patients(118). Six of 9 were considered insignificant based on criteria of <2 specimens positive for the same microorganism, but authors urged that further molecular study in AL is warranted. Conversely, Ryu et al.(126) showed in a retrospective study of 87 TKA revisions (with a major focus on PJI) that standard tissue cultures had a higher sensitivity than multiplex PCR tissue culture (69% versus 16%). However, these results need to be interpreted with caution and the authors also acknowledged limitations of the study that can be corrected and investigated in the future. Potential reasons for such a low sensitivity of PCR tissue culture in the study include that only a single tissue culture was taken for PCR analysis, that microorganisms are in biofilms and may not be equally distributed in the periprosthetic tissue, that implant membrane tissue was not the tissue used for PCR, and that specimen age may have played a role (retrospectively evaluated)(126). However, a large well-designed prospective multicenter study of 264 suspected THA/TKA PJI (215 confirmed PJI by Infectious Disease Society of America criteria [IDSA]) evaluating the ability of 16S rRNA PCR gene assays to diagnose PJI used multiple cultures (tissue/fluid) and showed poor sensitivity versus IDSA criteria and standard

tissue cultures(125). Additionally, the PCR assay used was not superior to cultures at detecting microorganism in AF cases, and was poor at detecting *C. acnes* (only 11% vs tissue cultures).

What is clear about the literature on molecular methods and microorganism in AF is that the data is controversial. To add to this controversy, two large studies using modern "Next-Generation Sequencing" (NGS) and "Shotgun Metagenomics" approaches again show conflicting results. In another study out of the Mayo Clinic, Thoendel et al.(128) applied a comprehensive metagenomic shotgun approach (all of nucleic acid in the sample extracted and sequenced in order to identify any potential organism) retrospectively to a large sample (408 revisions) of previously collected and stored aseptic and septic THA/TKA revisions. Again, using IDSA criteria for PJI, one of the major aims of the study was to investigate the potential role of unidentified microorganisms in otherwise seemingly aseptic failures. The authors found that only 3.6% (7/195) AL cases were considered microorganism positive using refined criteria (large number of contaminants common to metagenomic sequencing were excluded), thus not supporting the theory of a high prevalence of microorganisms in seemingly aseptic revisions. However, there were additional microorganisms identified in about 10% of culture-positive PJI, and microorganisms identified in in 43.9% of culture-negative PJI(128). Conversely, Tarabichi et al.(116) published a prospective evaluation of 65 revisions (with 17 primary THA/TKA controls) evaluating the accuracy of NGS in identifying microorganisms causing PJI, with a special interest in culture-negative PJI. NGS, similar the metagenomic shotgun methods, is capable of sequencing all DNA present in a sample. The authors found that the sensitivity of NGS was far superior to tissue culture in PJI, and that NGS identified microorganisms in 80% of culture-negative PJI. In MSIS PJI-negative aseptic revisions NGS identified microorganisms in 25% of patients (9/36) that were tissue culture negative, thus supporting the role of microorganisms in both culture-negative PJI and "aseptic" failures. However, both methods are suspect to contamination issues, and the clinical significance of the identified microorganisms in AF are largely unknown(116,128).

A lack of a gold standard for the diagnosis of PJI makes interpretation of these findings difficult. A majority of early studies did not use a widely accepted standard to diagnose PJI, (such as MSIS) instead using tissue cultures as the gold standard for diagnosis of PJI. The different PCR molecular methods used, criteria used for true-positive PCR specimens or PJI, and control of 'contaminants' makes comparing results difficult as well. Limitations of 16S rRNA

PCR, multiplex PCR, and newer whole DNA approaches are challenging as well (as outlined above). Additionally, the utility of sonication of implants is also inconclusive. Scraping implants has been shown to increase the diagnostic culture yield in revisions and to be a viable alternative to sonication, with the advantages of being less expensive and at less risk of contamination associated with sonication protocols(79,129). There have been conflicting results on the value of scraping material from implants and tissue samples with molecular techniques(78,115,126,129). However, the best combination of results with PCR molecular methods have been observed when at least 5 samples are studied, a limitation to most scraping and tissue sample studies with PCR(115,129,133). Additionally, sonication is also unable to determine if there are specific areas on prosthetic implants that microorganisms are more likely to be found (important information). A prospective molecular study with stringent control of contamination evaluating the material obtained by scraping multiple predetermined sites of prosthetic implants from presumed aseptic failures is thus warranted and may provide valuable information. There is a paucity of literature looking at the clinical outcomes or survival of presumed aseptic failures with microorganisms identified by molecular methods, and the clinical significance remains unclear(116,124,131). This clearly needs further evaluation.

The prevalence, role, and clinical significance of microorganisms identified by molecular methods in presumed aseptic failures in THA/TKA remains unclear. Given the enormity of the implication that microorganisms and/or unrecognized PJI may be associated with currently presumed aseptic failures, this area necessitates further study.

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Chapter 2

2. Thesis Rationale and Objectives

2.1 Rationale

The literature review in Chapter 1 revealed that the incidence, optimal treatment, clinical significance, and outcomes of UPC in presumed aseptic revision THA and TKA remains unclear. Given the reported prevalence and projected increases in revision surgery, this remains a clinically important and challenging problem. The literature is limited and conflicting, and larger studies are needed. This serves as the basis for the retrospective database portion of this thesis.

Furthermore, culture-independent molecular methods have several advantages over traditional cultures and have gained popularity because a significant proportion of PJI remains culture-negative (fails to identify a causative microorganism). Although the majority of molecular studies investigated the evaluation of PJI, molecular studies investigating presumed aseptic failures have identified microorganisms on failed implants. It has been postulated that microorganisms may play an important role in presumed aseptic failures and that a proportion of these may be undiagnosed PJI. However, the results of modern molecular studies in presumed aseptic revision THA and TKA, particularly those with stringent control of contamination, are contradictory. The prevalence, clinical significance, and role of microorganisms identified in presumed aseptic revisions using molecular techniques remains unclear. Additionally, no molecular study has investigated if there are specific areas on the prosthetic implants that microorganisms are more likely to be found. The implications of microorganisms having a role in previously presumed aseptic failures are enormous and this clearly deserves further investigation, serving as the basis for the prospective pilot molecular study portion of the current thesis.

2.2 Overview

This thesis is written in an integrated article format, with each chapter corresponding to a separate research study corresponding to the research objectives below. Chapter 3 and 4 will investigate the prevalence of UPC in presumed aseptic revision THA and TKA, as well as the infection-free implant survival for this patient population. Chapter 3 will be a study reporting on UPC in presumed aseptic revision THA and Chapter 4 will be a separate study reporting on UPC in presumed aseptic revision TKA. We elected to report on THA and TKA separately because the prognosis of hips and knees has recently been shown to differ in this patient population(1). These studies are retrospective reviews of a prospectively collected database at a single high-volume academic institution. To our knowledge, both will represent the largest series of UPC in revision THA and TKA in the literature and will be a valuable contribution to the current body of knowledge.

Chapter 5 is a prospective pilot study using modern molecular techniques in presumed aseptic revision THA and TKA at our institution, with an emphasis on stringent control of contamination. We will be investigating if and how frequently microorganisms are present on implants of presumed aseptic revisions, as well as where they are found on the implants and if their presence is associated with reason for revision. Our expectation is that data from this pilot study will be used to perform power calculations, design, and fund large definitive studies to determine the role and clinical implication of microorganisms identified by modern molecular techniques in presumed aseptic revisions. Due to the unprecedented COVID-19 pandemic and state of emergency, the completion of the molecular portion of this study has been postponed. All samples for molecular analysis are collected but we are awaiting lab access in order to complete the study. Please refer to Chapter 5 for full details and our progress to date.

Finally, Chapter 6 is the concluding chapter of this thesis. The main findings and conclusions of this work will be summarised, as well and limitations and future directions.

2.3 Specific Objectives

The primary purpose and secondary aims of each study are outlined below.

2.3.1 Prevalence and Outcomes of UPC in Presumed Aseptic Revision THA

The primary purpose of this study was to:

(I) Determine the prevalence of UPC in presumed aseptic revision THA and to report on the infection-free implant survival for this cohort.

Secondary aims included:

(I) Comparing infection-free implant survival between patients with 1 versus ≥2 UPCs.

(II) Comparing infection-free implant survival between patients with a single UPC treated with antibiotics versus not treated with antibiotics (considered a contaminant).

(III) Reporting on the infection-free implant survival for those with a single *Cutibacterium acnes* UPC.

2.3.2 Prevalence and Outcomes of UPC in Presumed Aseptic Revision TKA

The primary purpose of this study was to:

(I) Determine the prevalence of UPC in presumed aseptic revision TKA and the infection-free implant survival for this cohort.

Secondarily aims included comparing:

(I) The infection-free implant survival between patients with 1 versus ≥2 UPCs.

(II) The infection-free implant survival between patients treated with antibiotics versus not treated with antibiotics (considered a contaminant).

2.3.3 Do Microorganisms Have a Role in ‘Aseptic’ THA and TKA Implant Failure? A Prospective Molecular Study

Using modern molecular and sequencing methods with an emphasis on the stringent control of contamination our aims are to:

(I) Determine the frequency and type of bacterial microorganisms on prosthetic implants from presumed aseptic THA and TKA failures, and compare the microorganisms identified by molecular methods to those of standard cultures.
(II) Determine the type of implants and location on the implants that bacterial microorganisms are found.
(III) Determine if the presence of bacterial microorganisms is associated with the reason for revision.

2.4 References

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Chapter 3

3. The Prevalence and Outcomes of Unexpected Positive Intraoperative Cultures in Presumed Aseptic Revision Hip Arthroplasty

3.1 Introduction

Total hip arthroplasty (THA) is a highly successful and cost-effective treatment for end-stage arthritis(1,2). Approximately 500,000 THA are performed in North America annually(3,4), and this number is projected to increase substantially(4,5). Up to 12% of primary THA needs revision surgery by 10 years (6), and the number of revisions is also projected to increase markedly(4,5).

Periprosthetic joint infection (PJI) is a leading cause of revision at a rate of approximately 2% for primary THA(7–9). PJI is a dreaded complication associated with substantial morbidity and cost, and the rate has remained constant over time(7,8,10). Despite considerable scientific efforts, there remains no perfect test to diagnose PJI(11–13) and it has been postulated that a significant proportion of presumed aseptic failures are actually undiagnosed PJI(14–16). This results in the occurrence of unexpected positive intraoperative cultures (UPC) in presumed aseptic revision THA. UPC pose a challenging clinical problem because the surgical treatment of aseptic versus PJI-related failure differs substantially, and surgeons only become aware of the UPC after the presumed aseptic revision surgery.

The prevalence of UPC in presumed aseptic revision in the literature varies considerably (4-38%)(17). There is no consensus on the optimal treatment of UPC and the consequence in terms of infection-free implant survival remains unclear(14,17–21). The clinical significance of PJI diagnosed by UPC in presumed aseptic revision remains highly debated in the literature, and the significance of a single UPC is even more unclear(14,17–20,22). The literature on UPC is limited and larger studies are needed(17).

The primary purpose of this study was to determine the prevalence of UPC in presumed aseptic revision THA and to report on the infection-free implant survival for this cohort.

Secondary aims included (I) comparing infection-free implant survival between patients with a single UPC versus ≥2 UPCs, (II) comparing infection-free implant survival between patients with a single UPC treated with antibiotics versus not treated with antibiotics (considered a contaminant), and (III) reporting on infection-free implant survival for those with a single *Cutibacterium acnes* (*C. acnes*) UPC.

3.2 Patients and Methods

After obtaining ethics approval, we performed a retrospective review of operative notes and electronic medical records (EMRs) of all consecutive revision THA procedures contained in our prospective institutional database. We identified 2228 revision THA cases performed at our tertiary care academic center between January 2006 and April 2019, to allow for a minimum 1-year follow-up. Adult patients undergoing a single-stage presumed aseptic revision THA with intraoperative culture sample(s) taken during the procedure were eligible for inclusion in the study. Revisions were excluded if (I) PJI was known (including being on chronic antibiotic suppression) or suspected preoperatively, (II) the revision was part of treatment of PJI (debridement, antibiotics with implant retention procedure, one-stage or two-stage revision for PJI), (III) revisions of a hemiarthroplasty, or (IV) if no intraoperative samples were taken for culture or results were unavailable. Patients lost to follow-up less than 1-year from index study revision were excluded, unless this was secondary to a death (censored in survival analysis), subsequent aseptic revision (censored in survival analysis), or recurrent PJI (study endpoint) within 1-year. All single-stage presumed aseptic revisions meeting inclusion/exclusion criteria established the base cohort used to determine the prevalence of UPC. Of these, revisions with a minimum of 1 UPC (organism in broth or solid media) comprised the final study UPC cohort.

A detailed manual review of EMRs was performed to obtain patient, demographic, laboratory, microbiological, operative, treatment, and outcome data for the UPC study cohort (Table 3.1 and Table 3.2). There were 9 arthroplasty fellowship trained surgeons performing the surgeries with the aid of fellows and/or residents during the study period. Routine practice was to evaluate all failed revisions preoperatively for PJI both clinically and with serum c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). However, a joint fluid aspirate was performed only if these parameters were suspicious for PJI. Intraoperative samples for culture were taken using swab, fluid aspirate, or tissue samples at the discretion of the treating surgeon.

The number or type of samples taken was not standardized and varied based on surgeon preference. The microorganism, solid or broth status, and antibiotic sensitivity of each UPC was documented. Any cement used during revisions at our institution contains antibiotics (Bone Cement Antibiotic Simplex P with Tobramycin 1g; Stryker). Postoperatively patients received either standard 24 to 48-hour prophylactic antibiotics (cefazolin unless patient allergy), antibiotics until preliminary culture results were negative, or until cultures were negative out to 5-days (if in hospital), based on surgeon preference.

UPC treatment decisions were made by the treating surgeon, often in collaboration with infectious disease or microbiologist experts. Antibiotic and/or surgical treatment of the UPC was based on a combination of preoperative, patient, intraoperative/surgical, microorganism, and postoperative variables, as well as the number of UPCs. However, there was no predefined protocol for UPC treatment or routine multidisciplinary rounds at our institution.

Infection-related implant failure was defined as the occurrence of infection any time after the index study revision that required antibiotic treatment or revision surgery for PJI. Prior to 2012 the diagnosis of PJI was based on clinical, laboratory, and intraoperative variables, but not in a standardized or universally accepted fashion. Since 2012, the diagnosis of PJI at our institution was made based on the Musculoskeletal Infection Society (MSIS) definition for PJI criteria and updated versions(11,12). The microorganism(s) grown from cultures of the subsequent PJI was recorded and compared to the microorganism(s) of the index revision surgery UPC. All subsequent PJI were treated with surgery and antibiotic therapy, unless medically unfit for surgery. Any subsequent aseptic revision was documented, with the etiology and time from index revision surgery noted. Latest EMR clinical follow-up was used as latest follow-up, unless subsequent PJI, subsequent aseptic revision, or death occurred first (in order of occurrence).

The secondary aims were achieved by creating cohorts from the UPC study cohort: (I) a 1 UPC versus ≥2 UPC cohort based on number of UPCs during index revision surgery, (II) a 1 UPC cohort treated with antibiotics versus a 1 UPC cohort not treated with antibiotics (considered contaminant), and (III) a cohort of patients with a single UPC of *C. acnes* only.

3.2.1 Statistical Analysis

Statistical analysis was performed using SPSS v26.0 (IBM Inc., Armonk, NY). Descriptive statistics were used to report on variables and outcomes of interest. Means and standard deviations (SD), or medians and interquartile ranges (IQR), were used when appropriate. The prevalence of UPC was calculated. The Kaplan-Meier technique with 95% confidence intervals (CI) was used to determine the infection-free implant survival at 2 and 5-years for UPC study cohort, with subsequent PJI as the endpoint. Patients who died, underwent subsequent revision, or were lost to follow-up after the 1-year mark were censored. The 2- and 5-year Kaplan-Meier survival of the entire UPC cohort was repeated, using subsequent PJI by same microorganism as the UPC as the endpoint. The 5-year infection-free survival was calculated for all cohorts of interest, with subsequent PJI as the endpoint. The log-rank test was used to compare infection-free survival between the cohorts of interest. Categorical data was compared using the Pearson's chi-squared test or Fisher's exact test, when appropriate. Continuous data was compared using two-sample t tests or Wilcoxon rank-sum tests for parametric and nonparametric data, respectively. The Shapiro–Wilk test was used to test normality. Statistical significance was 2-tailed and set at a p-value ≤ 0.05.

3.3 Results

A flowchart of eligible revisions and number of revisions with UPC is shown in Figure 3.1. After exclusions there were 1336 eligible one-stage aseptic revision THAs. One-hundred and forty did not have intraoperative samples taken for culture, resulting in a base cohort of 1196 aseptic revisions to determine prevalence of UPC. The prevalence of ≥1 UPC in presumed aseptic revision THA was 9.2% (110/1196).

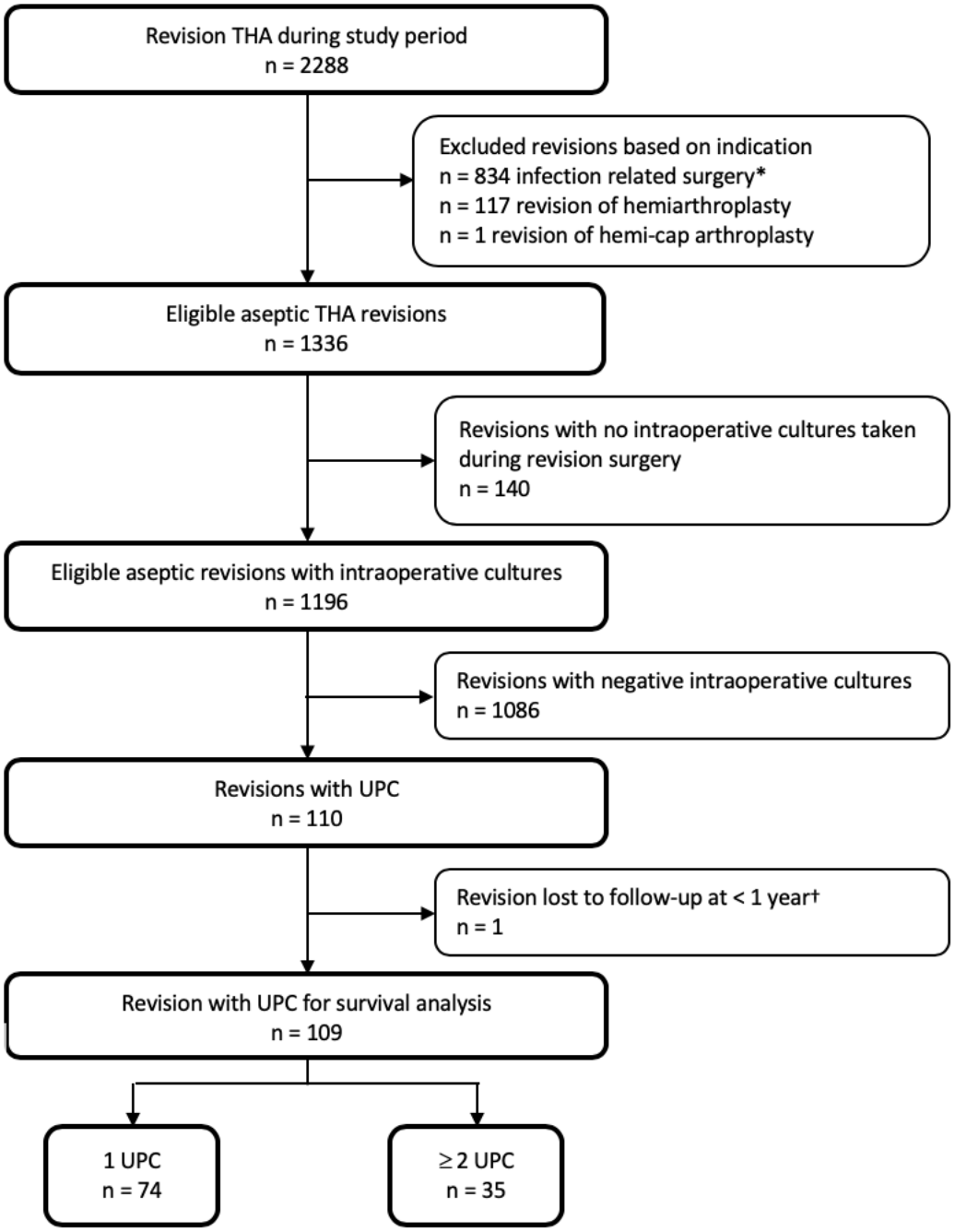

Figure 3.1. Flowchart of eligible aseptic THA revisions and revisions with UPC.
*Infection related surgeries include 1-stage, 2-stage, and debridement, antibiotics, and implant retention with modular exchange for periprosthetic joint infection, as well as revisions with known suppressed infection or those suspected of being infected. †Revisions that had the endpoint of recurrent infection, and those that had subsequent aseptic revision surgery or died prior to 1-year follow-up were not excluded from survival analysis. THA, total hip arthroplasty; UPC, unexpected positive intraoperative cultures.

In the 110 UPC cohort only 1 revision was lost to follow-up before 1-year for reasons other than death, a subsequent aseptic revision, or PJI. This patient's care was transferred closer to home immediately postoperatively to another city. This resulted in 109 UPC revisions included in the survival analysis. With time to subsequent PJI, subsequent aseptic revision, death, or latest clinical follow-up as the endpoint in order of occurrence, the median follow-up time was 3.3 years (IQR 1.5 to 6.4). Thirty-two revisions with UPC died at a mean of 3.7 years (SD 2.8). However, 28.1% (9/32) underwent a subsequent aseptic revision or had a PJI-related failure prior to death and were censored from survival analysis for those reasons. Of the 23 patients that died with no aseptic revision or subsequent PJI, 73.9% (17) died after the 1-year mark and 26.1% (6) died before the 1 year-mark.

Detailed baseline and operative data for entire UPC study cohort can be seen in Table 3.1. The dominant reason for revision was aseptic loosening, followed by polyethylene wear +/- osteolysis, instability, and periprosthetic fracture. Preoperative CRP and ESR were elevated in 18.3% (20) and 19.3% (21) of cases, respectively, and a preoperative aspiration were performed in 21.1% (23) of cases. Fifty-seven percent (62) of patients underwent a 1-component exchange during the study revision, and 33.5% (36) underwent modular head and liner exchange only.

Table 3.1. Baseline, demographic, and operative data of study population of 109 UPC revisions

Variable	
Age (years)*	72 (60 to 81)
Sex, F/M, n (%)	54/55 (49.5/50.5)
BMI (kg/m^2)*	29.2 (24.9 to 33.4)
ASA classification, n (%)	
1	0 (0)
2	25 (22.9)
3	66 (60.6)
4	18 (16.5)
Smoking, n (%)	24 (22.0)
Diabetes, n (%)	28 (25.7)
Anticoagulation, n (%)	13 (11.9)
Inflammatory condition, n (%)	14 (12.8)
Etiology for primary THA, n (%)	
Osteoarthritis	74 (67.9)
Dysplasia	9 (8.3)
Post-traumatic arthritis	5 (4.6)
Rheumatoid/inflammatory arthritis	6 (5.5)
Avascular necrosis	6 (5.5)
Other	9 (8.2)

Reasons for revision, n (%)	
Aseptic loosening	48 (44.0)
Instability	16 (14.7)
Polyethylene wear +/- osteolysis	22 (20.2)
Periprosthetic fracture	13 (11.9)
Adverse metal reaction	8 (7.3)
Other	2 (1.8)
Revision number*	1 (1 to 2)
History of previous THA revision in study joint, n (%)	35 (32.1)
Age of prosthesis (years)*	9.0 (2.6 to 17.0)
History of PJI in study joint, n (%)	5 (4.6)
Pre-operative serum CRP > 10mg/L, n (%)	20 (18.3)
Missing data CRP, n (%)	6 (5.5)
Pre-operative serum ESR > 30mm/h, n(%)	21 (19.3)
Missing data ESR, n (%)	7 (6.4)
Preoperative joint aspirate, n (%)	23 (21.1)
Type of revision, n (%)	
Modular exchange	36 (33.0)
1-component	62 (56.9)
2-component	11 (10.1)
Antibiotic cement used, n (%)	30 (27.5)

*Values are median (interquartile ranges). UPC, unexpected positive intraoperative cultures; F, female; M, male; BMI, body mass index; ASA, American society of anesthesiologists; THA, total hip arthroplasty; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate.

Detailed sampling, microbiological, treatment, and outcome data is shown in Table 3.2. A median of 4 samples (IQR 3 to 5) were taken per revision case. The most common sample type was tissue at 63.8% (270). Sixty-three percent (104) of UPC microorganisms were grown in solid medium, with the remainder grown in broth only. Sixty-eight percent (74) of the cohort had a single UPC and the remainder had ≥2 UPC. *C. acnes* was the most frequent microorganism at 37.6% (64). Coagulase-negative *Staphylococcus* species (CNS) comprised 33.5% (57) of all microorganisms, the most common being methicillin-resistant *Staphylococcus epidermidis* (MRSE) at 12.9% (22) (Table 3.2). Eleven percent (13) of the UPC revisions grew resistant microorganisms. Thirty-eight percent (41) of patients received antibiotic treatment for their UPC and the most frequent treatment duration was ≤ 6-weeks (46.3%). However, route and duration of antibiotic treatment varied (Table 3.2).

Eleven patients were diagnosed with a subsequent PJI at a median of 0.6 years (IQR 0.1 to 2.6). Of these, 6 occurred within 1-year, 4 occurred after the 2-year mark, and none occurred after 3.5 years. All were treated with surgery and antibiotics, except one palliative patient unfit

for surgery. Only 4/11 subsequent PJIs grew the same microorganism as the study revision UPC, 1/11 was mixed (subsequent PJI polymicrobial), and 6/11 grew a different microorganism.

Table 3.2. Sampling, microorganism, treatment, and outcome data for study population of 109 UPC revisions

Variable	
Number of samples taken in study revision*	4 (3 to 5)
Total samples taken, n	423
Swab samples, n (%)	135 (31.9)
Fluid samples, n (%)	18 (4.3)
Tissue samples, n (%)	270 (63.8)
Total number of UPC's, n	166
UPC broth, n (%)	62 (37.3)
UPC solid, n (%)	104 (62.7)
1 UPC vs ≥2 UPC, n (%)	
1 UPC	74 (67.9)
≥2 UPC	35 (32.1)
Microorganisms, n (%)	
C. acnes	64 (37.6)
MRSE	22 (12.9)
Other CNS	19 (11.2)
MSSE	16 (9.4)
Streptococcus sp	9 (5.3)
Micrococcus sp	8 (4.7)
Enterococcus sp	5 (2.9)
Corynebacterium sp	5 (2.9)
MSSA	4 (2.4)
E. coli	3 (1.8)
Bacillus sp.	3 (1.8)
Pseudomonas aeruginosa	3 (1.8)
Clostridium sp.	2 (1.2)
Others (7 species single occurrence)	7 (4.1)
Number of revisions resistant UPC, n (%)	13 (11.9)
Number revisions polymicrobial UPC, n (%)	13 (11.9)
Surgical treatment of UPC, n (%)	1 (0.9)
Antibiotic treatment of UPC, n (%)	41 (37.6)
Antibiotic route, n (%)	
Oral alone	10 (24.4)
IV alone	9 (21.9)
Combined IV and oral	22 (53.7)
Antibiotic duration, n (%)	
≤ 6 weeks	19 (46.3)
≤ 3 months	4 (9.8)
≤ 6 months	7 (17.1)
≤ 1 year	2 (4.9)
Chronic/lifelong suppression	9 (21.9)
Subsequent aseptic revision, n (%)†	9 (8.3)
Etiology subsequent aseptic revision, n (%)	
Instability	6 (66.7)

Aseptic loosening	3 (33.3)
Time to subsequent aseptic revision (years)‡	1.7 (1.2)
Subsequent PJI, n (%)	11 (10.1)
Time to subsequent PJI (years)*	0.6 (0.1 to 2.6)
Subsequent PJI microorganism, n (%)	
Same as UPC microorganism	4 (36.4)
Different than UPC microorganism	6 (54.5)
Mixed	1 (9.1)

*Values are median (interquartile ranges). †Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. ‡Values are mean (standard deviation). UPC, unexpected positive intraoperative cultures; *C. acnes*, *Cutibacterium acnes*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; CNS, coagulase-negative *Staphylococcus* species; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; sp, species; MSSA, methicillin-sensitive *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; IV, intravenous; PJI, periprosthetic joint infection.

The 2- and 5-year infection-free survival for the entire UPC cohort was 93.1% (95% CI 90.5% to 95.7%) and 86.8% (95% CI 82.9% to 90.7%), respectively (Figure 3.2). When considering only infection-related implant failure caused by the same microorganism as the UPC as the endpoint, the 2- and 5-year infection free survival for the entire UPC cohort was 95.8% (95% CI 93.7% to 97.9%) and 94.3% (95% CI 91.7% to 96.9%), respectively (Figure 3.3).

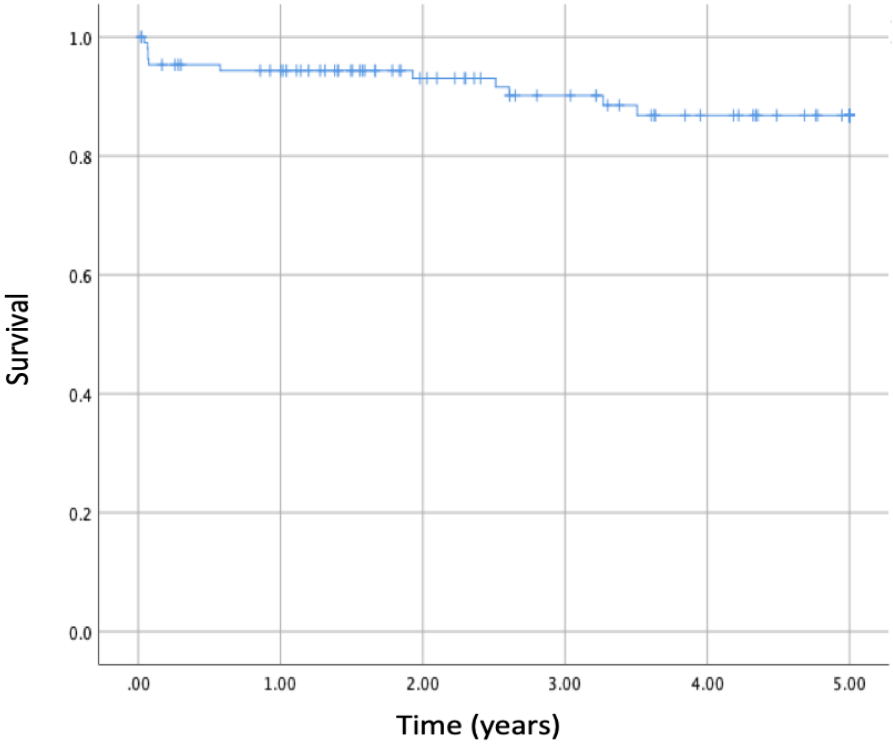

Figure 3.2. Figure 3.2. Kaplan-Meier 5-year infection-free survival for entire UPC cohort in presumed aseptic hip revisions.
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture.

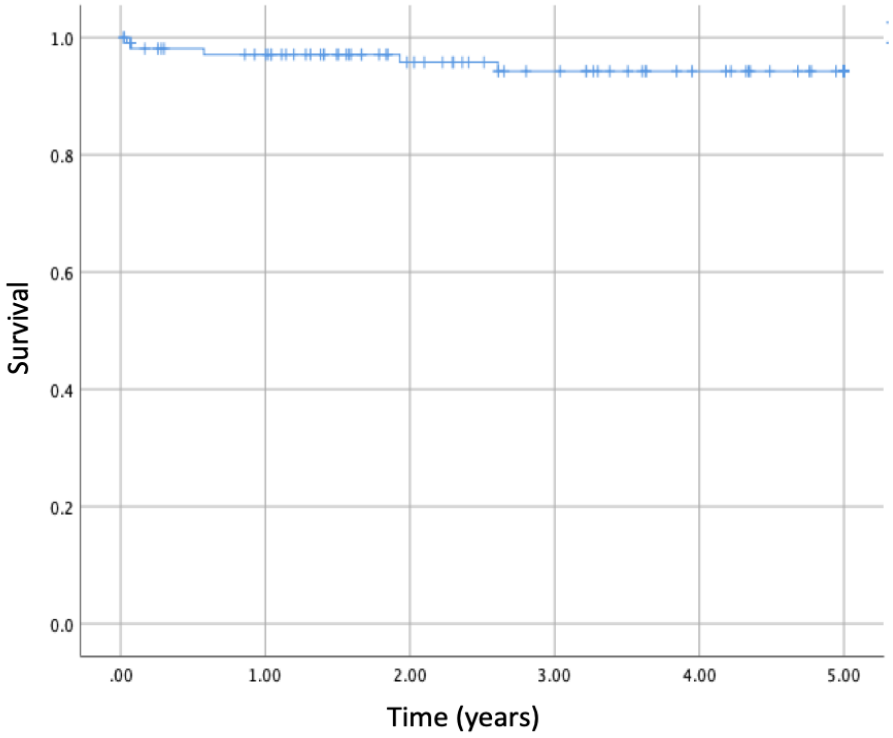

Figure 3. 3. Kaplan-Meier 5-year infection-free survival for entire UPC cohort with subsequent PJI by the same microorganism as the UPC as the endpoint.
Vertical spikes are censored data. UPC, Unexpected positive intraoperative culture; PJI, periprosthetic joint infection.

Detailed data for the 1 UPC versus ≥2 UPC cohorts is shown in Table 3.3. Most variables were similar between groups (p>0.05), however variability did exist (Table 3.3). *C. acnes* was the dominant microorganism in each cohort, however the ≥2 UPC cohort had a higher proportion of MRSE and proportions of other microorganisms differed between cohorts (p=0.002) (Table 3.3). The ≥2 UPC cohort had a higher proportion of resistant microorganisms (p=0.001) and were more likely to be treated with antibiotics versus the 1UPC cohort (74.3% versus 20.3%) (p=<0.001). The proportion of broth versus solid UPC was similar between cohorts (p=0.837). The shorter duration (p=0.096) and higher proportion of oral only antibiotic treatment (p=0.258) in the 1 UPC cohort was not statistically significant. The 5-year infection-free survival was similar for the 1 UPC versus ≥2 UPC cohorts, at 86.4% (95% CI 81.8% to 91.0%) and 88.5% (95% CI 82.0% to 95%), respectively (p=0.906) (Figure 3.4). Interestingly, 100% (3) of the subsequent PJIs in the ≥2 UPC cohort were caused by the same organism as the study revision

UPC, while only 25% (2) in the 1 UPC cohort were caused by same microorganism as the UPC (p=0.024).

Table 3.3. Baseline, demographic, operative, microbiological, treatment, and outcome data for revisions with 1 UPC versus ≥2 UPC

Variable	1 UPC (n = 74)	≥2 UPC (n = 35)	P value
Age (years)*	73.0 (63.8 to 82.0)	69.0 (59 to 80)	0.185[a]
Sex, F/M, n (%)	39/35 (52.7/47.3)	15/20 (42.9/57.1)	0.337[b]
BMI (kg/m^2)*	28.1 (24.8 to 33.7)	30.0 (25.1 to 33.2)	0.664[a]
ASA classification, n (%)			0.571[c]
1	0	0	
2	15 (20.3)	10 (28.6)	
3	47 (63.5)	19 (54.3)	
4	12 (16.2)	6 (17.1)	
Diabetes, n (%)	22 (29.7)	6 (17.1)	0.160[b]
Inflammatory condition, n (%)	12 (16.2)	2 (5.7)	0.218[c]
Etiology for primary THA, n (%)			0.917[b]
Osteoarthritis	50 (60.7)	24 (68.6)	
Other	24 (39.3)	11 (31.4)	
Reasons for revision, n (%)			0.940[c]
Aseptic loosening	32 (43.2)	16 (45.7)	
Instability	10 (13.5)	6 (17.1)	
Polyethylene wear +/- osteolysis	15 (20.3)	7 (20.0)	
Periprosthetic fracture	10 (13.5)	3 (8.6)	
Adverse metal reaction	6 (8.1)	2 (5.7)	
Other	1 (1.4)	1 (2.9)	
History of previous THA revision in study joint, n (%)	20 (27.0)	14 (40.0)	0.172[b]
Age of prosthesis (years)*	9.0 (2.1 to 17.0)	9.0 (3.6 to 22)	0.393[a]
History of PJI in study joint, n (%)	3 (4.1)	2 (5.7)	0.655[c]
Pre-operative serum CRP > 10mg/L, n (%)	14 (18.9)	6 (17.1)	0.828[b]
Pre-operative serum ESR > 30mm/h, n (%)	16 (21.6)	5 (14.3)	0.348[b]
Preoperative joint aspirate, n (%)	13 (17.6)	10 (28.6)	0.189[b]
Type of revision, n (%)			0.551[b]
Modular exchange	24 (32.4)	12 (34.3)	
1-component	44 (59.5)	18 (51.4)	
2-component	6 (8.1)	5 (14.3)	
Number of samples taken in study revision*	3.0 (3.0 to 5.0)	4.0 (3.0 to 5.0)	0.056[a]
Swab used for culture in revision, n (%)	51 (68.9)	21 (60.0)	0.359[b]
Fluid used for culture in revision, n (%)	9 (12.2)	7 (20.0)	0.280[b]
Tissue used for culture in revision, n (%)	64 (86.5)	29 (82.9)	0.617[b]
UPC broth or solid, n (%)			0.837[b]
Broth	27 (36.5)	35 (38.0)	
Solid	47 (63.5)	57 (62.0)	
Microorganisms, n (%)			0.002[c]
C. acnes	35 (46.1)	29 (30.9)	
MRSE	3 (3.9)	19 (20.2)	
MSSE	7 (9.2)	9 (9.6)	

Other CNS	12 (15.8)	7 (7.4)	
Micrococcus sp	2 (2.6)	6 (6.4)	
MSSA	0	4 (4.2)	
Streptococcus sp	5 (6.6)	4 (4.2)	
Enterococcus sp	1 (1.3)	4 (4.2)	
Corynebacterium sp	1 (1.3)	4 (4.2)	
Pseudomonas aeruginosa	0	3 (3.2)	
Others	10 (13.2)	5 (5.3)	
Number of revisions resistant UPC, n (%)	3 (4.1)	10 (28.6)	0.001[c]
Antibiotic treatment of UPC, n (%)	15 (20.3)	26 (74.3)	0.000[b]
Antibiotic route, n (%)			0.258[c]
Oral alone	6 (40)	4 (15.4)	
IV alone	3 (20)	6 (23.1)	
Combined IV and oral	6 (40)	16 (61.5)	
Antibiotic duration, n (%)			0.096[c]
≤ 6 weeks	11 (73.3)	8 (30.8)	
≤ 3 months	1 (6.7)	3 (11.5)	
≤ 6 months	2 (13.3)	5 (19.2)	
≤ 1 year	0	2 (7.7)	
Chronic/lifelong suppression	1 (6.7)	8 (30.8)	
Subsequent aseptic revision, n (%)†	8 (10.8)	1 (2.9)	0.267[c]
Subsequent PJI, n (%)	8 (10.8)	3 (8.6)	1.000[c]
Time to subsequent PJI (years)*	1.0 (0.1 to 3.1)	0.58 (0.1 to 1.1)	0.766[a]
Subsequent PJI microorganism, n (%)			0.024[c]
Same as UPC microorganism	1 (12.5)	3 (100)	
Different than UPC microorganism	6 (75.0)	0	
Mixed	1 (12.5)	0	

*Values are median (interquartile ranges). †Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. [a]Wilcoxon's rank-sum test. [b]Pearson's chi-squared test. [c]Fisher's exact test. UPC, unexpected positive intraoperative cultures; F, female; M, male; BMI, body mass index; ASA, American society of anesthesiologists; THA, total hip arthroplasty; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; *C. acnes*, *Cutibacterium acnes*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; CNS, coagulase-negative *Staphylococcus* species; sp, species; MSSA, methicillin-sensitive *Staphylococcus aureus*; IV, intravenous.

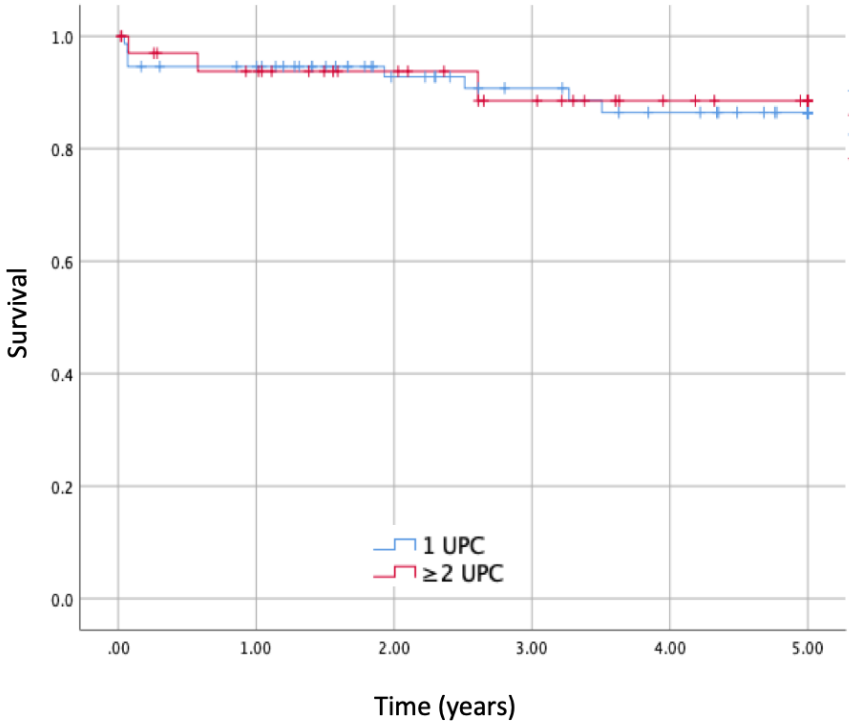

Figure 3.4. Kaplan-Meier 5-year infection-free survival for the 1 versus ≥2 UPC cohorts.
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture.

Detailed data for revisions with 1 UPC treated with antibiotics versus not treated with antibiotics is shown in Table 3.4. Eighty percent (59) of revisions with a single UPC did not receive antibiotic treatment. The majority of variables were similar between cohorts ($p>0.05$), but important differences were noted (Table 3.4). The antibiotic cohort had a higher proportion of patients with elevated preoperative serum CRP ($p=0.003$) and ESR ($p=0.008$). The differences in type of revision ($p=0.068$), reasons for revision ($p=0.054$), and higher proportion of patients with an inflammatory condition ($p=0.059$) in the antibiotic treatment were clinically important but did not reach statistical significance. The proportion of broth versus solid UPC between cohorts was similar (0.537). Differences in microorganisms were not statistically significant ($p=0.193$), however the no antibiotic cohort had a higher proportion of *C. acnes*. The 5-year infection free-survival was similar for the 1 UPC antibiotic versus no antibiotic treatment cohorts, at 84.8% (95% CI 74.8% to 94.8%) and 86.9% (81.8% to 92.0%), respectively ($p=0.706$) (Figure 3.5). All of the subsequent PJIs (2/2) in the antibiotic treatment cohort grew the same microorganism as the study revision UPC (1 same and 1 mixed), however none of the

subsequent PJIs (0/6) in the no antibiotic cohort were caused by the same microorganism as the UPC (p=0.036).

Table 3.4. Baseline, demographic, operative, microbiological, treatment, and outcome data for revisions with 1 UPC treated with antibiotics versus those not treated with antibiotics

Variable	Antibiotic treatment (n = 15)	No antibiotic treatment (n = 59)	P value
Age (years)*	78.0 (63.0 to 85.0)	73.0 (64.0 to 80.0)	0.459[a]
Sex, F/M, n (%)	10/5 (66.7/33.3)	29/30 (49.2/50.8)	0.225[b]
BMI (kg/m^2)*	26.7 (25.6 to 33.3)	29.7 (24.6 to 34.3)	0.568[a]
ASA classification, n (%)			1.000[c]
1	0	0	
2	3 (20.0)	12 (20.3)	
3	10 (66.7)	37 (62.7)	
4	2 (13.3)	10 (16.9)	
Diabetes, n (%)	2 (13.3)	20 (33.9)	0.205[c]
Inflammatory condition, n (%)	5 (33.3)	7 (11.9)	0.059[c]
Etiology for primary THA, n (%)			0.543[c]
Osteoarthritis	9 (60.0)	41 (69.5)	
Other	6 (40.0)	18 (30.5)	
Reasons for revision, n (%)			0.054[c]
Aseptic loosening	7 (46.7)	25 (42.4)	
Instability	2 (13.3)	8 (13.6)	
Polyethylene wear +/- osteolysis	0	15 (25.4)	
Periprosthetic fracture	5 (33.3)	5 (8.5)	
Adverse metal reaction	1 (6.7)	5 (8.5)	
Other	0	1 (1.7)	
History of previous THA revision in study joint, n (%)	2 (13.3)	18 (30.5)	0.328[c]
Age of prosthesis (years)*	11.0 (3.0 to 19.0)	9.0 (2.0 to 17.0)	0.793[a]
History of PJI in study joint, n (%)	0	3 (5.1)	1.000[c]
Pre-operative serum CRP > 10mg/L, n (%)	7 (46.7)	7 (11.9)	0.003[c]
Pre-operative serum ESR > 30mm/h, n (%)	7 (46.7)	9 (15.3)	0.008[c]
Preoperative joint aspirate, n (%)	4 (26.7)	9 (15.3)	0.458[c]
Type of revision, n (%)			0.068[c]
Modular exchange	2 (13.3)	22 (37.3)	
1-component	13 (86.7)	31 (52.5)	
2-component	0	6 (10.2)	
Number of samples taken in study revision*	4 (3.0 to 4.0)	3.0 (3.0 to 5.0)	0.978[a]
UPC from swab sample, n (%)	1 (6.7)	20 (33.9)	0.205[c]
UPC from fluid sample, n (%)	1 (6.7)	1 (1.7)	0.367[c]
UPC from tissue sample, n (%)	13 (86.7)	38 (64.4)	0.125[c]
UPC from broth or solid, n (%)			0.537[b]
Broth	7 (46.7)	20 (33.9)	
Solid	8 (53.3)	39 (66.1)	
Microorganisms, n (%)			0.193[c]
C. acnes	4 (25.0)	31 (51.7)	

Other CNS	5 (31.3)	7 (11.7)	
MSSE	2 (12.5)	5 (8.3)	
Streptococcus sp	2 (12.5)	3 (5.0)	
MRSE	1 (6.3)	2 (3.3)	
Micrococcus sp	0	2 (3.3)	
Others	2 (12.5)	10 (16.7)	
Number of revisions resistant UPC, n (%)	1 (6.7)	2 (3.4)	0.499[c]
Subsequent aseptic revision, n (%)	1 (6.7)	7 (11.9)	1.000[c]
Subsequent PJI, n (%)	2 (13.3)	6 (10.2)	0.660[c]
Time to subsequent PJI (years)*	1.0 (0.04 to 2.1)	1.3 (0.1 to 3.3)	0.429[a]
Subsequent PJI microorganism, n (%)			0.036[c]
Same as UPC microorganism	1 (50.0)	0	
Different than UPC microorganism	0	6 (100)	
Mixed	1 (50.0)	0	

*Values are median (interquartile ranges). †Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. [a]Wilcoxon's rank-sum test. [b]Pearson's chi-squared test. [c]Fisher's exact test. UPC, unexpected positive intraoperative cultures; F, female; M, male; BMI, body mass index; ASA, American society of anesthesiologists; THA, total hip arthroplasty; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; *C. acnes*, *Cutibacterium acnes*; CNS, coagulase-negative *Staphylococcus* species; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; sp, species; MRSE, methicillin-resistant *Staphylococcus epidermidis*.

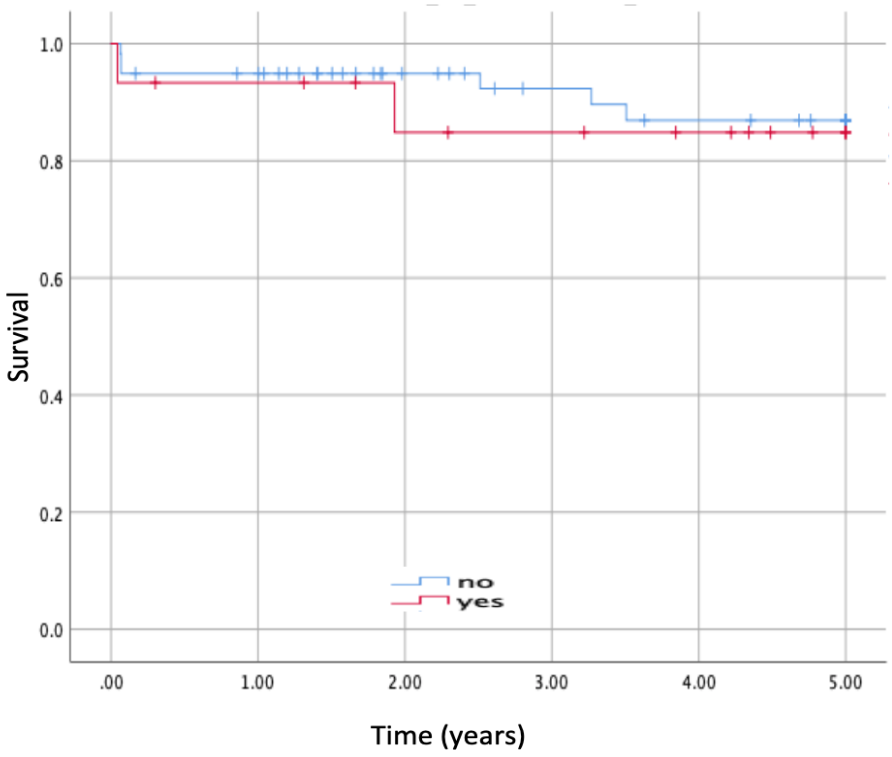

Figure 3.5. Kaplan-Meier 5-year infection-free survival for the 1 UPC cohort treated with antibiotics (yes) versus not treated with antibiotics (no).
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture.

Detailed data for the 1 UPC cohort with *C. acnes* is shown is Table 3.5. The 5-year infection-free survival was 83.3% (95% CI 76.3% to 90.3%). Only 4/35 patients were treated with antibiotics and all for ≤6 weeks duration. However, 4/5 of the subsequent PJIs were caused by different microorganisms than the UPC and only 1/5 of the subsequent PJIs grew *C. acnes* (polymicrobial).

Table 3.5. Baseline, demographic, operative, microbiological, treatment, and outcome data for revisions with a single UPC of *C. acnes* (n = 35)

Variable	
Age (years)*	69.0 (58.0 to 79.0)
Sex, F/M, n (%)	17/18 (48.6/51.4)
BMI (kg/m^2)*	28.4 (24.6 to 34.4)
ASA classification, n (%)	
1	0
2	8 (22.9)
3	21 (60.0)
4	6 (17.1)
Diabetes, n (%)	12 (34.3)
Inflammatory condition, n (%)	4 (11.4)
Etiology for primary THA, n (%)	
Osteoarthritis	23 (65.7)
Other	12 (34.3)
Reasons for revision, n (%)	
Aseptic loosening	17 (48.6)
Instability	4 (11.4)
Polyethylene wear +/- osteolysis	5 (14.3)
Periprosthetic fracture	5 (14.3)
Adverse metal reaction	4 (11.4)
Other	0
History of previous THA revision in study joint, n (%)	11 (31.4)
Age of prosthesis (years)*	9.0 (2.0 to 14.0)
History of PJI in study joint, n (%)	2 (5.7)
Pre-operative serum CRP > 10mg/L, n (%)	5 (14.3)
Pre-operative serum ESR > 30mm/h, n (%)	7 (20)
Preoperative joint aspirate, n (%)	7 (20.0)
Type of revision, n (%)	
Modular exchange	9 (25.7)
1-component	22 (62.9)
2-component	4 (11.4)
Number of samples taken in study revision*	3.0 (3.0 to 5.0)
UPC from a swab sample, n (%)	10 (28.6)
UPC from a tissue sample, n (%)	25 (71.4)
UPC broth, n (%)	13 (37.1)
UPC solid, n (%)	22 (62.9)
Number of revisions resistant UPC, n (%)	0

Antibiotic treatment of UPC, n (%)	4 (11.4)
Antibiotic route, n (%)	
Oral alone	3 (75.0)
IV alone	1 (25.0)
Combined IV and oral	0
Antibiotic duration, n (%)	
≤ 6 weeks	4 (100.0)
≤ 3 months	0
≤ 6 months	0
≤ 1 year	0
Chronic/lifelong suppression	0
Subsequent aseptic revision, n (%)†	3 (8.6)
Subsequent PJI, n (%)	5 (14.3)
Time to subsequent PJI (years)*	0.1 (0.5 to 2.9)
Subsequent PJI microorganism, n (%)	
Same as UPC microorganism	0
Different than UPC microorganism	4 (80.0)
Mixed	1 (20.0)

*Values are median (interquartile ranges).†Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. UPC, unexpected positive intraoperative cultures; *C. acnes*, *Cutibacterium acnes*; F, female; M, male; BMI, body mass index; ASA, American Society of Anesthesiologists classification; THA, total hip arthroplasty; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; IV, intravenous.

3.4 Discussion

The prevalence, clinical significance, and outcomes of UPC in presumed aseptic revision THA are unclear. Our aims were to report on the prevalence and infection-free survival in this patient population, as well as other clinical cohorts of interest. To our knowledge, this is the largest series of UPC in presumed aseptic revision THA in the literature.

We demonstrated that the prevalence of UPC in presumed aseptic revision THA was 9.2%, similar to that of 10.5% reported by a recent review of UPC in revision total knee (TKA) and THA(17). However, UPC was twice more common in THA than TKA and there was considerable variability (4-38%) between studies due to differences in preoperative evaluation protocols, aseptic revision cohort baseline data, sample size, follow-up, sampling and laboratory techniques, and definitions of UPC or PJI(17). We included broth only UPC because the specificity of these cultures have been shown to be high(23), and other studies have as well(18,20). Although studies including single UPCs tend report a higher incidence(19,20,24–27), the reported incidence varies when considering only ≥2 UPC as well(14,18,21,28–30).

Jacobs et al.(18) reported a 12.1% (26/214) incidence of PJI diagnosed by ≥2 UPC by the same microorganism in their THA cohort, excluding a single UPC as a contaminant or false-positive. In our institution the prevalence of UPC with ≥2 UPC was only 3.0% (36). *C. acnes* and CNS were the most common microorganisms, supporting the indolent nature of microorganisms common to UPC in the literature(17,18,20). *Staphylococcus epidermidis* and CNS are the most common microorganisms identified in the majority of early studies(14,19,20,28,31), however, *C. acnes* was also reported as the most common microorganism in a recent study with extended anaerobic incubation times(18). Our increased detection of *C. acnes* and may be due to the extended anerobic incubation time of 10-14 days for the majority of the study period and UPC revisions in our study, which has been shown to increase the detection of *C. acnes* substantially(32). Although infrequent, virulent and antibiotic resistant microorganisms did occur(17,20).

The 2- and 5-year infection-free survival for the entire UPC cohort was 93.1% (95% CI 90.5% to 95.7%) and 86.8% (95% CI 82.9% to 90.7%), respectively. We report on infection-free survival in hips separate from knees because the prognosis for these two cohorts has recently been show to differ(18). Additionally, we felt that reporting out to 5-years was important given the low virulence microorganisms common to UPC. No failure due to PJI were encountered after 3.5 years. The results of our large cohort are encouraging and consistent with a majority of the literature(17,18,20). In hips, Jacobs et al(18) found that the ≥2 UPC PJI cohort had similar infection free survival at 2-years as the aseptic cohort, however there were only 26 revisions in the UPC cohort. The majority of studies reporting 95-100% infection-free survival tended to have short follow-up or use advanced techniques such as implant sonication or molecular technology(14,27,31,33), both of which do not apply to our study. Even more encouraging is that when only considering infection-related failure caused by the same microorganism as the UPC, the 2- and 5-year infection-free survival for the entire UPC cohort was 95.8% (95% CI 93.7% to 97.9%) and 94.3% (95% CI 91.7% to 96.9%), respectively. Subsequent PJIs caused by different microorganisms likely represent a new infection independent of the UPCs, however it is possible that these microorganisms were present during the study revision but missed due to sensitivity limitations of cultures to identify microorganisms in PJI(34).

The infection-free survival between the 1 UPC and ≥2 UPC cohorts were similar in our study. However, this finding must be treated with caution. The ≥2 UPC had more patients treated

with antibiotics, differences in type of microorganisms, a higher proportion of resistant microorganisms, and a higher proportion on lifelong antibiotic suppression compared to the 1 UPC cohort. Saleh et al.(20) found that patients with a single UPC that did not meet institutional criteria for antibiotic treatment had lower rates of infection than those treated with antibiotics, whether the antibiotic cohort was MSIS criteria positive or not. Although the survival of ≥2 UPC is favorable in the literature(17–19), unacceptably high failure rates have been reported in this cohort(31,33). Our results suggest that the ≥2 UPC cohort is much more likely than the 1 UPC cohort to fail from ongoing or recurrent infection with the same microorganisms as the UPC (100% versus 25%). These findings agree with that the majority of the literature (17,20), but are not universal (18).

Nearly 80% (59) of patients with 1 UPC in our study did not receive antibiotic treatment and had similar-infection free survival as the 1UPC cohort treated with antibiotics. Similarly, these results must be interpreted with caution due to differences between cohorts and an important selection bias for those treated with antibiotics. We found that no subsequent PJI in the no antibiotic treatment cohort was caused by the same microorganism as the UPC, while all subsequent PJIs in the antibiotic cohort grew the same UPC microorganism. In the revisions with a single *C. acnes* UPC only 4/35 patients were treated with a short course of antibiotics and the 5-year infection-free survival was 83.3% (95% CI 76.3% to 90.3%). However, 4/5 of the subsequent PJIs were caused by different microorganisms than the UPC.

There is considerable controversy regarding the clinical significance of a single UPC. Several authors have suggested that in that the absence of other signs of infection single UPCs are likely contaminants and do not require treatment(14,19,26), a notion supported by the MSIS definition of PJI(11). *C. acnes* was a common single UPC in these studies and was specifically reported on by Dramis et al.(26). However, Saleh et al.(20) showed that even a single UPC with a high virulence microorganism not meeting MSIS criteria for PJI may truly be an infection and require antibiotic treatment. Revisions with a single UPC in our study had an extremely low risk of developing a recurrent infection with the same microorganism and those not treated with antibiotics had no recurrent infections by the UPC microorganism. Our results suggest that a single UPC without signs of infection is likely a contaminant and can be observed clinically without antibiotic treatment.

The current study has limitations. This is a single high-volume academic center and results may not be generalizable. However, the multiple surgeons included may help improve the generalizability of the results and most of our results are consistent with the literature. The retrospective design of this is subject to associated biases. There was no standardized preoperative PJI screening protocol, however all revisions were evaluated both clinically and with serum CRP and ESR. An aspirate was ordered selectively based suspicion for PJI, thus MSIS criteria could not be retrospectively applied. Consistent with the majority of UPC literature, the type and number of samples taken during revision surgery for culture was not standardized, both of which have been shown to be important for detecting microorganisms and PJI(24,35,36). Additionally, the lack of a standardized UPC treatment protocol introduced important selection biases. Lastly, our study was underpowered to detect differences between cohorts for secondary outcomes of interest. However, the UPC literature is limited and this study represents the largest series of UPC in presumed aseptic THA. Additionally, the inclusion of single UPC provides data a common and clinically controversial problem.

In conclusion, the prevalence of UPC in presumed aseptic revision THA was 9.2% and the infection-free survival at 2 and 5-years is encouraging. The infection-free implant survival when only considering PJI by the same UPC microorganism is excellent. Although we did not find a difference in infection-free implant survival between cohorts of interest, this must be interpreted with caution. Patients with ≥2 UPC or a single UPC that was deemed to require antibiotic treatment were more likely to have recurrent infection with the same microorganism as the UPC. Patients with a single UPC are unlikely to have recurrent infection by the same microorganism as the UPC and no patient with a single UPC not treated with antibiotics had an infection with the same microorganism. Thus, patients with a single UPC and no other signs of infection can be considered contaminants, and do not require antibiotic treatment.

3.5 References

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Chapter 4

4. The Prevalence and Outcomes of Unexpected Positive Intraoperative Cultures in Presumed Aseptic Revision Knee Arthroplasty

4.1 Introduction

Total knee arthroplasty (TKA) is a highly successful and cost-effective treatment for end-stage arthritis(1). Currently, over 1 million TKA are performed in North America annually(2,3), and these numbers are expected to increased markedly(2,4). At the 10-year mark, up to 12% of primary TKA require revision surgery(5), and the number of revisions is also projected to increase substantially(4).

Periprosthetic joint infection (PJI) occurs at a rate of approximately 2% for primary TKA and is a leading cause for revision(6–8). PJI is associated with enormous financial cost and morbidity, and the rate of this dreaded complication has remained constant over time(6,7,9,10). Despite great scientific effort there remains no perfect test to diagnose PJI in TKA(11–13), and a proportion of presumed aseptic failures may be undiagnosed PJI(14–16). Consequently, unexpected positive intraoperative cultures (UPC) in presumed aseptic revisions occur and can be expected to remain a problem. UPCs are clinically challenging because the surgeon becomes aware of the UPC after the presumed aseptic revision surgery, and the surgical management of aseptic failure differs significantly compared to PJI-related failure.

The prevalence of UPC in presumed aseptic revision TKA remains unclear (4-38%), as does the optimal treatment and clinical consequence in terms of infection-free survival(14,17–21). There is debate regarding the clinical significance of PJI diagnosed by UPC, and the significance of a single UPC in presumed aseptic revision is even more uncertain(17–19,21,22). The literature on UPC in revision TKA is inadequate and larger studies are needed(21).

Our primary aim was to determine the prevalence of UPC in presumed aseptic revision TKA and the infection-free implant survival for this cohort. Secondarily, we aimed to (I)

compare the infection-free implant survival between patients with 1 versus ≥2 UPCs and (II) compare the infection-free implant survival between patients treated with antibiotics versus not treated with antibiotics (considered a contaminant).

4.2 Patients and Methods

Our prospectively maintained institutional database was used to identify all 1795 consecutive revision TKA cases performed at our academic tertiary care center between January 2006 and April 2019. A retrospective review of operative notes and electronic medical records (EMRs) was performed to apply study inclusion and exclusion criteria. Adult patients that underwent presumed aseptic single-stage revision TKA with intraoperative culture samples(s) taken were eligible for inclusion. Revisions with no intraoperative samples taken for culture were excluded, as were revisions of patellofemoral or unicompartmental replacements. Patients on chronic antibiotic suppression for PJI were excluded. Revisions were excluded if PJI was known or suspected preoperatively, as were revisions that were part of treatment for PJI (debridement, antibiotics with retention of nonmodular implants, one-stage or two-stage revision for PJI). Patients lost to follow-up less than 1-year from the index study revision were excluded, unless this was secondary to a subsequent aseptic revision (censored in survival analysis) or recurrent PJI (study endpoint). The base cohort to determine the prevalence of UPC was comprised of all single-stage presumed aseptic revisions meeting inclusion/exclusion criteria. Of these, the final UPC study cohort was comprised of revisions with a minimum of 1 UPC (organism in broth or solid medium). Ethics approval was obtained from our institutional REB.

For the UPC study cohort a manual review of EMRs was performed to obtain patient, demographic, laboratory, microbiological, surgical, treatment, and outcome data (Table 4.1 and Table 4.2). Surgeries were performed by 9 fellowship trained arthroplasty surgeons with the assistance of residents and/or fellows. All revisions were evaluated preoperatively for PJI both clinically and with serum c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). However, a joint fluid aspirate was only obtained selectively when these parameters were suspicious of PJI. The number or type (swab, fluid aspirate, tissue) of intraoperative samples taken for culture was at the discretion of the treating surgeon and varied based on the preference

of the treating surgeon. For each UPC the microorganism, antibiotic sensitivities, and broth or solid medium status was documented.

All cement used in revisions contained antibiotics (Bone Cement Antibiotic Simplex P with Tobramycin 1g; Stryker). Postoperative antibiotic prophylaxis (1-2g cefazolin unless patient allergy) varied based on surgeon preference including standard 24-48 hours, antibiotics until preliminary culture results were negative, or antibiotics until 5-day culture results were negative (if in hospital).

There was no predefined treatment protocol for UPC at our institution, nor is there routine interdisciplinary rounds. The surgeon based the need for antibiotic and/or surgical treatment of UPC based on a combination of preoperative, patient, intraoperative/surgical, microorganism, and postoperative factors, as well as the number of UPCs. Infectious disease experts were often consulted to aid with treatment decisions.

Infection-related implant failure was defined as the occurrence of infection that required antibiotic treatment or revision surgery for PJI at any time after the index study revision. Since the year 2012, the diagnosis of PJI at our institution was made according to the Musculoskeletal Infection Society's (MSIS) definition for PJI criteria and updated versions(11,12). Prior to 2012 PJI was diagnosed based on clinical, laboratory, and intraoperative variables, but not in a universally accepted or standardized manner. The causative microorganism(s) of any subsequent PJI-related failure was recorded and compared to the microorganism(s) of the index revision surgery UPC. All subsequent PJI was treated with surgery and antibiotics. If a subsequent aseptic revision occurred it was documented, as well as the etiology and time from index study revision. Latest EMR clinical follow-up was used as latest follow-up, unless subsequent PJI, subsequent aseptic revision, or death occurred first (in order of occurrence).

Secondary study aims were accomplished by creating cohorts from the UPC study cohort: (I) a 1 UPC versus ≥2 UPC cohort based on number of UPCs during index revision surgery, and (II) an UPC treated with antibiotics cohort versus not treated with antibiotics cohort (considered contaminant).

4.2.1 Statistical Analysis.

Statistical analysis was performed using SPSS v26.0 (IBM Inc., Armonk, NY). The prevalence of UPC was calculated. Variables and outcomes of interest were reported on using descriptive statistics. Medians and interquartile ranges (IQR) or means and standard deviations (SD) were used, when appropriate. The Kaplan-Meier technique with 95% confidence intervals (CI) was used to determine the infection-free implant survival at 2 and 5-years for UPC study cohort, with subsequent PJI as the endpoint. Patients who died, underwent subsequent aseptic revision, or were lost to follow-up after the 1-year mark were censored. The 5-year Kaplan-Meier survival of the entire UPC cohort was repeated using subsequent PJI caused by same microorganism as the UPC as the endpoint. The 5-year infection-free survival was also calculated for all cohorts of interest, with subsequent PJI as the endpoint. Log-rank tests were used to compare infection-free survival between cohorts of interest. Continuous data was compared between cohorts using Mann-Whitney U tests or two-sample t tests for nonparametric and parametric data, respectively. The Shapiro–Wilk test was used to test normality. Categorical data was compared between cohorts using the Pearson's chi-squared test or Fisher's exact test, when appropriate. Statistical significance was 2-tailed and set at a p-value ≤ 0.05.

4.3 Results

After exclusions, the base cohort was comprised of 775 single-stage presumed aseptic revisions with intraoperative cultures taken (Figure 4.1). The prevalence of ≥ 1 UPC in presumed aseptic revision TKA was 9.8% (76/775). No revisions were lost to follow-up before 1-year for reasons other than subsequent aseptic revision or PJI. The median follow-up time was 3.6 years (IQR 2.0 to 6.2) with time to subsequent PJI, subsequent aseptic revision, death, or latest clinical follow-up as the endpoint (in order of occurrence). Ten revisions with UPC died at a mean of 5.3 years (SD 2.5), none before the 1-year mark.

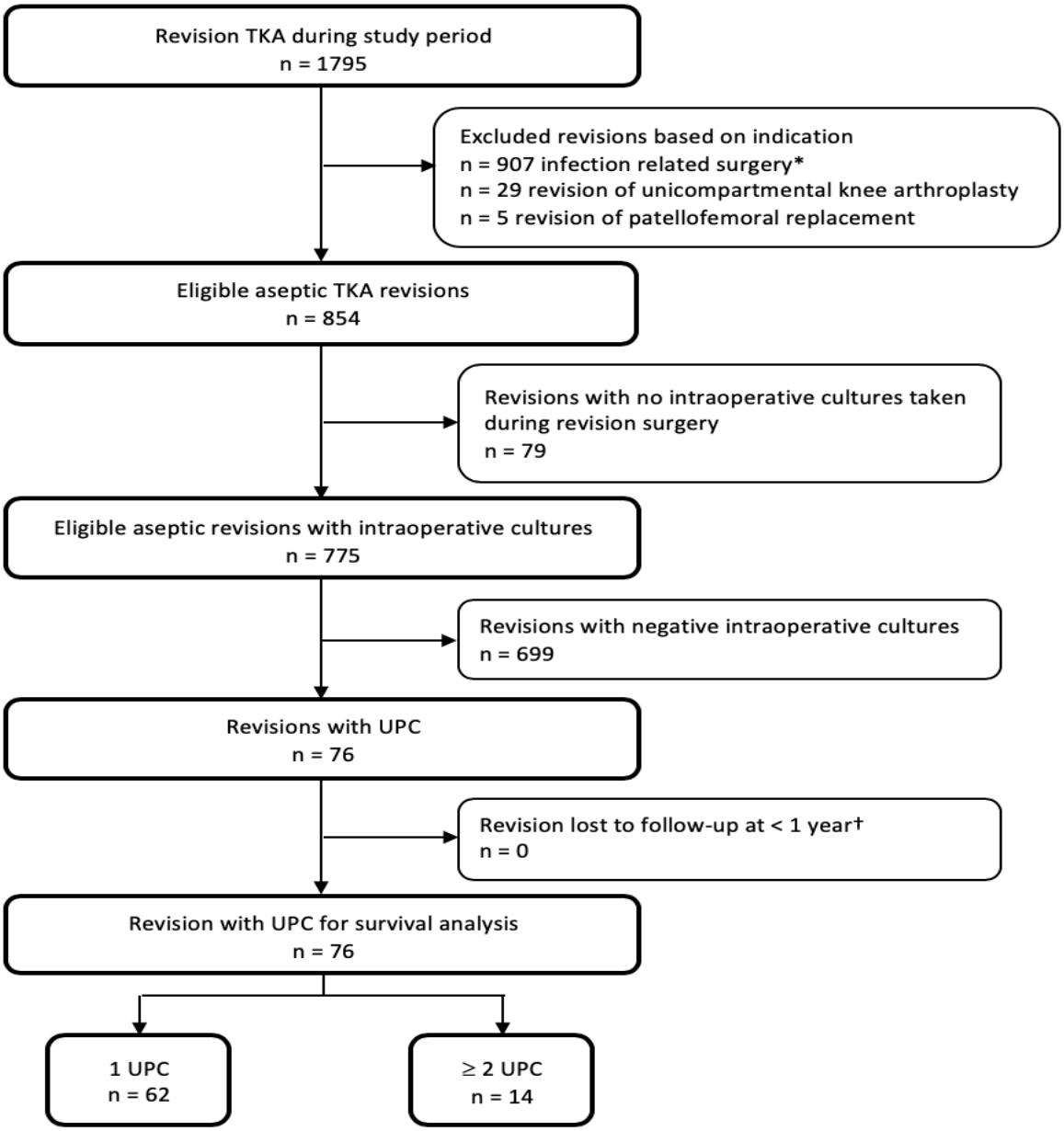

Figure 4.1. Flowchart of eligible aseptic TKA revisions and revisions with UPC.
*Infection related surgeries include 1-stage, 2-stage, and debridement, antibiotics, and implant retention with modular exchange for periprosthetic joint infection, as well as revisions with known suppressed infection or those suspected of being infected. †Revisions that had the endpoint of subsequent infection-related implant failure, or those that had subsequent aseptic revision surgery prior to 1-year follow-up were not excluded from survival analysis. TKA, total knee arthroplasty; UPC, unexpected positive intraoperative cultures.

Baseline and operative data for entire UPC study cohort can be seen in Table 4.1. Aseptic loosening and instability were the dominant modes of failure. Preoperative serum CRP and ESR were elevated in 11.8% (9) and 9.2% (7) of revisions. Thirty-two percent (24) of revisions had a preoperative joint aspirate performed. The majority (73.7%) of patients underwent a two-

component revision of the femur and tibia. Microbiological, treatment, and outcome data is shown in Table 4.2. Fifty-five percent (162) of operative samples for culture were tissue and a median 4 samples (IQR 3 to 5) were taken per revision. Nearly 82% (62) of the cohort had a single UPC and 51.6 % (48) of UPCs were grown in broth only. *Cutibacterium acnes (C. acnes)* was the most common microorganism (32.4%) identified followed by methicillin-sensitive *Staphylococcus epidermidis* (MSSE) (21.6%), however, coagulase-negative *Staphylococcus* (CNS) species comprised 45.1% of all microorganisms (Table 4.2). Only 35.5% (27) of patients received antibiotic treatment for their UPC, the vast majority (92.6%) for a duration of ≤6 weeks, though route of antibiotics varied (Table 4.2).

Table 4.1. Baseline, demographic, and operative data of study population of 76 UPC revisions

Variable	
Age (years)*	69.3 (9.0)
Sex, F/M, n (%)	47/29 (61.8/38.2)
BMI (kg/m^2)†	33.6 (28.6 to 37.8)
ASA classification, n (%)	
1	0 (0)
2	18 (23.7)
3	56 (73.7)
4	2 (2.6)
Smoking, n (%)	10 (13.2)
Diabetes, n (%)	18 (23.7)
Anticoagulation, n (%)	7 (9.2)
Inflammatory condition, n (%)	10 (13.2)
Etiology for primary TKA, n (%)	
Osteoarthritis	66 (86.8)
Rheumatoid/inflammatory arthritis	5 (6.6)
Avascular necrosis/SONK	2 (2.6)
Post-traumatic arthritis	2 (2.6)
Other	1 (1.3)
Reasons for revision, n (%)	
Aseptic loosening	34 (44.7)
Instability	22 (28.9)
Arthrofibrosis	6 (7.9)
Polyethylene wear +/- osteolysis	4 (5.3)
Patellar problem	4 (5.3)
Pain no known source	4 (5.3)
Periprosthetic fracture	1 (1.3)
Pain component malposition	1 (1.3)
Revision number†	1.0 (1.0 to 1.0)
History of prior TKA revision in study joint, n (%)	11 (14.5)
Age of prosthesis (years)†	8.9 (3.3 to 14.6)
History of PJI in study joint, n (%)	2 (2.6)

Preoperative serum CRP > 10mg/L, n (%)	9 (11.8)
Missing data CRP, n (%)	1 (1.3)
Preoperative serum ESR > 30mm/h. n (%)	7 (9.2)
Missing data ESR, n (%)	1 (1.3)
Preoperative joint aspirate, n (%)	24 (31.6)
Type of revision, n (%)	
Patella	4 (5.3)
Modular exchange	8 (10.5)
1-component	8 (10.5)
2-component	56 (73.7)
Antibiotic cement used, n (%)	70 (92.1)
Cemented stems used, n (%)	9 (11.8)

*Values are mean (standard deviation). †Values are median (interquartile range). UPC, unexpected positive intraoperative culture; F, female; M, male; BMI, body mass index; ASA, American society of anesthesiologists; TKA, total knee arthroplasty; SONK, spontaneous osteonecrosis of the knee; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate.

Table 4.2. Sampling, microorganism, treatment, and outcome data for study population of 76 UPC revisions

Variable	
Number of samples taken in study revision*	4 (3.0 to 5.0)
Total samples taken, n	295
Swab samples, n (%)	113 (38.3)
Fluid samples, n (%)	20 (6.8)
Tissue samples, n (%)	162 (54.9)
Total number of UPC's, n	93
UPC broth, n (%)	48 (51.6)
UPC solid, n (%)	45 (48.4)
1 UPC vs ≥2 UPC, n (%)	
1 UPC	62 (81.6)
≥2 UPC	14 (18.4)
Microorganisms, n (%)	
C. acnes	33 (32.4)
Other CNS	23 (22.5)
MSSE	22 (21.6)
MRSE	1 (1.0)
Streptococcus sp	8 (7.8)
Enterococcus sp	4 (3.9)
Bacillus sp.	3 (2.9)
Corynebacterium sp	2 (2.0)
Others (6 species)	6 (5.9)
Number of revisions resistant UPC, n (%)	4 (5.3)
Number revisions polymicrobial UPC, n (%)	12 (15.8)
Surgical treatment of UPC, n (%)	1 (1.3)
Antibiotic treatment of UPC, n (%)	27 (35.5)
Antibiotic route, n (%)	
Oral alone	12 (44.4)
IV alone	9 (33.3)

Combined IV and oral	6 (22.2)
Antibiotic duration, n (%)	
≤ 6 weeks	25 (92.6)
≤ 3 months	1 (3.7)
≤ 6 months	1 (3.7)
Subsequent aseptic revision, n (%)†	4 (5.3)
Etiology subsequent aseptic revision, n (%)	
Instability	1 (25.0)
Aseptic loosening	1 (25.0)
Periprosthetic fracture	1 (25.0)
Avascular necrosis patella	1 (25.0)
Time to subsequent aseptic revision (years)‡	3.5 (2.7)
Subsequent PJI, n (%)	3 (3.9)
Subsequent PJI microorganism, n (%)	
Same as UPC microorganism	0 (0)
Mixed	1 (33.3)
Different than UPC microorganism	2 (66.7)

Values are median (interquartile ranges). †Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. ‡Values are mean (standard deviation). UPC, unexpected positive intraoperative culture; C. acnes, Cutibacterium acnes*; CNS, coagulase-negative *Staphylococcus* species; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; sp, species; IV, intravenous; PJI, periprosthetic joint infection.

Three patients were diagnosed with a subsequent PJI at a mean of 1.1 years (SD 1.4) (Table 4.3). Of note, 2/3 of subsequent PJIs were caused by a different microorganism than the study revision UPC, and 1/3 was polymicrobial with one causative microorganism the same as the study revision UPC (Table 4.2 and Table 4.3). The 2- and 5-year infection-free survival for the entire UPC cohort was 97.4% (95% CI 95.6% to 99.2%) and 95.3% (92.6% to 98.0%), respectively (Figure 4.2). When considering only infection-related implant failure caused by the same microorganism as the UPC as the endpoint, the 5-year infection-free survival for the entire UPC cohort was 98.7% (95% CI 97.4% to 100%) (Figure 4.3).

Table 4.3. Patient, operative, microorganism, and treatment data for revisions with an UPC that had a subsequent PJI-related implant failure (n = 3)

Variable	Case 1	Case 2	Case 3
Age (years)	73	68	90
Sex	Female	Female	Male
BMI (kg/m^2)	30.7	55.8	27.1
Etiology for primary TKA	Osteoarthritis	Osteoarthritis	Osteoarthritis
Revision number	1	1	1
Age of prosthesis (years)	8	17	4
Reason for revision	Aseptic loosening	Aseptic loosening	Instability
History of PJI in study joint	No	No	No
Preoperative serum CRP (mg/L)	0.3	8.5	1.2
Preoperative serum ESR (mm/h)	9	13	7
Preoperative joint aspirate	No	Yes	No
Type of revision	2-component	2-component	Modular exchange
Number of UPC's	1	1	1
UPC solid or broth	Solid	Broth	Broth
UPC microorganism(s)	*C. acnes*	*Staph warneri*	MRSE
Surgical treatment UPC	No	No	No
Antibiotic treatment UPC	6 weeks oral	6 weeks oral	6 weeks oral
Time to subsequent PJI (years)	0.2	0.3	2.7
Microorganism(s) subsequent PJI	*C. acnes + Proteus Mirabilis*	MSSA	Culture-negative

UPC, unexpected positive intraoperative culture; PJI, periprosthetic joint infection; BMI, body mass index; TKA, total knee arthroplasty; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; *C. acnes, Cutibacterium acnes*; *Staph*, *Staphylococcus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MSSA, methicillin-sensitive *Staphylococcus aureus.*

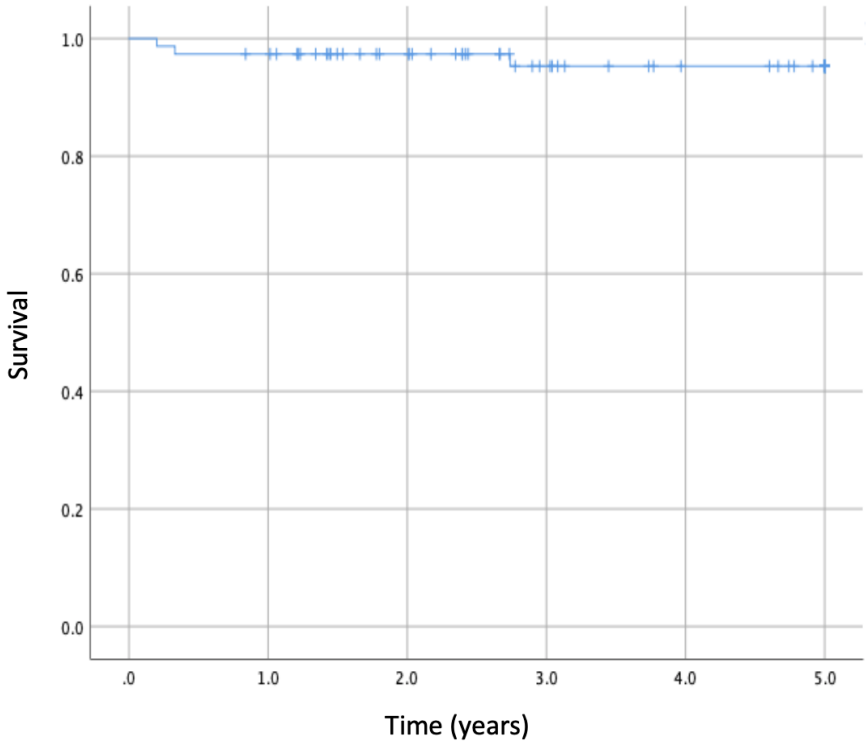

Figure 4.2. Kaplan-Meier 5-year infection-free survival for entire UPC cohort in presumed aseptic knee revisions.
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture.

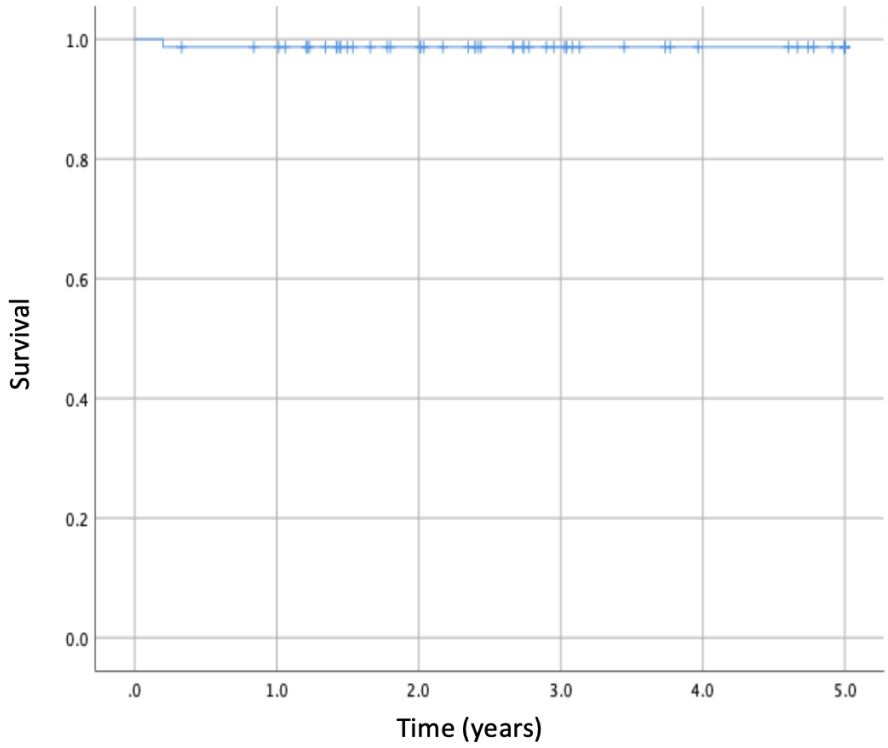

Figure 4.3. Kaplan-Meier 5-year infection-free survival for entire UPC cohort with subsequent PJI by the same microorganism as the UPC as the endpoint.
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture; PJI, periprosthetic joint infection.

Detailed data for the 1 UPC versus ≥2 UPC cohorts is shown in Table 4.4. The vast majority of variables showed no statistical difference between groups ($p > 0.05$), however there was variability (Table 4.4). *C. acnes* was the most common microorganism in the single UPC cohort whereas MSSE was the most common in the ≥2 UPC cohort, and the proportions of microorganisms differed between cohorts ($p = 0.029$). The ≥2 UPC cohort was more likely to receive antibiotic treatment of the UPC (64.3% versus 29.0%, $p = 0.027$). Although there was variability in the route ($p = 0.123$) and duration ($p = 0.103$) of antibiotic treatment between cohorts, these differences were not statistically significant. All 3 of the subsequent PJIs were in the single UPC cohort ($p = 1.000$). However, the 5-year infection-free survival was similar for the 1 UPC versus ≥2 UPC cohorts, at 94.3% (95% CI 91.0% to 97.6%) and 100%, respectively ($p = 0.416$) (Figure 4.4).

Table 4.4. Baseline, demographic, operative, microbiological, treatment, and outcome data for revisions with 1 UPC versus ≥2 UPC

Variable	1 UPC (n = 62)	≥2 UPC (n = 14)	P value
Age (years)*	69.2 (8.8)	69.6 (9.8)	0.872[a]
Sex, F/M, n (%)	36/26 (58.1/41.9)	11/3 (78.6/21.4)	0.154[b]
BMI (kg/m^2)†	33.3 (28.5 to 38.0)	34.7 (31.3 to 38.2)	0.445[c]
ASA classification, n (%)			0.294[d]
1	0 (0)	0 (0)	
2	16 (25.8)	2 (14.3)	
3	45 (72.6)	11 (78.6)	
4	1 (1.6)	1 (7.1)	
Diabetes, n (%)	15 (24.2)	3 (21.4)	1.000[d]
Inflammatory condition, n (%)	9 (14.5)	1 (7.1)	0.678[d]
Etiology for primary TKA, n (%)			0.678[d]
Osteoarthritis	53 (85.5)	13 (92.9)	
Other	9 (14.5)	1 (7.1)	
Reasons for revision, n (%)			0.926[d]
Aseptic loosening	29 (46.8)	5 (35.7)	
Instability	17 (27.4)	5 (35.7)	
Arthrofibrosis	5 (8.1)	1 (7.1)	
Polyethylene wear +/- osteolysis	3 (4.8)	1 (7.1)	
Patellar problem	3 (4.8)	1 (7.1)	
Pain no known source	3 (4.8)	1 (7.1)	
Periprosthetic fracture	1 (1.6)	0 (0)	
Pain component malposition	1 (1.6)	0 (0)	
History of prior TKA revision in study joint n (%)	10 (16.1)	1 (7.1)	0.678[d]
Age of prosthesis (years)†	8.7 (3.1 to 13.9)	12.0 (5.5 to 16.3)	0.366[c]
History of PJI in study joint, n (%)	2 (3.2)	0 (0)	1.000[d]
Pre-operative serum CRP > 10mg/L, n (%)	8 (12.9)	1 (7.1)	1.000[d]
Pre-operative serum ESR > 30mm/h, n (%)	6 (9.7)	1 (7.1)	1.000[d]
Preoperative joint aspirate, n (%)	20 (32.3)	4 (28.6)	1.000[d]
Type of revision, n (%)			0.391[d]
Patella	2 (3.2)	2 (14.3)	
Modular exchange	7 (11.3)	1 (7.1)	
1-component	7 (11.3)	1 (7.1)	
2-component	46 (74.2)	10 (71.4)	
Number of samples taken in study revision†	4.0 (3.0 to 5.0)	4.0 (3.0 to 5.0)	0.217[c]
Swab used for culture in revision, n (%)	43 (69.4)	11 (78.6)	0.745[d]
Fluid used for culture in revision, n (%)	16 (25.8)	4 (28.6)	1.000[d]
Tissue used for culture in revision, n (%)	50 (80.6)	9 (64.3)	0.284[d]
UPC broth or solid, n (%)			0.379[b]
Broth	30 (48.4)	18 (58.1)	
Solid	32 (51.6)	13 (41.9)	
Microorganisms, n (%)			0.029[d]
C. acnes	24 (36.9)	9 (24.3)	
Other CNS	16 (24.6)	7 (18.9)	
MSSE	8 (12.3)	14 (37.8)	
MRSE	1 (1.5)	0 (0)	
Streptococcus sp	6 (9.2)	2 (5.4)	

Enterococcus sp	1 (1.5)	3 (8.1)	
Others	9 (13.8)	2 (5.4)	
Number of revisions resistant UPC, n (%)	2 (3.2)	2 (14.3)	0.152[d]
Antibiotic treatment of UPC, n (%)	18 (29.0)	9 (64.3)	0.027[d]
Antibiotic route, n (%)			0.123[d]
Oral alone	10 (55.6)	2 (22.2)	
IV alone	6 (33.3)	3 (33.3)	
Combined IV and oral	2 (11.1)	4 (44.4)	
Antibiotic duration, n (%)			0.103[d]
≤ 6 weeks	18 (100.0)	7 (77.8)	
≤ 3 months	0 (0)	1 (11.1)	
≤ 6 months	0 (0)	1 (11.1)	
Subsequent aseptic revision, n (%)‡	2 (3.2)	2 (14.3)	0.152[d]
Subsequent PJI, n (%)	3 (4.8%)	0 (0)	1.000[d]
Subsequent PJI microorganism, n (%)			Not applicable
Same as UPC microorganism	0 (0)	Not applicable	
Mixed	1 (33.3)	Not applicable	
Different than UPC microorganism	2 (66.7)	Not applicable	

*Values are mean (standard deviation). †Values are median (interquartile ranges). ‡Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. [a]Two-sample t test. [b]Pearson's chi-squared test. [c]Mann-Whitney U test. [d]Fisher's exact test. UPC, unexpected positive intraoperative culture; F, female; M, male; BMI, body mass index; ASA, American society of anesthesiologists; TKA, total knee arthroplasty; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; *C. acnes*, *Cutibacterium acnes*; CNS, coagulase-negative *Staphylococcus* species; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; sp, species; IV, intravenous.

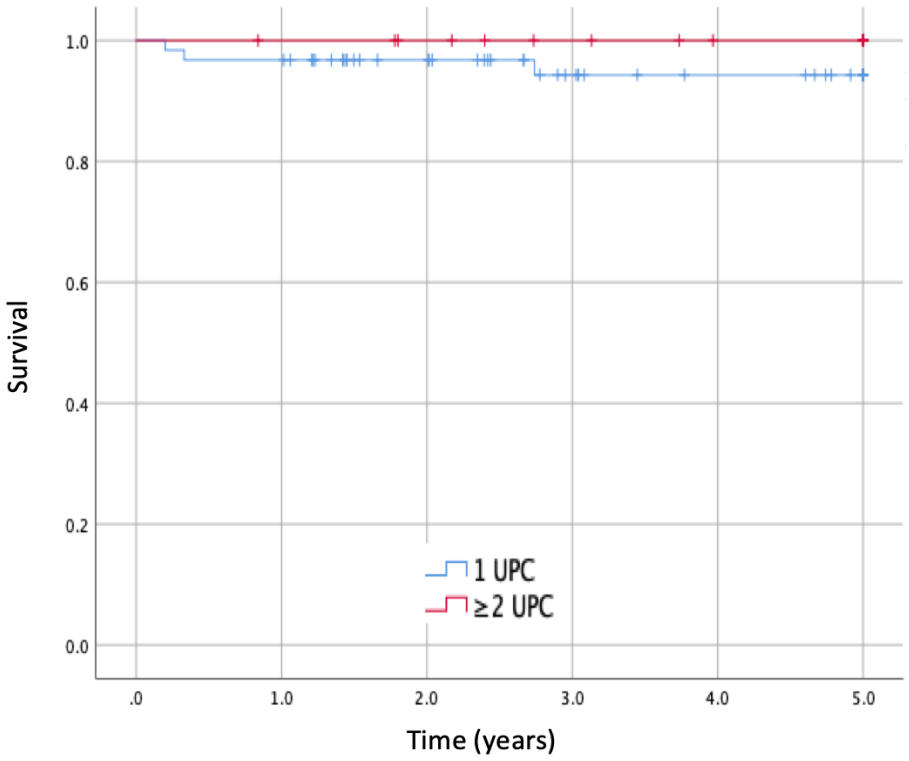

Figure 4.4. Kaplan-Meier 5-year infection-free survival for the 1 versus ≥2 UPC cohorts.
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture.

Detailed data for patients that had antibiotic treatment of their UPC(s) versus those that did not have antibiotic treatment of their UPC(s) is shown in Table 4.5. The vast majority of variables showed no statistical differences between cohorts ($p > 0.05$), however important differences were noted (Table 4.5). The antibiotic treatment cohort had a higher proportion of ≥2 UPCs (33.3% versus 10.2%, $p = 0.027$). The increased proportions of worse American Society of Anesthesiologists classification ($p = 0.078$), UPCs from swab samples ($p = 0.064$), and antibiotic resistant microorganisms ($p = 0.125$) in the antibiotic treatment cohort were not statistically significant, nor were differences in UPCs from tissue samples ($p = 0.094$) or microorganisms ($p = 0.100$). All 3 subsequent PJIs were in the antibiotic treatment cohort ($p = 0.042$) and the 5-year infection-free survival was worse for the antibiotic treatment cohort compared to the no antibiotic treatment cohort, at 87.4% (95% CI 80.5% to 94.3%) and 100%, respectively ($p = 0.021$) (Figure 4.5). However, no patient with a single UPC without antibiotic treatment had a subsequent PJI-related implant failure. Of note, there were no recurrent infections in patients with ≥2 UPCs, but the majority received antibiotic treatment and numbers were low.

Table 4.5. Baseline, demographic, operative, microbiological, treatment, and outcome data for UPC revisions treated with antibiotics versus those not treated with antibiotics

Variable	Antibiotic treatment (n = 27)	No antibiotic treatment (n =49)	P value
Age (years)*	69.6 (9.9)	69.1 (8.5)	0.808[a]
Sex, F/M, n (%)	19/8 (70.4/29.6)	28/21 (57.1/42.9)	0.256[b]
BMI (kg/m^2)†	34.2 (29.3 to 39.5)	32.8 (27.9 to 37.7)	0.259[c]
ASA classification, n (%)			0.078[d]
1	0 (0)	0 (0)	
2	4 (14.8)	14 (28.6)	
3	21 (77.8)	35 (71.4)	
4	2 (7.4)	0 (0)	
Diabetes, n (%)	6 (22.2)	12 (24.5)	0.824[b]
Inflammatory condition, n (%)	4 (14.8)	6 (12.2)	0.737[d]
Etiology for primary TKA, n (%)			0.737[d]
Osteoarthritis	23 (85.2)	43 (87.8)	
Other	4 (14.8)	6 (11.2)	
Reasons for revision, n (%)			0.684[d]
Aseptic loosening	14 (51.9)	20 (40.8)	
Instability	7 (25.9)	15 (30.6)	
Arthrofibrosis	1 (3.7)	5 (10.2)	
Polyethylene wear +/- osteolysis	3 (11.1)	1 (2.0)	
Patellar problem	1 (3.7)	3 (6.1)	
Pain no known source	1 (3.7)	3 (6.1)	
Periprosthetic fracture	0	1 (2.0)	
Pain component malposition	0	1 (2.0)	
History of prior TKA revision in study joint n (%)	3 (11.1)	8 (16.3)	0.737[d]
Age of prosthesis (years)†	10.9 (4.0 to 17.0)	8.6 (2.9 to 13.4)	0.373[c]
History of PJI in study joint, n (%)	1 (3.7)	1 (2.0)	1.000[d]
Pre-operative serum CRP > 10mg/L, n (%)	4 (14.8)	5 (10.2)	0.714[d]
Pre-operative serum ESR > 30mm/h, n (%)	2 (7.4)	5 (10.2)	1.000[d]
Preoperative joint aspirate, n (%)	8 (29.6)	16 (32.7)	0.786[b]
Type of revision, n (%)			0.690[d]
Patella	2 (7.4)	2 (4.1)	
Modular exchange	2 (7.4)	6 (12.2)	
1-component	4 (14.8)	4 (8.2)	
2-component	19 (70.4)	37 (75.5)	
Number of samples taken in study revision†	4 (3.0 to 5.0)	4 (3.0 to 5.0)	0.485[c]
1 UPC vs ≥2 UPC, n (%)			0.027[d]
1 UPC	18 (66.7)	44 (89.8)	
≥2 UPC	9 (33.3)	5 (10.2)	
UPC from swab sample, n (%)	22 (56.4)	20 (37.0)	0.064[b]
UPC from fluid sample, n (%)	0 (0)	1 (1.9)	1.000[d]
UPC from tissue sample, n (%)	17 (43.6)	33 (61.1)	0.094[b]
UPC broth or solid, n (%)			0.371[b]
Broth	18 (46.2)	30 (55.6)	
Solid	21 (53.8)	24 (44.4)	
Microorganisms, n (%)			0.100[d]

C. acnes	11 (24.4)	22 (38.6)	
Other CNS	11 (24.4)	12 (21.1)	
MSSE	14 (31.1)	8 (14.0)	
MRSE	1 (2.2)	0 (0)	
Streptococcus sp	2 (4.4)	6 (10.5)	
Enterococcus sp	3 (6.7)	1 (1.8)	
Others	3 (6.7)	8 (14.0)	
Number of revisions resistant UPC, n (%)	3 (11.0)	1 (2.0)	0.125[d]
Subsequent aseptic revision, n (%)‡	2 (7.4)	2 (4.1)	0.612[d]
Subsequent PJI, n (%)	3 (11.1)	0 (0)	0.042[d]
Subsequent PJI microorganism, n (%)			Not applicable
Same as UPC microorganism	0 (0)	Not applicable	
Mixed	1 (33.3)	Not applicable	
Different than UPC microorganism	2 (66.7)	Not applicable	

*Values are mean (standard deviation). †Values are median (interquartile ranges). ‡Subsequent aseptic revision after the study revision, censored out of survival analysis once occurs as subsequent PJI could be caused by subsequent aseptic revision. [a]Two-sample t test. [b]Pearson's chi-squared test. [c]Mann-Whitney U. [d]Fisher's exact test. UPC, unexpected positive intraoperative culture; F, female; M, male; BMI, body mass index; ASA, American society of anesthesiologists; TKA, total knee arthroplasty; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; *C. acnes*, *Cutibacterium acnes*; CNS, coagulase-negative *Staphylococcus* species; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; sp, species.

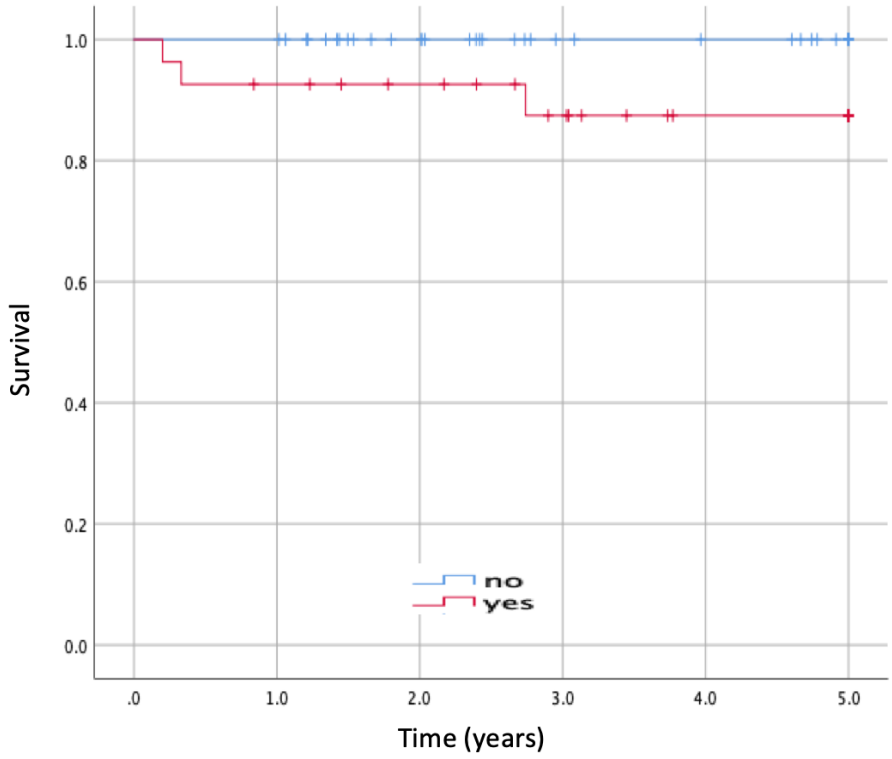

Figure 4.5. Kaplan-Meier 5-year infection-free survival for the UPC cohort treated with antibiotics (yes) versus not treated with antibiotics (no).
Vertical spikes are censored data. UPC, unexpected positive intraoperative culture.

4.4 Discussion

Literature on the prevalence, clinical significance, and outcomes of UPC in presumed aseptic revision TKA is limited, with no clear consensus. Our aims were to determine the prevalence of UPC in presumed aseptic revision TKA and the infection-free implant survival for this patient population, as well as other clinical cohorts of interest. To our knowledge, this study represents the largest series of UPC in presumed aseptic revision TKA in the literature.

The prevalence of UPC in presumed aseptic revision TKA in our study was 9.8%. This is consistent with the mean prevalence of 10.5% (379/3605) for revision total hip (THA) and TKA reported in literature, however the variability is substantial (4-38%), only 111 TKA with UPC were included, and UPC in THA was twice more common than TKA(21). The variability in the literature is due to significant heterogeneity between studies, including differing preoperative evaluation and definitions of UPC or PJI(21). We included broth only UPCs since the specificity of these cultures have been shown to be high(23), and other studies have as well(17–19). Studies that included a single UPC as opposed to only ≥2 UPCs tended to report a higher incidence(19,24–26), however, this is not universal(17), and those reporting on ≥2 UPC vary as well(21). Barrack et al.(17) reported a prevalence of UPC in presumed aseptic revision TKA of 5.9% with 29/41 having a single UPC, Saleh et al.(19) reported a combined prevalence of 10% for TKA and THA including those with a single UPC, and recently Jacobs et al.(18) reported a prevalence of 7.9% in TKA patients when only considering ≥2 UPC as significant. In our institution the prevalence of ≥2 UPC was only 1.8%. CNS species and *C. acnes* were the most common microorganisms, supporting the indolent nature of microorganisms in UPC(17–19). Virulent microorganisms did occur but were rare, as were antibiotic resistant microorganisms(19,21).

The 2- and 5-year infection-free survival for the entire UPC cohort was excellent at 97.4% (95% CI 95.6% to 99.2%) and 95.3% (92.6% to 98.0%), respectively. The majority of studies reporting similar infection-free survival in TKA and THA tended to be limited by short follow-up or use advanced techniques such as implant sonication or molecular techniques(14,26,29,30), both of which do not apply to the current study. The causative microorganism in 2/3 of the subsequent PJIs was different than the UPC and the 5-year

infection-free survival from a subsequent infection caused by the same UPC microorganism was outstanding at 98.7% (95% CI 97.4% to 100%). Although subsequent PJI caused by different microorganisms than the UPC likely represent a new infection, it is plausible that these microorganisms were present during the study revision but missed due to the limited sensitivity of cultures in PJI(27). A high proportion of subsequent PJI-related implant failure being caused by a different microorganism than the UPC is common, however factors associated with reinfection by the same microorganism have been identified(14,18,19,21). While most studies do not report on TKA and THA separately, we felt this was important because Jacobs et al.(18) showed that the prognosis for TKA is poorer than that of THA in this patient population. Additionally, we felt that reporting out to 5-years was important given the low virulence microorganisms common to UPC. The excellent infection-free survival of our large cohort was consistent with the majority of literature(18,19,21,25,26,28), however, only 2 studies report the survival of TKA separate from that of THA(17,18). Barrack et al.(17) reported at a mean of 45 months that only 2/41 of presumed aseptic TKA revisions with UPC went onto subsequent PJI. However, Jacobs et al.(18) reported a 2-year survival of 88% (95% CI 60 to 97) in 17 TKA with ≥2 UPC, which was lower than that of the true aseptic TKA cohort.

We found that the infection-free survival was similar for the 1 UPC versus ≥2 UPC cohorts. This was surprising and must be interpreted with caution due to important differences between cohorts (proportion treated with antibiotics and the type of microorganisms involved). Additionally, all of the subsequent infections were in the 1 UPC cohort, which is contrary to most literature(17,21), but not all prior research(19). Possible explanations for this in our study include differences in the proportion treated with antibiotics and causative microorganisms, the high proportion of 2-component revisions, the low sample size of the ≥2 UPC cohort (underpowered for comparisons), or other differences between cohorts not accounted for due to the retrospective nature of the study.

Treatment protocols have varied considerably in the literature(17,21,28). In our study only 35.5% (27) of patients received antibiotic treatment for their UPC(s). Of these, 92.6% (25) were treated for a duration of ≤6 weeks and no patient is on life-long suppression. Surprisingly, the infection-free survival was worse for the antibiotic treatment cohort. Similar results have been reported(14,28), however, one cannot conclude antibiotic treatment is associated with a higher risk of subsequent PJI based on our data. Differences between cohorts, lack of a

standardized UPC treatment protocol, and the retrospective nature of our study introduced a selection bias for those treated with antibiotics. Patients treated with antibiotics likely shared a higher degree of clinical of suspicion for PJI or other factors that influenced clinicians to treat medically.

There is debate regarding the clinical significance of a single UPC. Several studies excluded revisions with only a single UPC(18,21,28), and others have questioned their significance(17,21,25). No patient in our study with a single UPC deemed not to require antibiotic treatment had a subsequent PJI-related implant failure. These results suggest that a single UPC without signs of infection is likely a contaminant and does not require antibiotic treatment, and support the conclusions of Barrack et al(17). We are unable to draw any meaningful conclusions on antibiotic treatment and the significance of all ≥2 UPC in presumed aseptic revisions, however it has been shown that even a single UPC with a high virulence microorganism in a patient not meeting MSIS criteria may represent an infection and require antibiotic treatment(19).

Our study has several limitations. The lack of a standardized treatment protocol for UPCs and the retrospective design of this study is subject to associated biases, some of which are discussed above. The academic, high-volume, single center design may limit external validity of our results. However, our study included multiple surgeons at different points in their careers potentially improving the generalizability of our results and most of our results are consistent with the literature**.** Although it has been routine practice to order CRP and ESR for all failed revisions, there was no standardized preoperative protocol to screen for PJI. An aspirate was only ordered selectively, thus MSIS criteria could not be retrospectively applied. The type and number of samples taken during revision surgery for culture was not standardized, and although this is not uncommon to the UPC literature, both of have been shown to be important for detecting microorganisms and PJI(24,31,32). Lastly, our study was underpowered to detect differences between cohorts for secondary outcomes of interest and the low number of subsequent PJI limited comparisons between cohorts. However, this study is the largest series of UPC in presumed aseptic revision TKA in the literature, does not confound TKA results with those of THA, and inclusion of revisions with a single UPC provides data on a common and clinically relevant challenge for clinicians.

In conclusion, the prevalence of UPC in presumed aseptic revision TKA is 9.8% and the 2- and 5-year infection-free survival is excellent. Infection-free survival when only considering subsequent PJI caused by the same UPC microorganism is outstanding. The majority of subsequent PJI-related failures were caused by a different microorganism than that of the UPC. Infection-related survival was similar between the 1 and ≥2 UPC cohorts and the cohort treated with antibiotics had an inferior survival compared to those not treated with antibiotics, however, these findings must be interpreted with caution due to selection biases, differences between cohorts, and sample size limitations. No patient in our study with a single UPC deemed not to require antibiotic treatment had a subsequent PJI-related implant failure, strongly suggesting that a single UPC without signs of infection is likely a contaminant and does not require antibiotic treatment.

4.5 References

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Chapter 5

5. Do Microorganisms Have a Role in ‘Aseptic’ Total Hip and Knee arthroplasty Implant Failure? A Prospective Molecular Pilot Study

5.1 Postponement of Study Completion Due to the COVID-19 Pandemic and State of Emergency

Due to the unprecedented current situation, this study is presented in its preliminary format. The current state of emergency and Western University policies during the COVID-19 pandemic resulted in research laboratories being closed. Therefore, DNA extraction, PCR amplification, sample processing and sequencing has been postponed for an unknown period of time. However, all samples for molecular analysis have been collected and are safely stored. This study will be completed at a future date, but when this will occur is uncertain and out of our control. Thus, the introduction, methods, and preliminary results are presented in this chapter. The current progress of the study is outlined in the methods section. Chapter 6 will include discussion and future directions regarding this prospective of the thesis dissertation.

5.2 Introduction

Osteoarthritis is a leading cause of disability associated with significant patient suffering and economic cost(1–3). Total hip (THA) and knee arthroplasty (TKA) are cost effective and highly successful treatments for end-stage arthritis of the hip and knee(4,5). Over 1.5 million THA and TKA are performed annually in North America(6,7), and the demand is increasing greatly(7,8). Prosthetic implants can fail for a variety of reasons and require revision surgery to treat. As approximately 12% of primary THA and TKA require revision surgery by the 10-year mark(9), the number of revision surgeries will also increase substantially(7,8). Aseptic loosening and periprosthetic joint infection (PJI) are among the leading causes for both early and late

revision(6,10,11). PJI is a dreaded complication associated with substantial patient morbidity and economic cost(12–16). Unfortunately, the burden of PJI will likely continue to increase because the incidence has not decreased over time(16,17).

Several authors have questioned whether a proportion of 'aseptic' failures are actually undiagnosed PJI-related failure(18–21). Despite tremendous scientific effort, there remains no perfect test to diagnose PJI(22–24). Although identification of a causative microorganism remains the gold standard in the diagnosis of PJI, 20-40% of confirmed PJI remains culture-negative due to sensitivity limitations of standard culture methods(25,26). Additionally, unexpected positive intraoperative cultures (UPC) occur in approximately 10% of presumed aseptic revisions and molecular methods have identified bacterial microorganisms on implants of 'aseptic' failures (27–32).

Thus, there has been increased interest in culture-independent molecular methods that have several advantages over traditional cultures(33–35). The majority of these polymerase chain reaction (PCR) based molecular studies have focused on the diagnosis of PJI, with suboptimal results and an unclear role(33,34). Fewer molecular studies have evaluated the detection of microorganisms in 'aseptic' failures and results have been conflicting(29–32,35–40). Several authors question the significance of PCR based detection of microorganisms and others do not support detection of true positive microorganisms or undiagnosed PJI in presumed aseptic revisions, particularly those with a focus on the stringent control of contamination(35,37–39,41,42).

The prevalence, role, and clinical significance of microorganisms identified in presumed aseptic revisions using molecular techniques remains unclear. No molecular study has investigated if there are specific areas on prosthetic implants that microorganisms are more likely to be found and there have been conflicting results between studies using universal 16S ribosomal RNA (16S rRNA) PCR primers versus multiplex genus/microorganism specific primers(33,35,38,40–42). Therefore, we designed a prospective pilot study using modern molecular techniques, a stringent control of contamination, and several samples from predetermined sites of THA and TKA implants revised for presumed aseptic failure.

Using modern molecular and sequencing methods with an emphasis on the stringent control of contamination our aims were to: (I) determine the frequency and type of bacterial microorganisms on prosthetic implants from presumed aseptic THA and TKA failures, and

compare the microorganisms identified by molecular methods to those of standard cultures, (II) determine the type of implants and location on the implants that bacterial microorganisms are found, and (III) determine if the presence of bacterial microorganisms is associated with the reason for revision. We hypothesize that these implants will frequently be colonized by bacterial microorganisms, molecular techniques will identify more microorganisms versus standard intraoperative cultures, and that the presence of microorganisms will be associated with the implant type, location, and reason for revision.

5.3 Methods

5.3.1 Participants and Sample Collection

This prospective pilot study received ethical approval by the Western University Health Science Research Ethics Board (REB No 114030) and was funded in part by a Schulich Collaborative Seed Research grant. All participants provided written informed consent prior to inclusion in the study. Adult patients undergoing aseptic single-stage THA and TKA revision surgery at a single high-volume, tertiary, academic center (University Hospital – London Health Sciences Center) who provided informed consent were eligible for inclusion. Over an 8-month time period (August 2019 to March 2020) a total of 41 patients (20 THA and 21 TKA) were recruited for inclusion in this study. Revision surgeries were performed by one of seven fellowship trained subspecialty arthroplasty surgeons. The sample size of this pilot study was one of convenience (and funding) that will facilitate proper sample size power calculations for future grant proposals and larger studies. Exclusion criteria were (I) unable or unwilling to give consent, (II) known or suspected PJI, (III) on antibiotic suppression of a previous PJI, (IV) the second of a 2-stage revision for PJI, and (V) no prosthetic components removed. Preoperatively all patients were screened for PJI clinically and with serum erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP). A joint fluid synovial aspiration was performed only if clinical history and preoperative serum markers did not rule out PJI. The Musculoskeletal Infection Society definition of PJI was used to rule out infection when an aspiration was performed(23). Although it was preferable that all patients had revision of a metallic prosthetic component (acetabular cup or femoral stem for THA and femoral or tibial prosthesis in TKA), those that underwent modular exchange only were not excluded from the study.

Prior to skin incision all patients received routine weight-adjusted preoperative antibiotics (cefazolin unless allergic) and skin preparation (2% chlorhexidine-70% isopropyl alcohol or iodine-based) for postsurgical infection prophylaxis. All surgeries and sample collections were performed in operating rooms with vertical laminar flow. A minimum of 3 deep intraoperative samples for routine extended aerobic and anaerobic surgical cultures were performed, with a preference for tissue. All cultures were monitored, and the microbiologic details of any positive cultures documented. Routine sterile surgical technique was adhered to and surgeons were careful to only handle only the minimum portion of the implants required to remove during revision surgery. A member of the research team was present at each revision surgery for implant sample collection for molecular analysis. Once explanted the implants were placed directly onto a sterile tray by the surgeon to minimize the potential for contamination. Using sterile technique, new individual sterile scalpel blades were used to scrape predetermined areas of the implants (Appendix D). These sites were chosen to represent likely areas of biofilm formation on the implants that were unlikely to be touched by the surgeon during extraction. There were 3 sampling sites for each standard metallic implant, as well as additional sites for modular components (head and liner) (Appendix D). For revision or complex implants, all standard sites were sampled when able and additional sites sampled were documented. Individual sterile swabs were used for sampling on sites not amenable to scraping. Each scraping sample was placed in individual sterile prelabelled Eppendorf tubes for storage and transport. Swab samples were placed back into their individual sterile tubes. A separate sterile Eppendorf tube was left open to air during the revision procedures to serve as an aerosol control. All implant samples and aerosol controls were sealed, immediately deidentified for protection of patient privacy, stored at -20 °C until DNA extraction, and transported to the Canadian Centre for Human Microbiome and Probiotics at St. Joseph's Health Care, London, ON. Baseline patient, demographic, preoperative, operative, and microbiologic data of interest were collected for further analysis.

Currently, all implant samples are collected and stored at -20 °C at the Canadian Centre for Human Microbiome and Probiotics at St. Joseph's Health Care, London, ON. For the molecular methodology described below it is important that all samples be prepared, processed and sequenced at the same time. We were ready to proceed with DNA extraction, PCR amplification, and sequencing, however, were postponed due to unforeseen and uncontrollable circumstances. Unfortunately, due to the COVID-19 state of emergency and Lawson Health

Research Institute policies during this pandemic, research laboratories have been closed and further molecular preparation and analysis has been postponed. I have described the planned molecular methodology below.

5.3.2 DNA Extraction, PCR Amplification, Sequencing, and Data Analysis

Total DNA extraction (microbial and human) will be performed for each implant sample in a biological safety cabinet using sterile tools pre-treated with RNase AWAY™ Surface Decontaminant solution (Thermo Fisher Scientific Inc, Waltham, MA), as described in a previous a previous publication(43). DNA from all samples (including aerosol controls) will be extracted using the DNeasy PowerSoil HTP 96 Kit® (Qiagen, Germantown, MD). DNA blank controls, containing only reagents used for DNA extraction, will be included in order to detect any microbial contamination from the DNA isolation kits or reagents used in subsequent procedures. Additionally, gram-negative, gram-positive, and PCR blank controls will be used. DNA samples will be stored at - 20 °C until PCR amplification. A detailed protocol of the DNA extraction is available at https://www.qiagen.com/gb/resources/resourcedetail?id=fd3fa52e-3a66-4d55-a9cd-7ed20ea046d9&lang=en.

A BioMek®3000 Laboratory Automation Workstation will be used to maximize the accuracy and precision of the following PCR amplification procedures. DNA samples will be aseptically transferred to 96-well plates containing forward and reverse PCR primers. Two types of PCR primers will be used. The first will be a “universal” or “broad-range” PCR primer that targets and amplifies the V4 hypervariable region of the bacterial 16S rRNA gene. This highly preserved gene is universal and specific to bacterial microorganisms. These primers are all different because they contain individual bar codes incorporated into the sequence. Thirty-two primers (16 left and 16 right) with unique barcodes into 96 will be used. Amplifications of the V4 region of the 16S ribosomal RNA gene will be carried out with the primers (5′-3′) ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNxxxxxxxxGTGCCAGCMGCCGCGGTAA and (5′-3′) CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNxxxxxxxxGGACTACHVG

GGTWTCTAAT (xxxxxxxx is a sample specific nucleotide barcode and the preceding sequence is a portion of the Illumina adapter sequence for library construction).

However, it has been identified that universal 16S rRNA PCR primers have only moderate sensitivity for coagulase-negative *Staphylococcus* (CNS) species and very poor sensitivity to detect anerobic bacteria such as *Cutibacterium acnes* (*C. acnes*)(35). This is problematic because *C. acnes* and CNS species were the most common microorganisms identified from UPC in presumed aseptic revision THA and TKA at our institution (Chapter 3 and Chapter 4). Therefore, we will also be using genus or group specific primers that target and amplify genes specific to *C. acnes* and CNS species. This combined approach will allow us to detect rare or unexpected microorganisms and maintain the ability to detect expected microorganisms with a high sensitivity. The Illumina adapter sequences and unique barcode sequences appended to the 5' end of the primers will allow us to unmistakably identify each sample.

A detailed protocol for preparation of the plates for gene sequencing can be found in Appendix E. Prior to sequencing, the amplified DNA samples will be quantified using a Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen), pooled at equimolar concentrations and cleaned using the QIAquick PCR Purification Kit (Qiagen, Germantown, MD). An Illumina MiSeq (2x220 cycles) will be used to sequence the purified samples at the London Regional Genomics Centre, Robarts Research Institute and the output data processed at the Canadian Centre for Human Microbiome and Probiotics, Lawson Health Research Institute. A PANDAseq analysis tool will be used to remove the unpaired or low-quality reads, and the remaining high-quality reads will be collated into "operational taxonomic units" (OTUs) based on 97% or greater sequence identity. Putative taxonomies will be assigned by comparing the OTUs to the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/).

Only UTOs assigned to various families, genera, or species of microbiota will be included, excluding all those assigned to human, other eukaryotic, mitochondrial, chloroplast, or unclassified sequences. OTUs that implant samples and controls (blank DNA extraction controls and surgical aerosol controls) have in common will be considered contamination and excluded. Furthermore, to minimize the likelihood of false-positive findings, OTUs that were less than 1% of the total number of reads in each sample will be excluded.

5.3.3 Statistical Analysis

Statistical analysis of OTUs will be performed using R software as previously described (43). Counts and proportions, medians and interquartile ranges (IQR) or means and standard deviations (SD) will be used to report on outcomes of interest when appropriate. The frequency of positive molecular microorganism identification on implants and the frequency of different types of microorganisms will be determined. The frequency of UPC from standard intraoperative cultures will be determined and these results compared to molecular findings. Microorganism identification will be compared between those with and without UPC. The frequency of microorganism identification on different types of implants and different locations on the implants will be determined. We will compare these frequencies between reasons for revision using an ANOVA (parametric) or Kruskal-Wallis (nonparametric) test depending on the normality of the data and sample size. Categorical data will be compared using the Pearson's chi-squared test or Fisher's exact test, when appropriate. Continuous data between two independent cohorts will be compared using two-sample t tests or Mann-Whitney U tests for parametric and nonparametric data, respectively. The Shapiro–Wilk test was used to test normality. Statistical significance was 2-tailed and set at a p-value ≤ 0.05.

5.4 Preliminary Results

Baseline and preoperative variables for all 41 presumed aseptic revisions are shown in Table 5.1. The entire cohort had a median age of 70.0 years (IQR 63.0 to 74.0) and BMI of 30.1 (27.0 to 35.8). Fifty-six percent (23) of the patients were female and the dominant reasons for revision were aseptic loosening (39.0%) and instability (19.5%). Nearly one-third of patients had a prior revision in the study joint. One patient (TKA) patient had a history of an acute postoperative methicillin-sensitive *Staphylococcus aureus* PJI in the study joint successfully treated with debridement, modular exchange, antibiotics and implant retention 4 years prior (Table 5.1). This same patient later underwent subsequent aseptic revisions for aseptic loosening and arthrofibrosis, but preoperative infection workup and intraoperative cultures in our study were negative. Roughly one-tenth of patients had an elevated CRP (9.8%) and ESR (12.2%), and 24.4% (10) underwent a preoperative joint fluid aspiration to rule out PJI. There was some variability between THA and TKA cohorts in variables examined (Table 5.1).

Table 5.1. Baseline and preoperative data for all patients and by joint type

Variable	Total (n = 41)	THA (n = 20)	TKA (n = 21)
Age (years)*	70.0 (63.0 to 74.0)	66.5 (61.5 to 76.75)	71.0 (66.0 to 74.0)
Sex, n (%)			
Female	23 (56.1)	11 (55.0)	12 (57.1)
Male	18 (43.9)	9 (45.0)	9 (42.9)
BMI (kg/m^2)*	30.1 (27.0 to 35.8)	28.3 (26.2 to 30.1)	35.8 (29.7 to 40.7)
ASA, n (%)			
2	8 (19.5)	5 (25.0)	3 (14.3)
3	33 (80.5)	15 (75.0)	18 (85.7)
Inflammatory condition, n (%)	1 (2.4)	1 (5.0)	0 (0)
Etiology for primary, n (%)			
Osteoarthritis	35 (85.4)	15 (75.0)	20 (95.2)
Avascular necrosis	3 (7.3)	2 (10.0)	1 (4.8)
Neck of femur fracture	1 (2.4)	1 (5.0)	n/a
Dysplasia	1 (2.4)	1 (5.0)	n/a
Perthes	1 (2.4)	1 (5.0)	n/a
Reasons for revision, n (%)			
Aseptic loosening	16 (39.0)	8 (40.0)	8 (38.1)
Instability	8 (19.5)	3 (15.0)	5 (23.8)
Arthrofibrosis	5 (12.2)	0 (0)	5 (23.8)
Polyethylene wear +/- osteolysis	4 (9.8)	3 (15.0)	1 (4.8)
Adverse metal reaction	4 (9.8)	4 (20.0)	0 (0)
Metal allergy	1 (2.4)	0 (0)	1 (4.8)
Implant fracture	1 (2.4)	1 (5.0)	0 (0)
Pain/mechanical symptoms	1 (2.4)	1 (5.0)	0 (0)
Chronic patella dislocation	1 (2.4)	n/a	1 (4.8)
Prior revision in study joint, n (%)	13 (31.7)	4 (20.0)	9 (42.9)
History of PJI in study Joint, n (%)	1 (2.44)	0 (0)	1(4.8)
Preoperative serum CRP $\geq$ 10mg/L, n (%)	4 (9.8)	3 (15.0)	1 (4.8)
Preoperative serum ESR $\geq$ 30mm/h. n (%)	5 (12.2)	3 (15.0)	2 (9.5)
Preoperative joint aspirate, n (%)	10 (24.4)	5 (25.0)	5 (23.8)

*Values are median (interquartile ranges). THA, total hip arthroplasty; TKA, total knee arthroplasty; BMI, body mass index; ASA, American society of Anesthesiologists classification; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate.

Two-component exchange was the most common type of revision (41.5%), followed by 1-component exchange (34.1%), and modular exchange only (24.4%) (Table 5.2). In the THA cohort 1-component exchange (55.0%) was the most common type of revision performed followed by modular exchange (30.0%), and for TKA it was 2-component exchange (66.7%)

followed by modular exchange (19.0%) (Table 5.2). A median of 4 (IQR 4 to 5) intraoperative surgical samples for standard cultures were taken per revision and a majority of these samples were tissue (80.6%) (Table 5.2).

Table 5.2. Type of surgery and standard laboratory culture data for all patients and by joint type

Variable	Total (n = 41)	THA (n = 20)	TKA (n = 21)
Type of revision, n (%)			
Modular exchange	10 (24.4)	6 (30.0)	4 (19.0)
1-component	14 (34.1)	11 (55.0)‡	3 (14.3)‡
2-component	17 (41.5)	3 (15.0)	14 (66.7)
Total number of intraoperative samples taken for standard culture*	186	83	103
Swab samples, n (%)	19 (10.2)	13 (15.7)	6 (5.8)
Fluid samples, n (%)	17 (9.1)	4 (4.8)	13 (12.6)
Tissue samples, n (%)	150 (80.6)	66 (79.5)	84 (81.6)
Number of intraoperative samples taken per revision for standard culture†	4 (4 to 5)	4 (3.75 to 5)	4 (4 to 5)

*All intraoperative samples taken were send for aerobic, anerobic, and extended (14 days) cultures in the hospital laboratory. †Values are median (interquartile ranges). ‡In the 1-component exchanges there were 6 acetabulum & 5 femurs for THA and 1 femur & 2 tibias for TKA. THA, total hip arthroplasty; TKA, total knee arthroplasty.

The prevalence of UPC was 9.8% (4/41) for the entire cohort, 10.0% (2/20) for THA and 9.5% (2/21) for TKA. Details of the 4 patients with UPC can be seen in Table 5.3. Only 1 patient had a history of a prior revision in the study joint and no patients had a history of PJI. Preoperative screening serum CRP and ESR were normal and no patient underwent a preoperative joint aspirate. Each patient had a single UPC (one with 2 microorganisms) and all microorganisms were isolated from broth only (Table 5.3). *C. acnes* was the most common microorganism (3/4 patients), followed by *Staphylococcus Epidermidis* (1/4), and *Anaerococcus octavius* (1/4) (Table 5.3).

Table 5.3. Details of the 4 patients with unexpected positive intraoperative cultures

Variable	Case 1	Case 2	Case 3	Case 4
Joint type	THA	THA	TKA	TKA
Age (years)	62	26	74	66
Sex	Male	Female	Female	Male
BMI (kg/m^2)	26.0	33	40.7	47.3
Etiology for primary	Osteoarthritis	DDH	Osteoarthritis	Osteoarthritis
Reason for revision	Aseptic loosening	Instability	Aseptic loosening	Aseptic loosening
Prior revision in study joint	No	Yes (instability)	No	No
History of PJI in study joint	No	No	No	No
Preoperative serum CRP (mg/L)	4.2	2.9	2.2	1.3
Preoperative serum ESR (mm/h)	7	11	14	8
Preoperative joint aspirate	No	No	No	No
Type of revision	1-component	Modular	1-component	1-component
Number of samples taken for standard culture*	4	7	5	5
Number of UPCs	1	1	1	1
UPC solid or broth	Broth	Broth	Broth	Broth
UPC sample type	Tissue (aerobic)	Swab (anaerobic)	Tissue (anaerobic)	Tissue (anaerobic)
UPC microorganism(s)	*Staphylococcus Epidermidis*	*Cutibacterium acnes*	*Cutibacterium acnes + Anaerococcus octavius*	*Cutibacterium acnes*

*All intraoperative samples taken were send for aerobic, anerobic, and extended (14 days) cultures in the hospital laboratory. THA, total hip arthroplasty; TKA, total knee replacement; BMI, body mass index; DDH, developmental dysplasia of the hip; PJI, periprosthetic joint infection; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; UPC, unexpected positive intraoperative culture.

A total of 248 implant samples and 41 operative aerosol controls to be included in the molecular analysis are currently being stored at the Canadian Centre for Human Microbiome and Probiotics at St. Joseph's Health Care, London, ON. Complete results are pending post COVID-19 crisis.

5.5 Discussion

Pending completed results.

5.6 Conclusions

Pending completed results.

5.7 References

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Chapter 6

6. Conclusions

6.1 Unexpected Positive Intraoperative Cultures in Presumed Aseptic Revision THA and TKA

This thesis represents the largest series of unexpected positive intraoperative cultures (UPC) in presumed aseptic revision for both total hip (THA) and knee arthroplasty (TKA). Our results are a valuable addition to the literature and can be used by clinicians to counsel patients on expected outcomes and as an aid in decision making.

6.1.1 UPC in Presumed Aseptic Revision THA

We showed that the prevalence of UPC was 9.2% and that the 2- and 5-year infection-free survival was encouraging (93.1% and 86.8%, respectively). Infection-free survival when only considering infection-related implant failure by the same UPC microorganism is excellent (95.8% and 94.3%). We did not find a difference in infection-free survival between cohorts of interest (1 versus with ≥2 UPC and 1 UPC treated with antibiotics versus not treated with antibiotics), but this must be interpreted with caution. Patients with ≥2 UPC and those with a single UPC treated with antibiotics were more likely to have recurrent infection-related implant failure caused by the same UPC microorganism (100% and 100%, respectively). Patients with a single UPC are unlikely to have recurrent infection by the same UPC microorganism (25% in antibiotic treatment versus 0% in the no antibiotic treatment cohorts). Finally, patients with a single UPC and no other signs of infection can be considered contaminants, and do not require antibiotic treatment. However, this may not be absolute or universal to all cases and host status, reason for revision, microorganism, and surgical factors need to be considered when making treatment decisions.

6.1.2 UPC in Presumed Aseptic Revision TKA

Similarly, we demonstrated that the prevalence of UPC was 9.8% and the 2- and 5-year infection free survival is excellent (97.4% and 95.3%, respectively). For TKA the infection-free survival when considering only infection-related implant failure by the same UPC microorganism is outstanding (98.7%). The majority of subsequent infection-related failures were caused by a different microorganism than the UPC (66.7%). Infection-free survival was similar for the 1 versus ≥2 UPC cohorts, however, it was poorer for the cohort treated with antibiotics versus those not treated with antibiotics. Again, these comparisons between cohorts must be interpreted with caution due to selection biases, differences between cohorts, and sample size limitations. No patient with a single UPC that was deemed not to require antibiotic treatment had a subsequent infection-related implant failure, strongly suggesting that a single UPC without signs of infection is likely a contaminant and does not require antibiotic treatment. However, this may not be absolute or universal to all cases and host status, reason for revision, microorganism, and surgical factors need to be considered when making treatment decisions.

6.1.3 Limitations and Future Directions

The major limitations of these studies were the retrospective and single center design, lack of a standardized UPC treatment protocol, absence of a standardized intraoperative sampling protocol (type, number), and sample size limitations between cohorts for secondary outcomes of interest. A selection bias existed for those deemed to require antibiotic treatment and limited our ability to establish UPC treatment protocol recommendations.

Many authors have advised that prospective evaluation of UPC in presumed aseptic revision THA and TKA is unlikely to occur because of the infrequent nature of the problem. It is the current author’s opinion that this is wrong. We showed that the prevalence of UPC is approximately 10% (consistent with the mean prevalence in the literature). The number of revision cases will increase substantially and periprosthetic joint infection remains a devastating complication with no perfect diagnostic test. Thus, a well-designed, multicenter, prospective study on this subject matter is feasible and required to establish the clinical significance and proper treatment protocols for UPC in presumed aseptic revision. Additionally, since there is not

universal agreement that all revisions require intraoperative cultures (especially those with a low index of suspicion for infection), there is a potential need for future cost-effectiveness analyses.

6.2 Do Microorganisms Have a Role in ‘Aseptic’ Total Hip and Knee arthroplasty Implant Failure? A Prospective Molecular Pilot Study

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We hypothesized that these implants will frequently be colonized by bacterial microorganisms, molecular techniques will identify more microorganisms versus standard intraoperative cultures, and that the presence of microorganisms will be associated with the implant type, location, and reason for revision. Our specific aims were to use modern molecular and sequencing methods with an emphasis on the stringent control of contamination to, (I) determine the frequency and type of bacterial microorganisms on prosthetic implants from presumed aseptic THA and TKA failures, and compare the microorganisms identified by molecular methods to those of standard cultures, (II) determine the type of implants and location on the implants that bacterial microorganisms are found, and (III) determine if the presence of bacterial microorganisms is associated with the reason for revision.

As previously described, all samples for molecular analysis are collected and safely stored. However, due to the unprecedented COVID-19 pandemic and state of emergency, the completion of the molecular portion of this study has been postponed.

The UPC microorganisms identified in our database studies were helpful to the molecular methodology of this prospective pilot study and led to the utilization of both universal and genus/species specific PCR primers. Additionally, the approximately 40/60 split for etiology for revision (aseptic loosening versus other aseptic failure modes) will aid in the potential identification of an association between molecular identification of microorganisms and reason for revision.

Preliminary results from the prospective molecular study supports our work on the prevalence of UPC (standard surgical cultures) in presumed aseptic revision THA and TKA. The indolent nature of the microorganisms identified is also consistent with our previous work. The

normal preoperative clinical history, serum C-reactive protein and erythrocyte sedimentation rate values in patients with UPC shows how unpredictable these positive cultures can be.

No molecular study has investigated if there are specific areas on prosthetic implants that microorganisms are more likely to be found. If these locations were to be discovered, this would have important implications**.** The prevalence and role and of microorganisms identified in presumed aseptic revisions using molecular techniques remains unclear, and the clinical significance in terms of implant survival and functional outcomes is virtually unknown**.** Our pilot study will not be able to answer these questions definitively, nor was it designed to. Data from this pilot study will be used to perform appropriate power calculations, obtain funding, and design large studies that can definitively determine the role and clinical implications of microorganisms identified by modern molecular techniques in presumed aseptic revisions**.** If we identify that 'aseptic' failures are frequently colonized with microorganisms, future molecular studies should follow these patients longitudinally to evaluate the clinical significance of this.

The role of microorganisms in presumed aseptic revision THA and TKA may have been underestimated and this clearly necessitates ongoing investigation.

Appendices

Appendix A. REB Approval

Western Research

Date: 17 September 2019

To: Dr. Edward Vasarhelyi

Project ID: 113707

Study Title: The prevalence and outcomes of unexpected positive intraoperative cultures in presumed aseptic revision knee and hip arthroplasty

Application Type: HSREB Initial Application

Review Type: Delegated

Meeting Date / Full Board Reporting Date: 01/Oct/2019

Date Approval Issued: 17/Sep/2019

REB Approval Expiry Date: 17/Sep/2020

Dear Dr. Edward Vasarhelyi

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
UPIC - Data Collection Form 13-Aug-2019	Other Data Collection Instruments	13/Aug/2019	1.0
v3.1.Study Protocol UPC Aseptic Revision Arthoplasty_27August2019	Protocol	27/Aug/2019	3.1

Documents Acknowledged:

Document Name	Document Type	Document Date
UPIC - References	References	13/Aug/2019

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

LAWSON FINAL APPROVAL NOTICE

LAWSON APPROVAL NUMBER: R-19-495

PROJECT TITLE: The prevalence and outcomes of unexpected positive intraoperative cultures in presumed aseptic revision knee and hip arthroplasty

PRINCIPAL INVESTIGATOR: Dr. Edward Vasarhelyi

LAWSON APPROVAL DATE: 17/09/2019

ReDA ID: 6229

Overall Study Status: Active

Please be advised that the above project was reviewed by Lawson Administration and the project was approved.

Please provide your Lawson Approval Number (R#) to the appropriate contact(s) in supporting departments (eg. Lab Services, Diagnostic Imaging, etc.) to inform them that your study is starting. The Lawson Approval Number must be provided each time services are requested.

Dr. David Hill
V.P. Research
Lawson Health Research Institute

Appendix B. REB Approval

Western Research

Date: 10 July 2019

To: Dr. Edward Vasarhelyi

Project ID: 114030

Study Title: Potential roles for microorganisms in hip and knee joint implant failures

Application Type: HSREB Initial Application

Review Type: Delegated

Full Board Reporting Date: 06Aug2019

Date Approval Issued: 10/Jul/2019 10:23

REB Approval Expiry Date: 10/Jul/2020

Dear Dr. Edward Vasarhelyi

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
114030 - Data Collection Form 06-May-2019	Other Data Collection Instruments	06/May/2019	
114030 - Letter of Information and Consent 25-June-2019 Final	Written Consent/Assent	25/Jun/2019	2
Implant Infection Sampling 25-Jun-19 Final	Protocol	25/Jun/2019	2

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Nicola Geoghegan-Morphet, Ethics Officer on behalf of Dr. Philip Jones, HSREB Vice-Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

LAWSON FINAL APPROVAL NOTICE

LAWSON APPROVAL NUMBER: R-19-495

PROJECT TITLE: The prevalence and outcomes of unexpected positive intraoperative cultures in presumed aseptic revision knee and hip arthroplasty

PRINCIPAL INVESTIGATOR: Dr. Edward Vasarhelyi

LAWSON APPROVAL DATE: 17/09/2019

ReDA ID: 6229

Overall Study Status: Active

Please be advised that the above project was reviewed by Lawson Administration and the project was approved.

Please provide your Lawson Approval Number (R#) to the appropriate contact(s) in supporting departments (eg. Lab Services, Diagnostic Imaging, etc.) to inform them that your study is starting. The Lawson Approval Number must be provided each time services are requested.

Dr. David Hill
V.P. Research
Lawson Health Research Institute

Appendix C. Letter of Information and Consent Form

Letter of Information and Consent Form

Potential Roles for Microorganisms in Hip and Knee Joint Implant Failures

Principal Investigator:
Dr. Edward Vasarhelyi

Co-Investigators:
Dr. Brent Lanting
Dr. David O'Gorman
Dr. Jeremy Burton
Dr. Matthew Teeter
Dr. Douglas Naudie
Dr. James Howard
Dr. Steven MacDonald
Dr. Richard McCalden
Dr. Emil Schemitsch

Study Coordinator:

You are being invited to participate in a research study designed for patients who will receive a revision total knee or hip replacement under Dr. Edward Vasarhelyi or Dr. Brent Lanting's care. This letter of information describes the research study and your role as a participant. The purpose of this letter is to provide you with the information you require to make an informed decision about participating in this research. Please read this form carefully.

Study Purpose

Periprosthetic joint infection (infection in the joint after hip or knee replacement) occurs in around 2% of patients, and can be a challenge to treat for both patients and surgeons. When a severe, chronic infection occurs, patients must undergo a two-stage procedure to remove the infected implant, clear the infection, and re-implant a new joint replacement. In some patients undergoing revision total joint replacement without clinical signs of infection, microorganisms (bacteria that may cause infection) have still been identified on the removed implants. It is suspected that some clinically non-infected revision joint replacements are actually infected. The goal of this study is to investigate whether microorganisms are present on removed implants when infection is not the reason for revision joint replacement. We also aim to investigate the type of microorganisms that are present, along with their location, on hip and knee implants removed from patients during revision surgery (for both infected and non-infected patients). We hope that a better understanding of the presence, location and type of microorganisms present during revision joint replacement can lead to better treatments for infection in the future.

Procedure

If you decide to participate in this study your surgeon will take up to nine (9) samples from the implants removed during your revision hip or knee replacement procedure. The samples are taken after your implant has been removed when you are in the OR. The samples will involve scraping up to nine (9) locations on your implant with a scalpel. These samples are tissue samples and although are primarily bacterial samples may contain your tissue. Your samples will be stored in tubes and will be taken to Dr. O'Gorman's lab at St. Joseph's Hospital. Samples collected for the study will be analyzed in the lab in order to investigate the presence, type and location of microorganisms on your removed implants. No personal information will be attached to your samples; the tubes will be labeled with a number (ie. sample 1, 2, 3, etc.). Study samples will be kept only until the DNA is extracted (DNA is taken from the samples to identify any microorganisms are present), at which time the samples will be destroyed. Although the extraction will focus on bacterial DNA it is possible that your DNA will be part of the sample but this will be ignored in the analysis. The DNA (both bacterial and human) will be kept for 10 years after which it will be disposed of.

As part of the standard of care at University Hospital, after your implants are removed, they are sterilized (cleaned) and taken to the Implant Retrieval Lab (at University Hospital) for storage. For this study, we will also look at your implants to evaluate any damage that may be present to determine how the implants were functioning in your body.

Approximately 40 patients will be asked to participate in this study.

Risk

There are no additional risks of this study outside of the standard risks associated with a revision joint replacement procedure.

Benefits

Participation in this study will provide no known benefit to you. Information learned from this study may help lead to improvements in treatments for periprosthetic infections in the future.

Compensation

There will be no compensation for your participation in this study.

Voluntary Participation

Your participation in this study is voluntary. You may refuse to participate or discontinue your participation at any time without affecting the care being provided to you. Should you choose to withdraw; no further information will be collected. The data you have contributed to that point will be used to help answer our research question. Once the samples have been collected and sent to the lab, the samples cannot be withdrawn.

Confidentiality

All information will be kept confidential to the best of our ability. No identifiers will be directly attached to your sample. A master list linking your study ID to identifiable information will be kept separately from your sample data. This list will be maintained for 15 years at university hospital. There is the possibility that the sample extracted from the implant will have include parts of your tissue, although this will be ignored in extraction of DNA and analysis. There is always a remote chance that your information, including consent forms or the participant log, could be breached by someone without permission to your information. The chance that this information will be accidentally released is minimal. In any publication, presentation or report, all results will be de-identified and any information that would reveal your identity will not be published.

You will be given a copy of this letter of information and consent form once it has been signed. You do not waive any legal rights by signing the consent form. Representatives of The University of Western Ontario Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research.

Qualified representatives of the Lawson Quality Assurance Education Program may look at your medical/clinical study records at the site where these records are held, for quality assurance (to check that the information collected for the study is correct and follows proper laws and guidelines).

If you have any questions about your rights as a research participant or the conduct of the study you may contact the Patient Relations Office at LHSC [redacted]

If you have any questions about your surgery, please contact your orthopaedic surgeon. If you have any questions about this research, please contact the principal investigator Dr. Edward Vasarhelyi at [redacted]

London Health Sciences Centre

Caring for You. Innovating for the World.®

Potential Roles for Microorganisms in Hip and Knee Joint Implant Failures

Principal Investigator: Dr. Edward Vasarhelyi

Informed Consent Form

Agreement of Participating Subject

I have read the accompanying letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Print Participant's Full Name

Participant's Signature Date

Name of Person Obtaining Consent

Signature of Person Obtaining Consent Date

☐ The person signing below acted as a translator or witness for the participant during the consent process and attests that the study as set out in this form was accurately translated/communicated and has had any questions answered.

Print Name of Translator/Witness Signature Date

Language

Appendix D. Molecular Sample Collection Sheet

Potential Role of Microorganisms on Implant Failure

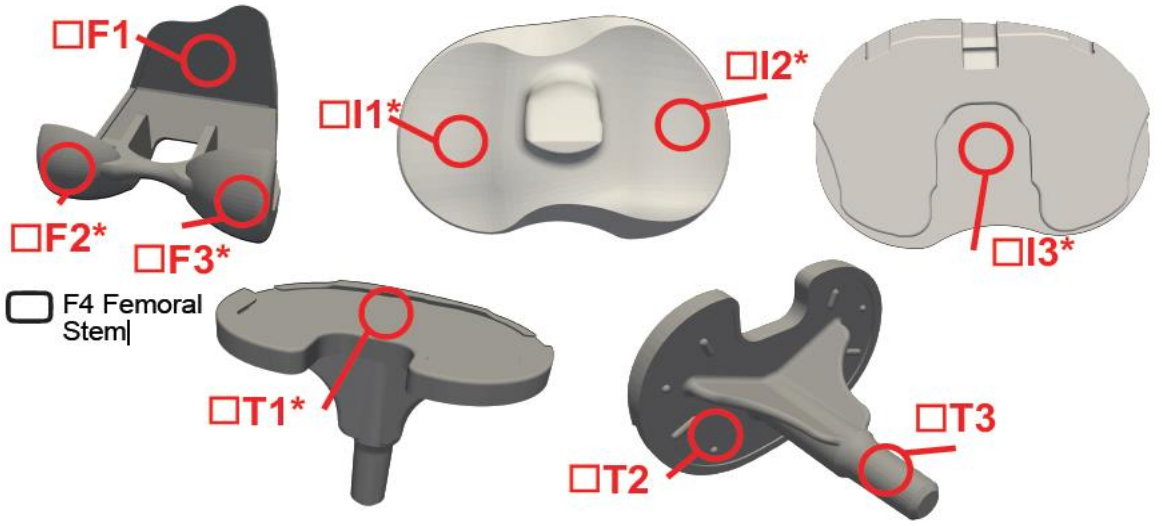
PIN: _____ Date: _____

IRL ID: _____ Surgeon: _____

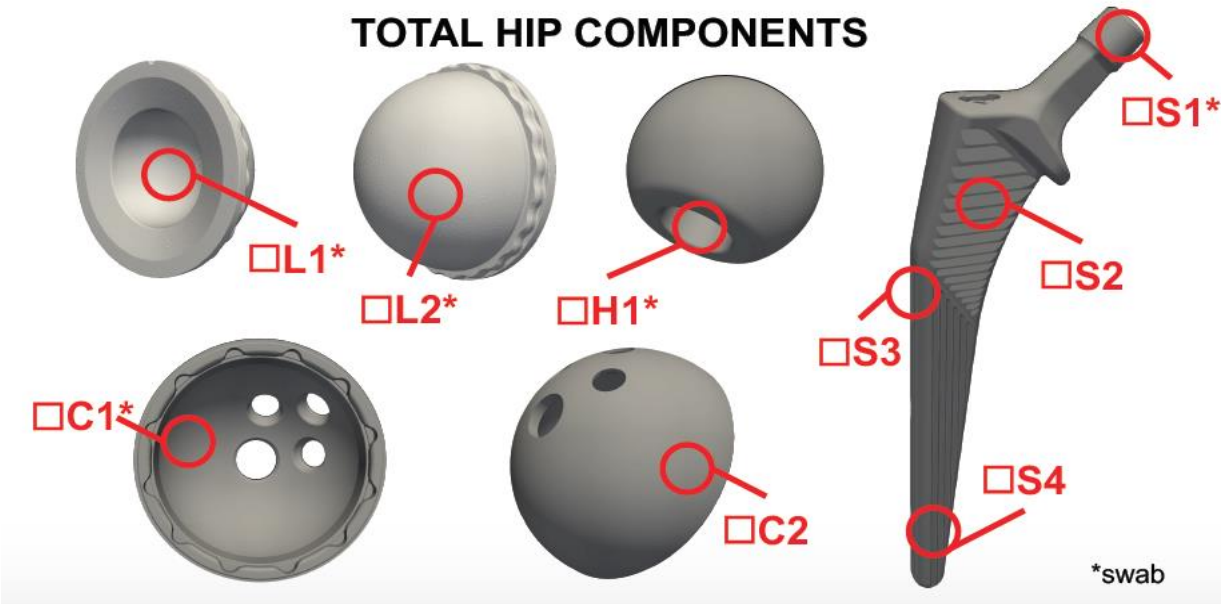
Revision For: _____ Side: _____

Time In Vivo: _____ Collected By: _____

TOTAL KNEE COMPONENTS



TOTAL HIP COMPONENTS



Appendix E. Preparation of Primer Plate for Gene Sequencing Protocol (Manufacturer)

Preparation of Golay Primer Plates for V4 16S rRNA gene sequencing

- LifeTech primer stocks made to 200 µM (pMole/uL) and frozen at -80 °C (as per Greg's Illumina SOP https://github.com/ggloor/miseq_bin/blob/master/Illumina_SOP.pdf)

doi: 10.1128/mSystems.00009-15, Improved bacterial 16S rRNA gene V4 and V4-5 and fungal internal transcribed spacer maker gene primers for microbial community surveys

- Each PCR reaction requires 10 µL of each L and R unique-barcoded primers at 3.2 µM
- Prep 5 sets of each plate for stock
- There are 24 L and 24 R nucleotide-balanced barcoded Golay primers (see sequences attached), therefore we have 576 unique combinations, or 6 X 96-well plates
- Each primer will be used 24 times in one set of plates
- 10 µL x 24 combinations per primer x 5 sets of plates = 1200 µL of each primer required
- Add 50 µL of extra primer for robot pipetting allowance
- $C_1 \cdot V_1 = C_2 \cdot V_2$
  200 µM · V_1 = 3.2 µM · 1250 µL
  V_1 = 20 µL
  Therefore we add 20 µL of 200 µM primer stock to 1230 µL nuclease-free water
- Label the side/front of DNase/RNase free Eppendorfs with L1-24 and R1-24
- Once 1250 µL aliquots of 3.2 µM primers are prepared in the tubes, centrifuge briefly.
- Cut the tops off the Eppendorf tubes with RNaseZap'ed/ UV'ed scissors inside biosafety cabinet
- Place L1-24 in robot Eppendorf tube rack inside the biosafety cabinet, then transfer to robot cabinet before removing the cut lids (tube labels should be facing forward)
- First portion L-primers using KA_L_Pri_Golay Beckman protocol, followed by the same process for R primers, KA_R_Pri_Golay. Order is important!
- Use the appropriate tips marked for these protocols stored inside the robot cabinet
- These protocols generate 1 "stock" plate of all 6 unique layouts, each well within plate contains 100 µL (50 µL of each L and R unique primer combo). Can be stored at -80 before aliquoting.
- Finally, (if frozen, thaw and) centrifuge plates briefly and portion 20 µL from the "stock" plates into 4 duplicates so you end with 5 x Plate 1- Plate 6 and each plate contains 20 µL (10 µL x L, 10 µL x R) using protocol Golay_primer_aliquots

Left-Illumina-adaptornnnnccaaggttLeft-primer

Right-Illumina-adaptornnnnccaaggttRight-primer

FIGURE 1. Structure of the barcoded amplification primers. The 5' end of each primer contains the left- or right-side Illumina adaptor (black), this is followed by four degenerate nucleotides (dark blue), then by the 8-mer barcode (red) and finally the amplification primer (light blue).

←12-mer (not 8) barcodes for Golay (V4EMB primers in Gloor demultiplex pipeline)

Oligo Name	Oligo Sequence (5'-3') 12-mer barcodes amplifying F 515 and R 806
Golay_L1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGCATACACTGGGTGCCAGCMGCCGCGGTAA
Golay_L2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACTCACAGGAATGTGCCAGCMGCCGCGGTAA
Golay_L3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTAGGTGCTTACGTGCCAGCMGCCGCGGTAA
Golay_L4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGTCGTTAAGAGTGCCAGCMGCCGCGGTAA
Golay_L5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACTACGCTAGAGTGCCAGCMGCCGCGGTAA
Golay_L6	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCTCGAAGATTCGTGCCAGCMGCCGCGGTAA
Golay_L7	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAACGTTGGATGTGCCAGCMGCCGCGGTAA
Golay_L8	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGGTTCACCCGGTGCCAGCMGCCGCGGTAA
Golay_L9	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCGAGGGAAAGTCGTGCCAGCMGCCGCGGTAA
Golay_L10	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTACTACGTGGCCGTGCCAGCMGCCGCGGTAA
Golay_L11	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTTCCTCCATTAGTGCCAGCMGCCGCGGTAA
Golay_L12	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGATATGGTCAGTGCCAGCMGCCGCGGTAA
Golay_L13	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTATCGACACAAGGTGCCAGCMGCCGCGGTAA
Golay_L14	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGCATGTCCCGTGTGCCAGCMGCCGCGGTAA
Golay_L15	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGATATAGCAGTGCCAGCMGCCGCGGTAA
Golay_L16	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGTCCGGATTCGTGCCAGCMGCCGCGGTAA
Golay_L17	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATCGCACAGTAAGTGCCAGCMGCCGCGGTAA
Golay_L18	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCATCAGCGTGCCAGCMGCCGCGGTAA
Golay_L19	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCATATGCACTGGTGCCAGCMGCCGCGGTAA
Golay_L20	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGTAGGTGTGCTGTGCCAGCMGCCGCGGTAA
Golay_L21	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGAGACTGATTGTGCCAGCMGCCGCGGTAA
Golay_L22	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCATCAGTACGCCGTGCCAGCMGCCGCGGTAA
Golay_L23	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTATCTGCGCGTGTGCCAGCMGCCGCGGTAA

Golay_L24	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGCGTCAGCTACGTGCCAGCMGCCGCGGTAA
Golay_R1	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCGAGGGAAAGTCGGACTACHVGGGTWTCTAAT
Golay_R2	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNTACTACGTGGCCGGACTACHVGGGTWTCTAAT
Golay_R3	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGTTCCTCCATTAGGACTACHVGGGTWTCTAAT
Golay_R4	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNACGATATGGTCAGGACTACHVGGGTWTCTAAT
Golay_R5	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNTATCGACACAAGGGACTACHVGGGTWTCTAAT
Golay_R6	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNAGCATGTCCCGTGGACTACHVGGGTWTCTAAT
Golay_R7	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCCAGATATAGCAGGACTACHVGGGTWTCTAAT
Golay_R8	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGTGTCCGGATTCGGACTACHVGGGTWTCTAAT
Golay_R9	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNATCGCACAGTAAGGACTACHVGGGTWTCTAAT
Golay_R10	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCAGCTCATCAGCGGACTACHVGGGTWTCTAAT
Golay_R11	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGCATATGCACTGGGACTACHVGGGTWTCTAAT
Golay_R12	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNTGTAGGTGTGCTGGACTACHVGGGTWTCTAAT
Golay_R13	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNACGAGACTGATTGGACTACHVGGGTWTCTAAT
Golay_R14	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCATCAGTACGCCGGACTACHVGGGTWTCTAAT
Golay_R15	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGTATCTGCGCGTGGACTACHVGGGTWTCTAAT
Golay_R16	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNTGCGTCAGCTACGGACTACHVGGGTWTCTAAT
Golay_R17	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGTAGATCGTGTAGGACTACHVGGGTWTCTAAT
Golay_R18	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCAGCTGGTTCAAGGACTACHVGGGTWTCTAAT
Golay_R19	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNAGCTGATAGTTGGGACTACHVGGGTWTCTAAT
Golay_R20	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNTCTACGGCACGTGGACTACHVGGGTWTCTAAT
Golay_R21	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNGCATAAACGACTGGACTACHVGGGTWTCTAAT
Golay_R22	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNAAGGCGCTCCTTGGACTACHVGGGTWTCTAAT
Golay_R23	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNTGCCTAAGATCGGGACTACHVGGGTWTCTAAT
Golay_R24	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNCTTAGCTACTCTGGACTACHVGGGTWTCTAAT

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Golay_L1-Golay_R1	Golay_L1-Golay_R2	Golay_L1-Golay_R3	Golay_L1-Golay_R4	Golay_L1-Golay_R5	Golay_L1-Golay_R6	Golay_L1-Golay_R7	Golay_L1-Golay_R8	Golay_L1-Golay_R9	Golay_L1-Golay_R10	Golay_L1-Golay_R11	Golay_L1-Golay_R12
B	Golay_L1-Golay_R13	Golay_L1-Golay_R14	Golay_L1-Golay_R15	Golay_L1-Golay_R16	Golay_L1-Golay_R17	Golay_L1-Golay_R18	Golay_L1-Golay_R19	Golay_L1-Golay_R20	Golay_L1-Golay_R21	Golay_L1-Golay_R22	Golay_L1-Golay_R23	Golay_L1-Golay_R24
C	Golay_L2-Golay_R1	Golay_L2-Golay_R2	Golay_L2-Golay_R3	Golay_L2-Golay_R4	Golay_L2-Golay_R5	Golay_L2-Golay_R6	Golay_L2-Golay_R7	Golay_L2-Golay_R8	Golay_L2-Golay_R9	Golay_L2-Golay_R10	Golay_L2-Golay_R11	Golay_L2-Golay_R12
D	Golay_L2-Golay_R13	Golay_L2-Golay_R14	Golay_L2-Golay_R15	Golay_L2-Golay_R16	Golay_L2-Golay_R17	Golay_L2-Golay_R18	Golay_L2-Golay_R19	Golay_L2-Golay_R20	Golay_L2-Golay_R21	Golay_L2-Golay_R22	Golay_L2-Golay_R23	Golay_L2-Golay_R24
E	Golay_L3-Golay_R1	Golay_L3-Golay_R2	Golay_L3-Golay_R3	Golay_L3-Golay_R4	Golay_L3-Golay_R5	Golay_L3-Golay_R6	Golay_L3-Golay_R7	Golay_L3-Golay_R8	Golay_L3-Golay_R9	Golay_L3-Golay_R10	Golay_L3-Golay_R11	Golay_L3-Golay_R12
F	Golay_L3-Golay_R13	Golay_L3-Golay_R14	Golay_L3-Golay_R15	Golay_L3-Golay_R16	Golay_L3-Golay_R17	Golay_L3-Golay_R18	Golay_L3-Golay_R19	Golay_L3-Golay_R20	Golay_L3-Golay_R21	Golay_L3-Golay_R22	Golay_L3-Golay_R23	Golay_L3-Golay_R24
G	Golay_L4-Golay_R1	Golay_L4-Golay_R2	Golay_L4-Golay_R3	Golay_L4-Golay_R4	Golay_L4-Golay_R5	Golay_L4-Golay_R6	Golay_L4-Golay_R7	Golay_L4-Golay_R8	Golay_L4-Golay_R9	Golay_L4-Golay_R10	Golay_L4-Golay_R11	Golay_L4-Golay_R12
H	Golay_L4-Golay_R13	Golay_L4-Golay_R14	Golay_L4-Golay_R15	Golay_L4-Golay_R16	Golay_L4-Golay_R17	Golay_L4-Golay_R18	Golay_L4-Golay_R19	Golay_L4-Golay_R20	Golay_L4-Golay_R21	Golay_L4-Golay_R22	Golay_L4-Golay_R23	Golay_L4-Golay_R24

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Golay_L5-Golay_R1	Golay_L5-Golay_R2	Golay_L5-Golay_R3	Golay_L5-Golay_R4	Golay_L5-Golay_R5	Golay_L5-Golay_R6	Golay_L5-Golay_R7	Golay_L5-Golay_R8	Golay_L5-Golay_R9	Golay_L5-Golay_R10	Golay_L5-Golay_R11	Golay_L5-Golay_R12
B	Golay_L5-Golay_R13	Golay_L5-Golay_R14	Golay_L5-Golay_R15	Golay_L5-Golay_R16	Golay_L5-Golay_R17	Golay_L5-Golay_R18	Golay_L5-Golay_R19	Golay_L5-Golay_R20	Golay_L5-Golay_R21	Golay_L5-Golay_R22	Golay_L5-Golay_R23	Golay_L5-Golay_R24
C	Golay_L6-Golay_R1	Golay_L6-Golay_R2	Golay_L6-Golay_R3	Golay_L6-Golay_R4	Golay_L6-Golay_R5	Golay_L6-Golay_R6	Golay_L6-Golay_R7	Golay_L6-Golay_R8	Golay_L6-Golay_R9	Golay_L6-Golay_R10	Golay_L6-Golay_R11	Golay_L6-Golay_R12
D	Golay_L6-Golay_R13	Golay_L6-Golay_R14	Golay_L6-Golay_R15	Golay_L6-Golay_R16	Golay_L6-Golay_R17	Golay_L6-Golay_R18	Golay_L6-Golay_R19	Golay_L6-Golay_R20	Golay_L6-Golay_R21	Golay_L6-Golay_R22	Golay_L6-Golay_R23	Golay_L6-Golay_R24
E	Golay_L7-Golay_R1	Golay_L7-Golay_R2	Golay_L7-Golay_R3	Golay_L7-Golay_R4	Golay_L7-Golay_R5	Golay_L7-Golay_R6	Golay_L7-Golay_R7	Golay_L7-Golay_R8	Golay_L7-Golay_R9	Golay_L7-Golay_R10	Golay_L7-Golay_R11	Golay_L7-Golay_R12
F	Golay_L7-Golay_R13	Golay_L7-Golay_R14	Golay_L7-Golay_R15	Golay_L7-Golay_R16	Golay_L7-Golay_R17	Golay_L7-Golay_R18	Golay_L7-Golay_R19	Golay_L7-Golay_R20	Golay_L7-Golay_R21	Golay_L7-Golay_R22	Golay_L7-Golay_R23	Golay_L7-Golay_R24
G	Golay_L8-Golay_R1	Golay_L8-Golay_R2	Golay_L8-Golay_R3	Golay_L8-Golay_R4	Golay_L8-Golay_R5	Golay_L8-Golay_R6	Golay_L8-Golay_R7	Golay_L8-Golay_R8	Golay_L8-Golay_R9	Golay_L8-Golay_R10	Golay_L8-Golay_R11	Golay_L8-Golay_R12
H	Golay_L8-Golay_R13	Golay_L8-Golay_R14	Golay_L8-Golay_R15	Golay_L8-Golay_R16	Golay_L8-Golay_R17	Golay_L8-Golay_R18	Golay_L8-Golay_R19	Golay_L8-Golay_R20	Golay_L8-Golay_R21	Golay_L8-Golay_R22	Golay_L8-Golay_R23	Golay_L8-Golay_R24

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Golay_L9-Golay_R1	Golay_L9-Golay_R2	Golay_L9-Golay_R3	Golay_L9-Golay_R4	Golay_L9-Golay_R5	Golay_L9-Golay_R6	Golay_L9-Golay_R7	Golay_L9-Golay_R8	Golay_L9-Golay_R9	Golay_L9-Golay_R10	Golay_L9-Golay_R11	Golay_L9-Golay_R12
B	Golay_L9-Golay_R13	Golay_L9-Golay_R14	Golay_L9-Golay_R15	Golay_L9-Golay_R16	Golay_L9-Golay_R17	Golay_L9-Golay_R18	Golay_L9-Golay_R19	Golay_L9-Golay_R20	Golay_L9-Golay_R21	Golay_L9-Golay_R22	Golay_L9-Golay_R23	Golay_L9-Golay_R24
C	Golay_L10-Golay_R1	Golay_L10-Golay_R2	Golay_L10-Golay_R3	Golay_L10-Golay_R4	Golay_L10-Golay_R5	Golay_L10-Golay_R6	Golay_L10-Golay_R7	Golay_L10-Golay_R8	Golay_L10-Golay_R9	Golay_L10-Golay_R10	Golay_L10-Golay_R11	Golay_L10-Golay_R12
D	Golay_L10-Golay_R13	Golay_L10-Golay_R14	Golay_L10-Golay_R15	Golay_L10-Golay_R16	Golay_L10-Golay_R17	Golay_L10-Golay_R18	Golay_L10-Golay_R19	Golay_L10-Golay_R20	Golay_L10-Golay_R21	Golay_L10-Golay_R22	Golay_L10-Golay_R23	Golay_L10-Golay_R24
E	Golay_L11-Golay_R1	Golay_L11-Golay_R2	Golay_L11-Golay_R3	Golay_L11-Golay_R4	Golay_L11-Golay_R5	Golay_L11-Golay_R6	Golay_L11-Golay_R7	Golay_L11-Golay_R8	Golay_L11-Golay_R9	Golay_L11-Golay_R10	Golay_L11-Golay_R11	Golay_L11-Golay_R12
F	Golay_L11-Golay_R13	Golay_L11-Golay_R14	Golay_L11-Golay_R15	Golay_L11-Golay_R16	Golay_L11-Golay_R17	Golay_L11-Golay_R18	Golay_L11-Golay_R19	Golay_L11-Golay_R20	Golay_L11-Golay_R21	Golay_L11-Golay_R22	Golay_L11-Golay_R23	Golay_L11-Golay_R24
G	Golay_L12-Golay_R1	Golay_L12-Golay_R2	Golay_L12-Golay_R3	Golay_L12-Golay_R4	Golay_L12-Golay_R5	Golay_L12-Golay_R6	Golay_L12-Golay_R7	Golay_L12-Golay_R8	Golay_L12-Golay_R9	Golay_L12-Golay_R10	Golay_L12-Golay_R11	Golay_L12-Golay_R12
H	Golay_L12-Golay_R13	Golay_L12-Golay_R14	Golay_L12-Golay_R15	Golay_L12-Golay_R16	Golay_L12-Golay_R17	Golay_L12-Golay_R18	Golay_L12-Golay_R19	Golay_L12-Golay_R20	Golay_L12-Golay_R21	Golay_L12-Golay_R22	Golay_L12-Golay_R23	Golay_L12-Golay_R24

Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	Golay_L13-Golay_R1	Golay_L13-Golay_R2	Golay_L13-Golay_R3	Golay_L13-Golay_R4	Golay_L13-Golay_R5	Golay_L13-Golay_R6	Golay_L13-Golay_R7	Golay_L13-Golay_R8	Golay_L13-Golay_R9	Golay_L13-Golay_R10	Golay_L13-Golay_R11	Golay_L13-Golay_R12
B	Golay_L13-Golay_R13	Golay_L13-Golay_R14	Golay_L13-Golay_R15	Golay_L13-Golay_R16	Golay_L13-Golay_R17	Golay_L13-Golay_R18	Golay_L13-Golay_R19	Golay_L13-Golay_R20	Golay_L13-Golay_R21	Golay_L13-Golay_R22	Golay_L13-Golay_R23	Golay_L13-Golay_R24
C	Golay_L14-Golay_R1	Golay_L14-Golay_R2	Golay_L14-Golay_R3	Golay_L14-Golay_R4	Golay_L14-Golay_R5	Golay_L14-Golay_R6	Golay_L14-Golay_R7	Golay_L14-Golay_R8	Golay_L14-Golay_R9	Golay_L14-Golay_R10	Golay_L14-Golay_R11	Golay_L14-Golay_R12
D	Golay_L14-Golay_R13	Golay_L14-Golay_R14	Golay_L14-Golay_R15	Golay_L14-Golay_R16	Golay_L14-Golay_R17	Golay_L14-Golay_R18	Golay_L14-Golay_R19	Golay_L14-Golay_R20	Golay_L14-Golay_R21	Golay_L14-Golay_R22	Golay_L14-Golay_R23	Golay_L14-Golay_R24
E	Golay_L15-Golay_R1	Golay_L15-Golay_R2	Golay_L15-Golay_R3	Golay_L15-Golay_R4	Golay_L15-Golay_R5	Golay_L15-Golay_R6	Golay_L15-Golay_R7	Golay_L15-Golay_R8	Golay_L15-Golay_R9	Golay_L15-Golay_R10	Golay_L15-Golay_R11	Golay_L15-Golay_R12
F	Golay_L15-Golay_R13	Golay_L15-Golay_R14	Golay_L15-Golay_R15	Golay_L15-Golay_R16	Golay_L15-Golay_R17	Golay_L15-Golay_R18	Golay_L15-Golay_R19	Golay_L15-Golay_R20	Golay_L15-Golay_R21	Golay_L15-Golay_R22	Golay_L15-Golay_R23	Golay_L15-Golay_R24
G	Golay_L16-Golay_R1	Golay_L16-Golay_R2	Golay_L16-Golay_R3	Golay_L16-Golay_R4	Golay_L16-Golay_R5	Golay_L16-Golay_R6	Golay_L16-Golay_R7	Golay_L16-Golay_R8	Golay_L16-Golay_R9	Golay_L16-Golay_R10	Golay_L16-Golay_R11	Golay_L16-Golay_R12
H	Golay_L16-Golay_R13	Golay_L16-Golay_R14	Golay_L16-Golay_R15	Golay_L16-Golay_R16	Golay_L16-Golay_R17	Golay_L16-Golay_R18	Golay_L16-Golay_R19	Golay_L16-Golay_R20	Golay_L16-Golay_R21	Golay_L16-Golay_R22	Golay_L16-Golay_R23	Golay_L16-Golay_R24

Plate 5

	1	2	3	4	5	6	7	8	9	10	11	12
A	Golay_L17-Golay_R1	Golay_L17-Golay_R2	Golay_L17-Golay_R3	Golay_L17-Golay_R4	Golay_L17-Golay_R5	Golay_L17-Golay_R6	Golay_L17-Golay_R7	Golay_L17-Golay_R8	Golay_L17-Golay_R9	Golay_L17-Golay_R10	Golay_L17-Golay_R11	Golay_L17-Golay_R12
B	Golay_L17-Golay_R13	Golay_L17-Golay_R14	Golay_L17-Golay_R15	Golay_L17-Golay_R16	Golay_L17-Golay_R17	Golay_L17-Golay_R18	Golay_L17-Golay_R19	Golay_L17-Golay_R20	Golay_L17-Golay_R21	Golay_L17-Golay_R22	Golay_L17-Golay_R23	Golay_L17-Golay_R24
C	Golay_L18-Golay_R1	Golay_L18-Golay_R2	Golay_L18-Golay_R3	Golay_L18-Golay_R4	Golay_L18-Golay_R5	Golay_L18-Golay_R6	Golay_L18-Golay_R7	Golay_L18-Golay_R8	Golay_L18-Golay_R9	Golay_L18-Golay_R10	Golay_L18-Golay_R11	Golay_L18-Golay_R12
D	Golay_L18-Golay_R13	Golay_L18-Golay_R14	Golay_L18-Golay_R15	Golay_L18-Golay_R16	Golay_L18-Golay_R17	Golay_L18-Golay_R18	Golay_L18-Golay_R19	Golay_L18-Golay_R20	Golay_L18-Golay_R21	Golay_L18-Golay_R22	Golay_L18-Golay_R23	Golay_L18-Golay_R24
E	Golay_L19-Golay_R1	Golay_L19-Golay_R2	Golay_L19-Golay_R3	Golay_L19-Golay_R4	Golay_L19-Golay_R5	Golay_L19-Golay_R6	Golay_L19-Golay_R7	Golay_L19-Golay_R8	Golay_L19-Golay_R9	Golay_L19-Golay_R10	Golay_L19-Golay_R11	Golay_L19-Golay_R12
F	Golay_L19-Golay_R13	Golay_L19-Golay_R14	Golay_L19-Golay_R15	Golay_L19-Golay_R16	Golay_L19-Golay_R17	Golay_L19-Golay_R18	Golay_L19-Golay_R19	Golay_L19-Golay_R20	Golay_L19-Golay_R21	Golay_L19-Golay_R22	Golay_L19-Golay_R23	Golay_L19-Golay_R24
G	Golay_L20-Golay_R1	Golay_L20-Golay_R2	Golay_L20-Golay_R3	Golay_L20-Golay_R4	Golay_L20-Golay_R5	Golay_L20-Golay_R6	Golay_L20-Golay_R7	Golay_L20-Golay_R8	Golay_L20-Golay_R9	Golay_L20-Golay_R10	Golay_L20-Golay_R11	Golay_L20-Golay_R12
H	Golay_L20-Golay_R13	Golay_L20-Golay_R14	Golay_L20-Golay_R15	Golay_L20-Golay_R16	Golay_L20-Golay_R17	Golay_L20-Golay_R18	Golay_L20-Golay_R19	Golay_L20-Golay_R20	Golay_L20-Golay_R21	Golay_L20-Golay_R22	Golay_L20-Golay_R23	Golay_L20-Golay_R24

Plate 6

	1	2	3	4	5	6	7	8	9	10	11	12
A	Golay_L21-Golay_R1	Golay_L21-Golay_R2	Golay_L21-Golay_R3	Golay_L21-Golay_R4	Golay_L21-Golay_R5	Golay_L21-Golay_R6	Golay_L21-Golay_R7	Golay_L21-Golay_R8	Golay_L21-Golay_R9	Golay_L21-Golay_R10	Golay_L21-Golay_R11	Golay_L21-Golay_R12
B	Golay_L21-Golay_R13	Golay_L21-Golay_R14	Golay_L21-Golay_R15	Golay_L21-Golay_R16	Golay_L21-Golay_R17	Golay_L21-Golay_R18	Golay_L21-Golay_R19	Golay_L21-Golay_R20	Golay_L21-Golay_R21	Golay_L21-Golay_R22	Golay_L21-Golay_R23	Golay_L21-Golay_R24
C	Golay_L22-Golay_R1	Golay_L22-Golay_R2	Golay_L22-Golay_R3	Golay_L22-Golay_R4	Golay_L22-Golay_R5	Golay_L22-Golay_R6	Golay_L22-Golay_R7	Golay_L22-Golay_R8	Golay_L22-Golay_R9	Golay_L22-Golay_R10	Golay_L22-Golay_R11	Golay_L22-Golay_R12
D	Golay_L22-Golay_R13	Golay_L22-Golay_R14	Golay_L22-Golay_R15	Golay_L22-Golay_R16	Golay_L22-Golay_R17	Golay_L22-Golay_R18	Golay_L22-Golay_R19	Golay_L22-Golay_R20	Golay_L22-Golay_R21	Golay_L22-Golay_R22	Golay_L22-Golay_R23	Golay_L22-Golay_R24
E	Golay_L23-Golay_R1	Golay_L23-Golay_R2	Golay_L23-Golay_R3	Golay_L23-Golay_R4	Golay_L23-Golay_R5	Golay_L23-Golay_R6	Golay_L23-Golay_R7	Golay_L23-Golay_R8	Golay_L23-Golay_R9	Golay_L23-Golay_R10	Golay_L23-Golay_R11	Golay_L23-Golay_R12
F	Golay_L23-Golay_R13	Golay_L23-Golay_R14	Golay_L23-Golay_R15	Golay_L23-Golay_R16	Golay_L23-Golay_R17	Golay_L23-Golay_R18	Golay_L23-Golay_R19	Golay_L23-Golay_R20	Golay_L23-Golay_R21	Golay_L23-Golay_R22	Golay_L23-Golay_R23	Golay_L23-Golay_R24
G	Golay_L24-Golay_R1	Golay_L24-Golay_R2	Golay_L24-Golay_R3	Golay_L24-Golay_R4	Golay_L24-Golay_R5	Golay_L24-Golay_R6	Golay_L24-Golay_R7	Golay_L24-Golay_R8	Golay_L24-Golay_R9	Golay_L24-Golay_R10	Golay_L24-Golay_R11	Golay_L24-Golay_R12
H	Golay_L24-Golay_R13	Golay_L24-Golay_R14	Golay_L24-Golay_R15	Golay_L24-Golay_R16	Golay_L24-Golay_R17	Golay_L24-Golay_R18	Golay_L24-Golay_R19	Golay_L24-Golay_R20	Golay_L24-Golay_R21	Golay_L24-Golay_R22	Golay_L24-Golay_R23	Golay_L24-Golay_R24

Curriculum Vitae

Michael E. Neufeld

Orthopaedic Fellow
Department of Surgery, Division of Orthopaedic Surgery
Western University
London, ON

EDUCATION

Accepted & pending	**The Hip Society / M.E Müller Foundation of North America European Fellowship** • Application accepted for 2020 (postponed due to COVID-19) • Hamburg, Germany • London UK
2019 - present	**Master's of Science in Surgery Candidate** • Western University, London, ON
2019 - present	**Adult Hip and Knee Reconstruction Surgery Arthroplasty Fellowship** • Joint Replacement Institute, London Health Sciences Centre, University Hospital, London, ON, Canada
2014 – 2019	**Orthopaedic Surgery Residency Program (FRCSC)** • University of British Columbia, Vancouver, BC
2010 - 2014	**Doctor of Medicine** • University of Toronto, Toronto, ON
2003 - 2008	**Bachelor of Science (Honours) in Kinesiology** • Brock University, St. Catharines, ON

QUALIFICATIONS, CERTIFICATIONS, AND MEMBERSHIP

2019 - present	**The Bone & Joint Journal Scientific Manuscript Reviewer**
2019 - present	**Ontario Medical Association (OMA)**
2019 - present	**College of Physicians & Surgeons of Ontario (CPSO) Independent Practice License/Certificate**

- License number: 114052

2019 - present **Fellow of the Royal College of Surgeons of Canada (FRCSC) Orthopaedic Surgery Examination**
- Ottawa, ON

2018 - present **World Association against Infection in Orthopaedics and Trauma (WAIOT)**

2017 **College of Physicians & Surgeons of Ontario (CPSO) Postgraduate Education Certificate for a Resident Elective**
- LHSC University Hospital, Division of Orthopaedic Surgery, London, ON
- License number: 114052

2016 **29th Annual Tom Smallman Canadian Orthopaedic Association Basic Science Course**
- Ottawa, ON

2016 **Royal College of Physicians and Surgeons of Canada Surgical Foundations Examination**
- University of British Columbia, Vancouver, BC

2015 **Licensure of the Medical Council of Canada (LMCC)**
- 2015 MCCQE II – Vancouver, BC
- 2014 MCCQE I - Toronto, ON

2015 **The 9th Annual Principles and Practice of Clinical Research (PPCR) Course**
- Burlington, ON

2015 **AO North America AOTrauma Course – Basic Principles of Fracture Management**
- Bellevue, WA, USA

2014 - present **Canadian Orthopaedic Association (COA)**

2014 – 2019 **College of Physicians & Surgeons of British Columbia (CPSBC) Resident Physician License**
License number: 38343

2014 - 2019 **Resident Member, American Academy of Orthopaedic Surgeons (AAOS)**

2014 - 2019 **Canadian Orthopaedic Residency Association (CORA)**

2014 - 2019	**Doctors of British Columbia (formerly BCMA)**
2014	**Advanced Trauma Life Support (ATLS) Certification** • Vancouver, BC
2014	**Advanced Cardiac Life Support (ACLS) Certification** • Toronto, ON
2010 - present	**Canadian Medical Association (CMA)**
2010 - 2014	**Ontario Medical Association (OMA)**

ACADEMIC AWARDS & ACCOMPLISHMENTS

2019 (Accepted)	**The Hip Society / M.E Müller Foundation of North America European Fellowship** • Competitive application process for North American trained Orthopaedic Surgeons; my application was successful, and I awarded the funded fellowship for 2020 • Funding: $40,000 (USD)
2018	**UBC Resident Research Day Competition – Second Prize** • Awarded to the resident with the second-best podium presentation at the annual research day, Department of Orthopaedics, University of British Columbia
2014 – 2018	**Orthopaedic in-Training Examination (OITE)** • PGY-5 100th percentile all takers (Canadian & US) • PGY-3 99th percentile • PGY-2 97th percentile • PGY-1 92^{cnd} percentile
2016	**"Blue Ribbon Article" – Selected by the Editors of *Orthopedics* as a significant contribution to the literature** • Neufeld ME, O'Hara NN, Zhan M, Zhai Y, Broekhuyse HM, Lefaivre KA, Abzug JM, Slobogean GP. Timing of Hip Fracture Surgery and 30-Day Outcomes. *Orthopedics*. 2016; 39(6):361-368.
2015	**UBC Resident Research Day Competition – Second Prize** • Awarded to the resident with the second-best podium presentation at the annual research day, Department of Orthopaedics, University of British Columbia
2014	**Dr. Kenzie Takahashi Scholarship in Medicine and Surgery**

- Graduation scholarship awarded to the student with highest academic standing in medicine and surgery during clerkship in the graduating class, Faculty of Medicine, University of Toronto

2014 **Irving Heward Cameron Undergraduate Scholarship in Surgery**

- Graduation scholarship awarded to the students who achieved the highest academic standing in Surgery during clerkship in the graduating class, Faculty of Medicine, University of Toronto

2013 **Dr. F.J. Colling O.B.E. Memorial Scholarship**

- In recognition of high academic standing in the third year of medical school, Faculty of Medicine, University of Toronto

2012 **CREMS (Comprehensive Research Experience for Medical Students) - Summer Program**

- CIHR Scholarship Recipient - University of Toronto

2012 **Dr. F.J. Colling O.B.E. Memorial Scholarship**

- In recognition of high academic standing in the second year of medical school, Faculty of Medicine, University of Toronto

2008 **CSEP (Canadian Society for Exercise Physiology) Undergraduate Award - Brock University**

- Presented by the CSEP each year to the student graduating in that academic year in physical education, human kinetics, or related disciplines with the highest standing in the scientific portion of the curriculum in their respective Canadian university.

2008 **First Class Honours – BSc (Honours) in Kinesiology**

- Brock University, St. Catharines, ON

2003 – 2008 **Dean's Honour List – BSc (Honours) in Kinesiology**

- Brock University, St. Catharines, ON

2003 – 2008 **Student Athlete Scholarship**

- For CIS (Canadian Interuniversity Sport) and academic achievement
- Brock University, St. Catharines, ON

2006 **R.M. Davis Surgite Award**

- Awarded to the male varsity athlete that has the highest academic grade point average and best combines athletics and academics
- Brock University, St. Catharines, ON

2003 **Brock Scholars Award**

- $14, 000 to the student that on admission to Brock University has an admission grade point average of 93.0% and above
- Brock University, St. Catharines, ON

2003 **Governor General's Academic Medal**

- Awarded to the student with the highest grade point average in their graduating secondary school class
- Governor Simcoe Secondary School, St. Catharines, ON

GRANTS

1. **Canadian Institutes of Health Research (CIHR): Canada Graduate Scholarships-Master's (CGS M) Frederick Banting and Charles Best Scholarship**

- Competitive annual national CIHR scholarship competition open to Canadian Master's applicants, I was awarded this based on the merit of my MSC in Surgery project
- $17,500 (CAD)
- 2019

2. **Canadian Institutes of Health Research (CIHR)**

- Awarded upon successful application to the University of Toronto's CREMS (Comprehensive Research Experience for Medical Students) Summer Program to support the study "The Impact of Socioeconomic Status on Implant Selection for Patients Undergoing Hip Arthroplasty"
- $2750 (CAD)
- 2012

SCIENTIFIC PRESENTATIONS

2019 **Can the Oxford Hip and Knee Score Identify Patients that Don't Require Total Knee or Hip Arthroplasty?**

- Podium Presentation
- Canadian Orthopaedic Association (COA) Annual Meeting, Montreal, ON

2018 **Can the Oxford Knee and Hip Scores Identify Patients that Don't Require Total Knee or Hip Arthroplasty?**
- Podium presentation (presented by coauthor Dr. BA Masri - closed meeting to Hip Society members)
- The Hip Society 2018 Summer Meeting, New York, NY,

2018 **The Longitudinal Short, Medium, and Long-Term Functional Recovery after Unstable Pelvic Ring Injuries**
- Podium Presentation
- Canadian Orthopaedic Association (COA) Annual Meeting, Victoria, BC

2018 **Can the Oxford Hip and Knee Score Identify Patients that Don't Require Total Knee or Hip Arthroplasty?**
- Podium Presentation
- UBC Department of Orthopaedics Annual Research Day, Orthopaedic Update and BCOA Annual Meeting, Vancouver, BC

2017 **The Longitudinal Short, Medium, and Long-Term Functional Recovery after Unstable Pelvic Ring Injuries**
- Podium Presentation
- Orthopaedic Trauma Association (OTA) Annual Meeting, Vancouver, BC

2017 **A Comparison of Mobile and Fixed-Bearing Unicompartmental Knee Arthroplasty at Minimum 10-Year Follow-up**
- Podium Presentation
- Canadian Orthopaedic Association (COA) Annual Meeting, Ottawa, ON

2017 **The Longitudinal Short, Medium, and Long-Term Functional Recovery after Unstable Pelvic Ring Injuries**
- Podium Presentation
- UBC Department of Orthopaedics Annual Research Day, Orthopaedic Update and BCOA Annual Meeting, Vancouver, BC

2016 **Does the Surgical Treatment of Hip Fractures within Two-Days of Injury Improve Patient Outcomes? An Analysis of 26,066 Cases from ACS-NSQIP**
- Podium Presentation (presented by coauthor Nathan O'Hara)

- Canadian Orthopaedic Association (COA) Annual Meeting, Quebec City, Quebec

2016 **Survivorship and Outcomes of Unicompartmental Knee Arthroplasty at Minimum 10-Year Follow-up**

- Podium Presentation
- UBC Department of Orthopaedics Annual Research Day, Orthopaedic Update and BCOA Annual Meeting, Vancouver, BC

2015 **US Hospitals Frequently Miss the British NICE Benchmark for Time to Hip Fracture Surgery: Does it Matter?**

- Podium Presentation
- Canadian Orthopaedic Residents Association (CORA) Annual Meeting, Vancouver, BC

2015 **US Hospitals Frequently Miss the British NICE Benchmark for Time to Hip Fracture Surgery: Does it Matter?**

- UBC Department of Orthopaedics Grand Rounds

2015 **US Hospitals Frequently Miss the British NICE Benchmark for Time to Hip Fracture Surgery: Does it Matter?**

- Podium Presentation PGY-1
- UBC Department of Orthopaedics Annual Research Day, Orthopaedic Update and BCOA Annual Meeting, Vancouver, BC

2014 **The Impact of Socioeconomic Status on Access to Care for Patients Undergoing Hip Arthroplasty**

- Poster Presentation
- American Academy of Orthopaedic Surgeons (AAOS) Annual Meeting, New Orleans, Louisiana, USA

2013 **The Impact of Socioeconomic Status on Access to Care for Patients Undergoing Hip Arthroplasty**

- Podium Presentation (presented by coauthor Dr. Michael Olsen)
- Canadian Orthopaedic Association (COA) Annual Meeting, Winnipeg, Manitoba

2013 **The Impact of Socioeconomic Status on Access to Care for Patients Undergoing Hip Arthroplasty**

- Poster Presentation (presented by coauthor Dr. Emil Schemitsch)
- Orthopaedic Research Society (ORS) Annual Meeting, San Antonio, Texas, USA

2013 **The Impact of Socioeconomic Status on Access to Care for Patients Undergoing Hip Arthroplasty**

- Poster Presentation
- University of Toronto Medical Student Research Day, Toronto, ON

2012 **The Impact of Socioeconomic Status on Access to Care for Patients Undergoing Hip Arthroplasty**

- Podium Presentation (presented by coauthor Dr. Emil Schemitsch – closed meeting to fellows and staff)
- Canadian Orthopaedic Arthroplasty Society (COAS) Inaugural Meeting, London, ON

PUBLICATIONS

1. (*Accepted for publication*) Garceau S, Igbokwe E, Warschawski Y, Neufeld ME, Wade JP, Guy P, Safir O, Wolfstadt JI. Management Options and Outcomes for Patients with Femur Fractures with Post-Polio Syndrome of the Lower Extremity: A Critical Analysis review. JBJS Reviews. *Accepted for publication 23 April 2020.*

2. Neufeld ME, Broekhuyse HM, O'Brien PJ, Guy P, Lefaivre KA. The Longitudinal Short, Medium, and Long-Term Functional Recovery After Unstable Pelvic Ring Injuries. J Orthop Trauma. 2019 Dec;33(12):608-613.

3. Neufeld ME & Masri BA. Can the Oxford Knee and Hip Score Identify Patients that Don't Require Total Knee or Hip Arthroplasty? Bone Joint J. 2019 June;101-B(6_Supple_B):23-30.

4. Neufeld ME, Albers A, Greidanus NV, Garbuz DS, Masri BA. A Comparison of Mobile and Fixed-Bearing Unicompartmental Knee Arthroplasty at Minimum 10-Year Follow-up. J Arthroplasty. 2018 Jun;33(6):1713-1718.

5. Neufeld ME, O'Hara NN, Zhan M, Zhai Y, Broekhuyse HM, Lefaivre KA, Abzug JM, Slobogean GP. Timing of Hip Fracture Surgery and 30-Day Outcomes. *Orthopedics*. 2016; 39(6):361-368.

6. Olsen M, Neufeld ME, Sellan M, Morison Z, Schemitsch EH. The Impact of Socioeconomic Status on Implant Selection for Patients Undergoing Hip Arthroplasty. *UTMJ*. 2015; 92(2):39-43.

BOOK CHAPTERS

1. Elserafi, J., Neufeld, M**.,** Ravichandiran, K, and Stockton, D. (Chapter Editors). Orthopedics. Toronto Notes 2014, 30th edition, Type and Graphics.