

Novel Approaches to Prosthetic Joint Infection

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Abstract

Prosthetic joint infection is a devastating complication affecting around 1% of joint replacements. Patients affected by this frequently require multiple surgeries to try to eradicate the infection in addition to prolonged courses of antibiotics.

The diagnosis of infection in itself can be challenging. This work has explored novel approaches to diagnosing joint replacement infection using the Fc receptor CD64 found on neutrophils in peripheral blood as well as new diagnostic markers in synovial fluid using Alpha Defensin.

The treatment of joint replacement infection is controversial and this thesis explores the results of patients treated with a less invasive approach known as Debridement Antibiotics and Implant Retention (DAIR).

In addition prevention strategies have also been explored with a particular focus on the use of laminar flow operating theatres and an exploration of their effectiveness in the presence of operating theatre lights.

Dedication

I dedicate this work to my family and friends who have helped and supported me over the past three and a half years. In particular I must say a special thanks to my dad for all his kind words of encouragement throughout but particularly for grounding me during manic moments when I was writing up and to Clare my beautiful wife to be for her patience especially with all the late finishes when things in the lab invariably took twice as long as anticipated (usually on Friday nights)!

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Declaration

This thesis is based on research performed in the Institute of Cellular Medicine, Newcastle University. I performed all experiments, measurements and data analysis of these with the exception of chapter 5 (The effect of operating theatre lights on laminar flow). The experiments in chapter 5 were performed with the assistance of both Paul Rushton and Paul McGovern. Data processing was performed with the assistance of Daniel Thompson and statistical analysis was kindly performed by Ignazio Serrano-Pedraza.

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Abbreviations

AAHKS	American Association of Hip and Knee Surgeons
ABC	Antibodies Bound per Cell
ADCC	Antibody-dependent cell mediated cytotoxicity
AUC	Area Under the curve
BES	Body Exhaust suit
BMI	Body Mass Index
BOA	British Orthopaedic Association
CD	Cluster of Differentiation
CDC	Centre for disease control
CFU	Colony Forming Units
CRP	C-reactive protein
CT	Computed Tomography
DAIR	Debridement Antibiotics and Implant Retention
ECL	Electrochemiluminescence
ELISA	enzyme-linked immunosorbent assay
ESR	Erythrocyte Sedimentation Rate
Fc	Fragment Crystallisable
GCSF	Granulocyte Colony Stimulating Factor
HEPA	High Efficiency Particulate air
HPA	Health Protection Agency
IC	Immune Complexes
Ig	Immunoglobulin
IL	Interleukin
MDT	Multidisciplinary Meeting
MRI	Magnetic Resonance Imaging
MSD	Mesoscale Discovery

MSIS	Muskuloskeletal Infection Society
NHS	National Health Service
NJR	National Joint Registry
OHS	Oxford Hip Score
OKS	Oxford Knee Score
PCR	Polymerase Chain Reaction
PE	Phycoerithrin
PerCP	Peridinin Chlorophyll Protein
PET	Positron Emission Tomography
PHE	Public Health England
PJI	Periprosthetic Joint Infection
PMN	Polymorphonuclear cells
PROMS	Patient Reported Outcome Measures
SEM	Standard Error of the Mean
SIGN	Scottish Intercollegiate Network
SSI	Surgical site infection
TNF	Tumour Necrosis Factor
WCC	White Cell Count

Chapter 1 Introduction

1.1 Background

Joint replacement surgery remains the only viable treatment option to restore functional loss and relieve the pain that results from osteoarthritis. As such there are over 170000 joint replacements performed each year in the UK¹. The most frequent procedures performed are hip and knee replacements which offer significant improvement in quality of life and are a cost effective treatment for the pain and disability caused by degenerative arthritis^{2,3,4}.

Although joint replacement surgery is an extremely successful procedure it is not without complications. One of the most devastating complications is infection. Estimates of infection rates range from 0.6%⁵ up to 3.3%⁶. Infection rates are however difficult to interpret in the absence of standardised diagnostic criteria. The Health Protection Agency compile data from the self-reporting of surgical site infection by NHS trusts in England and Wales. Their last report quoted infection rates of 0.7% for hip and 0.6% for knee replacements respectively⁵. The true figure may well be higher with other infection surveillance programs reporting figures of 3.0 % for hips and 3.3% for knees⁶. The reality is infection rates will vary from country to country and institution to institution as a result of different surgical practices as well as different bacterial flora. The British Orthopaedic Association (BOA) recommends surgeons inform patients of a 1-2% risk of infection for knee replacement and a 1-2.5% risk for hip replacements⁷.

The lifespan of a joint replacement is dependent on multiple factors including how active the patient is, which bearing surfaces are used and how well aligned the joint replacement is. When a joint replacement fails it has to be “revised”. The process of revision involves removing the existing prosthesis and replacing it with a new set of components. Over 16,000 revision joint replacements were carried out in the UK in 2013. Infection was the second commonest reason for revision accounting for 12% of hips and 22% of knees¹.

1.2 Pathogenesis of prosthetic joint infection:

When Charnley wrote about prosthetic joint infection in 1969 he stated there was “still uncertainty as to how often a wound is infected in the operating room and how often at a later date during the healing of the wound.”⁸ Wound contaminants may arise from the patient's own skin, from the surgical personnel or from the surgical instrumentation itself^{9,10}. It is likely that almost all surgical wounds are in fact contaminated to a degree because skin preparation techniques with either bactericidal or bacteriostatic agents will only decontaminate the skin surface and bacteria will remain in deeper layers of the skin^{7,11}. Whilst therefore many wounds will be contaminated at the time of surgery it is also recognised that infective organisms can seed to the implant via the patient's blood stream e.g. in the context of a systemic infection such as a urinary tract infection or chest infection where a bacteraemia develops - so called “metastatic infection”¹². The most common infecting organisms are Gram positive and *Staphylococcus aureus* (*S.aureus*) accounts for at least a third of PJIs in England and Wales^{5,13}.

However not all surgical wounds become clinically infected and this is a result of the complex interplay between the host's immune system, the surgical implant and the organism itself. In the presence of foreign material such as a prosthetic joint the number of colony forming units (cfu) of bacteria required to produce a clinical infection is significantly reduced¹⁴. In addition, the formation of biofilms on the implant by pathogenic organisms may add to the complexity of these interactions. As bacteria adhere to a foreign body the formation of a biofilm acts as a defence barrier shielding the organisms from both the hosts immune system and antibiotics^{15 16} thus an organism's ability to form a biofilm will also impact on the likelihood of developing an infection.

1.3 Risk factors and prevention strategies:

Information regarding risk factors can be compiled from a number of sources each with their own strengths and weaknesses. Many countries around the world have now set up joint registries. These large databases are used to record joint replacement surgery. Various models have been developed to collect and analyse data from such sources. In England and Wales joint replacements are recorded in the National joint registry (NJR). If patients subsequently develop an infection and require revision surgery this is captured by the NJR database and can provide useful information about risk factors. Information from joint registries tends to be disseminated through annual reporting¹ One issue with reporting through registries is that patients who do not have formal revision surgery but are treated for infection either by surgery or with antibiotics will not be captured in this way.

Data on infection rates are also generated through local and national surveillance programs^{6 5} institutional¹⁷ and insurance based databases^{18,19} as well as case series.^{20,21,22,23,24}

Prevention strategies can be broadly considered as follows:

1. Modification and optimisation of patient related risk factors
2. Antibiotic Prophylaxis
3. Management of the surgical environment
4. Wound management

An alternative would be to consider these in relation to the operation as pre, peri or post operative interventions.

1.3.1 Modification and optimisation of patient related risk factors:

A number of common comorbid conditions have been shown to have an association with PJI. Recognising and managing these in the peri-operative period is a key tactic in the prevention of PJI. Patients with rheumatoid and inflammatory arthritis are frequently treated with immunosuppressive medication placing them at higher risk of joint replacement infection particularly if such medications are not stopped pre-operatively^{20,21,22}. Although no formal national guidelines exist in the UK it is generally accepted that patients should cease the more recently developed immunosuppressive 'biologic' medications pre-operatively²⁵. Other groups at increased risk for PJI include those with diabetes in whom poor glycaemic control has been shown to be a key factor in conveying this increased risk^{23 24}. A large proportion of diabetic patients are obese with their obesity contributing to insulin resistance and further complicating this factor. Obesity remains a contentious issue however with evidence from a recent meta-analysis showing that obese patients are at increased risk for PJI²⁶. The American Association of Hip and Knee Surgeons (AAHKS) have suggested a cut off body mass index (BMI) of 40kg/m² (morbid obesity) above which joint replacement should be deferred²⁷. At present in the UK no such consensus exists and the decision whether or not to offer arthroplasty often remains at the discretion of the operating surgeon or based on local departmental policies.

Other risk factors including smoking, excess alcohol consumption and intravenous drug abuse have all shown an association with PJI^{5,28,29}. Individual units and indeed surgeons may have policies on how best to deal with these, however there is currently no national guidance on optimising these factors. Smoking cessation programs have been shown to help reduce the incidence of complications particularly those related to the vascular sequelae of smoking such as wound healing, infection and cardiac complications^{30,31}

Patients undergoing joint replacement surgery are routinely screened for Methicillin Resistant Staph Aureus (MRSA) yet perhaps as important as MRSA is Methicillin Sensitive Staphylococcus Aureus (MSSA). Whilst screening for MSSA is not currently mandatory, evidence from both orthopaedic surgery and other surgical specialties supports screening

and eradication therapy for MSSA both in terms of risk reduction and cost effectiveness.^{32,33}

1.3.2 Antibiotic Prophylaxis

The administration of pre-operative intravenous antibiotic prophylaxis is established practice in orthopaedics and this is supported by evidence from a meta-analysis of 3065 patients which showed a significant risk reduction in the incidence of PJI in patients who received antibiotic prophylaxis.³⁴ There remains however some controversy over the optimal timing of antibiotic delivery with some studies suggesting that administration close to the time of incision is best and others concluding that antibiotics should be delivered 1-2 hours prior to the skin incision. The latest Scottish Intercollegiate Guidelines Network (SIGN) guidelines on antibiotic prophylaxis recommend administration as close to the time of incision as possible³⁵. Perhaps just as important however as the timing of administration is the type of antibiotic used. The choice of antibiotic should be guided by local resistance patterns which will lead to variation in prophylaxis choice from region to region and country to country. A further consideration in cemented joint replacement surgery is whether to use antibiotic impregnated cement. Antibiotic loaded cement has been shown repeatedly to reduce the incidence of PJI^{36,37} yet whilst its use is widespread in the UK it's use in North America still remains limited³⁸. There are also concerns about antibiotic loading of cement in terms of driving antibiotic resistance³⁸.

1.3.3 The day of surgery

Care and attention must be given to every step of the patient's journey from their first clinic visit until discharge after their operation. Once a patient arrives in theatre the following are factors that can have a bearing on their risk of infection:

1. Hand scrubbing
2. Surgical gloves
3. Surgical Drapes
4. Surgical gowns
5. Patient skin preparation
6. Patient warming
7. Operating theatre air flow
8. Theatre access
9. Surgical instruments

1.3.3.1 Surgeon factors: Hand Scrubbing, gloves, gowns and drapes

Prior to commencing a surgical procedure, surgeons scrub their hands followed by donning on gloves which protects both the patient and the surgeon. Alcohol based preparations have shown the highest efficacy at reducing bacterial hand counts following application. In most orthopaedic operations surgeons wear two pairs of gloves – 'double gloving'³⁹. Double gloving commonly involves the use of an "indicator" system. This is a system of two different coloured gloves, for example a green underglove with an opaque lighter coloured overglove. This has the advantage of providing a visual early warning system to alert the surgeon to glove perforations and has been shown to allow faster identification of perforations than in non indicator systems⁴⁰. Frequent changes of the outer glove have also been shown to reduce the risk of perforation and thus contamination of the underglove.⁴¹

All members of the surgical team wear a sterile gown. These can be either single use disposable gowns or textile reusable gowns that are washed and sterilised between uses. Charnley introduced his body exhaust suit (BES) in 1973⁴² which was subsequently claimed by Lidwell to significantly reduce infection rates in hip replacement by up to 90%^{43,44}. This initial body suit was connected to both inlet and outlet tubes which created a negative pressure inside the suit thereby removing any shed skin from the wearer. Despite its success the system was cumbersome and its use has waned in favour of modern so called space suit designs. These incorporate a helmet with a built in fan above the surgeon's head. The fans draw cool air through the hood and it passes through the gown and is expelled at the bottom, close to the floor. This is in effect a positive pressure system. Recently there has been emerging evidence to suggest that wearing space suits during arthroplasty surgery may in fact lead to higher infection rates.⁴⁵

Similar to gowns, drapes are generally either single use plastic and paper drapes or textile drapes that can be sterilised and reused. In orthopaedics single use adhesive paper drapes are standard practice. Although there is evidence demonstrating that single use plastic adhesive drapes lead to lower levels of wound contamination compared to textile drapes⁴⁶ this concept has been challenged more recently with the Cochrane group suggesting that in fact there is no evidence supporting the notion that they reduce infection and in fact there is a suggestion that they may in fact increase rates of infection.

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1.3.3.2 Patient factors – skin preparation and patient warming

The aim of skin preparation is to reduce the number of microorganisms on the skin prior to making an incision, however this must be balanced against preserving the integrity of the skin so as not to impair wound healing. The available preparations of skin antiseptics are iodine based, alcohol based, chlorhexidine based or combinations of these. A 2003 meta-analysis from the Cochrane wounds group concluded that there was insufficient evidence to demonstrate a difference between the different type of skin preparation agents.⁴⁸ However following the findings of a large randomised controlled trial comparing povidone-iodine and chlorhexidine-alcohol which showed significantly fewer SSIs in the alcohol chlorhexidine group,⁴⁹ a subsequent Cochrane review on skin preparation agrees

that alcohol based products are likely to be the most effective agents to reduce the risk of SSI.⁵⁰

1.3.3.3 *The theatre environment*

The theatre environment should remain as clean as possible with consideration to anything that could increase the risk of patients developing SSIs. Particular consideration should be given to the hygiene of theatre floors which have been shown to account for up to 15% of airborne bacteria in an operating theatre⁵¹ and theatre shoes which have also been shown to carry pathogenic bacteria⁵². Based on this it is unsurprising that increased numbers of staff circulating through the operating theatre have been shown to increase the levels of airborne pathogens^{53,54} as has leaving theatre doors open⁵⁵. Great care must also be taken when handling surgical instruments. Minimising the time these are open, covering them when not in use and opening instruments within the laminar flow canopy are commonly performed steps that are perceived to lessen the chance of contamination.

Perhaps most controversial of all in terms of optimising the theatre environment is the use of laminar flow in operating theatres. Laminar flow was initially introduced by Charnley in the 1970s but became widespread following the work of Lidwell et al. who demonstrated reduced deep infections following the use of ultraclean air theatres in hip replacements^{44,56}. Air is initially filtered through a High Efficiency Particulate Air (HEPA) filter removing 99.97% of all particles larger than 0.3 micrometres in size⁵⁷. The system then streams this ultraclean air over the operating field. This has been shown to reduce the number of airborne particles, airborne bacteria and wound bacteria counts significantly^{44,58,59}. For these reasons the use of laminar flow theatres for lower limb arthroplasty is well established and widely used⁶⁰

Recently however several authors have challenged the use of laminar flow, demonstrating similar or increased infection and revision rates with laminar flow compared to conventional theatres^{45,61,62,63}. Thus whilst it currently remains the gold-standard in theatres undertaking joint replacement procedures, some feel its use should be abandoned.⁶⁰

In summary, there is evidence to support many areas of best practice. Also equally as important however is the prevalent culture in theatre. In order for best practices to be adhered to all members of the team must understand the importance of their individual roles and surgeons as managers should create an environment in which all members of the team feel they have a voice that will be listened to.

1.4 Classification and diagnosis:

1.4.1 General thoughts

The diagnosis of PJI remains a challenge. When trying to better understand PJI a major issue lies in the classification used to describe case series in the literature. Many series do not make explicit which system if indeed any is being used. This renders comparisons between series challenging. Diagnostic criteria often incorporate a classification systems as is the cases with the modified Centre for Disease Control (CDC) definition as used currently for reporting of PJI in the UK and the MSIS criteria.

1.4.2 Classification systems

Tsukayama et al^{64,65} described one of the few formal classification systems for PJI. They described four types of both hip and knee arthroplasty-associated infections according to the most common presenting patterns :

- Type I – occult with positive intra operative cultures at revision for presumed aseptic causes.
- Type II - Early postoperative infection occurring within 4 weeks of the index procedure.
- Type III – Acute haematogenous infection. A sudden presentation of infection in a previously well functioning implant often following an infection elsewhere in the body, for example following a urinary tract infection.

- Type IV – Late deep infection that presents later than 1 month following the index procedure.

Other authors have suggested that type II infections be extended from 4 to the 6 week⁶⁶ cut off used by the MSIS⁶⁷.

1.4.3 Diagnostic criteria

The diagnosis of PJI is based on a combination of clinical signs and symptoms, hospital laboratory blood tests and microbial culture from synovial fluid and or joint tissue specimens. Histological evaluation may also be used as a further adjunct to these.

It is widely accepted that a sinus communicating with a joint and / or the growth on microbial culture of an organism from tissue or fluid specimens taken from directly around the implant are diagnostic of infection^{68,69}. However the possibility of false positives due contamination either in the operating room at the time of sampling or in the microbiology laboratory itself during handling of the samples or indeed false negatives due to a low virulence organism mean that neither method is 100% sensitive or specific⁶⁹.

In England and Wales the reporting of PJI is co-ordinated by Public Health England (PHE). The reporting of surgical site infection (SSI) is based on the Centre for Disease Control (CDC) definition⁷⁰.

1.4.4 CDC Definition

The CDC definition distinguishes two types of surgical site infection relevant to orthopaedic surgery: superficial incisional and deep incisional. The criteria used to establish each diagnosis are listed in table 1. The defining criteria are based on clinical presentation and microbial cultures.

Superficial incisional	Deep incisional
Only skin and subcutaneous tissue involved and one of the following:	Appears to be related to the operation and infection involves deep soft tissue and at least one of the following
< 30 days from operation	Infection within 1 year in the presence of an implant
Purulent drainage	Purulent drainage
Positive tissue or fluid culture from the incision	A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localised pain or tenderness, unless incision is culture-negative.
1 or more clinical signs of infection (swelling, redness, heat)	An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
Superficial incision is deliberately opened by surgeon unless incision is culture-negative	Diagnosis of deep incisional SSI made by clinician
Diagnosis of superficial incisional made by clinician	

Figure 1-1 CDC definition of surgical site infection ⁷⁰

The classification is relatively simple and has the advantage of not requiring many specialist tests.

1.4.5 MSIS Criteria

At the annual meeting of The Musculoskeletal Infection Society (MSIS) in 2011 a “workgroup” was established with the aim of creating a “gold standard” definition that could be used by all the stakeholders in PJI ⁶⁷. The criteria that were proposed following this meeting and are as follows:

1.4.5.1 Major Criteria:

- 1 – There is a sinus tract communicating with the prosthesis
- 2 – A pathogen is isolated by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joint

1.4.5.2 Minor Criteria:

- 1 – Elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration
- 2 - Elevated synovial fluid leukocyte count
- 3 - Elevated synovial fluid neutrophil percentage (PMN%)
- 4 - Presence of purulence in the affected joint
- 5 - Isolation of a microorganism in one culture of periprosthetic tissue or fluid
- 6 - Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at x400 magnification.

The presence of a single major criteria or the presence of four out of six minor criteria is said to be diagnostic with the caveat that infection could still be present with fewer than four minor criteria.

This definition is certainly more specific to orthopaedics than the CDC definition as a means of identifying PJIs and has the potential to facilitate better comparisons by researchers in the field of PJI. The workgroup considered all relevant literature in this

area in formulating these criteria. The criteria as a whole however requires validation in larger series and was initially met with some scepticism particularly in the UK ⁷¹ where it was thought that the suggested thresholds for CRP (10mg/l) and ESR (30 mm/hr) were too low and whilst they might be sufficiently sensitive would compromise on the specificity of the criteria. The initial criteria also failed to address the distinction between acute and chronic infections.

Some of these concerns have since been addressed and following the international consensus meeting on PJI held in 2013 some changes were made to the definition. In particular, the consensus group have now defined specific cut-off values for the minor criteria based on whether the tests are performed within 6 weeks of the first procedure or after this period and specific cut offs have also been proposed (see table 2.) In addition the presence of purulence as a minor criteria has now been omitted because of the relative subjectivity of this sign ⁶⁸

	< 6 weeks	> 6 weeks
ESR	“Not useful at this timepoint”	30 mm/hr
CRP	100 mg/l	10 mg/l
Synovial fluid WCC	> 10,000 cells/ μ L	> 3,000 cells/ μ L
Synovial fluid % Neutrophils	> 90%.	> 80%.

Figure 1-2 revised thresholds for MSIS criteria ⁶⁸

Subsequent to these changes the definition is now starting to be used in orthopaedic research and several recent publications have used the definition as their diagnostic standard ^{72 73}. In addition, thresholds for ESR and CRP may still have poor discriminatory ability as both have been shown to remain elevated above the suggested levels as part of the normal response to surgery ⁷⁴ and may remain high well beyond 6 weeks after surgery ⁷⁵. Furthermore, at a cut-off of 100 mg/l, CRP will have a reduced sensitivity for

PJI. At the lower concentration suggested beyond 6 weeks, CRP is likely to be sensitive but will lack specificity. Another consideration is the huge variation in the quoted thresholds for ESR and CRP within the literature and the uncertainty around the significance of these levels in patients with inflammatory conditions like rheumatoid arthritis⁷⁶. Finally, to fully evaluate patients using this criteria necessitates the collection of several different sample types (blood, synovial fluid and tissue). There are also issues of reproducibility particularly with cell counting⁷⁷ which is prone to inter-observer error and is also challenging in the context of blood stained synovial fluid samples which occur frequently⁷¹. These limitations have now begun to be acknowledged in recent publications⁷⁸. Furthermore thresholds for cell counting vary widely in the literature, most likely due to the issues described above and it is also well-recognised that samples from joints with metal on metal articulations can skew the results of cell counting.

Whilst a universal definition is clearly favourable particularly to allow for accurate comparison of outcomes between series, the application of the MSIS criteria remains problematic particularly as the group have not published methodology on total white cell counting and performing differential counts. In addition these techniques are particularly difficult to reproduce particularly across different laboratories⁷⁷. Furthermore the presence of blood contamination in synovial fluid aspirates occurs frequently and has been shown to cause false positive results for cell counts and differentials in up to 25% of patients³⁶.

Ultimately treatment can only be accurately guided by microbiological diagnosis in order to target the relevant infecting organisms based on accurate antimicrobial sensitivity profiles. It is widely accepted as reflected in the MSIS criteria and popularized by the Oxford study group that 2 or more positive samples of the same organism from 5-6 samples taken intraoperatively is diagnostic of infection⁷⁹.

Whilst the MSIS definition provides us with more criteria on which to base our diagnosis it is yet to be validated and does not help guide treatment in the same way that microbiological diagnosis can.

As a result of the limitations of our current diagnostic criteria there is a keen interest in developing biomarkers to simplify the diagnosis of infection. This work has been particularly focused on synovial fluid biomarkers however there is also a need to develop improved markers for peripheral blood testing.

1.5 Biomarkers of prosthetic joint replacement infection:

1.5.1 Peripheral blood biomarkers current evidence:

A biological marker or biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”⁸⁰. In the case of PJI this can be applied to the pathogenic interaction between the infecting organism and the host immune system and theoretically any by-products of this interaction may serve as biomarkers. PJI biomarkers can be obtained from peripheral blood or from the synovial fluid in the joint itself. The measurement of peripheral blood CRP is established practice in the assessment of patients with suspected joint replacement infection and multiple studies have evaluated the performance of this marker in the diagnosis of PJI⁸¹. CRP has the advantage of being familiar to clinicians, widely available and with relatively standardised assays across centres. The main concern with its use is that CRP remains significantly elevated post-operatively for around 3 weeks⁷⁴ and in fact does not normalise for as long as three months⁷⁵ which is precisely the time period that many patients present with wound problems and signs that are suspicious of infection. In patients with inflammatory conditions such as rheumatoid arthritis it is particularly difficult to determine whether a persistently raised CRP is due to a PJI or a flare their disease process⁸².

Other markers that have been assessed in peripheral blood include procalcitonin, IL-6, Tumour Necrosis factor Alpha, soluble intercellular adhesion molecule 1 and IgG to lipoteichoic acid^{81,83,84,85,86}. A recent meta-analysis of biomarkers of PJI comparing IL-6, CRP ESR and WCC found that IL-6 performed best but the authors noted that only a small number of studies looking at IL-6 were available for analysis⁸⁶. It should also be noted this study only included patients having revision surgery and did not include acute presentations that are often treated by Debridement Antibiotics and Implant Retention (DAIR). There is in fact very little literature on the use of either synovial fluid and/or peripheral blood biomarkers in acute presentations of PJI. Two retrospective case series are however worth noting. Bedair et al⁶⁷ identified 19 early PJIs and found serum CRP to have an area under the curve (AUC) of 73% while Yi et al's⁸⁷ series of 36 joints

demonstrated CRP had an AUC of 93% when evaluating early infection (<6 weeks). Aside from these two retrospective series there is little in the way of literature evaluating novel diagnostic tools in early / acute infections. In addition few studies have evaluated blood and synovial fluid markers simultaneously. Randau et al compared serum and synovial fluid IL-6 demonstrating that IL-6 in synovial fluid performed better than serum IL-6 with an AUC of 0.72 in synovial fluid compared with 0.76 in the aspirate. In another study Golwitzer et al studied proinflammatory cytokines and antimicrobial peptides in both serum and synovial fluid in a series of 15 infections. Again they found that markers in synovial fluid showed higher area under the curve values compared to their levels in peripheral blood.

1.5.2 Synovial fluid biomarkers

In recent years there has been a growing interest in the evaluation of synovial fluid based biomarkers in joint replacements. Studies have tended to measure pro-inflammatory cytokines and antimicrobial peptides. In addition, synovial CRP has also been evaluated and was shown by Parvizi et al ⁷¹ in a cohort of 20 infections to have an AUC of 0.92. Several studies have looked at large panels of synovial fluid markers in head to head comparisons. Lonner et al looked at a panel of 24 synovial fluid proteins in 14 infections demonstrating that IL-1 and IL-6 had an AUC of 1 in this series. Subsequent studies have also shown that synovial IL-1 and IL-6 levels are consistently elevated in infected cases ⁶⁷. Perhaps the most exciting development however came with the first publication evaluating Alpha Defensin in synovial fluid from patients with PJI ⁷³. 29 infections were evaluated using a panel of 16 biomarkers. Alpha defensin showed an AUC of 1. In this series IL 1 alpha, IL 1 beta, IL-6, IL-8, IL-10 and IL-17 also showed areas under the curve greater than 0.9. Subsequent to this work tests for alpha defensin are now commercially available as both a lateral flow device that can be used in the operating theatre and an enzyme linked immunosorbent assay (ELISA) based test that is reported from a central reference laboratory. Another potential rapid diagnostic is the leucocyte esterase strip. Leukocyte esterase is an enzyme produced by white blood cells which has been routinely used as a diagnostic performed on urine samples for some time. The repurposing of urine

dipsticks for use on synovial fluid was popularised by Parvizi et al ⁶⁷ showing these could be used to diagnose PJI effectively by applying synovial fluid to the leucocyte esterase strip.

Since the launch of the alpha defensin test there has been a flurry of publications showing very promising results and most recently a meta-analysis showing a pooled area under the curve of 0.99 ⁸⁸.

1.5.3 Additional approaches and emerging technologies

Whilst the mainstay of diagnostics are centred around identifying living bacteria and the hosts response to these in synovial fluid or blood there are a number of alternative approaches. These include the use of imaging techniques such as MRI, CT, radioisotope bone scans and more advanced techniques such as PET CT. In addition, molecular tools such as PCR for amplification of bacterial nucleic acids as well as high throughput techniques such as metabolomics may well improve our understanding and ability to diagnose PJI.

1.5.4 CD64 a potential new target peripheral blood biomarker

Cluster of differentiation (CD)64 is a cell surface receptor found on many cell types. The measurement of CD64 on the surface of neutrophils has been used as an infection biomarker in multiple disease states ^{71,89} including a series of various musculoskeletal infections that included two prosthetic joints in which CD64 showed overall better discriminatory characteristics than CRP ⁹⁰.

CD64 is part of the Fragment crystallisable (Fc) receptor family. CD64 is also known as Fc Gamma receptor 1 (FcγRI) and is part of the immunoglobulin (Ig) supergene family. Fc receptors are proteins found on the surface of the majority of immune cells. They are classified according to their antibody binding properties and thus Fc Gamma receptors

(FcγR) bind IgG, Fc Alpha (FcαR) receptors bind IgA and Fc Epsilon receptors (FcεR) bind IgE. There are three FcγR subtypes and multiple isotypes (see figure 3). The activation of Fc receptors will lead to either cell activation or cell inhibition depending on whether the Fc receptor is linked to an immunoreceptor tyrosine-based activation motif (ITAM) or an immunoreceptor tyrosine-based inhibitory motif (ITIM)⁹¹. The main role of these FcγRs is in the regulation of both innate and adaptive immune responses⁹².

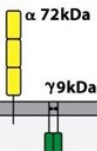
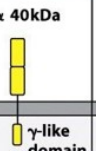
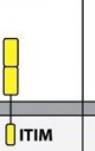
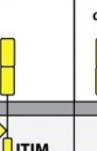
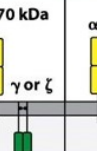
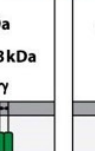
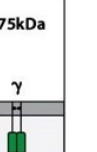
Ligand	IgG					IgE	IgA
Receptor name	FcγRI	FcγRIIA	FcγRIIB2	FcγRIIB1	FcγRIII	FcεRI	FcαRI
Receptor structure							
Activating	+	+			+	+	+
Inhibitory			+	+			
Macrophage	+	+	+		+		+
Neutrophil	(+)	+	+		+		+
Eosinophil	(+)	+	+		+	(+)	(+)
Mast cell				+		+	
Basophil						+	
B cell				+			
Dendritic cell	+						+
Langerhans' cell		+					
Platelet		+					
NK cells					+		
CD number	CD64	CD32			CD16	not assigned	CD89
Gene location	Chromosome 1						Chromosome 19

Figure 1-3 Fc Receptor family⁹³

CD64 is expressed on the cell surface of macrophages, neutrophils, eosinophils and dendritic cells (see figure 4). Crucially its expression is inducible on neutrophils and eosinophils. CD64 binds monomeric IgG1 with the highest affinity of any FcγR (see figure 4) and plays a key role in antibody-dependent cell mediated cytotoxicity (ADCC), clearance of immune complexes (IC) and modulates the release of cytokines including IL-1, IL-6 and TNFα. In the resting neutrophil CD64 is expressed at low levels however up-regulation is induced by interferon gamma⁹⁴ granulocyte colony stimulating factor (GCSF)

^{95 96} and lipopolysaccharide (LPS) ⁹⁷. Increases are reported to occur rapidly within 4 – 6 hours. These properties have led to the investigation of neutrophil surface expression of CD64 as a potential biomarker of infectious disease in various patient groups and infectious disease processes.

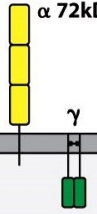
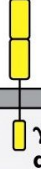
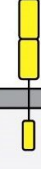
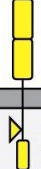


Receptor	FcγRI (CD64)	FcγRIIA (CD32)	FcγRIIB2 (CD32)	FcγRIIB1 (CD32)	FcγRIIIa (CD16)	FcγRIIIb (CD16)
Structure	 α 72kDa γ	 α 40kDa γ-like domain	 ITIM	 ITIM	 α 70kDa γ or ζ	 α 50kDa γ or ζ
IgG subclass specificity	3>1>4>>>2	R131:3>1>>>2,4 H131:3>1,2>>>4	3>1>4>>2	3>1>4>>2	1,3>>>2,4	1,3>>>2,4
Relative binding strength to IgG1	200	4	4	4	1	1
Effect of ligation	Activation	Activation	Inhibition	Inhibition	Activation	Activation

Figure 1-4 Fc gamma receptor properties ⁹³

1.5.5 Potential advantage of CD64 over CRP

The expression of CD64 does not appear to be induced by inflammatory processes such as active rheumatoid arthritis ⁹⁸ unlike other biomarkers including CRP. In fact CRP which is known to be raised in a wide range of inflammatory states including rheumatoid arthritis is frequently used as a marker of disease activity ⁹⁹.

Consequently interpreting CRP levels in patients with rheumatoid arthritis presenting with possible infection can be difficult and may lead to false positive diagnoses. Tohma and colleagues ¹⁰⁰ compared the performance of CRP and CD64 as infection markers in a series of patients with rheumatoid arthritis. They found that at a cut-off of 2000 molecules/cell CD64 had a sensitivity of 92.7% and specificity of 96.5% for diagnosing infection in patients with rheumatoid arthritis. This initial study included patients with a variety of infective presentations (chest infection, urine infection). Subsequent to this the group have published work comparing CD64 with CRP specifically in the diagnosis of local

musculoskeletal infection⁹⁰. 141 patients with a range of musculoskeletal infective diagnoses (including two PJIs) were assessed for infection and the levels of CRP and CD64 correlated with bacterial culture results. At the previously described cut-off of 2000 molecules/cell CD64 had a sensitivity of 60.9% and specificity of 97.9%. The overall area under the curve (AUC) for CD64 was 0.791 which compared favourably with CRP (CRP AUC 0.641).

Inflammatory markers such as CRP have been shown to rise significantly in response to joint replacement surgery^{74,101}. Whilst CD64 has also been shown to rise in response to joint replacement surgery the changes are reportedly short lived with a peak level on the third post-operative day and no statistical difference between levels measured at baseline and days 5, 7 and 14¹⁰².

The clinical presentation of PJI makes the diagnosis a challenge. In acute cases, the clinical signs of redness, swelling, pain and even wound discharge may all represent a normal post-operative response. To compound this the biomarkers that are currently used are all raised as part of the systemic response to surgery and therefore difficult to interpret. A clinician must decide whether to investigate the patient further (open exploration or aspiration), observe the patient or prescribe antibiotics. It is also well known that many patients will present to primary care initially and receive antibiotics without the surgical team being notified. Apart from causing issues with future management if symptoms persist, the prescription of these antibiotics may be unnecessary and contributes to anti-microbial resistance. Therefore, biomarkers that can reliably exclude infection in this situation would be welcome.

In a meta-analysis of 13 studies which included a wide range of sources of infection, Cid et al⁸⁹ found a pooled sensitivity of 79%, pooled specificity of 91%, and summary receiver operating characteristic character curve (SROC) area under the curve of 0.94 for diagnosing infection. Similar results were replicated in a subsequent meta-analysis from 2013 which included 26 studies and found a pooled sensitivity of 76%, pooled specificity of 85%, and SROC area under the curve of 0.92⁷¹.

1.5.6 Panel of biomarkers selected for evaluation

Based on the most promising biomarkers described in the literature we selected a panel made of the following biomarkers: CRP, IL-6, IL-8, IL-10, IL-17, IL-1, IL-1beta and alpha defensin.

These are all largely secreted as part of the innate immune response. The innate immune response is a complex interaction between host defences (physical and cellular) and infective agents. It is said to be the first line of defence against invading pathogens and within this macrophages play a key role¹⁰³. Macrophages are activated by Pathogen Associated Molecular Patterns (PAMPS). PAMPS are a broad group of molecular motifs that are associated with particular groups of microbes e.g.

bacterial Lipopolysaccharides (LPS) are PAMPs found on the cell membranes of gram negative bacteria¹⁰⁴ which activate macrophages via TLR-4 receptors. Activated macrophages subsequently secrete a number of pro-inflammatory cytokines including tumor necrosis factor (TNF), IL-1, IL-6, IL-8, and IL-12 as well as anti-inflammatory cytokines including IL-10 and TGF-Beta¹⁰⁵. IL-1, IL-6, IL-8 and IL-10 have all been reported as joint replacement infection markers¹⁰⁶. In response to the secretion of these cytokines, particularly IL-6, the liver produces a range of products termed acute phase proteins¹⁰⁷. These include perhaps most notably C- reactive protein (CRP). CRP is known to facilitate phagocytosis acting as an opsonin and binding to dead or dying bacteria. The use of CRP in clinical practice as a marker of infection is well established across a wide range of different infections including PJI⁸⁴. IL-17 is another pro-inflammatory mediator best known for its role in chronic inflammation where it is secreted by CD4+ T cells but has also been shown to be secreted as part of the innate immune response particularly by $\gamma\delta$ T cells, a small subset of T cells said to represent around 1 - 5% of total lymphocytes¹⁰⁸. IL-17 has also shown promising results as a potential biomarker of joint replacement infection¹⁰⁹. In addition Alpha defensin is an anti-microbial peptide which is particularly active against Gram-negative and Gram-positive bacteria. Alpha defensin is said to be secreted by activated neutrophils and through a range of mechanisms disrupt the cell membrane of bacteria facilitating their lysis¹¹⁰.

Treatment of PJI

There are several potential treatment strategies for prosthetic joint infection (PJI). The chosen strategy will depend on patient factors, the infecting organism and the clinical presentation. More and more centres are adopting a multi disciplinary approach to these complex cases so as to improve the decision making process. Broadly speaking the options are to suppress the infection with antimicrobial therapy which has a high chance of failure over time or attempt to surgically eradicate the infection in combination with antimicrobial therapy. The choice of treatment will vary greatly depending on both patient and pathogen factors. For example, in the context of a frail elderly patient with multiple co-morbidities major surgery could potentially be life threatening and the most appropriate management may be suppressive antibiotics with no surgery. Another option in patients with low functional demands and multiple co-morbidities would be to remove the prosthesis entirely – so called excision arthroplasty. This has the advantage of being a single procedure which is relatively quick and leaves the joint free from foreign material. Alternatively, in fitter individuals revision surgery may be considered. This involves removing the infected joint replacement along with the surrounding infected bone and soft tissue and implanting a new prosthesis. This can be done in a single sitting, so called single stage revision or in two sittings – two stage revision. For two stage revision the initial procedure involves removal of the implant and placement of a spacer to minimise soft tissue contracture. This is often made from antibiotic loaded cement and is left in place to help treat the infection during the interval leading up the second stage. Antibiotics are given systemically intravenously and/or orally until the clinical team is confident the infection has been eradicated. Depending on the type of spacer used some movement of the joint and weight bearing through it may be possible. Following this interval the second stage is carried out. This involves the removal of the spacer and the implantation of a new joint. Two stage revision is often referred to as the gold standard for infection eradication but there is controversy over whether this is truly superior to the single stage approach ¹¹¹ and a large multicentre trial is currently underway comparing these approaches in the hope of clarifying this perennial question ¹¹². An alternative surgical approach is known as Debridement, Antibiotics and Implant Retention (DAIR)¹¹³. This technique involves surgical removal of any infected material from around the

prosthetic joint (debridement), the joint is then thoroughly irrigated to further disinfect and clean the area. Subsequently the patient is treated with antibiotics to further lower any bacterial burden. This is clearly an attractive approach and if successful means that patients can keep their original joint replacement. Success rates with this approach vary widely in the literature however. This is in part due to the different definitions of failure and success with many studies considering a repeat DAIR procedure as a failure. In addition there is little in the way of literature exploring the functional outcomes from patients treated by DAIR.^{114 115} Furthermore there is little in the way of literature exploring the functional outcomes from patients treated by DAIR and certainly no high level evidence comparing the alternative treatment strategies.

1.6 Impact of PJI

There is clear evidence that joint replacement infection carries a huge burden not only for individual patients but also the wider healthcare system.

Patients with joint replacement infection have now been shown to be at increased risk of death in the year following their operation¹¹⁶ and it is unsurprising that recent research has shown PJI to have a negative impact on patient's quality of life^{117,118}. At the healthcare system level treating PJI carries a great financial burden and in fact under the current NHS remuneration system NHS trusts may in fact be treating patients at a loss¹¹⁹ compared to revision for other indications.

1.7 Northumbria healthcare and PJI

The culture around joint replacement infection prevention at Northumbria Healthcare is noticeably different to other trusts in the NHS. This stems from a difficult period in which the Orthopaedic department there had become outlier due to its high infection rates in joint replacements and had been the subject of HPA warnings. These became the catalyst for a sustained program of improvements that led to a significant reduction in infection rates from as high as 5% percent to levels consistently below 1%.

Widespread changes were introduced across pre, peri and post-operative practice. The aim of the improvement program was to use the best evidence possible to drive a shift in culture which would in turn lead to improvements in practice. Changes to antibiotic prophylaxis, skin preparation, wound dressings as well theatre shoes and dress code were implemented. Maintenance schedules for laminar flow and theatres were increased. A program of research was also used to drive the changes. This included two large randomised controlled trials studying antibiotic impregnated cement and antibiotic loaded sutures as well as research into alternative thromboprophylaxis regimes. Perhaps the biggest change came from work exploring the effect of warming patients using forced air devices and the interactions of these devices with laminar flow. This led to a wholesale change from forced air warming which had become the norm in UK operating theatres to conductive warming devices.

1.8 Summary

Joint replacement infection is a devastating yet thankfully relatively rare complication. The unfortunate patients who develop a PJI face great challenges in overcoming the physical and psychological burden this poses on them. Patients place great trust in the clinicians who strive to prevent this dreaded complication. Yet for clinicians there is uncertainty about the best approaches to prevention, diagnosis and treatment of infection in the face of a heterogeneous and often vague evidence base. There is a clear need for ongoing research to evaluate and further validate new and existing approaches to the overall management of PJI right from the start of the patient journey. Ideally a more collaborative approach should be fostered. This would facilitate a better understanding through higher powered studies at each stage of this problem.

1.9 Aims of PhD

1. To evaluate the effectiveness of treating patients by DAIR specifically with regards to infection eradication rates and functional outcomes.
2. To evaluate the impact of the positioning of operating theatre lights in laminar flow operating theatres.
3. To evaluate the use of CD64 as a peripheral blood joint replacement infection marker and comparing its performance to the currently recommended test CRP as well as IL-6, IL-8, IL-10, IL-17, IL-1alpha and IL-1beta
4. To evaluate the use of Alpha defensin as a synovial fluid joint replacement infection marker and compare its performance to CRP, IL-6, IL-8, IL-10, IL-17, IL-1alpha and IL-1beta

Chapter 2 Materials and methods

2.1 Clinical data collection for evaluating current practice

Caldicott approval was obtained to review the data of patients treated for PJI at Northumbria Healthcare. Data obtained from the coding department, paper and electronic theatre log books as well as data collected by the infection surveillance team were used to identify patients who had been treated for suspected infection. The Northumbria infection surveillance program follows all primary arthroplasty patients from the day of surgery for 1 year post op. They make calls direct to the patient at day 3 and day 30 after surgery as well as providing an orthopaedic helpline service which patients can call for advice and early review in case of concern. A retrospective review of both electronic and paper case notes was then conducted in order to create a definitive list of patients and collect relevant data e.g. causative organism, time since replacement antibiotic regimen and treatment. Patients were also invited to complete a patient reported outcome score (oxford hip score(OHS) or Oxford knee score (OKS). The OHS and OKS are the outcome scores currently collected as part of routine follow up of joint replacement patients in England and Wales and for the first 6 months after surgery.

2.2 Patients and samples for Biomarker study

Ethical approval was obtained for a prospective cohort study to investigate the potential of CD64 as marker of PJI (see Appendix A). A grant from Orthopaedics Research UK (ORUK) was awarded through open competition for this prospective cohort study which also adopted by the NIHR portfolio. Following local Research and Development approval recruitment commenced on the 7th January 2014 with the study closing for recruitment on the 30th June 2016. Patients were screened using theatre planning diaries and sent patient information sheets (PIS) by post. Patients were then approached on the day of intervention and invited to give informed consent as per Good Clinical Practice (GCP) guidelines. Up to 4ml of whole blood was collected into purple-top EDTA tubes by the research nurses before patients went to theatre followed by the day 1 and 7 post-operative samples as appropriate. For the day 7 samples, most patients had been discharged and therefore funding was secured to pay for research nurse time and travel

to visit the patients at home for the sample. Synovial fluid samples were collected by surgical practitioners in theatre during 'aspiration lists' run by a single orthopaedic practitioner¹²⁰. All samples were then securely transported to the Royal Victoria Infirmary, Newcastle upon Tyne specimen reception for collection and processing. Clinical data were collected by research nurses and all results were collected by the lead researcher. All data were stored in a secure database for subsequent analysis.

2.3 Sample Processing and storage at Newcastle University

Collected samples were stained with CD64/CD45 quantibrite antibody as per protocol (see below) excess blood was then centrifuged at 1000g for 10 mins and the resultant plasma aliquots frozen at -80°C for subsequent analysis. Synovial fluid samples were collected and centrifuged at 1000g for 10 mins prior to aliquoting and storage at -80°C.

2.4 Flow Cytometry

2.4.1 Principles of flow cytometry

Flow cytometry is a technique that can measure the fluorescence and physical properties (size and internal complexity) of single cells or other particles. The main components of a flow cytometer are the fluidics, optics and the electronics (see figure 2-1 below). Typically stained cell suspensions are passed along with sheath fluid through the beam of the cytometer's lasers. Fluorescence as well as the properties of forward and side scatter are then detected by the cytometer allowing cells to be identified. In whole blood the properties of forward and side scatter alone allow for the identification of major leukocyte subsets (see figure 2-2 below). Fluorescent dyes (fluorophores) bound to antibodies can in turn be bound to particular cell surface proteins to allow the expression of these proteins to be measured. As the suspension of stained cells is passed through the cytometer's laser the fluorophores are excited and emit fluorescence at a given wavelength. The intensity of this fluorescence is then measured by the cytometer which in turn gives an estimate of the expression of the protein of interest.

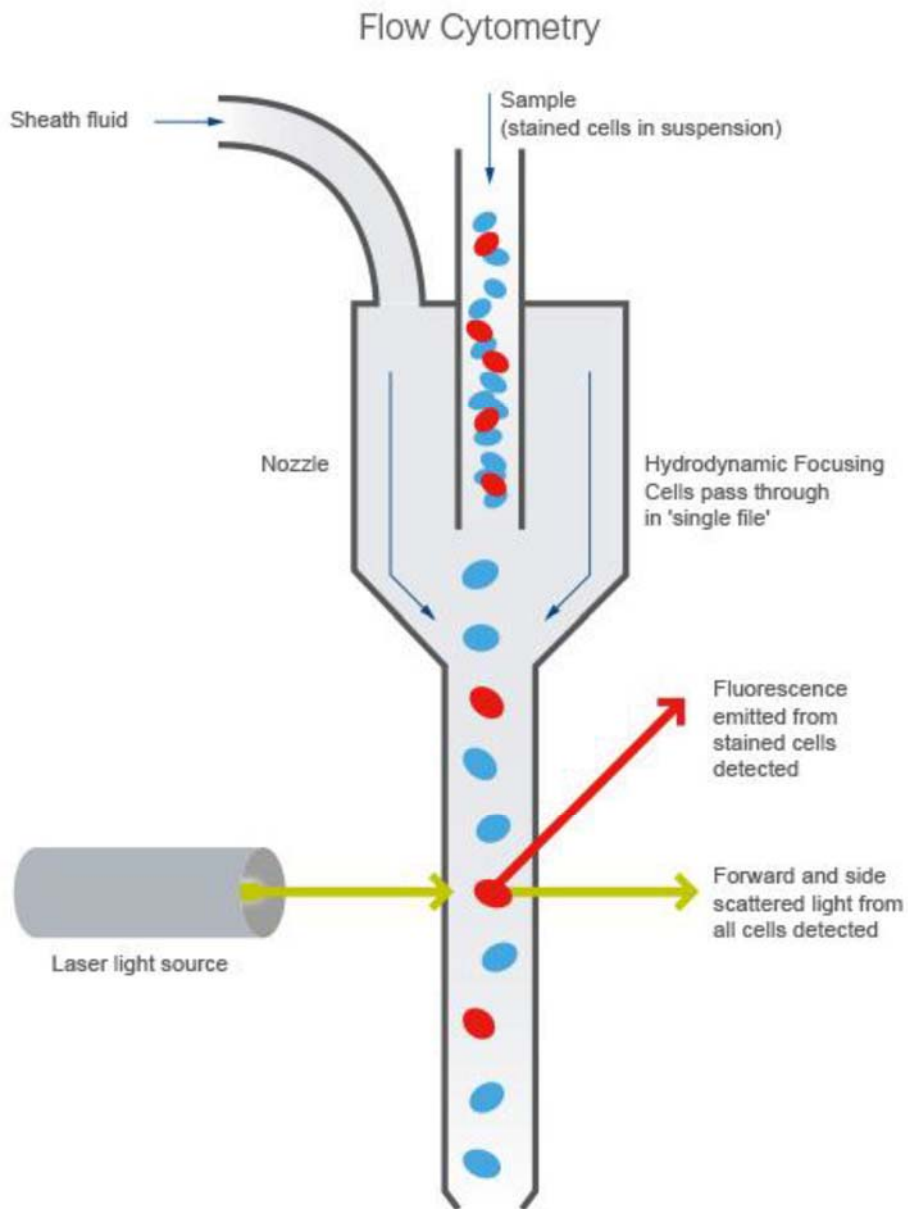


Figure 2-1. Overview of the structure of a flow cytometer.

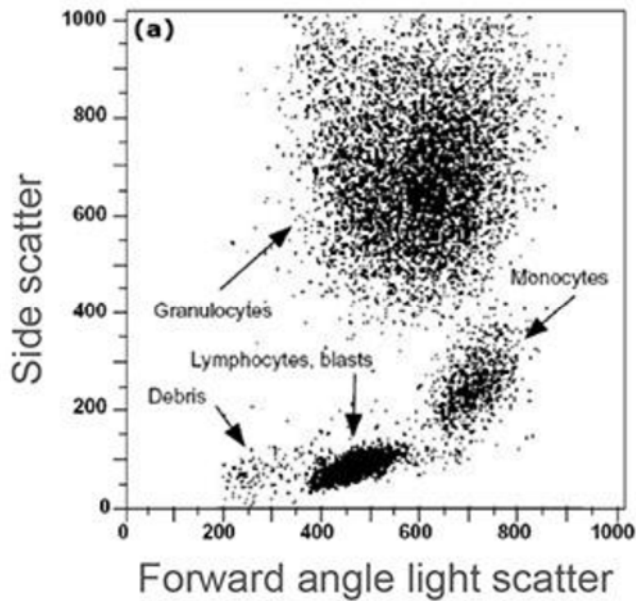


Figure 2-2 Dot plot of forward scatter vs side scatter showing the major leucocyte subsets.

2.4.2 The Quantibrite system

The BD biosciences Quantibrite system was used to quantitatively measure Neutrophil CD 64 expression in whole blood from study participants. The Quantibrite system is a reproducible and validated method¹²¹ that allows for the number of antibodies bound per cell (ABC) to be calculated for a given surface antigen - in this case neutrophil CD64. The system requires the use of Quantibrite phycoerythrin (PE) beads and Quantibrite PE antibodies (BD biosciences). Quantibrite beads are a suspension of beads that are conjugated to PE at 4 known concentrations (Low, medium low, medium high and high). Quantibrite CD64PE/CD45PerCP antibodies are bound in a known ratio of 1 fluorophore to each antibody. When acquiring the beads the mean fluorescence intensity for the beads and the neutrophil population is recorded. A standard curve can then be created and resolved to give an accurate measure of the number of CD64 molecules per neutrophil.

2.4.3 Flow cytometry reagents

1. Quantibrite PE beads
2. Quantibrite CD64PE/CD45PerCP
3. 10xFACS Lysing solution
4. Phosphate buffered saline (PBS) with azide
5. Distilled water

2.4.4 Flow cytometry protocol

1. 500 μ l of whole blood was added to 20 μ l of CD64PE/CD45PerCP Quantibrite antibody were mixed in a polypropylene tube
2. The mixture was gently vortexed before incubation for 60 minutes in the dark
3. 2ml of 1x FACS lysing solution was then added
4. After 60 minutes samples were acquired on a FACS CANTO flow cytometer (BD biosciences)
5. Shortly prior to sample acquisition Quantibrite PE beads were reconstituted by adding 0.5 mls of PBS with Azide and vortexed.
6. Quantibrite beads were acquired with a gate created around bead singlets. 10000 events were recorded.
7. Prepared patient samples were then acquired with 10000 events recorded for the Granulocyte population identified by CD45 and Side scatter.
8. Flow cytometry analysis

All analyses were performed using FACS DIVA software (BD biosciences). In brief a histogram plot is generated for the bead singlets and values of PE fluorescence intensity gathered for each peak representing the beads at each concentration i.e. Low, Medium Low, Medium High and High, see figure 1. For patient samples the granulocyte population was identified by its CD45 and Side scatter profile, see figure 2. Fluorescence intensity of PE of this population was subsequently recorded.

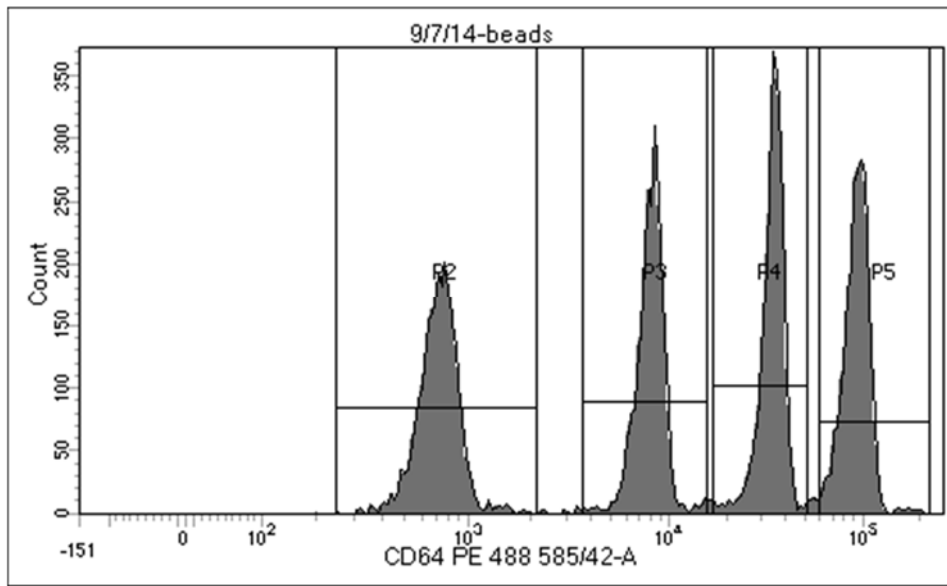


Figure 2-3 Histogram plot of Quantibrite PE beads. The four peaks P2, P3, P4 and P5 represent the four discrete bead populations - low, Medium Low, Medium High and High.

Values of CD64 molecules per cell were then calculated for each sample using Microsoft Excel 2013. The Logs of the 4 known concentrations of Quantibrite PE beads (provided by the manufacturer - Log PE molecules per bead) and the logs of the recorded geometric mean values obtained for each bead concentration (Log PE fluorescence) were calculated. Using the equation ($y=mx + c$) a linear regression of Log PE molecules per bead against Log PE fluorescence is then plotted where y equals Log PE fluorescence and x equals Log PE molecules per bead. The equation is then resolved for x which is then converted to its antilog which corresponds to the number of antibodies bound per cell.

2.5 Mesoscale discovery assays

2.5.1 Principles of Mesoscale discovery assays

Mesoscale discovery (MSD) assays are Electrochemiluminescence (ECL) based assays which allow the simultaneous quantitation of multiple analytes within a single 96 well plate well. They offer high precision and sensitivity across a wide range of analytes. The bottom of each well of an MSD multi spot plate is divided into distinct independent electrically conductive regions or “spots”. Combinations of up to 10 analytes from pre defined groups are available in so called “V-PLex” assays. Individual spots within the MSD plate are coated with capture antibodies specific to the analyte of interest.

2.5.2 Overview of MSD protocol

Suitably diluted samples followed by detection antibodies conjugated with electrochemiluminescent (MSD SULFO-TAG) labels are added to the MSD plate over the course of two separate incubations. This allows analytes of interest within the sample to bind to the capture antibodies on the relevant spot. The recruitment of capture antibodies by the bound analytes then completes a sandwich. Finally a read buffer specifically optimised to facilitate ECL is added prior to loading the plate into the MSD instrument. A voltage is then applied to the plate electrodes which causes the capture antibody to emit light. The intensity of this light signal is then measured providing a quantitative measure of the intensity of the analytes in the sample.

2.5.3 MSD Reagents

1. V-PLex Vascular injury panel 2 (human) – CRP
2. V plex Proinflammatory Panel 1 (human) – IL-1 β , IL-6, IL-8, IL-10
3. V plex Cytokine Panel 1 (human) – IL-1 α , IL-17A
4. Vascular injury panel 2 (human) calibrator blend (20X)
5. SULFO-TAG Anti-hu CRP Antibody
6. Proinflammatory Panel 1 (human) calibrator blend (Lyophilized)
7. SULFO-TAG Anti-hu IL-1 β Antibody
8. SULFO-TAG Anti-hu IL-6 Antibody
9. SULFO-TAG Anti-hu IL-8 Antibody
10. SULFO-TAG Anti-hu IL-10 Antibody
11. Cytokine Panel 1 (human) calibrator blend (Lyophilized)
12. SULFO-TAG Anti-hu IL-1 α Antibody
13. SULFO-TAG Anti-hu IL-17A Antibody
14. Diluent 43
15. Diluent 2
16. Diluent 3
17. Diluent 101
18. Read Buffer T (4x)
19. Wash Buffer (20x)

2.5.4 V-PLex Vascular injury panel 2 (human) – CRP Protocol

1. Sample preparation: Patient samples were centrifuged at 1000g before dilution. A 100 fold dilution was performed in two steps. 10 μ l of sample was added to 190 μ l of diluent 101 (20 fold dilution) before 10 μ l of the 20 fold dilution was added to 490 μ l of diluent 101 (50 fold dilution).
2. Detection antibody preparation: 60 μ l of 50x SULFO-TAG Anti-hu CRP Antibody was added to 2940 μ l of diluent 101.
3. Calibrator preparation and dilution: A serial dilution was performed. 10 μ l of the 20x stock calibrator was added to 190 μ l of Diluent 101. This mixture was labelled calibrator 1 and vortexed. In turn 40 μ l of calibrator 1 was transferred to 160 μ l of Diluent 101 creating calibrator 2. This was repeated a further 6 times creating calibrators 3 – 7 in turn. Calibrator 8 was made up of diluent 101 without addition of calibrator mix.
4. Wash buffer preparation: 15 ml of 20x wash buffer was added to 285ml of deionized water.
5. Read buffer preparation: 5 ml of 4x read buffer was added to 15 ml deionized water.
6. Plate preparation: the plate was washed and dried 3 times with 150 μ l per well of wash buffer prior to addition of samples. 25 μ l of each of calibrators 1 – 8 were added in duplicate to the first two vertical rows of the plate. 25 μ l of diluted sample was then added to each of the remaining 80 wells.
7. The plate was sealed with an adhesive plate sealer and incubated with shaking at 300 revolutions per minute (rpm) for 2 hours at room temperature.
8. Plate wash: the contents of the plate were discarded after incubation. Each well was filled with 150 μ l of wash buffer which was then discarded. This sequence was repeated 3 times and following the last wash the plate wash inverted and blotted with paper towels.
9. Addition of detection antibody: 25 μ l of detection antibody was then added to each well. The plate was sealed using an adhesive plate sealer and incubated for 1 hour at room temperature with shaking at 300 rpm.

10. Plate wash: the contents of the plate were discarded after incubation. Each well was filled with 150 μ l of wash buffer which was then discarded. This sequence was repeated 3 times. Following the last wash the plate was inverted and blotted with paper towels
11. Plate reading: 150 μ l of read buffer is added to each well before reading the plate on the MSD instrument.

2.5.5 V plex Proinflammatory Panel 1 (human) – IL-1 β , IL-6, IL-8, IL-10 Protocol

1. Sample preparation: Patient samples were centrifuged at 1000g before dilution. A 1 in 2 dilution was performed by adding 60 μ l of sample to 60 μ l of Diluent 2.
2. Detection antibody preparation: 60 μ l of each of SULFO-TAG Anti-hu IL-1 β Antibody, SULFO-TAG Anti-hu IL-6 Antibody, SULFO-TAG Anti-hu IL-8 Antibody, SULFO-TAG Anti-hu IL-10 Antibody were added to 2760 μ l of Diluent 3.
3. Calibrator preparation and dilution: The lyophilised calibrator blend (calibrator 1) was reconstituted with 1000 μ l of Diluent 2, vortexed and allowed to stand for 5 minutes. 100 μ l of the reconstituted calibrator 1 was added to 300 μ l of Diluent 2 making Calibrator 2. The process was repeated a further 5 times to create Calibrators 3 to 7. Calibrator 8 was made up of Diluent 2 without the addition of calibrator blend.
4. Wash buffer preparation: 15 ml of 20x wash buffer was added to 285ml of deionized water.
5. Read buffer preparation: 5 ml of 4x read buffer was added to 15 ml deionized water.
6. Plate preparation: 50 μ l of each of calibrators 1 – 8 were added in duplicate to the first two Vertical rows of the plate. 50 μ l of diluted sample was then added to each of the remaining 80 wells.
7. The plate was sealed with an adhesive plate sealer and incubated with shaking at 300 revolutions per minute (rpm) for 2 hours at room temperature.
8. Plate wash: the contents of the plate were discarded after incubation. Each well was filled with 150 μ l of wash buffer which was then discarded. This sequence was repeated 3 times. Following the last wash the plate wash inverted and blotted with paper towels.
9. Addition of detection antibody: 25 μ l of detection antibody was then added to each well. The plate was sealed using an adhesive plate sealer and incubated for 2 hours at room temperature with shaking at 300 rpm.
10. Plate wash: the contents of the plate were discarded after incubation. Each well was filled with 150 μ l of wash buffer which was then discarded. This

sequence was repeated 3 times. Following the last wash the plate wash inverted and blotted with paper towels

11. Plate reading: 150 μ l of read buffer was added to each well before reading the plate on the MSD instrument.

2.5.6 V plex Cytokine Panel 1 (human) – IL-1 α , IL-17A Protocol

1. Sample preparation: Patient samples were centrifuged at 1000g before dilution. A 1 in 2 dilution was performed by adding 60 μ l of sample to 60 μ l of Diluent 43.
2. Detection antibody preparation: 60 μ l of SULFO-TAG Anti-hu IL-1 α and 60 μ l of SULFO-TAG Anti hu IL-17A were added to 2880 μ l of Diluent 3.
3. Calibrator preparation and dilution: The lyophilised calibrator blend (calibrator 1) was reconstituted with 1000 μ l of Diluent 43, vortexed and allowed to stand for 5 minutes. 100 μ l of the reconstituted calibrator 1 was added to 300 μ l of Diluent 43 making Calibrator 2. The process was repeated a further 5 times to create Calibrators 3 to 7. Calibrator 8 was made up of Diluent 43 without the addition of calibrator blend.
4. Wash buffer preparation: 15 ml of 20x wash buffer was added to 285ml of deionized water.
5. Read buffer preparation: 5 ml of 4x read buffer was added to 15 ml deionized water.
6. Plate preparation: 50 μ l of each of calibrators 1 – 8 were added in duplicate to the first two Vertical rows of the plate. 50 μ l of diluted sample was then added to each of the remaining 80 wells.
7. The plate was sealed with an adhesive plate sealer and incubated with shaking at 300 revolutions per minute (rpm) for 2 hours at room temperature.
8. Plate wash: the contents of the plate were discarded after incubation. Each well was filled with 150 μ l of wash buffer which was then discarded. This sequence was repeated 3 times. Following the last wash the plate wash inverted and blotted with paper towels.
9. Addition of detection antibody: 25 μ l of detection antibody was then added to each well. The plate was sealed using an adhesive plate sealer and incubated for 2 hours at room temperature with shaking at 300 rpm.
10. Plate wash: the contents of the plate were discarded after incubation. Each well was filled with 150 μ l of wash buffer which was then discarded. This

sequence was repeated 3 times. Following the last wash the plate wash inverted and blotted with paper towels

11. Plate reading: 150 μ l of read buffer was added to each well before reading the plate on the MSD instrument.

2.5.7 MSD data interpretation

MSD data is interpreted using proprietary MSD Discovery Workbench software that is supplied with the MSD reading instrument. The lot specific calibrator concentrations for each analyte are entered into the software and a standard curve (see figure below) is generated for each analyte plotting the intensity of the signal that is measured against the known concentrations. The standard curves are then used to generate values for each analyte of each sample.

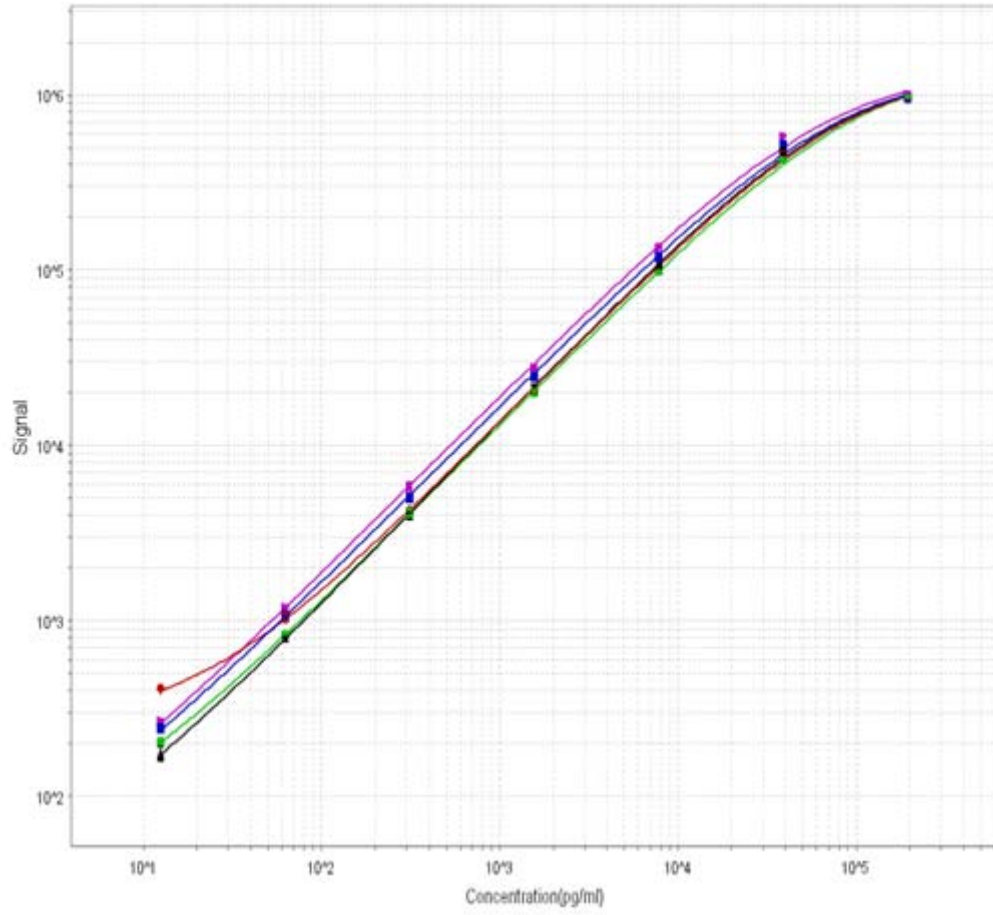


Figure 2-4 Example standard curves for CRP generated by MSD workbench software. Each line representing a standard curve generated on a different run of the CRP assay.

Alpha Defensin ELISA

2.6.1 Principles of ELISA

Enzyme linked immunosorbent assay (ELISA) is a biological test that measures the concentration of a protein in a biological fluid. This uses the principles of antibody antigen binding. There are various types of ELISA. The alpha defensin ELISA used here is a sandwich type of non-competitive ELISA providing results for alpha defensin that are directly proportional to the concentration of alpha defensin antigen.

2.6.2 Alpha Defensin ELISA reagents (CD Diagnostics)

1. Alpha Defensin coated microwell plate
2. Calibrator
3. Standards 1 – 7
4. Lyophilised QC 1, alpha defensin
5. Lyophilised QC 2, alpha defensin
6. Lyophilised QC3, alpha defensin
7. Lyophilised biotin conjugate 100x
8. Streptavidin-Peroxidase conjugate
9. Assay Diluent 5x
10. Wash buffer 10x
11. TMB colour solution
12. Stop solution

2.6.3 Alpha Defensin ELISA Protocol

1. All reagents and samples were brought to room temperature prior to use.
2. Calibrator was rehydrated with 250 µl of deionized water and mixed thoroughly with a vortex
3. QC 1, QC2 and QC3 were rehydrated with 250 µl of deionized water and mixed thoroughly using a vortex
4. 100 ml 10x wash buffer concentrate was added to 900 ml of deionized water and gently mixed
5. 40 ml 5x assay diluent was added to 160 ml deionized water and gently mixed

6. Biotin conjugate was rehydrated with 250 μ l of deionized water.
7. Three step dilution for samples, calibrator, QCs and standards: 50 μ l is added to 950 μ l of assay diluent to make 1 in 20 dilution. 50 μ l of 1 in 20 dilution is then added to 950 μ l of assay diluent to make 1 in 400 dilution. 50 μ l of in 1 in 400 dilution is then added to 950 μ l of assay diluent to make 1 in 8000 dilution. Samples are mixed thoroughly at each step.
8. Add 100 μ l of either sample or calibrator or QC or standard to each well as per plate map.
9. Cover the plate with a plate seal and incubate for 1 hour at room temperature.
10. Remove the plate seal and discard the contents of the wells before washing each well with 250 μ l of wash solution. Repeat four times then blot and dry with clean towels.
11. Add 100 μ l of biotinylated antibody to each well.
12. Cover the plate with a plate seal and incubate for 30 minutes at room temperature
13. Remove the plate seal and discard the contents of the wells before washing each well with 250 μ l of wash solution. Repeat four times then blot and dry with clean towels.
14. Add 100 μ l of Streptavidin-Peroxidase to each well.
15. Cover the plate with a plate seal and incubate for 30 minutes at room temperature
16. Remove the plate seal and discard the contents of the wells before washing each well with 250 μ l of wash solution. Repeat four times then blot and dry with clean towels.
17. Add 100 μ l of TMB solution to each well.
18. Incubate at room temperature in the dark for 30 minutes
19. Add 100 μ l of Stop solution to all wells
20. Read plate using a plate reader at a wavelength of 450nm

2.6.4 Alpha Defensin ELISA analysis

A second order polynomial (quadratic) function was used to generate a standard curve using the measured absorbance values for the known concentrations of the standards and in turn work out concentration using the absorbance values of the samples.

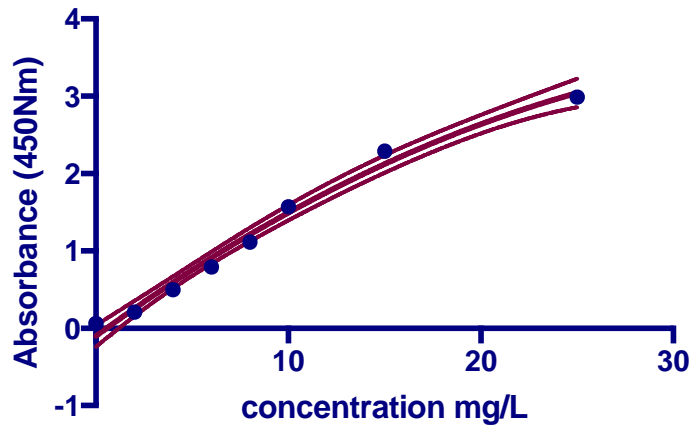


Figure 2-5 Example standard curve for Alpha Defensin ELISA generated using Graphpad Prism software second order polynomial function

2.7 The SAI model 5 neutrally buoyant helium bubble generator

The SAI™ Model 5 Bubble Generator generates helium-filled, neutrally-buoyant bubbles of uniform size see figures 1-4, 1-5 and 1-6 below.



Figure 2-6 The SAI Model 5 bubble generator console

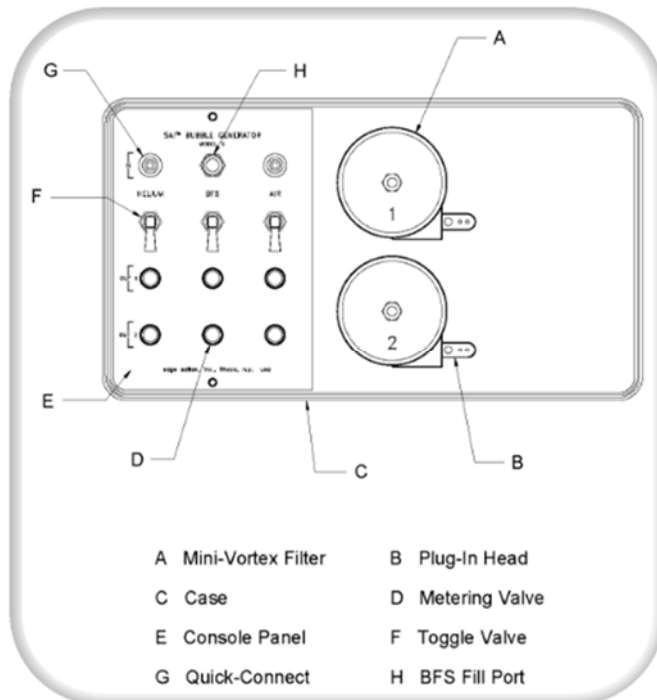


Figure 2-7 Schematic of the Model 5 bubble generator console

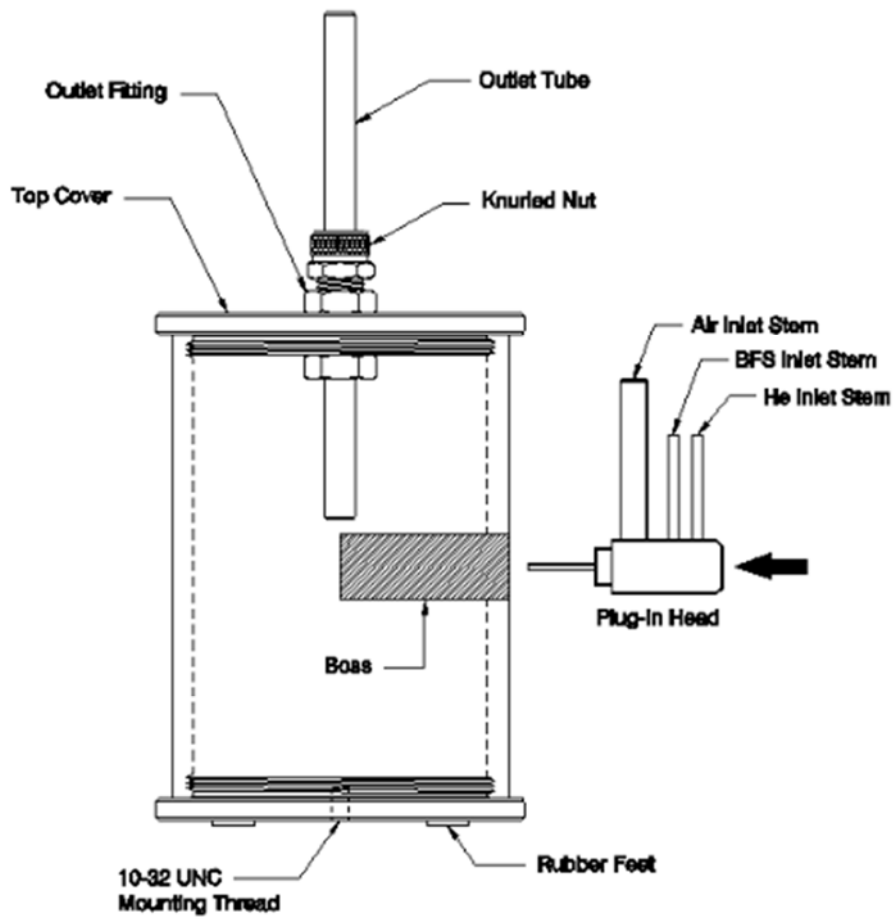


Figure 2-8 Schematic view of plug in head and mini vortex filter

2.7.1 Operating procedure for SAI model 5 bubble generator

1. Fill console with BFS.
2. Connect helium and air supplies
3. Attach paratubing to relevant inlets on the plug inn head
4. Attach plug in head to boss on the mini vortex filter
5. Attach outlet tube to chrome fitting on top of mini vortex filter
6. Turn on external air supply and set to 30 PSI
7. Turn on external helium supply and set to 20 PSI
8. Slowly open helium toggle valves
9. Open air toggle valves to begin bubble generation

2.7.2 Data presentation

Box and Whisker plots are used where possible throughout the text to present quantitative data. The box and whisker plots used here follow the method of drawing box and whiskers as first described by Tukey where the box spans the 25th to the 75th percentile and a horizontal line in the box denotes the median. The upper whisker corresponds to the data point closest to the sum of the 75th percentile and 1.5 times the interquartile range and the lower whisker corresponds to the data point closest to the 25th percentile minus 1.5 times the interquartile range. Any individual data points that fall outside the span of the box and whiskers are then plotted individually.¹²²

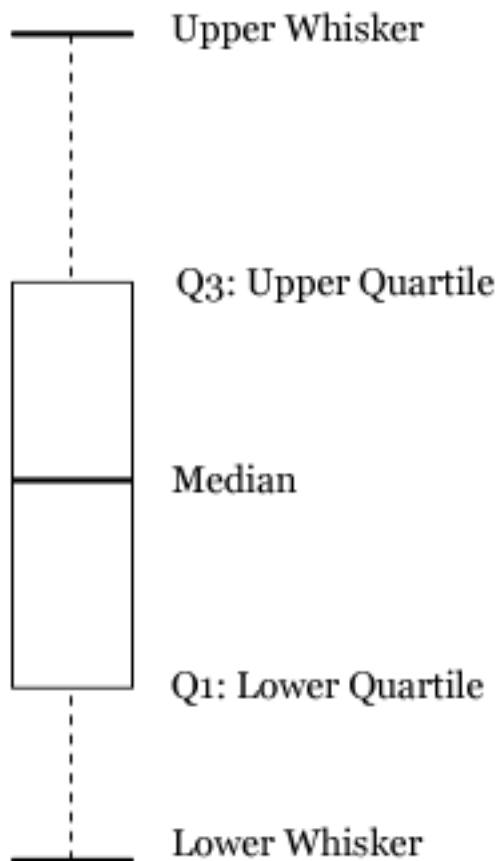


Figure 2-9 Tukey' box and whisker plot

Chapter 3 Debridement antibiotics and implant retention for the treatment of hip and knee replacement infection

3.1 Introduction

The treatment of joint replacement infection remains controversial. Two stage and single stage revision both have infection eradication success rates reported to be greater than 90% ¹¹¹ yet whilst the infection eradication rates of Debridement Antibiotics and Implant Retention so called DAIR ¹¹³ are rarely quoted above 80% ¹²³ DAIR is often regarded as the first treatment option for patients with PJI, particularly acute PJI. The cut-off time beyond which this treatment should be foregone in favour of formal revision procedures is much debated. ^{124 125 126}.

Intuitively DAIR has many advantages over formal revision surgery. The procedure is faster and less of a physiological insult to patients. Preserving the original joint replacement also preserves bone stock. Patients are also likely to require shorter hospital stays and the combination of these along with the fact that only new easily exchangeable modular implant components are required may represent cost savings over either single or 2 stage revision, particularly if a single DAIR procedure is successful. A further consideration is that the skill set required to perform a DAIR procedure is more of a general one meaning that on call surgeons can perform DAIR in emergency situations without the need for specific revision experience and the level of planning and surgical kit required for revision surgery. It is often believed that DAIR may offer the additional benefit of better function of the joint replacement compared to revision joint replacements yet whilst this may seem a logical assumption there have been no randomised controlled trials comparing these interventions.

3.2 Aims and Objectives

The aim of this study was to review the clinical results of DAIR performed on patients with primary joint replacements within a year of their primary surgery. Performance was measured by the infection eradication rate and where possible patient reported functional outcome score (Oxford hip score and Oxford knee score). The Oxford hip and knee scores are both validated patient reported outcome scores for the hip and knee joint respectively. They each contain 12 questions that are specific to a particular joint. Each question has 5 possible answers and is scored between 0 and 4. 4 being the best possible level and 0 the lowest. Giving a maximum score of 48 and a lowest score of 0. An example questionnaires are shown in appendix B.

3.3 Northumbria healthcare DAIR protocol

A consultant-led procedure is performed. Open exploration of the joint is performed. In well fixed implants, 5 or 6 fluid and tissue samples obtained for microbiology. This is followed by radical debridement in order to remove all infected and non viable tissues. Irrigation then follows with high volume warmed saline using a pulsed lavage system, typically delivering 9 litres of fluid to the joint and surrounding tissues. Any modular components are routinely exchanged and drains are used selectively. If the organism is not known pre-operatively the patients are placed on empirical antibiotics (typically Teicoplanin and Gentamicin) until culture results can be used to guide antibiotic therapy. All patients treated for suspected infection are discussed at the weekly infection MDT either pre or post operatively.

3.4 Methods

Patients who had undergone DAIR procedures for confirmed deep infection between March 2009 and April 2014 were identified using SSI reporting data, operating theatre logs and hospital episode coding data. Patient details were collected from patient notes and by postal questionnaire. Information was also collected from a large national patient reported outcomes (PROMS) database of patients undergoing routine primary hip and knee replacement procedures at Northumbria healthcare between December 2012 and January 2016.

3.5 Results

Between March 2009 and April 2014 90 deep infections treated by DAIR were identified. At latest review 13 patients were found to have died. The patients that were found to have died had all undergone a single DAIR procedure and in all but 1 case had completed their antibiotic treatment. Deaths occurred at an average of 27 months from the time of initial DAIR surgery (2-60). All deaths occurred in the community and are not thought to directly relate to ongoing problems with joint replacement infection. Where patients died less than 24 months following DAIR (n=7) they were excluded from the analysis because DAIR was considered to be successful after a minimum of 24 months free of infection. Of the remaining 6 patients who had died, death occurred on average 44 months after their DAIR procedure (range 24-69 months). Therefore of the total 90 deep infections treated by DAIR 83 were included in this analysis.

3.5.1 Summary of patients included in the study:

There were 44 female and 39 male patients. This included 43 hip replacements (23 female, 20 male), 40 knee replacements (21 female, 19 male). The average age of patients was 67 years (39 – 89). The prevalence of comorbidities was as follows: hypertension 49%, atrial fibrillation 6%, diabetes 6%, rheumatoid arthritis 4%, COPD 1.2% and smoking 14.5%. The mean BMI was 34.7 (range 20 - 51) and the median ASA was 2 (mean 2.1, range; 1-3). The mean pre-operative CRP in all infections was 77.1 (range 1 – 421) and the mean time from primary joint replacement to DAIR was 27 days (range 6 – 276). Infections were predominantly acute occurring within the first 6 weeks after surgery in 83% of cases (n=69). The mean preoperative CRP in these acute cases was 76.1 (range 1 – 421) and the mean length of antibiotic treatment was 12 weeks (range 6 – 50). 35 infections were caused by gram +ve organisms, 19 by gram –ve organisms and 29 were caused by polymicrobial infections.

3.5.2 Success of infection eradication by DAIR

The trajectory of patients undergoing DAIR treatment is summarized in Figure 3-1 below:

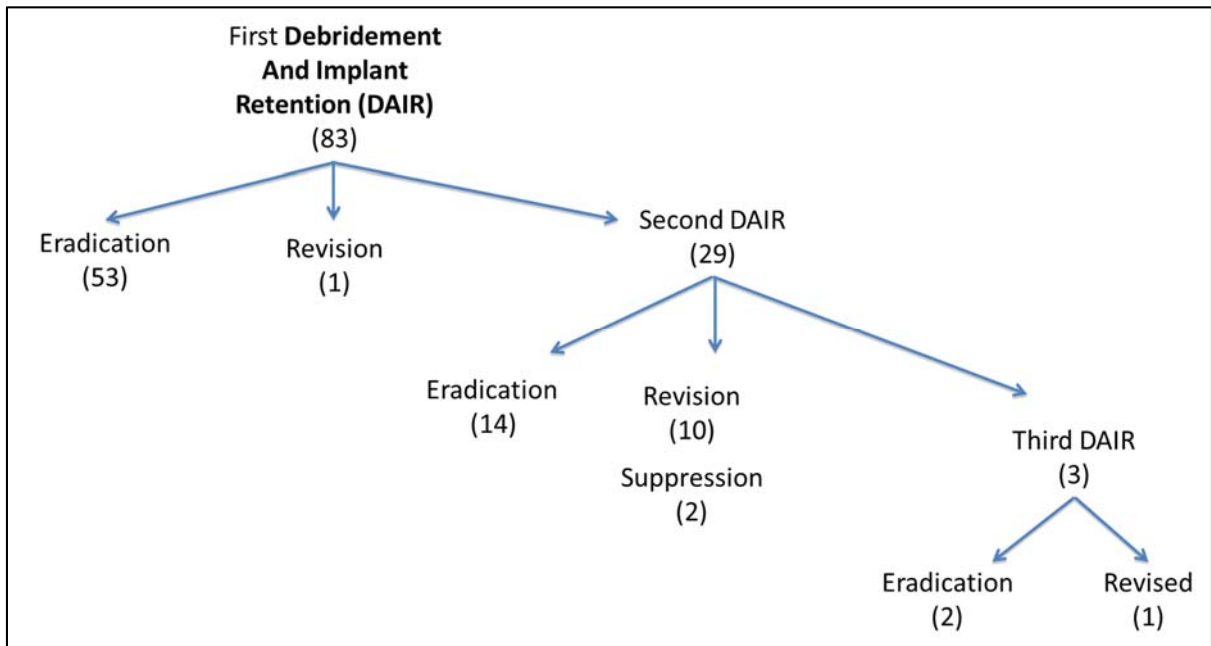


Figure 3-1 Flowchart of patients treated for deep infection by DAIR

Summary of patients treated by DAIR

Single DAIR: 54 patients had a single DAIR procedure. 53 of these infections were successfully eradicated with the single DAIR. One patient went on to receive a two stage revision.

Two DAIRs: 25 patients had two DAIR procedures in total. In 14 patients infection was eradicated. Nine went on to have a two stage revision and 2 were treated with suppressive antibiotics.

Three DAIR procedures: 3 patients underwent a third DAIR procedure. Two of these were successful and one went on to 2 stage revision.

The overall infection eradication rate for DAIR in this series was 83% at a mean follow up of 54 months (range 27 – 78). Patients should be aware to obtain these success rates they may require 1 or 2 or 3 procedures.

3.5.3 Risk factors for failure

Statistical analysis of the characteristics of patients successfully treated by DAIR and those who failed DAIR was performed using Fisher's exact test for categorical variables and Mann Whitney U test for continuous variables. Failure of DAIR was associated with multiple debridement ($p < 0.001$), gram +ve infections ($p = 0.0058$) and infections caused by *Staphylococcus aureus* ($p = 0.0104$). There were no statistical differences in comorbidities, preoperative CRP or the length of time from index surgery to debridement between successfully treated patients and those who failed DAIR treatment.

3.6 Patient reported outcomes following DAIR

The collection of patient reported outcome scores is now routine in England and Wales. The oxford hip and knee scores are used as patient reported outcome scores to estimate the effectiveness of hip and knee replacement surgery. Pre-operative and post-operative scores at 6 months are routinely collected¹²⁷.

Northumbria outcomes team regularly collects outcomes on multiple procedures for the purpose of service improvement - Caldicott "An Outcomes Project for the Trust" Ref C2437 (RPI- 59). All available patients were invited to complete a patient reported outcome score (n=77). A total of 64 completed questionnaires were returned. Of these 53 patients had successful eradication of infection (26 knees and 27 hips), 10 had failed infection eradication therapy and had gone on to 2 stage revision (9 hips and 1 knee) and 1 patient remained on long term suppression therapy (1 knee).

From the 64 patients who completed outcome questionnaires it was possible to link there episode back to the national PROMS database for 20 patients (11 knees and 9 hips) all of whom had had successful DAIR treatment (16 single DAIR, 3 two DAIRS and one patient had 3 DAIRS).

Figures 1-2 and 1-3 below show a comparison of absolute Oxford hip and knee scores compared with patients who had uncomplicated joint replacement surgery at Northumbria. In addition figures 1-3 and 1-4 show comparisons of the change in Oxford hip and knee scores for patients undergoing DAIR treatment compared to patients undergoing uncomplicated hip and knee replacements at Northumbria.

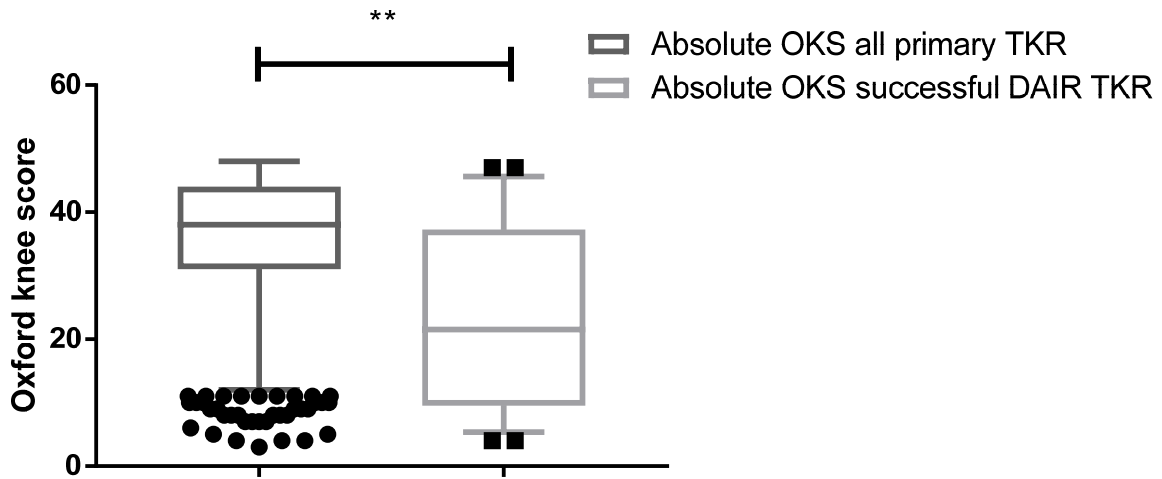


Figure 3-2 Absolute Oxford knee scores in patients undergoing routine total knee replacement surgery (n=2277) and patients treated by DAIR for knee replacement infection (n=26) median OKS in routine total knee replacement surgery at 6 months post op was 38 (mean 36.27, range 3 – 48, SEM 0.1948). Median OKS in patients treated by DAIR was 21.5 (mean 23.77, range 4 – 47, SEM 2.846) statistical analysis was performed by two tailed Mann-Whitney test where statistical significance is $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

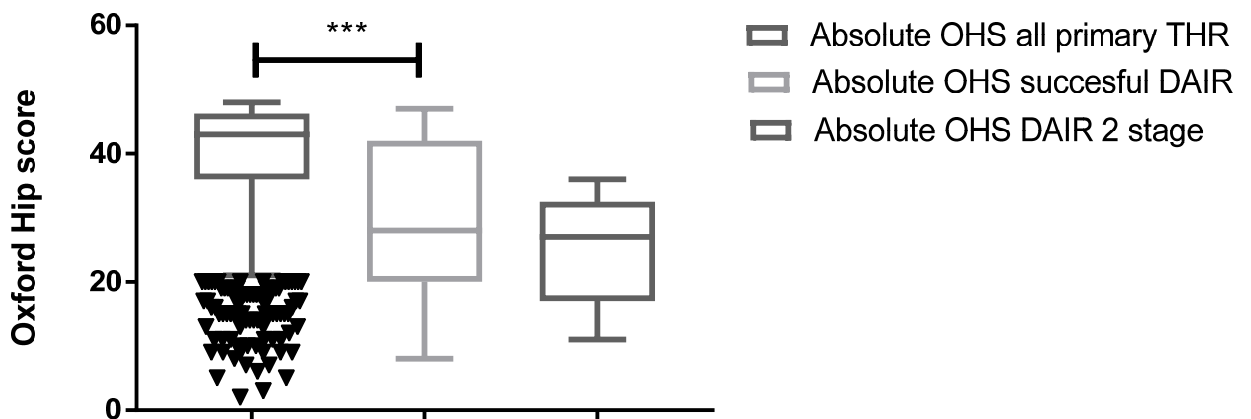


Figure 3-3 Absolute Oxford hip scores in patients undergoing routine total hip replacement surgery (n=1978), patients treated by DAIR for hip replacement infection (n=27) and patients who failed DAIR before going onto 2 stage revision (n=9). Median OHS in routine total hip replacement surgery at 6 months post op was 43 (mean 39.91, range 2 – 48, SEM 0.1967). Median OHS in patients successfully treated by DAIR was 28 (mean 27, range 8 – 47, SEM 2.374). Median OHS in patients who failed DAIR and had 2 stage revision was 27 (mean 25.44, range 11 – 36, SEM 2.954) statistical

analysis was performed by two tailed Mann-Whitney test where statistical significance is $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

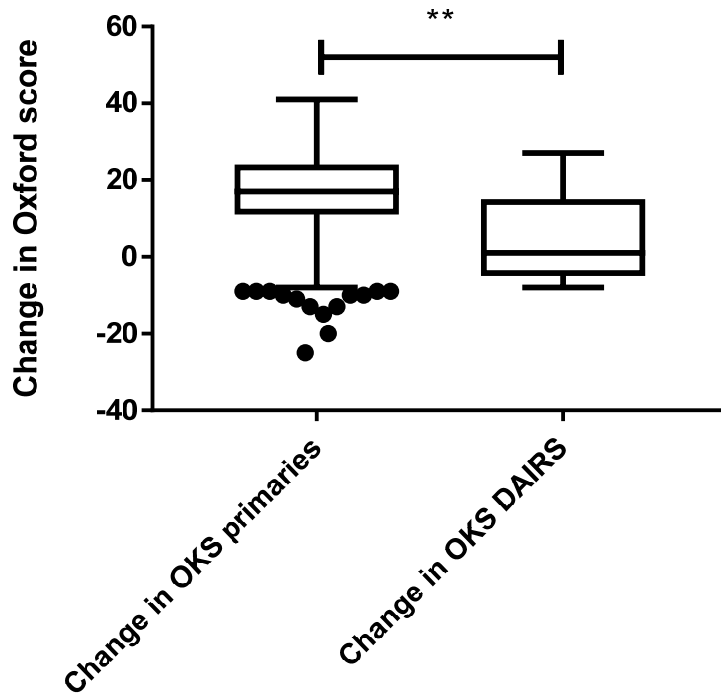


Figure 3-4 Change in Oxford knee scores in patients undergoing routine total knee replacement surgery ($n=2277$) and patients treated by DAIR for knee replacement infection ($n=11$). Median change in OKS in routine total knee replacement surgery at 6 months post op was 17 (mean 16.94, range -25 – 41, SEM 0.1986). Median change in OKS in patients successfully treated by DAIR was 1 (mean 5.72, range -8 – 27, SEM 3.39) statistical analysis was performed by two tailed Mann-Whitney test where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

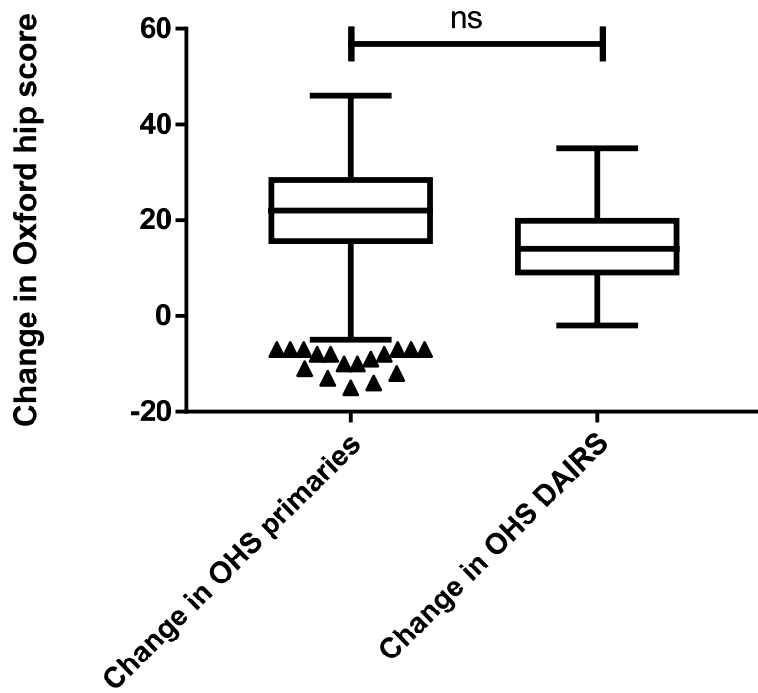


Figure 3-5 Change in Oxford hip scores in patients undergoing routine total hip replacement surgery (n=1978) and patients successfully treated by DAIR for hip replacement infection (n=9). Median change in OHS in routine total hip replacement surgery at 6 months post op was 22 (mean 21.7, range -15 – 46, SEM 0.219). Median change in OHS in patients successfully treated by DAIR was 14 (mean 14.89, range -2 – 35, SEM 3.45) statistical analysis was performed by two tailed Mann-Whitney test where statistical significance $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$, ns (not significant).

3.7 Discussion

The results in this DAIR cohort compare favourably with other series in the literature with a high infection eradication rate of 83% overall. This is comparable to those demonstrated by Byren et al¹¹³, Choi et al²⁹ and Laffer et al¹²⁸. Our identification of *Staphylococcus aureus* and multiple debridement as risk factors for treatment failure is also well described in the literature¹²³. The practice of exchanging modular components was the standard of care of in this series and this too has been shown to confer an advantage in terms of infection eradication²⁹. Where this study differs is in the analysis of patient reported outcome scores. Although a limited number of studies have drawn comparisons between DAIR and two stage revision none have assessed the change in patient reported outcome following treatment^{29,124} and none have drawn comparisons to primary joint replacement surgery. The use of absolute scores particularly with regards the Oxford hip and knee scores must be treated with caution and the recommendation from the group that developed these scoring systems is that they should typically be used to assess the change in score as a measure of the effect of the intervention¹²⁷.

When considering absolute scores in this series we see that patients with infected knee replacements successfully treated by DAIR have significantly worse absolute OKS knee scores as well as a significantly lower change in OKS compared to uncomplicated primary knee replacement patients. When looking at hip replacements treated by DAIR we see that the median OHS in DAIR patients is statistically significantly lower than in patients having uncomplicated primary joint replacement surgery but not statistically different from patients who failed DAIR and went onto to 2 stage revision. However when we look at the change in score in patients with hip replacement infections successfully treated by DAIR we see that there is no statistical difference with the change in OHS for routine primary hip replacement patients at Northumbria healthcare.

Limitations

These findings must obviously be treated with caution given the small number of patients with both pre-operative and post-operative PROMS in addition to the fact that the PROMS were collected at different time points. This is however the first analysis of its

kind and provides potential evidence that DAIR may be more effective in hip replacements than in knee replacements with regards to patient reported outcome scores. Further validation in a prospective study is however required.

Chapter 4 Improving the diagnosis of joint replacement infection – Biomarkers of joint replacement infection – a prospective cohort study

4.1 Overview of the study

As described in chapter 2 ethical approval was obtained for an observational study with a focus on the use of CD64 as a potential novel marker for joint replacement infection. The initial phase of the study was to establish the sequential changes seen in CD64 expression around the time of routine joint replacement surgery. CD64 levels were measured at Baseline (on the day of surgery) and on the 1st and 7th post-operative days. The aim of this was to understand the changes in the expression of CD64 and concentrations of IL-1 α , IL-1 β , IL-6, IL-8, IL-10 and IL-17 and CRP that result from surgery. CRP, as previously described, remains elevated following surgery for around three weeks, which potentially affects its discriminatory ability in the early post-operative phase.

CD64 was analysed prospectively and plasma was stored at -80^oC for subsequent analysis. Patients who were not captured at the time of their initial surgery but were suspected of having infection were enrolled at the time they returned to hospital.

After commencing the study it became clear that biomarkers for PJI, particularly in synovial fluid, were rapidly evolving. In particular alpha defensin as previously described appeared to have almost the perfect characteristics as a biomarker. We were approached by CD diagnostics, a commercial company, that manufacture an ELISA to measure alpha defensin in synovial fluid about performing a study to evaluate this marker and through an ethical amendment we were able to expand our original observational study to include the collection of excess synovial fluid from patients having investigation for suspected infection. Control synovial fluid samples were obtained from patients undergoing routine primary knee replacement at the Freeman Hospital Newcastle upon Tyne under the Newcastle University Biobank ethics.

Peripheral blood samples were prospectively analysed for CD64 by flow cytometry with the remaining blood processed and plasma supernatants stored for subsequent measurements of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 and CRP. In addition synovial fluid samples from patients with confirmed infection were analysed for alpha defensin, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 and CRP alongside an age and sex matched control group.

The diagnosis of infection in our study cohort is based on the MSIS major criteria.

4.2 Patient demographics

A total of 2040 patients were recruited. Patients were enrolled at the time of planned surgery (n=1711; 1636 primary joint replacements, 75 revision joint replacements) or suspected infection (n=366). There were 1102 females and 938 males. This included 1026 hip joints and 1014 knee joints. The mean age was 67 (range; 21-93). Mean BMI was 30 (range; 20 – 53). A summary of patient co-morbidities including diabetes, atrial fibrillation, hypertension, smoking, chronic obstructive pulmonary disease and Inflammatory arthritis, is shown below in Figure 4-1.

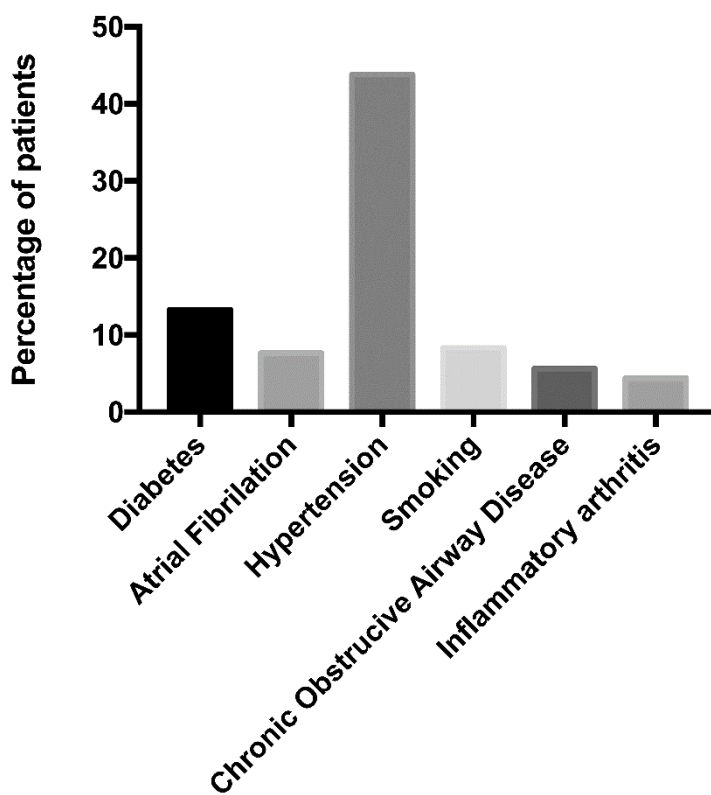


Figure 4-1 Incidence of co-morbidities in patients undergoing routine primary joint replacement . Diabetes (13.3%), Atrial fibrillation (7.6%), Hypertension (43.8%), Smoking (8.3%), Chronic Obstructive Airway Disease (5.6%), Inflammatory arthritis (4.4%) for all study patients shown as a percentage of all study patients.

4.2.1 Patients with infection

A total of 35 infections were confirmed by microbiological culture during the study period. This included 13 female and 22 male patients with an average age of 66 (range; 46 – 88) 15 knees (11 primary knee replacements, 4 revision knee replacements) and 20 hips (17 primary hips, 2 revision hips and 1 metal on metal hip). Mean BMI was 35.5 (range; 24 - 49). Incidence of other co-morbidities is illustrated below in Figure 4-2.

Infections were classified as acute or chronic based on time since implantation (mean 27 months; range 0.5 - 180 months) acute n = 12, chronic =23 and by duration of symptoms (mean 5 months; range 0.25 - 72) acute n=23, chronic n=12. Infections were caused by 26 gram positive organisms, 7 gram negative organisms and 2 polymicrobial infections see Figure 4-3 below which lists the actual causative organisms

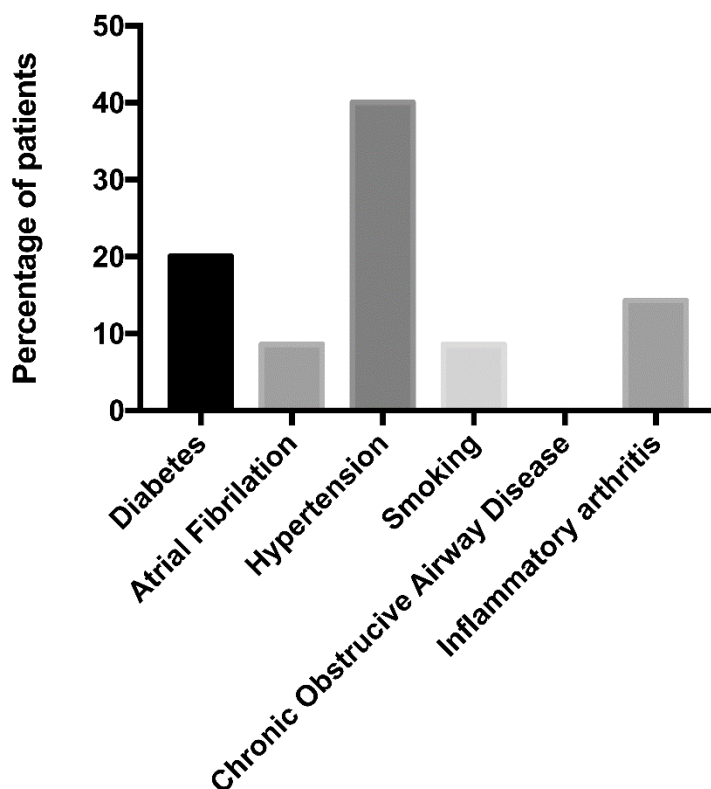


Figure 4-2 Incidence of co-morbidities in patients with confirmed infection. Diabetes (20%), Atrial fibrillation (8.6%), Hypertension (40%), Smoking (8.6%), Chronic Obstructive Airway Disease (0%), Inflammatory arthritis (14.3%) for all study patients shown as a percentage of all study patients

Gram positive organisms	
Organism	Count of organism
<i>Coagulase negative Staphylococci</i>	1
<i>Corynebacterium striatum</i>	1
<i>Haemolytic streptococcus group G</i>	1
<i>Staphylococcus aureus</i>	7
<i>Staphylococcus caprae</i>	1
<i>Staphylococcus epidermidis</i>	9
<i>Staphylococcus lugdunensis</i>	2
<i>Streptococcus dysgalactiae</i>	1
<i>Streptococcus parasanguinus</i>	1
<i>Streptococcus pneumonia</i>	1
<i>Streptococcus salivarius</i>	1
Gram negative organisms	
Organism	Count of organism
<i>Escherichia coli</i>	4
<i>Klebsiella pneumoniae</i>	1
<i>Pseudomonas aeruginosa</i>	1
<i>Serratia marcescens</i>	1
Polymicrobial organisms	
Organism combination	Count of combination
<i>Proteus mirabilis and Escherichia coli</i>	1
<i>Staphylococcus epidermidis and Staphylococcus aureus</i>	1

Figure 4-3 Summary of causative organisms for patients diagnosed with PJI

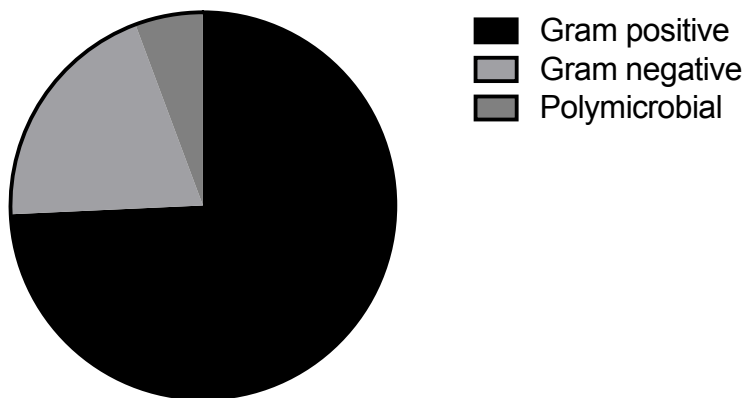


Figure 4-4 Proportion of infections caused by grouping of organism. Gram positive organisms n=26, gram negative organisms n= 7 and polymicrobial infections n= 2

4.2.2 Controls

A group of age and sex matched controls (1.5 controls per infection) n= 54 were selected from patients who underwent synovial fluid sampling but failed to meet the infection criteria. There was no significant difference in age between the groups (Two tailed Mann Whitney test p =0.5996). Mean age of controls was 66.1 (min = 45.49, max = 88), mean age of patients with infection was 64.5 (min = 43, max = 92) as shown in figure below. There was also no statistical difference (two tailed Chi squared test p=05110) in the distribution of sexes between the groups. The control group was made up of 29 males and 25 females whilst the infection group contained 22 males and 13 females). Mean BMI was 30.3 (range; 19-44). The remaining comorbidities for the control group are summarized below in Figure 4-6.

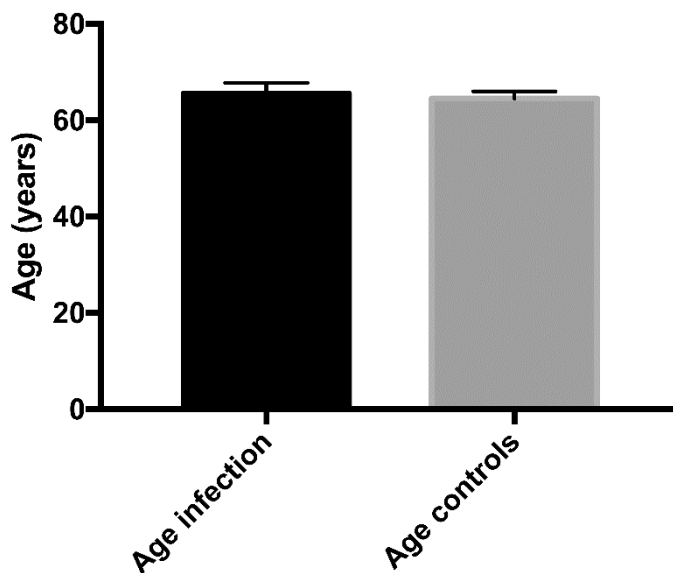


Figure 4-5 Age of patients in control and infection groups displayed as mean and SEM. The mean age in infected group was 65.58 (n=35, range 45.49 – 88, SEM 2.203) and the mean age in the control group was 64.5 (n= 54, range 43 – 92, SEM 1.482)

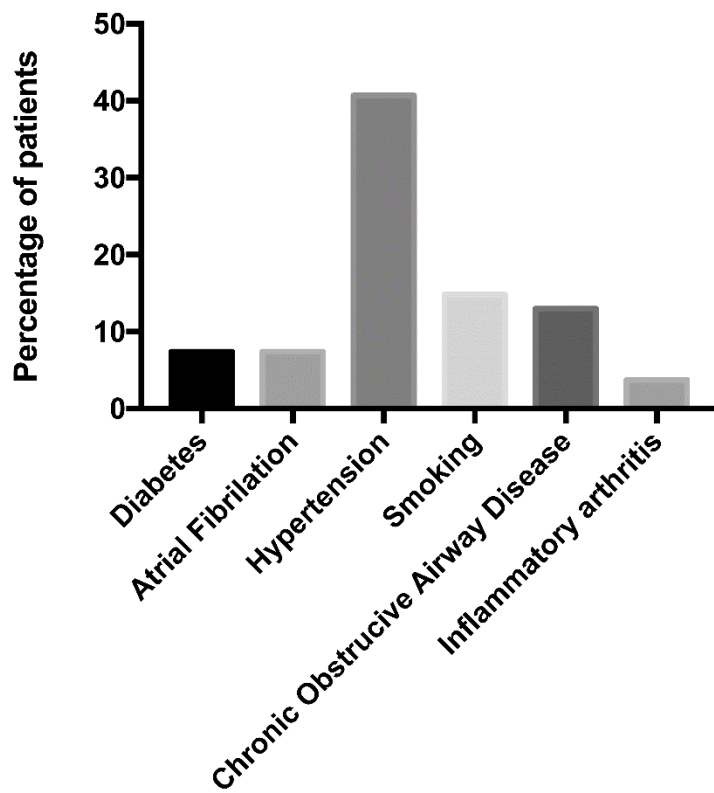


Figure 4-6 Incidence of co-morbidities for control patients Shown as a percentage of all control patients. Diabetes 7.4%, Atrial fibrillation 7.4%, Hypertension 40.7%, Smoking (9%), Chronic Obstructive Airway Disease 12.9%, Inflammatory arthritis 3.7%

4.2.3 Sequential changes cohort demographics

The sequential changes group n=78 was made up of 44 female and 34 male patients.

Mean age was 67.8 years (range; 50 – 85), mean BMI was 28.9 (range; 20 – 47.7).

Incidence of co-morbidities is summarized in figure below. This group of patients had their CD64 and cytokine levels measured pre-operatively (Baseline), on the first day after surgery (Day 1) and on the 7th post-operative day (Day 7).

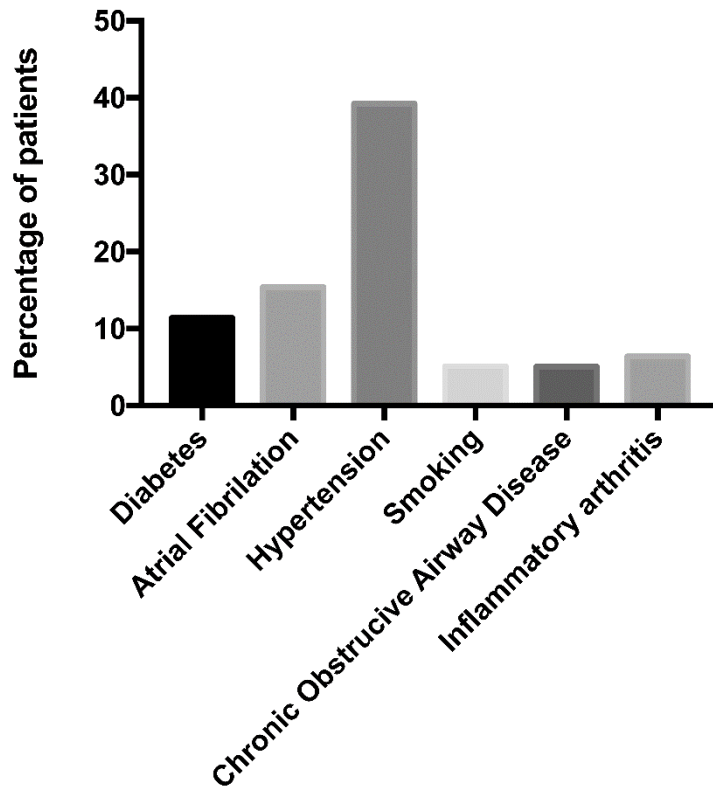


Figure 4-7 co-morbidities for patients included in the sequential changes group As a percentage of the group. Diabetes 11.4%, Atrial fibrillation 16.5%, Hypertension 39.2%, Smoking 6.3%, Chronic Obstructive Airway Disease 5.1%, Inflammatory arthritis 7.6%

4.3 Results – sequential changes in peripheral blood CD64, IL-1 α , IL-1 β , IL-6, IL-8, IL-10 and IL-17 in response to joint replacement surgery

One of the concerns with the use of CRP as an infection marker is that the levels of CRP are elevated as part of a normal inflammatory response. As such elevated CRP levels are seen in patients with inflammatory conditions such as rheumatoid arthritis or in patients who are undergoing an inflammatory response following surgery. Alternative markers are often suggested on the basis that they show a less marked and/or shorter response to inflammation.

To establish the typical response to surgery for our panel of infection markers we measured their sequential levels in a group of patients receiving routine primary joint replacement surgery n= 78. Measures of CD64, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 and CRP were made as previously described by flow cytometry and MSD respectively. Samples from each patient taken at baseline (day of surgery), Day 1 after surgery and on the 7th post-operative day were analysed.

Statistical differences were calculated using the Friedman test (Two tailed) and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

Results are summarized in the figures below.

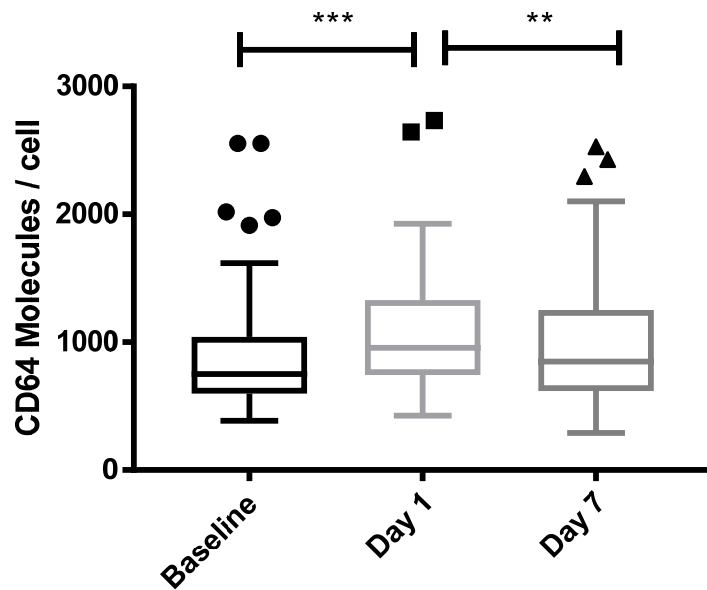


Figure 4-8 CD64 levels in patients undergoing routine joint replacement surgery (n=78). Median 752.4 Molecules/cell (Mean 921.9 mol/cell, Range 387.3 - 3253, SEM 59.27), Median Day 1, 956.5 mol/cell (Mean 1113 mol/cell, Range 427.3 - 3105, SEM 62.26), Mean Day 7 levels 995.3 mol/cell (mean 995.3, Range 290.3 - 2528, SEM 57.76). Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

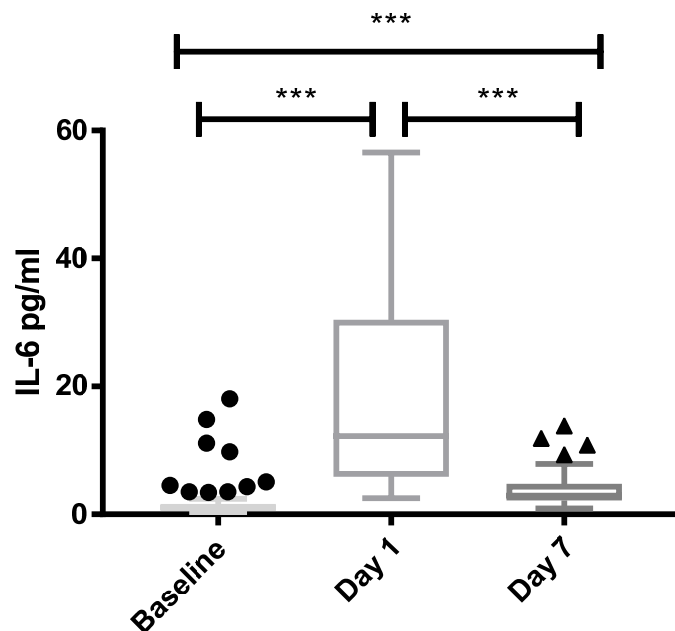


Figure 4-9 Levels of IL-6 in patients undergoing routine joint replacement surgery (n=78) measured by MSD on plasma supernatants. Median baseline 0.9565 pg/ml (Mean 1.849 pg/ml Range 0.2428 - 18.07, SEM 0.3367), median day 1 12.24 pg/ml (mean 21.03 pg/ml, Range 2.52 - 144.3, SEM 0.2671), median day 7 levels 2.909 (Mean 3.782 Range 0.9063 - 13.84, SEM 0.2761) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

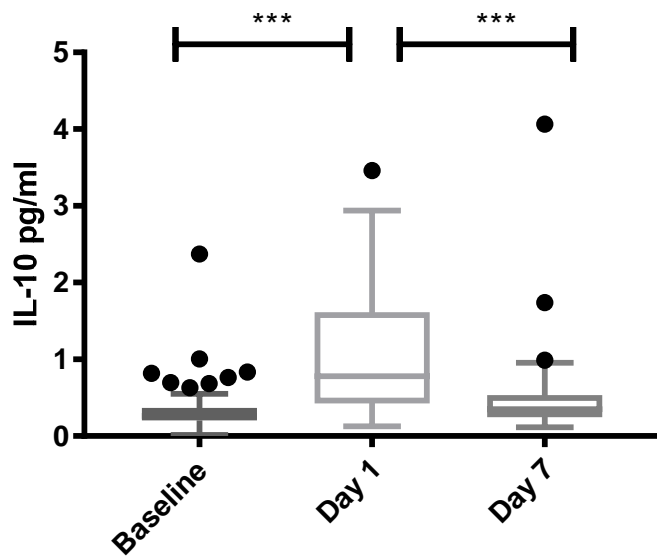


Figure 4-10 IL-10 levels in patients undergoing routine joint replacement surgery (n=78), median baseline 0.2656 pg/ml (Mean 0.337 pg/ml, Range 0.0151 - 2.374, SEM 0.0369), median day 1 0.7796, (Mean 1.52 pg/ml, Range 0.1268 – 15.43, SEM 0.2474), median Day 7 levels 0.3529 (mean 0.4563 Range 0.1152 – 4.066, SEM 0.05525) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

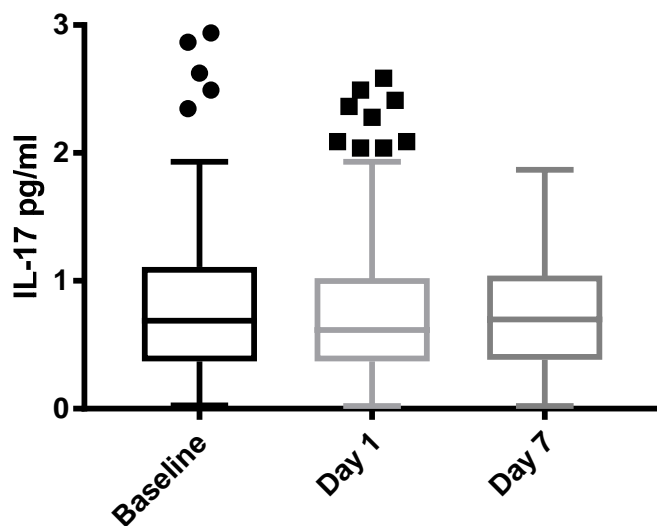


Figure 4-11 IL-17 levels in patients undergoing routine joint replacement surgery (n=78), median 0.6886, mean 1.032 pg/ml Range 0.02647 – 7.84, SEM 0.1327), median day 1, 0.6175 pg/ml (Mean 0.9379 pg/ml Range 0.02071 – 4.99, SEM 0.1327), Median Day 7 levels 0.3529 (mean, 0.9776 pg/ml Range 0.0271 – 7.52, SEM 0.1278) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

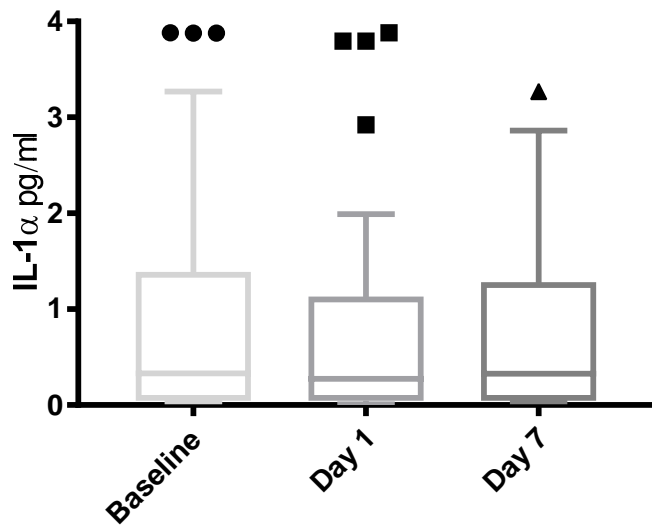


Figure 4-4-12 IL-1 α levels in patients undergoing routine joint replacement surgery (n=78), median 0.3317(mean 1.08 pg/ml, Range 0.03813 – 7.903, SEM 0.1788), median day 1, 0.2756 (mean 1.159 pg/ml Range 0.007204 – 11.71, SEM 0.2399), median day 7, 0.3273 (mean, 1.151 Range 0.002564 – 15.34, SEM 0.2787) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

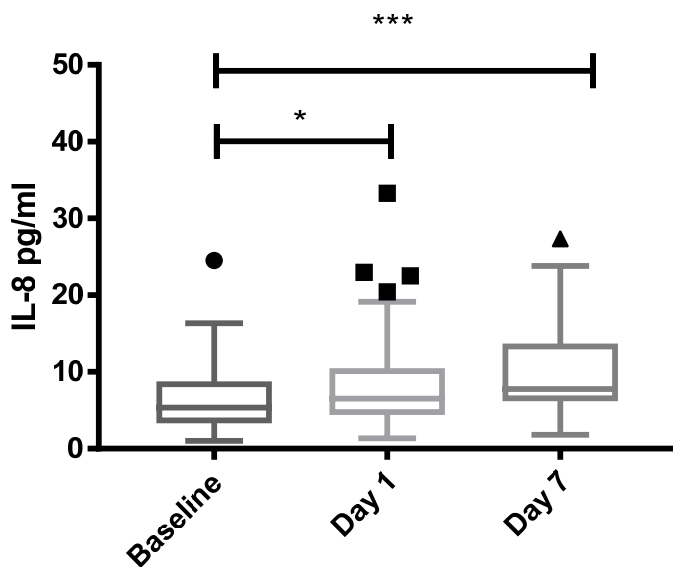


Figure 4-13 IL-8 levels in patients undergoing routine joint replacement surgery (n=78), median baseline 5.637(mean 26.91 pg/ml Range 1.007 – 220.2, SEM 3.047), median day 1, 6.525pg/ml (mean, 9.692 pg/ml (Range 1.358 – 93.05, SEM 1.367), median day 7 levels 7.789 (mean 15.9 Range 1.83 – 227.4, SEM 3.419) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

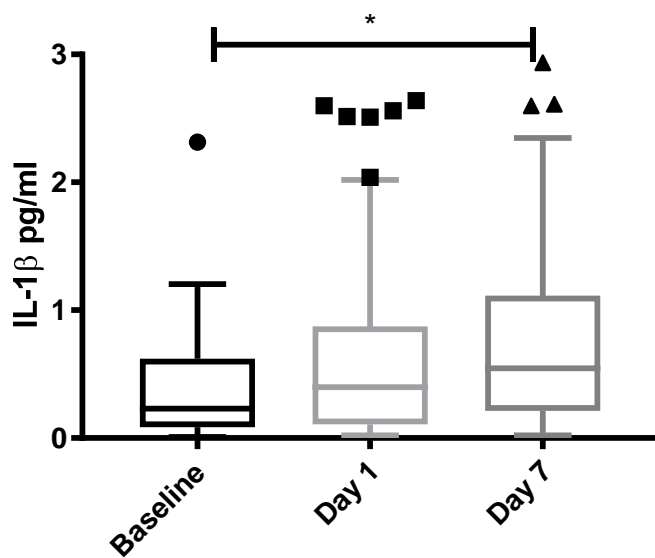


Figure 4-14 IL-1 β levels in patients undergoing routine joint replacement surgery (n=78), median baseline 0.2324 pg/ml, (mean 0.9305 pg/ml, Range 0.009367 – 22.01, SEM 0.312), median day 1, 0.3992 (mean Day 1 levels 0.9228, Range 0.0295 - 9.232, SEM 0.312), median day 7, 0.5465 (mean Day 7 levels 0.988, Range 0.0212 – 10.14, SEM 0.1683) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

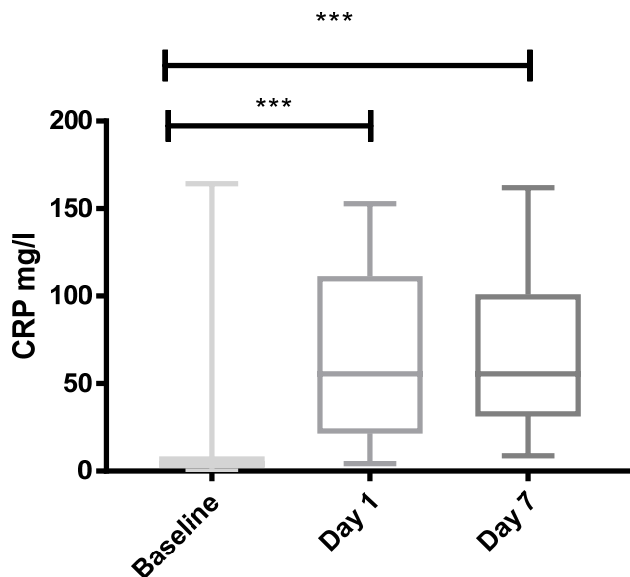


Figure 4-15 CRP levels in patients undergoing routine joint replacement surgery (n=78), median 4.155 mg/l (mean 12.51 mg/l Range 0.001991 - 164.3, SEM 3.364), median day 1, 55.55 mg/l (Mean 65.3 mg/l Range 4.228 – 152.7, SEM 5.488), median day 7, 55.57 mg/l (mean 66.62 Range 8.778 - 162, SEM 4.843) Statistical analysis was performed by two tailed Friedman test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

4.4 Discussion – sequential changes results

Having an understanding of the changes in a potential infection marker and in particular its likely concentrations following surgery is important. The ideal marker in this respect would not change in response surgery but would show highly elevated levels in infection. Such a marker remains elusive.

A frequent criticism of CRP which is the most commonly used serological marker measured as part of the assessment of patients with infection in joint replacements as well as other types of infection is that not only does it rise in response to surgery but that its levels remain highly elevated after surgery¹⁰¹ and in fact do not return to normal for around three months post-operatively. Our results confirm this observation as shown in figure 1-14 above where we see a significant rise at day 1 which persists up until day 7.

IL-10, IL-6 and CD64 also showed a marked positive response to surgery. In the case of CD64 and IL-10 a statistically significant increase at day 1 was followed by a statistically significant decrease between day 1 and day 7 with no statistical difference between baseline and day 7 indicating a return to baseline levels. IL-6 also showed a similar pattern with a statistically significant rise from baseline to day 1 followed by a statistically significant fall between day 1 and day 7. However, levels of IL-6 at day 7 were still significantly higher than levels at baseline but with a clear decreasing trend as demonstrated by significantly lower levels than at day 1. These patterns are in keeping with other reports on the response to joint replacement surgery^{102,129,130}.

Of the cytokines measured only IL-1 α and IL-17 showed no statistical change in their levels at baseline, day 1 and day 7. This is in keeping with other reports in the literature that have shown that these cytokines did not change in response to joint replacement surgery¹²⁹. IL-1 β showed a similar response to IL-1 α and IL-17 but levels of IL-1 α at Day 7 were marginally higher than baseline. The mechanism for this is unclear. It is worth noting that when an alternative statistical approach was taken and the IL-1 α dataset was analysed using Enova with Bonferroni correction for multiple comparisons that no statistical difference was observed.

The pattern of changes seen in IL-8 was unexpected with a decrease from Baseline to day 1 followed by an increase between day 1 and day 7 with levels at Day 7 significantly higher than their baseline levels. A closer inspection of the data from Reikeras et al ¹³¹ shows that IL-8 levels peaked at 6 hours after surgery and that they had returned to normal by day 1. By day 6 they had shown that levels had again increased to levels that were higher than baseline. In summary, CD64 and IL-10 were the most reliable markers in the context of return to near baseline levels by day 7 and the next step was to assess their utility and compare them to the other markers in patients with proven PJI.

4.5 Results – Markers of joint replacement infection

A total of 35 infections were identified during the study period. 32 samples were available for complete analysis of all peripheral blood markers. CD64, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 were measured. A total of 24 synovial fluid samples for analysis of alpha defensin, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 were available from these patients.

There are multiple ways to classify joint replacement infections. It is unclear whether current tests perform differently with different types of infection. The following analysis has been conducted by grouping the infections into the following groups:

1. Infections occurring within the first 4 weeks of the index operation (acute <4weeks),
2. Infections occurring within the first 6 weeks of the index operation (acute <6weeks),
3. Infections where symptoms had been present for less than six weeks
4. Chronic infections occurring more than 6 weeks from the original operation
5. Infections caused by Gram positive organisms
6. Infections caused by gram negative organisms

A receiver operating characteristic curve was created for each marker for each of the above classifications. Figure 4-16 below is an example of the graphical representation of ROC curves for multiple markers for comparison. Because of the overlapping lines these can be difficult to interpret. In order to better display the areas under the curve for each marker I have constructed supplementary bar graphs to compare markers (see Figure 4-25).

ROC curves all infections - blood markers

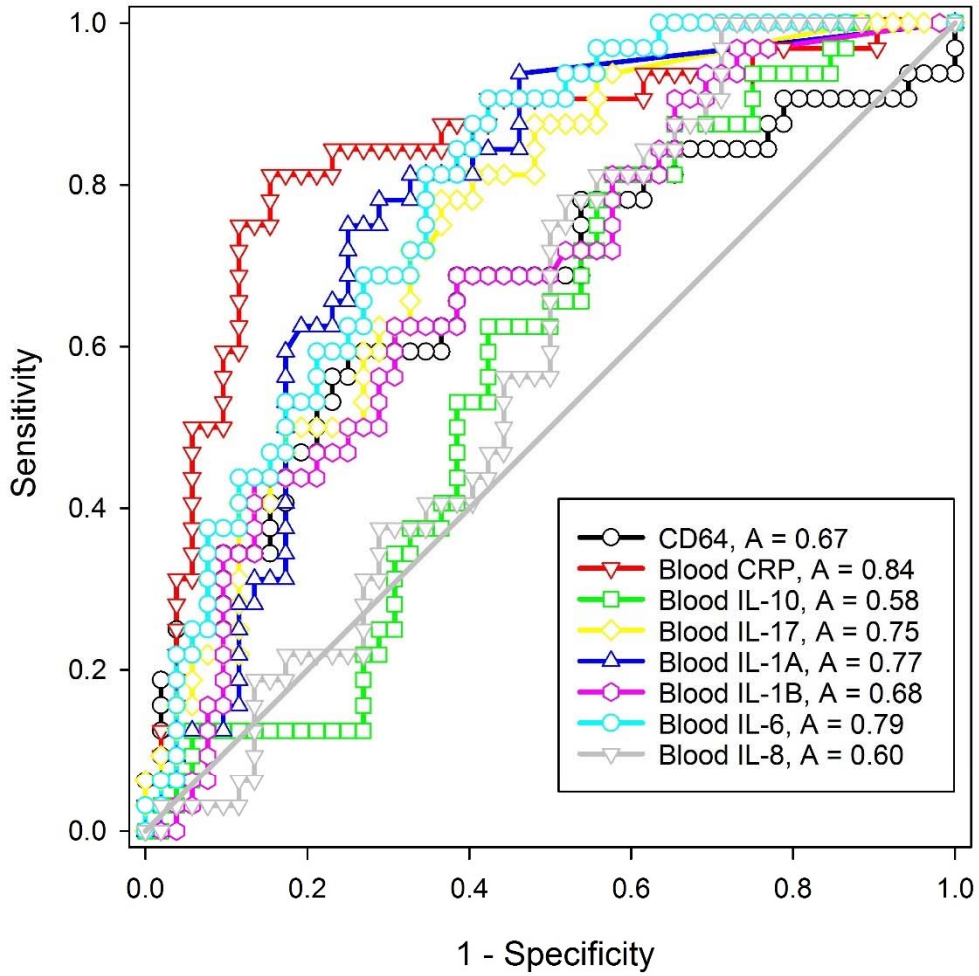


Figure 4-16 Combined ROC curves for all blood markers (CD64, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17) for all infections (n=32)

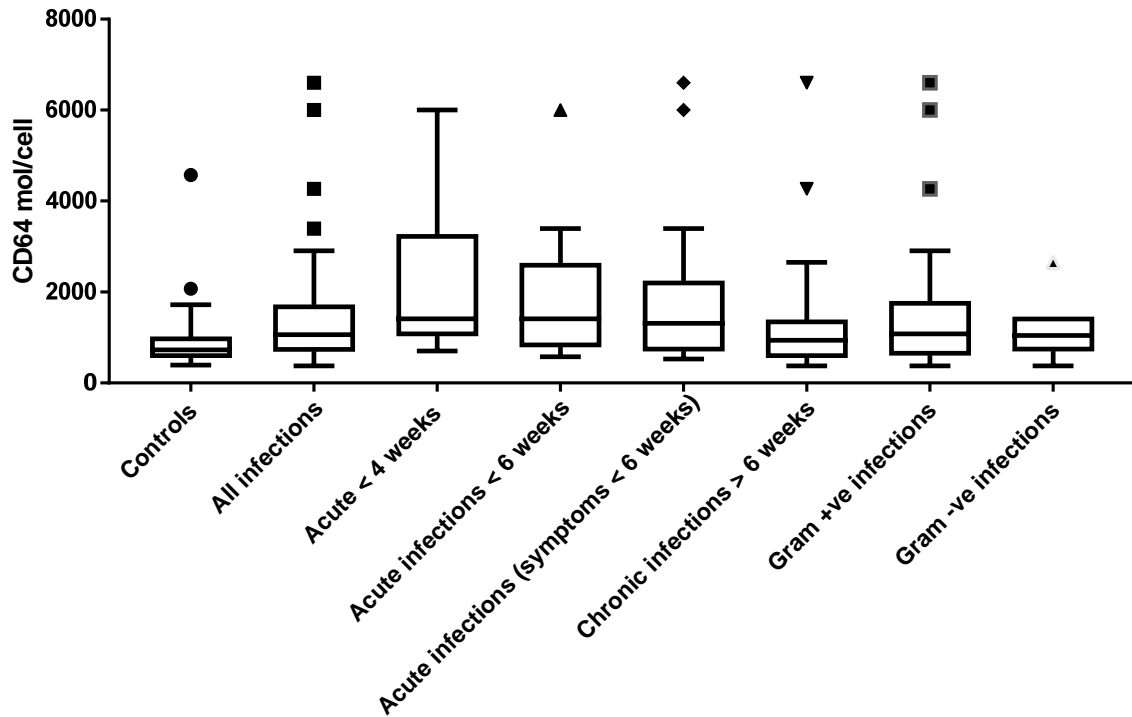


Figure 4-17 CD64 levels in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median CD64 level in controls (n=54) was 726.3 mol/cell (Range; 395.8 – 4569, Mean 901.7, SD 631.2, SEM 85.89), median CD64 level in all infections (n=32) was 1060 mol/cell (Range; 375.9 – 6600, Mean 1594, SD 1528, SEM 270.1), median CD64 levels in acute infections occurring less than 4 weeks from surgery (n=8) was 1413 mol/cell (Range; 705 – 6003, Mean 2237, SD 1797, SEM 635.2), Median CD64 level in acute infections occurring in less than 6 weeks after surgery (n=12) was 1413 mol/cell (Range; 578.3 – 6003, Mean 1900, SD 1559, SEM 450.1), Median CD64 level in acute infections with symptom duration less than 6 weeks (n=21) was 1313 mol/cell (Range; 526.7 – 6600, Mean 1829, SD 1688, SEM 368.4), Median CD64 level in chronic infections (n=20) was 940.3 mol/cell (Range; 375.9 – 6600, Mean 1410, SD 1519, SEM 339.6), Median CD64 level in infections caused by Gram stain positive organisms (n=23) was 1076 mol/cell (Range; 375.9 – 2650, Mean 1208, SD 726, SEM 274.4), Median CD64 level in infections caused by gram stain negative organisms (n=7) was 1044 mol/cell (Range; 375.9 – 2650, Mean 1208, SD 726, SEM 274.4). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

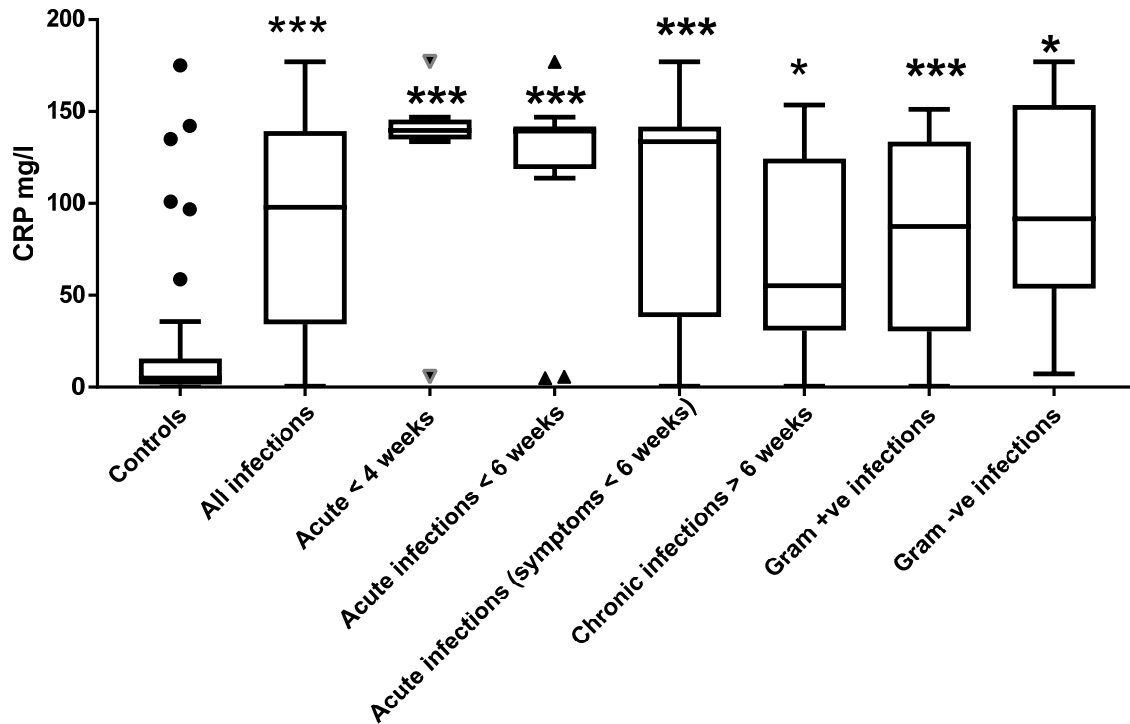


Figure 4-18 CRP concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median CRP concentration in controls (n=54) was 5 mg/l (Range; 0.065 – 175.1, Mean 19.97, SD 38.21, SEM 5.2), median CRP concentration in all infections (n=32) was 97.98 mg/l (Range; 0.5141 – 177.1, Mean 87.64, SD 56.84, SEM 10.05), median CRP concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 139.8 mg/l (Range; 5.694 – 127.9, Mean 127.9, SD 51.18, SEM 18.1), Median CRP concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 139.3 mg/l (Range; 4.964 – 177.1, Mean 118.6, SD 54.72, SEM 15.8), Median CRP concentration in acute infections with symptom duration less than 6 weeks (n=21) was 133.6 mg/l (Range; 0.5141 – 177.1 Mean 101.5, SD 59.83, SEM 13.06), Median CRP concentration in chronic infections (n=20) was 55.23 mg/l (Range; 0.5141 – 153.5, Mean 69.07, SD 50.69, SEM 11.34), Median CRP concentration in infections caused by Gram stain positive organisms (n=23) was 87.41 mg/l (Range; 0.5141 – 151.2, Mean 79.44, SD 56.23, SEM 11.72), Median CRP concentration in infections caused by gram stain negative organisms (n=7) was 91.61 mg/l (Range; 7.198 – 177.1, Mean 98.85, SD 60.68, SEM 22.93). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

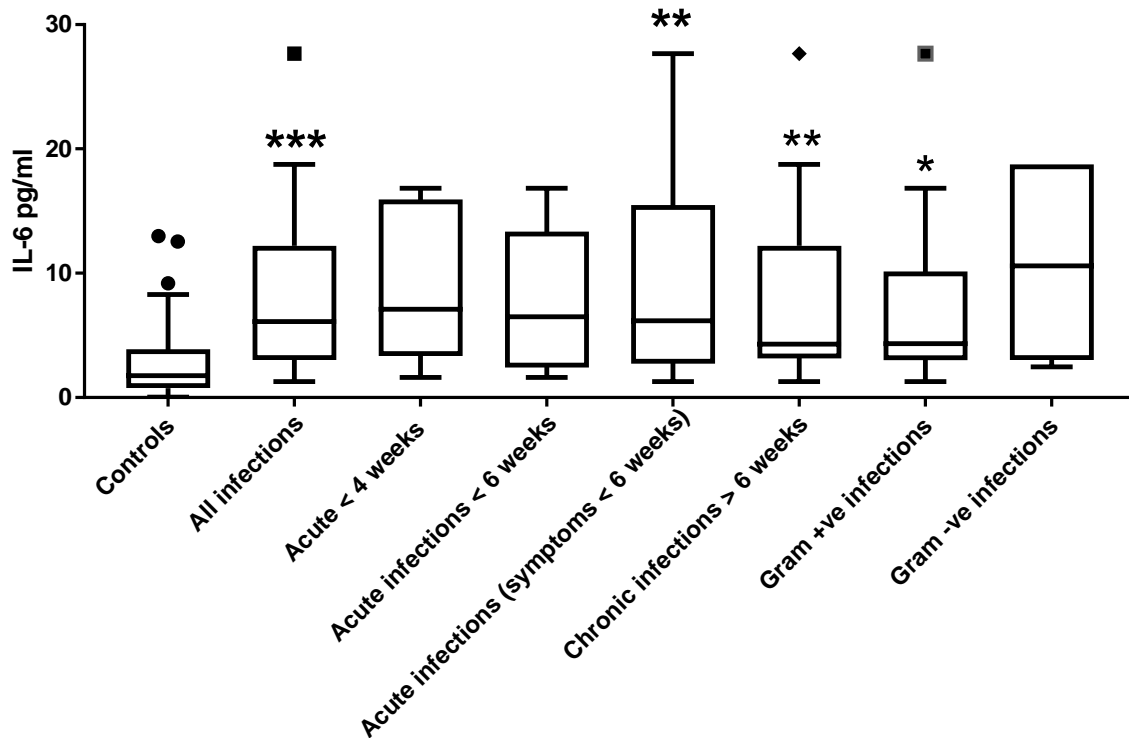


Figure 4-19 IL-6 concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-6 concentration in controls (n=54) was 1.75 pg/ml (Range; 0.03 – 65.05, Mean 4.434, SD 9.776, SEM 1.33), median IL-6 concentration in all infections (n=32) was 6.112 pg/ml (Range; 1.28 – 165.4, Mean 14.22, SD 29.89, SEM 5.284), median IL-6 concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 7.096 pg/ml (Range; 1.62 – 16.84, Mean 9.017, SD 6.101, SEM 2.157), Median IL-6 concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 6.5 pg/ml (Range; 1.62 – 16.84, Mean 7.765, SD 5.59, SEM 1.614), Median IL-6 concentration in acute infections with symptom duration less than 6 weeks (n=21) was 6.175 pg/ml (Range; 1.28 – 165.4, Mean 17.79, SD 36.5, SEM 7.965), Median IL-6 concentration in chronic infections (n=20) was 4.305 pg/ml (Range; 1.28 – 165.4, Mean 18.15, SD 37.37, SEM 8.355), Median IL-6 concentration in infections caused by Gram stain positive organisms (n=23) was 4.337 pg/ml (Range; 1.28 – 165.4, Mean 13.79, SD 33.6, SEM 7.006), Median IL-6 concentration in infections caused by gram stain negative organisms (n=7) was 10.58 pg/ml (Range; 2.464 – 62.84, Mean 16.47, SD 21.41, SEM 8.092). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

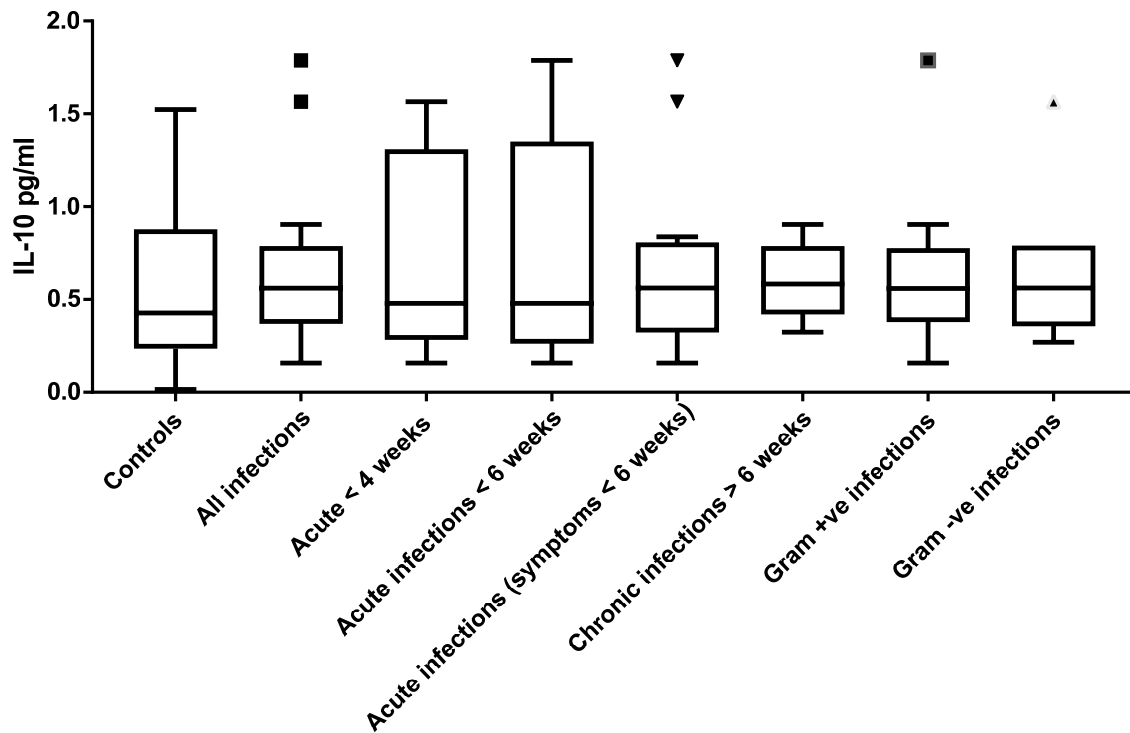


Figure 4-20 IL-10 concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-10 concentration in controls (n=54) was 0.4279 pg/ml (Range; 0.01438 – 7.156, Mean 0.6768 SD 1.045, SEM 0.1435), median IL-10 concentration in all infections (n=32) was 0.5162 pg/ml (Range; 0.157 – 10.28, Mean 0.9965, SD 1.803, SEM 0.3187), median IL-10 concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 0.4789 pg/ml (Range; 0.157 – 3.493, Mean 0.9136, SD 1.13, SEM 0.3995), Median IL-10 concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 0.4789 pg/ml (Range; 0.157 – 3.493, Mean 0.855, SD 0.9837, SEM 0.284), Median IL-10 concentration in acute infections with symptom duration less than 6 weeks (n=21) was 0.5261 pg/ml (Range; 0.157 – 10.28, Mean 1.218, SD 2.206, SEM 0.4815), Median IL-10 concentration in chronic infections (n=20) was 0.5848 pg/ml (Range; 0.3234 – 10.28, Mean 1.081, SD 2.173, SEM 0.4859), Median IL-10 concentration in infections caused by Gram stain positive organisms (n=23) was 0.5602 pg/ml (Range; 0.157 – 10.28, Mean 1.018, SD 2.047, SEM 0.4268), Median IL-10 concentration in infections caused by gram stain negative organisms (n=7) was 0.5621 pg/ml (Range; 0.2693 – 1.565, Mean 0.6658, SD 0.4304, SEM 0.1627). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

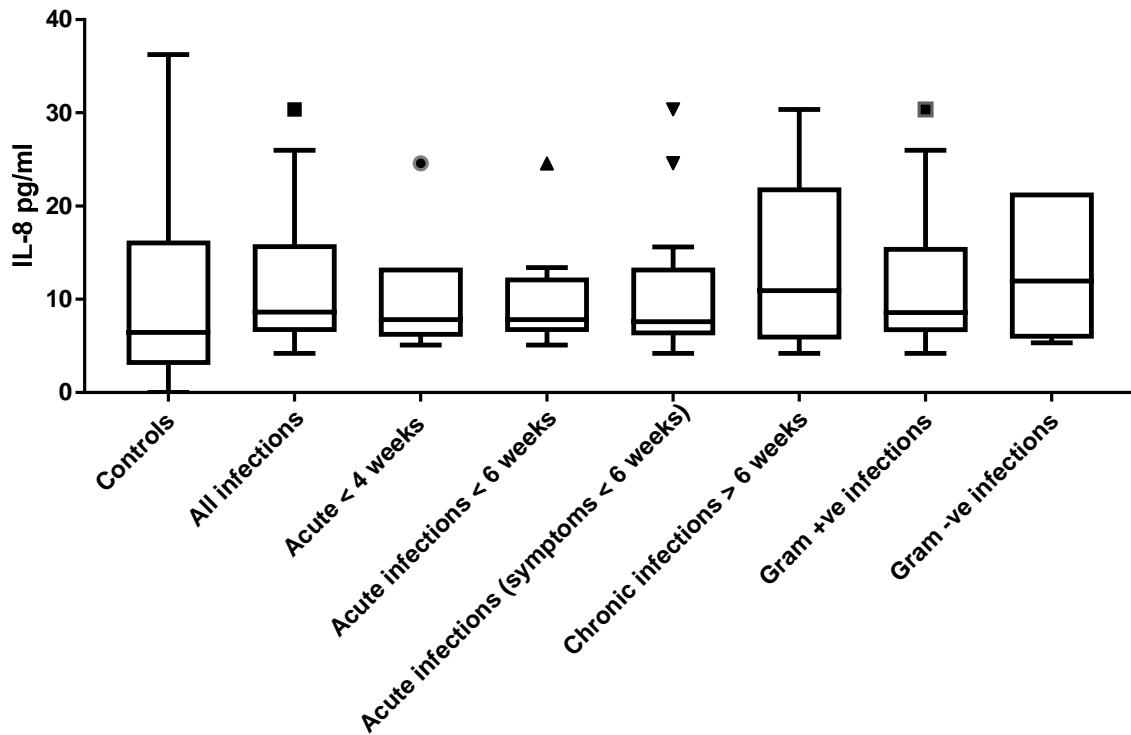


Figure 4-21 IL-8 concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-8 concentration in controls (n=54) was 6.449 pg/ml (Range;0.02 – 1014, Mean 31.55, SD 137.8, SEM 18.75), median IL-8 concentration in all infections (n=32) was 8.648 pg/ml (Range; 4.197 – 202.4, Mean 17.67, SD 34.51, SEM 6.1), median IL-8 concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 7.827 pg/ml (Range; 5.093 – 24.57, Mean 10.57, SD 6.505, SEM 2.3), Median IL-8 concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 7.827 pg/ml (Range;5.093 – 24.57, Mean 9.649, SD 5.407, SEM 1.561), Median IL-8 concentration in acute infections with symptom duration less than 6 weeks (n=21) was 7.593 pg/ml (Range; 4.197 – 202.4, Mean 19.09, SD 42.52, SEM 9.279), Median IL-8 concentration in chronic infections (n=20) was 10.93 pg/ml (Range; 4.197 – 202.4, Mean 22.48, SD 43.14, SEM 9.647), Median IL-8 concentration in infections caused by Gram stain positive organisms (n=23) was 11.98 pg/ml (Range; 5.236 – 202.4, Mean 38.78, SD 72.39, SEM 27.36), Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

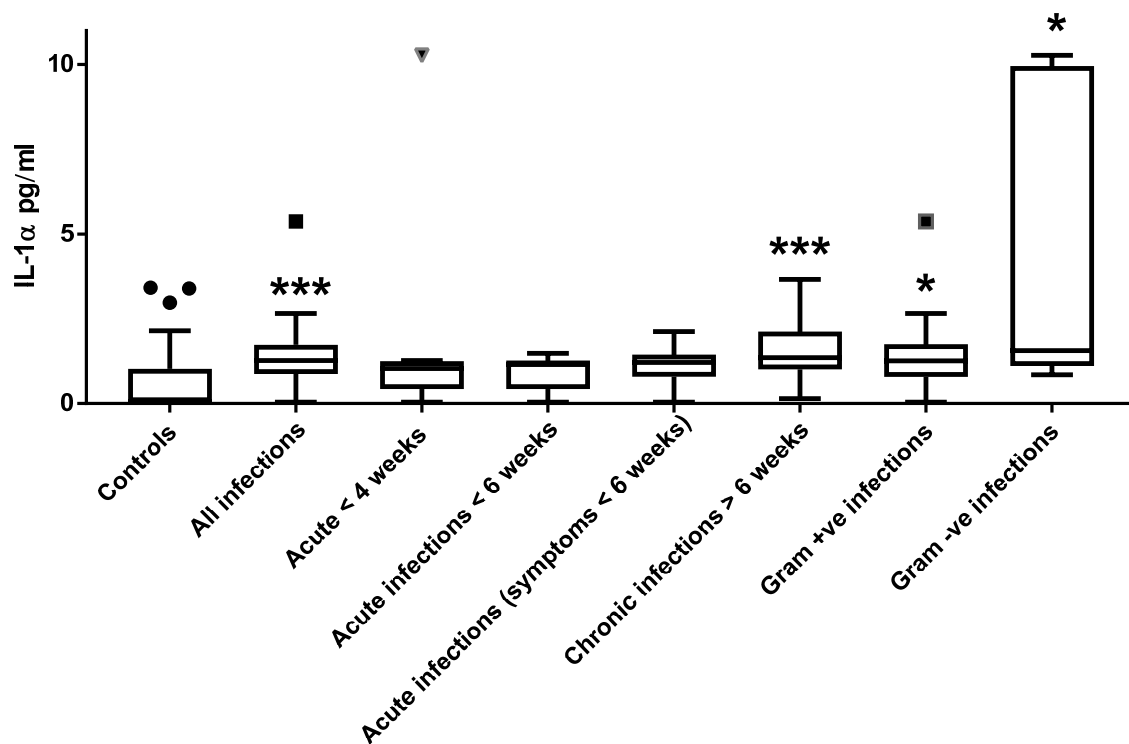


Figure 4-22 IL-1 α concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-1 α concentration in controls (n=54) was 0.045 pg/ml (Range; 0.045 – 15.73, Mean 1.04, SD 2.475, SEM 0.3432), median IL-1 α concentration in all infections (n=32) was 1.267 pg/ml (Range; 0.045 – 10.27, Mean 1.92, SD 2.385, SEM 0.4215), median IL-1 α concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 1.029 pg/ml (Range; 0.045 – 10.27, Mean 1.98, SD 3.375, SEM 1.193), Median IL-1 α concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 1.151 pg/ml (Range; 0.045 – 10.27, Mean 1.653, SD 2.756, SEM 0.7957), Median IL-1 α concentration in acute infections with symptom duration less than 6 weeks (n=21) was 1.212 pg/ml (Range; 0.045 – 10.27 Mean 1.534, SD 2.084, SEM 0.4548), Median IL-1 α concentration in chronic infections (n=20) was 1.357 pg/ml (Range; 0.146 – 9.956, Mean 2.079, SD 2.192, SEM 0.4903), Median IL-1 α concentration in infections caused by Gram stain positive organisms (n=23) was 1.263 pg/ml (Range; 0.045 – 5.368, Mean 1.44, SD 1.195, SEM 0.2492), Median IL-1 α concentration in infections caused by gram stain negative organisms (n=7) was 1.56 pg/ml (Range; 0.8507 – 10.27, Mean 3.822, SD 4.306, SEM 1.628). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

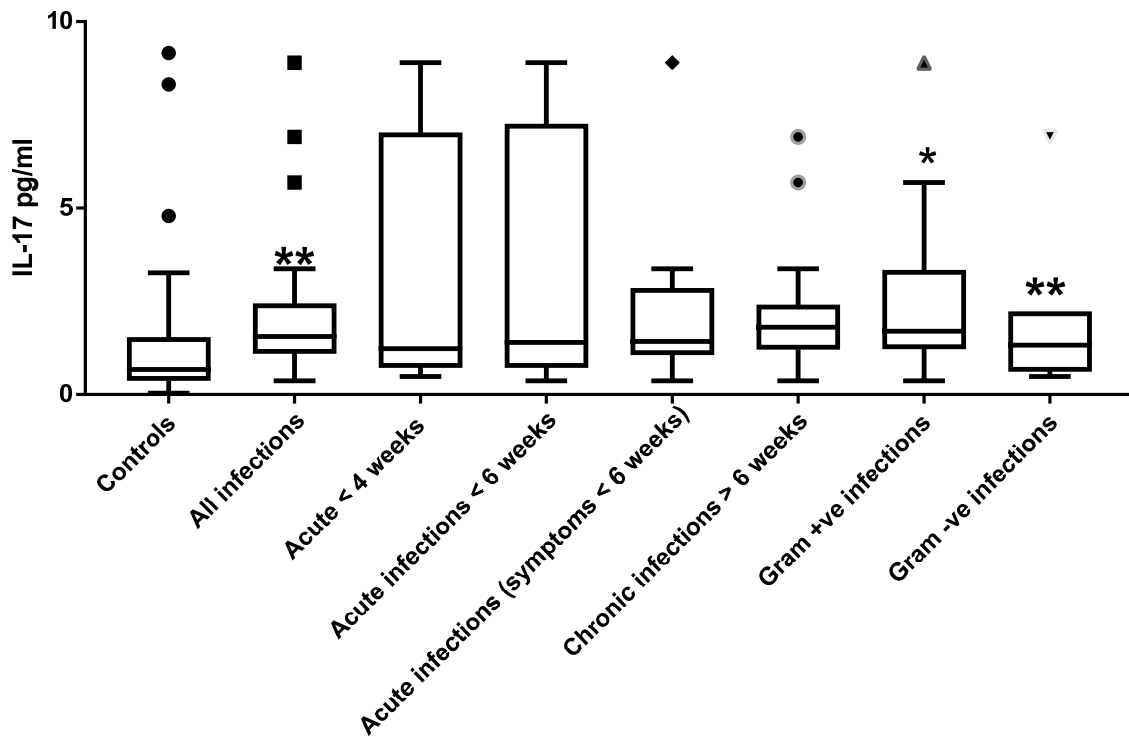


Figure 4-23 IL-17 concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-17 concentration in controls (n=54) was 0.6752 pg/ml (Range; 0.04152 – 9.155, Mean 1.284, SD 1.764, SEM 0.2446), median IL-17 concentration in all infections (n=32) was 1.556 pg/ml (Range; 0.37 – 58.72, Mean 4.832, SD 11.39, SEM 2.014), median IL-17 concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 1.234 pg/ml (Range; 0.483 – 32.91, Mean 5.978, SD 11.23, SEM 3.971), Median IL-17 concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 1.401 pg/ml (Range; 0.37 – 58.72, Mean 9.248, SD 18.09, SEM 5.222), Median IL-17 concentration in acute infections with symptom duration less than 6 weeks (n=21) was 1.428 pg/ml (Range; 0.37 – 58.72, Mean 6.07, SD 13.95, SEM 3.043), Median IL-17 concentration in chronic infections (n=20) was 1.806 pg/ml (Range; 0.37 – 6.904, Mean 2.183, SD 1.63, SEM 0.3645), Median IL-17 concentration in infections caused by Gram stain positive organisms (n=23) was 1.698 pg/ml (Range; 0.37 – 58.72, Mean 6.003, SD 13.28, SEM 2.769), Median IL-17 concentration in infections caused by gram stain negative organisms (n=7) was 1.326 pg/ml (Range; 0.483 – 6.904, Mean 2.065, SD 2.255, SEM 0.8533). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

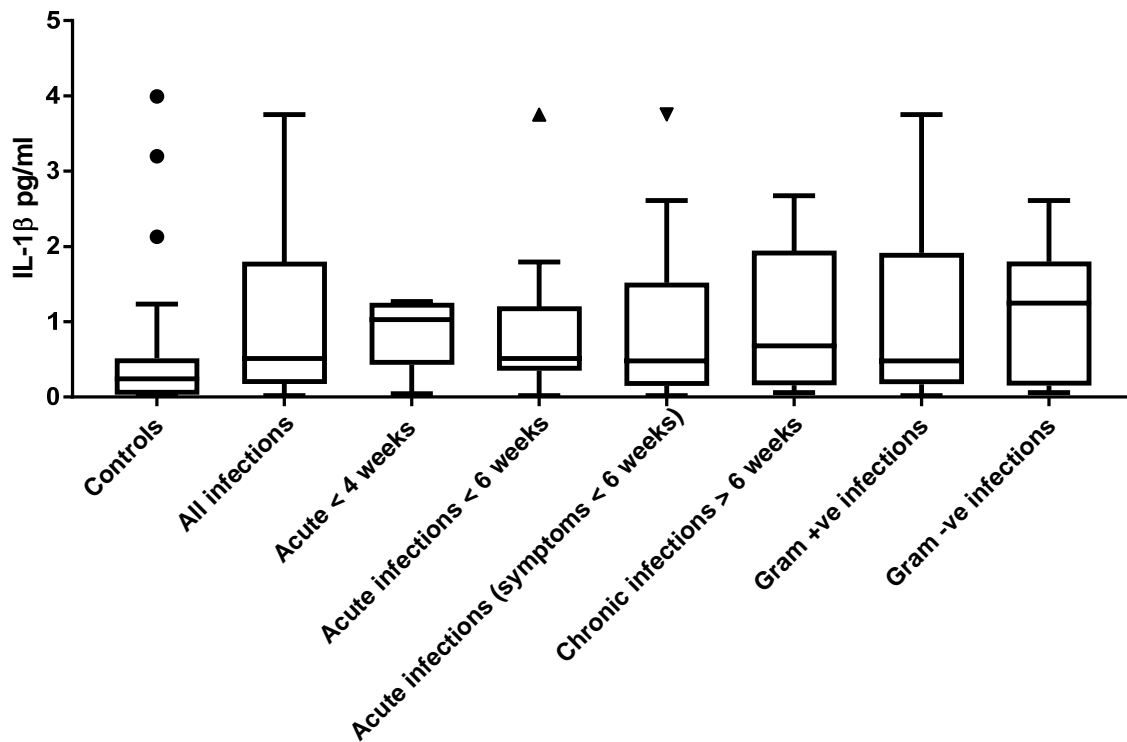
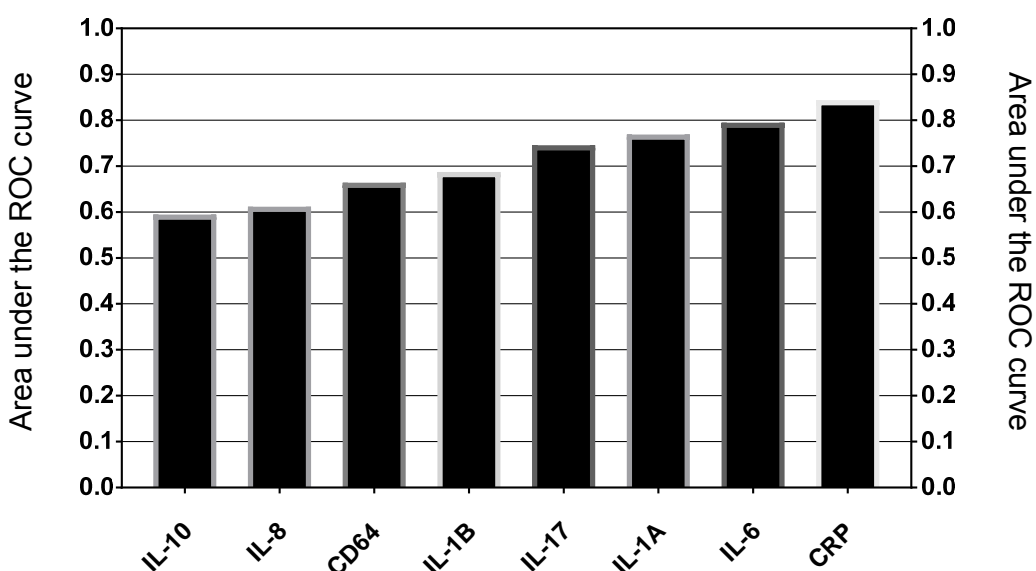


Figure 4-24 IL-1 β concentration in infected patients (n=32) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-1 BETA concentration in controls (n=54) was 0.246 pg/ml (Range; 0.01344 – 7.64 , Mean 0.5732 , SD 1.236 , SEM 0.1697), median IL-1 BETA concentration in all infections (n=32) was 0.5103 pg/ml (Range; 0.02 – 5.42 , Mean 1.095 , SD 1.244 , SEM 0.2199), median IL-1 BETA concentration in acute infections occurring less than 4 weeks from surgery (n=8) was 1.029 pg/ml (Range; 0.045 – 10.27 , Mean 1.98 , SD 3.375 , SEM 1.193), Median IL-1 BETA concentration in acute infections occurring in less than 6 weeks after surgery (n=12) was 0.5103 pg/ml (Range; 0.02 – 3.753 , Mean 0.8956 , SD 1.032 , SEM 0.2979), Median IL-1 BETA concentration in acute infections with symptom duration less than 6 weeks (n=21) was 0.4806 pg/ml (Range; 0.02 – 3.753 Mean 0.9434 , SD 1.016 , SEM 0.2217), Median IL-1 BETA concentration in chronic infections (n=20) was 0.6806 pg/ml (Range; 0.05918 – 5.42 , Mean 1.215 , SD 1.367 , SEM 0.3056), Median IL-1 BETA concentration in infections caused by Gram stain positive organisms (n=23) was 0.4806 pg/ml (Range; 0.02 – 5.42, Mean 1.115 , SD 1.374 , SEM 0.2865), Median IL-1 BETA concentration in infections caused by gram stain negative organisms (n=7) was 1.248 pg/ml (Range; 0.05918 – 2.609, Mean 1.132 , SD 0.9956 , SEM 0.3763). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

4.5.1 ROC curve analysis peripheral blood markers of joint replacement infection

ROC curve areas for peripheral blood infection markers are summarized below for each infection classification.

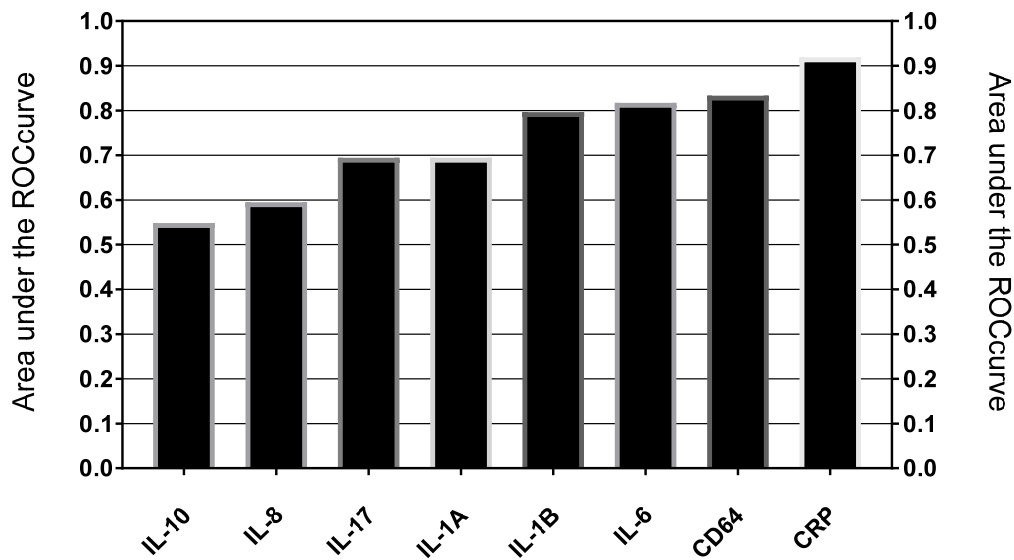
Summary of areas under ROC curves in peripheral blood - all infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-10	0.59	0.474 - 0.714	0.36	81.25	44.44
IL-8	0.61	0.494 - 0.729	3.49	100.00	31.48
CD64	0.66	0.554 - 0.762	996.36	59.38	74.07
IL-1B	0.69	0.572 - 0.802	0.39	62.50	70.37
IL-17	0.75	0.641 - 0.849	1.03	78.12	63.46
IL-1A	0.77	0.666 - 0.871	0.88	75.00	75.00
IL-6	0.79	0.701 - 0.887	2.34	90.62	59.26
CRP	0.84	0.750 - 0.913	29.92	81.25	85.19

Figure 4-25 Summary of diagnostic performance of peripheral blood infection markers for all infections. Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for all infections (n=32).

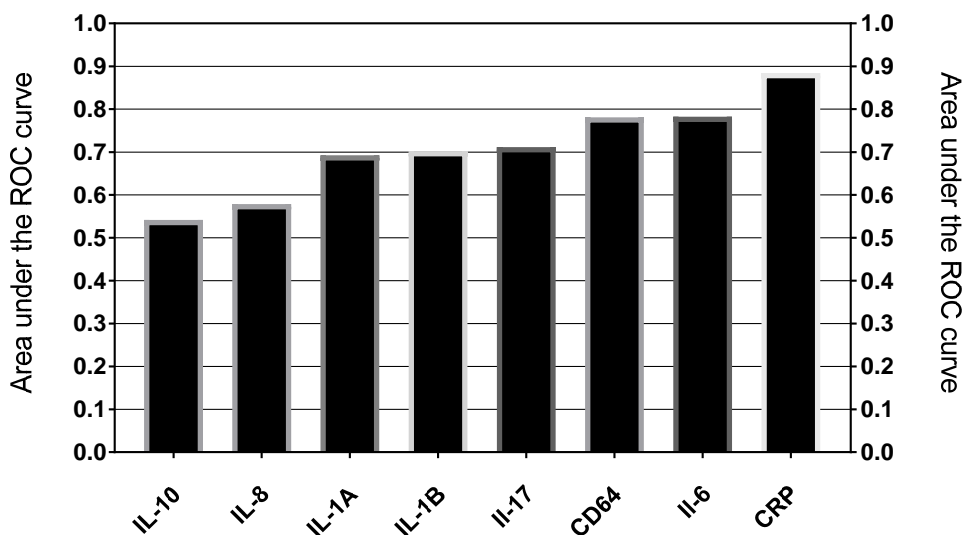
Summary of areas under ROC curves in peripheral blood - acute infections < 4weeks



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-10	0.548611	0.331 - 0.766	1.523689	25	94.44
IL-8	0.594907	0.439 - 0.750	4.866257	100	37.04
IL-17	0.694712	0.523 - 0.866	0.475669	100	44.23
IL-1A	0.694712	0.519 - 0.870	0.386888	100	70.37
IL-1B	0.796296	0.686 - 0.906	5.936378	75	83.33
IL-6	0.81713	0.673 - 0.961	5.936378	75	83.33
CD64	0.833333	0.692 - 0.973	1017.41	87.5	75.93
CRP	0.918981	0.812 - 1.023	100.9117	87.5	94.44

Figure 4-26 Summary of diagnostic performance of peripheral blood infection markers acute infections (<4weeks) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for infections diagnosed within the first 4 weeks after surgery (n=8).

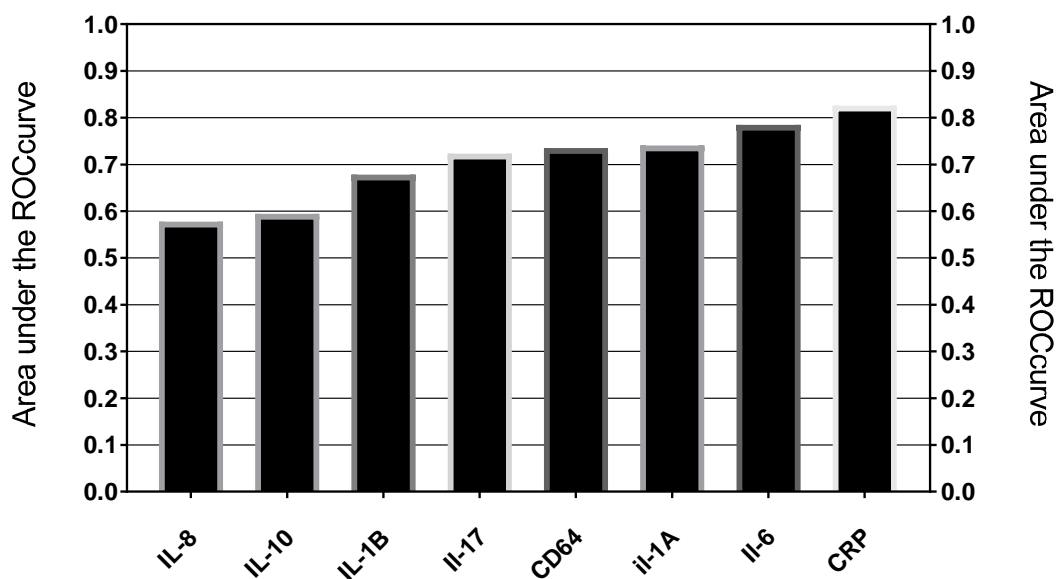
Summary of areas under ROC curves in peripheral blood - acute infections < 6weeks



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-10	0.54	0.355 - 0.727	1.52	25.00	94.44
IL-8	0.58	0.442 - 0.714	5.76	91.67	46.30
IL-1A	0.69	0.537 - 0.847	1.71	87.50	81.48
IL-1B	0.70	0.543 - 0.860	0.32	83.33	62.96
IL-17	0.71	0.555 - 0.867	0.48	91.67	44.23
CD64	0.78	0.632 - 0.929	1017.41	75.00	75.93
IL-6	0.78	0.653 - 0.911	5.94	66.67	83.33
CRP	0.88	0.766 - 1.00	100.91	83.33	94.44

Figure 4-27 Summary of diagnostic performance of peripheral blood infection markers acute infections (<6weeks) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for infections diagnosed within the first 6 weeks after surgery (n=12).

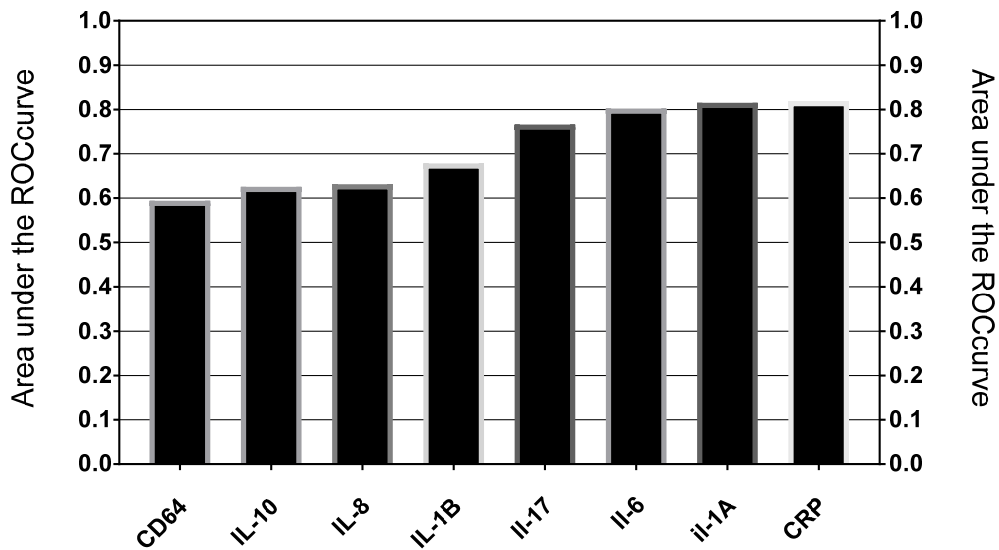
Summary of areas under ROC curves in peripheral blood - symptoms <6 weeks



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-8	0.58	0.448 - 0.707	3.49	100.00	31.48
IL-10	0.59	0.456 - 0.732	0.53	61.90	59.26
IL-1B	0.68	0.545 0.810	0.39	66.67	70.37
IL-17	0.72	0.600 0.845	0.94	80.95	59.62
CD64	0.74	0.619 - 0.832	1017.41	66.67	76.92
IL-1A	0.74	0.621 - 0.859	0.88	76.19	75.00
IL-6	0.78	0.678 - 0.891	2.34	85.71	59.26
CRP	0.83	0.719 - 0.905	35.69	76.19	88.46

Figure 4-28 Summary of diagnostic performance of peripheral blood infection markers acute infections (symptoms <6weeks) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for infections where symptom duration was less than 6 weeks (n=21).

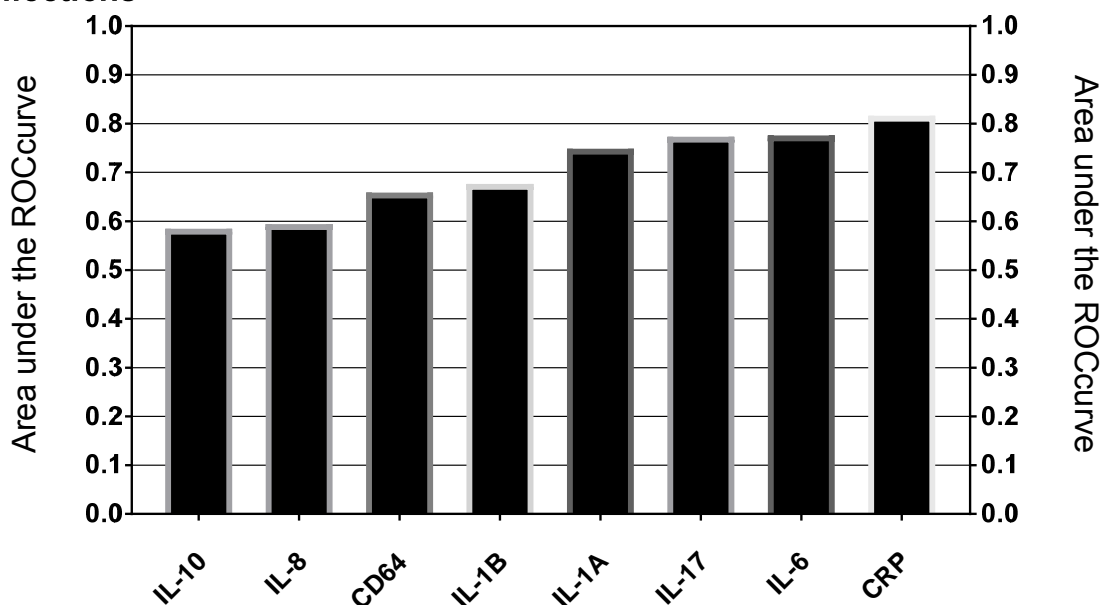
Summary of areas under ROC curves in peripheral blood - chronic infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
CD64	0.59	0.473 - 0.706	770.00	65.00	61.11
IL-10	0.63	0.5020 - 0.749	0.36	95.00	44.44
IL-8	0.63	0.499 - 0.763	3.49	100.00	31.48
IL-1B	0.68	0.541 - 0.814	0.83	50.00	87.04
IL-17	0.77	0.653 - 0.878	1.03	85.00	63.46
IL-6	0.80	0.700 - 0.903	2.54	95.00	62.96
IL-1A	0.81	0.717 - 0.912	0.77	90.00	67.31
CRP	0.82	0.713 - 0.899	29.92	80.00	85.19

Figure 4-29 Summary of diagnostic performance of peripheral blood infection markers chronic infections (>6weeks since surgery) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for infections diagnosed more than 6 weeks after surgery, chronic infections (n=20).

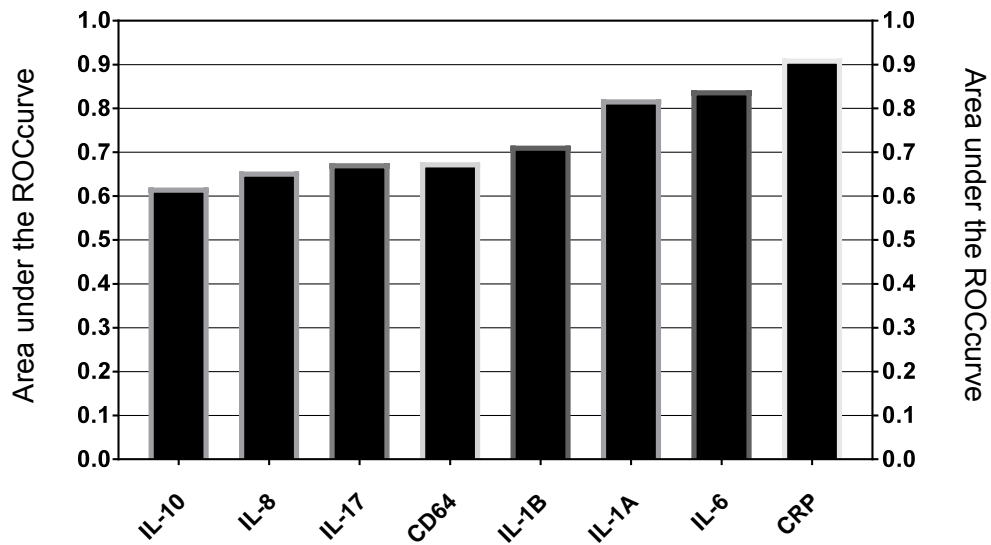
Summary of areas under ROC curves in peripheral blood - Gram +ve infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested Cut-off	Sensitivity	Specificity
IL-10	0.58	0.455 - 0.713	0.36	82.61	46.30
IL-8	0.59	0.467 - 0.721	3.49	100.00	31.48
CD64	0.66	0.542 - 0.763	770.00	69.57	61.11
IL-1B	0.68	0.547 - 0.805	0.32	69.57	62.96
IL-1A	0.75	0.634 - 0.863	0.88	73.91	75.00
IL-17	0.77	0.662 - 0.883	1.03	86.96	63.46
IL-6	0.78	0.672 - 0.881	2.34	86.96	59.26
CRP	0.82	0.712 - 0.896	29.92	78.26	85.19

Figure 4-30 Summary of diagnostic performance of peripheral blood infection markers for infections caused by Gram +ve infections. Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from Youden's J statistic and sensitivity and specificity values at this cut-off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for infections caused by gram positive bacteria (n=23).

Summary of areas under ROC curves in peripheral blood - Gram -ve infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut off	Sensitivity	Specificity
IL-10	0.619342	0.441 - 0.797	0.258619	100	27.78
IL-8	0.656379	0.502 - 0.810	5.19236	100	40.74
IL-17	0.675214	0.516 - 0.834	0.475669	100	44.23
CD64	0.676955	0.456 - 0.897	1017.41	66.67	75.93
IL-1B	0.715021	0.535 - 0.894	0.834565	55.56	87.04
IL-1A	0.820513	0.702 - 0.938	0.803372	88.89	71.15
IL-6	0.841564	0.723 - 0.959	2.435243	100	61.11
CRP	0.91358	0.825 - 1.01	35.69125	88.89	88.89

Figure 4-31 Summary of diagnostic performance of peripheral blood infection markers for infections caused by Gram -ve infections Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for CD64, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in peripheral blood for infections caused by gram negative bacteria (n=7).

4.5.2 Discussion peripheral blood markers of joint replacement infection

Of the makers evaluated CD64, IL-10, IL-8 and IL-1B showed no statistical differences between their levels in control patients and patients with any sub category of infection.

IL 1 α and IL-17 showed some statistical differences between levels in controls and patients with infection. IL-1 α levels were significantly higher when considering all infections together (n=32) and when considering chronic infections together (n=20) compared to controls (p<0.001). In addition modest statistical differences were noted between IL-1 α levels in controls compared to patients who had infections caused by gram +ve bacteria (n=23) and gram -ve bacteria (n=7) (p<0.05). IL-17 showed statistically higher concentrations in all infections (p<0.01) in chronic infections (n=20) (p<0.01) and in gram -ve infections (n=7) (p<0.05).

CRP and IL-6 showed a more consistent pattern of changes in infection. In the case of CRP highly statistically significant differences between all infections overall (n=32), acute infections less than 4 weeks from surgery (n=8), acute infections less than 6 weeks from surgery (n=12), infections with symptoms of less than 6 weeks duration (n=21), and gram positive infections (n=23) all (p<0.001). More modest statistical differences were noted when comparing CRP levels in controls to chronic infections (n=20) and gram -ve infections (n=7) (p<0.05). IL-6 levels showed a less consistent pattern of differences which is reflected in consistently lower AUCs.

The performance of each marker as measured using receiver operating characteristic curves is a reflection of how well they can differentiate between patients with infection and controls. It therefore follows that markers such as IL-6 and CRP which showed the most consistent differences showed consistently high areas under the curve. CRP had the greatest area under the curve in all infections and in every category of infection. IL-6 also performed consistently well. CD64 showed its highest area under the curve in acute infections less than 4 weeks (n=8) with an area under the curve of 0.833 meaning in this group of infections its performance was ahead of IL-6 (AUC 0.817) but still behind CRP (AUC 0.919).

CRP and IL-6 were consistently the best discriminatory markers across this series. Even in early infection at less than 4 weeks the theoretical disadvantage of CRP and its sustained elevation following surgery did not impair its ability to differentiate between patients with infection and controls. Of note CD64 had its lowest areas under the curve in chronic infection where it was the worst performing marker (AUC 0.59). Also of note was that IL-1 α showed its highest performance in chronic infections (AUC 0.81) outperforming IL-6 (AUC 0.80) but still inferior to CRP (AUC 0.82).

This series is the first to evaluate CD64, IL-1 α , IL-1 β , IL-17, IL-8 and IL-10 in peripheral blood from patients with joint replacement infection. As such there are no previous studies against which to benchmark our results. In the case of CD64 there had been a previous study evaluating its use in diagnosing local musculoskeletal infection e.g. cellulitis in patients with rheumatoid arthritis. This series was particularly heterogeneous and had included two patients with joint replacements. The authors in that study compared CD64 to CRP as a diagnostic marker showing that CD64 had a higher area under the curve⁹⁰. Other than this most studies evaluating CD64 have been in neonatal sepsis where it has shown varying results. Perhaps the most relevant study in adults comes from the Mayo clinic which included 120 ITU patients with sepsis and compared CRP with CD64. In this series CRP with an AUC of 0.86 outperformed CD64 (AUC 0.83)¹³². Our patient group at 4 weeks from surgery is the closest comparator we have to this kind of level of inflammatory insult and to some extent our findings mirror these with CD64 showing a similar AUC.

The evaluation of IL-1 α , IL-1 β , IL-17, IL-8 and IL-10 in peripheral blood was driven by their consistently excellent performance as diagnostic markers in synovial fluid. However they did not show increases in peripheral blood that were significant and therefore did not show high AUCs in this cohort. Similar findings have been seen in other analogous clinical situations. For example Conway Morris et al¹³³ looked at cytokine levels in bronchoalveolar lavage (BAL) samples from intensive care patients as a potential means of distinguishing ventilator associated pneumonia (VAP) (infective) to other syndromes that mimic this condition. Although they found significantly higher levels of IL-8 and IL-1 β in BAL samples there were no differences in peripheral blood of the intensive care patients compared to healthy controls. This suggests that similar to joint replacement

infection patients local levels in a closed compartment are not necessarily mirrored in the systemic circulation, the same phenomenon may explain the poor performance of IL-17, IL-10 and IL-1 α overall. The mechanisms underlying the increase observed in IL-1 α concentrations in chronic infection are however unclear.

In summary CRP was consistently the best performing biomarker in peripheral blood across all infection types. Despite the known elevation of CRP in the early post-operative period this remained the case even in infections occurring within the first 4 weeks of surgery where CRP demonstrated an AUC of 0.92 and at the optimal cut off of 101 a sensitivity of 87.5 % and specificity of 94.4 %. These findings mirror those from recent work by Yi et al ⁸⁷ who reviewed hip replacement patients who had an aspiration of their joint within 6 weeks of their primary procedure. In their series which included 36 infections from 6033 consecutive total hip replacements they found CRP to have an AUC of 0.93 for diagnosing infection and at their optimal cut off of 93 mg/l a sensitivity of 88% and specificity of 100% were reported.

It is also important to consider these data in the context of the individual assay's performance parameters and where the measures fall in relation to these. The lower limit of detection (LLOD) describes the lowest concentration of analyte that an assay can discriminate from analytical noise ¹³⁴, in the case of the MSD V plex assays this is defined as the calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). Perhaps more importantly however is the lower limit of quantitation (LLOQ) which can be defined as the lowest concentration at which a biomarker can be reliably measured within a predefined range for total analytical error ¹³⁵. In the case of the MSD V plex assays this has been defined as the lowest concentration at which the coefficient of variation of the calculated concentration is less than 20% and the recovery of each analyte is within 80% to 120% of the known value. The ideal biomarker would be absent in the absence of disease and present in great abundance in disease states. The biomarkers which consistently performed best here namely CRP and IL-6 came closest to this with statistically different levels in patients with infection and those without. The median concentrations measured of these markers were also consistently above the lower limit of quantitation for their respective assays in both infected and non-infected cases allowing us to have greater confidence in these measures

and subsequent conclusions relating to them. In the case of IL-8 values in infected and non-infected cases were also consistently above the LLOQ. In the case of IL-1 β , IL-10, IL-1 α and IL-17 the median values for both infected and non-infected cases were all above the LLOD but fell below the LLOQ. As such the findings for these biomarkers must be interpreted with caution as values below the LLOQ are more susceptible to error and therefore potentially less accurate.

4.6 Levels of synovial alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17in joint replacement infection

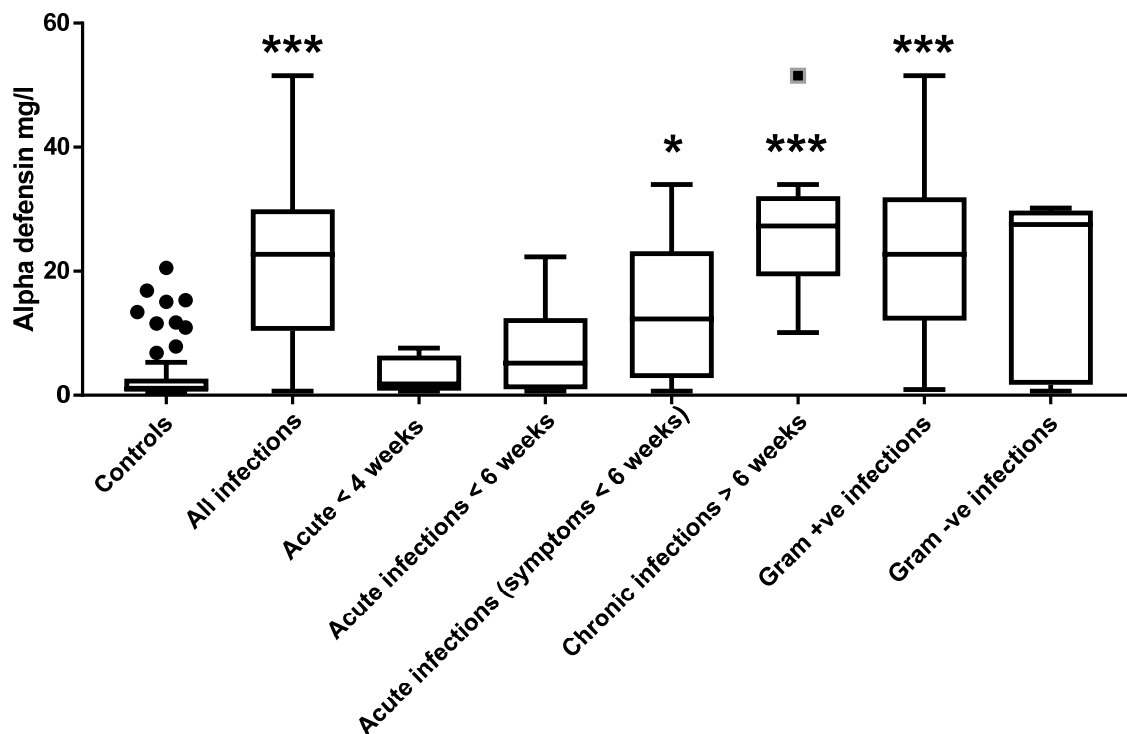


Figure 4-32 Synovial Alpha defensin concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median Alpha defensin concentration in controls (n=54) was 0.9551pg/ml (Range; 0.5175–20.54, Mean 3.335, SD 5.033, SEM 0.6849), median Alpha defensin concentration in all infections (n=24) was 22.74pg/ml (Range; 0.6799–51.53, Mean 20.21, SD 12.99, SEM 2.652), median Alpha defensin concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 1.853pg/ml (Range; 0.6799–7.595, Mean 2.995, SD 3.204, SEM 1.602), Median Alpha defensin concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 5.18pg/ml (Range; 0.6799–22.29, Mean 7.425, SD 7.688, SEM 2.718), Median Alpha defensin concentration in acute infections with symptom duration less than 6 weeks (n=15) was 12.31pg/ml (Range; 0.6799–33.98, Mean 14.36, SD 11.19, SEM 2.889), Median Alpha defensin concentration in chronic infections (n=16) was 27.31pg/ml (Range; 10.13–51.53, Mean 26.6, SD 10.02, SEM 2.506), Median Alpha defensin concentration in infections caused by Gram stain positive organisms (n=18) was 22.74pg/ml (Range; 0.942–51.53, Mean 21.49, SD 12.77, SEM 3.009), Median Alpha defensin concentration in infections caused by gram stain negative organisms (n=5) was 27.5pg/ml (Range; 0.6799–30.18, Mean 18.09, SD 14.99, SEM 6.703). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

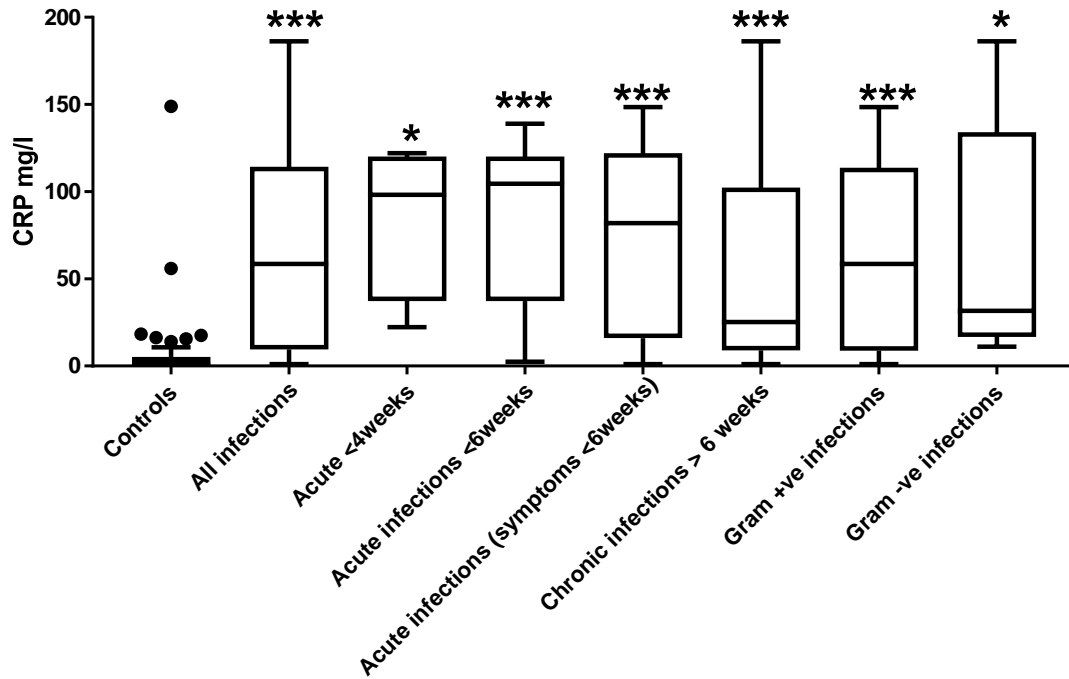


Figure 4-33 Synovial CRP concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median CRP concentration in controls (n=54) was 0.8461pg/ml (Range; 0.003556–148.9, Mean 7.404, SD 22.79, SEM 3.29), median CRP concentration in all infections (n=24) was 58.49pg/ml (Range; 1.051–186.3, Mean 65.1, SD 57, SEM 11.63), median CRP concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 98.27 pg/ml (Range;22.32-122, Mean 85.22, SD 45.39, SEM 22.7), Median CRP concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 104.5pg/ml (Range; 2.528-139, Mean 86.44, SD 48.78, SEM 17.25), Median CRP concentration in acute infections with symptom duration less than 6 weeks (n=15) was 82pg/ml (Range; 10.51-148.5, Mean 74.39, SD 53.89, SEM 13.91), Median CRP concentration in chronic infections (n=16) was 25.23pg/ml (Range; 1.051–186.3, Mean 54.43, SD 59.21, SEM 4.8), Median CRP concentration in infections caused by Gram stain positive organisms (n=18) was 58.49pg/ml (Range; 1.051–148.5, Mean 61.49, SD 54.45, SEM 12.83), Median CRP concentration in infections caused by gram stain negative organisms (n=5) was 31.69pg/ml (Range; 11.17-186.3, Mean 66.7, SD 72.16, SEM 32.27). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

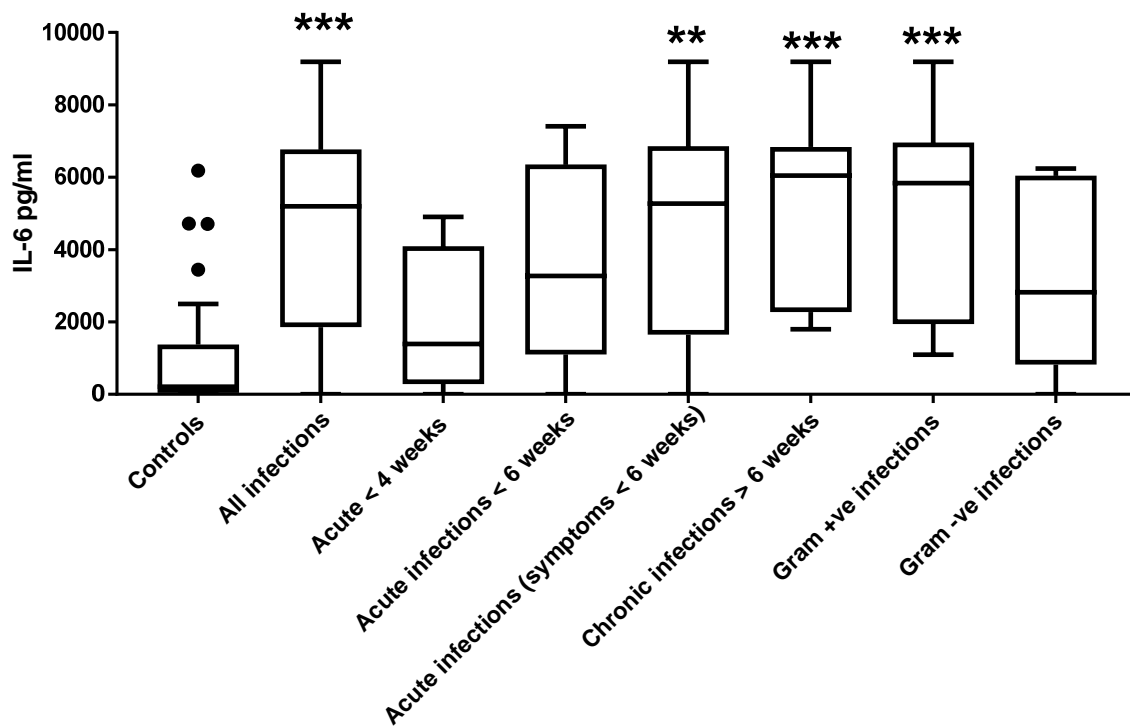


Figure 4-34 Synovial IL-6 concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-6 concentration in controls (n=54) was 209.2pg/ml (Range; 0.03–9314, Mean 1114, SD 1957, SEM 226.3), median IL-6 concentration in all infections (n=24) was 5196pg/ml (Range; 4.162–9189, Mean 4636, SD 2604, SEM 531.6), median IL-6 concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 1390pg/ml (Range; 4.162 – 4905, Mean 1922, SD 2104, SEM 1052), Median IL-6 concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 3275pg/ml (Range; 4.162–7407, Mean 3522, SD 2872, SEM 1015), Median IL-6 concentration in acute infections with symptom duration less than 6 weeks (n=15) was 5272pg/ml (Range; 4.162– 9189 Mean 4534, SD 2961, SEM 764.6), Median IL-6 concentration in chronic infections (n=16) was 6048pg/ml (Range; 1797–9189, Mean 5193, SD 2358, SEM 589.4), Median IL-6 concentration in infections caused by Gram stain positive organisms (n=18) was 5839pg/ml (Range; 1094–9189, Mean 1989, SD 2612, SEM 615.6), Median IL-6 concentration in infections caused by gram stain negative organisms (n=5) was 2821pg/ml (Range; 4.162–,6236 Mean 3313, SD 2693, SEM 1204). Statistical analysis was performed by Kruskal Wallis test and Dunn’s test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

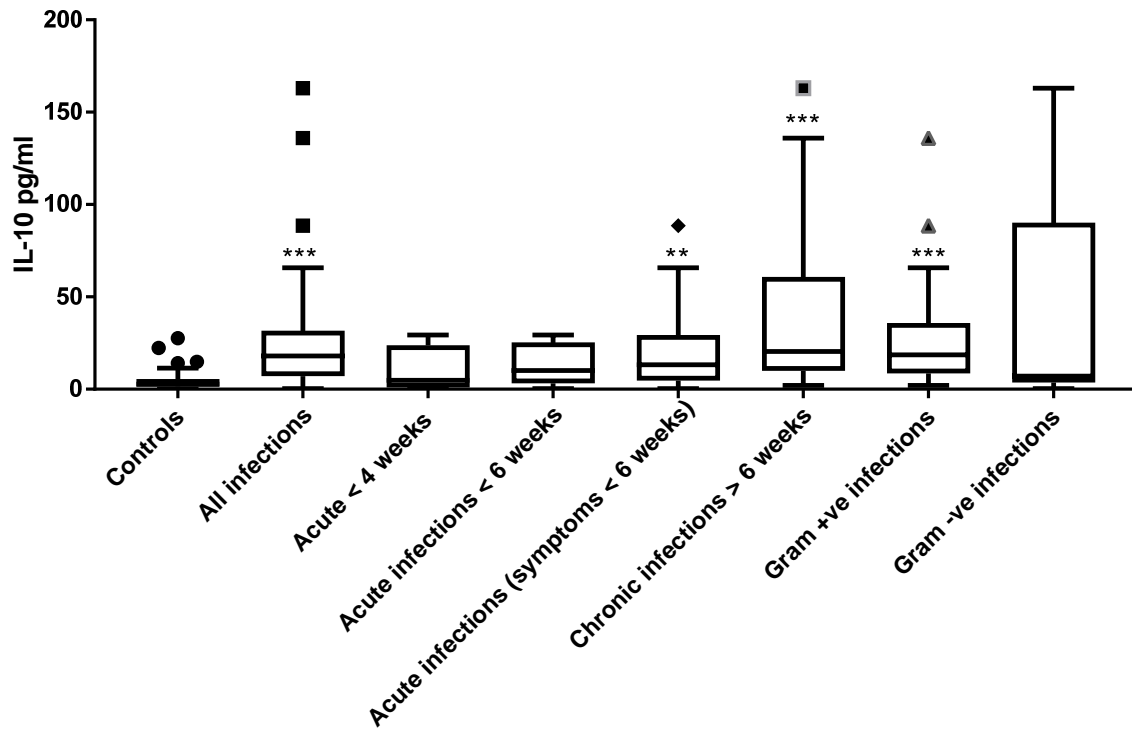


Figure 4-35 Synovial IL-10 concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-10 concentration in controls (n=54) was 2.851pg/ml (Range; 0.015-27.71, Mean 4.392, SD 5.267, SEM 0.7168), median IL-10 concentration in all infections (n=24) was 18.05pg/ml (Range; 0.3879-163, Mean 32.35, SD 41.73, SEM 8.517), median IL-10 concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 4.856pg/ml (Range; 0.3879-29.33, Mean 9.857, SD 13.26, SEM 6.632), Median IL-10 concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 10.15pg/ml (Range; 0.3879-29.33, Mean 12.97, SD 11.22, SEM 3.965), Median IL-10 concentration in acute infections with symptom duration less than 6 weeks (n=15) was 13.32pg/ml (Range; 0.3879-88.58 Mean 22.32, SD 25.07, SEM 6.472), Median IL-10 concentration in chronic infections (n=16) was 20.44pg/ml (Range; 2.028-163, Mean 42.03, SD 48.07, SEM 12.02), Median IL-10 concentration in infections caused by Gram stain positive organisms (n=18) was 18.54pg/ml (Range; 2.028-136.1, Mean 30.68, SD 34.64, SEM 8.165), Median IL-10 concentration in infections caused by gram stain negative organisms (n=5) was 7.125pg/ml (Range; 0.3879-163 Mean 38.94, SD 69.59, SEM 31.12). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

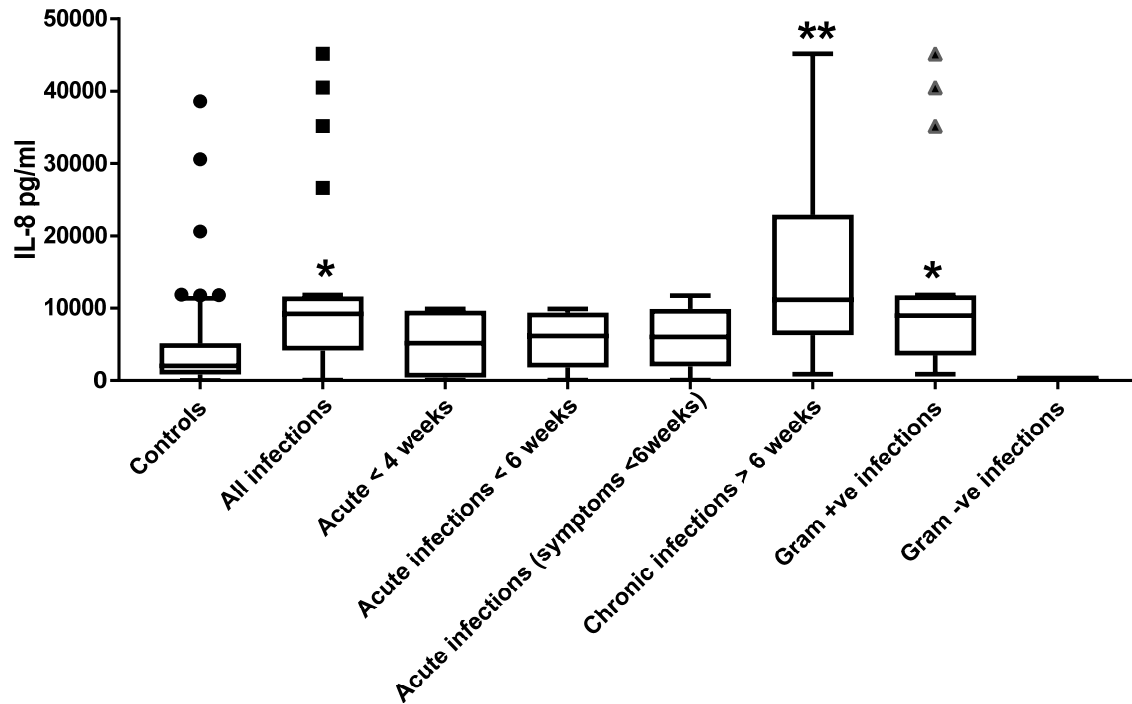


Figure 4-36 Synovial IL-8 concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-8 concentration in controls (n=54) was 2020pg/ml (Range; 0.02–38584 , Mean 4864, SD 7364, SEM 1002), median IL-8 concentration in all infections (n=24) was 9204pg/ml (Range; 51.17-45182, Mean 12005, SD 12275, SEM 2508), median IL-8 concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 5138pg/ml (Range; 51.17–9903, Mean 5058, SD 5015, SEM 2508), Median IL-8 concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 6156pg/ml (Range; 51.17–9903 , Mean 5622, SD 3810, SEM 1347), Median IL-8 concentration in acute infections with symptom duration less than 6 weeks (n=15) was 6009pg/ml (Range; 51.17–11713 Mean 6103, SD 4112, SEM 1062), Median IL-8 concentration in chronic infections (n=16) was 11168pg/ml (Range; 862.9–45182, Mean 15197, SD 13844, SEM 3461), Median IL-8 concentration in infections caused by Gram stain positive organisms (n=18) was 8971pg/ml (Range; 862.9–45182 , Mean 12514, SD 13420, SEM 3163), Median IL-8 concentration in infections caused by gram stain negative organisms (n=5) was 11.98pg/ml (Range; 5.786–21.44, Mean 12.75, SD 6.185, SEM 2.766). Statistical analysis was performed by Kruskal Wallis test and Dunn’s test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

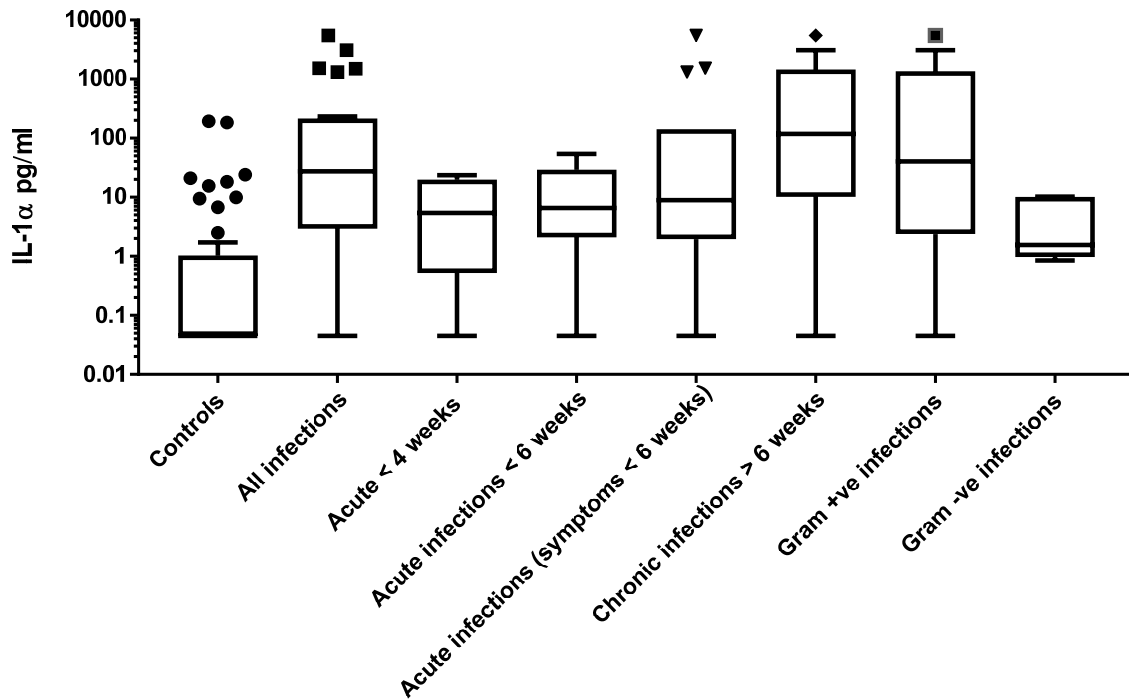


Figure 4-37 Synovial IL-1A concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-1A concentration in controls (n=54) was 0.045pg/ml (Range; 0.045–5503, Mean 9.106, SD 35.96, SEM 4.893), median IL-1A concentration in all infections (n=24) was 27.43pg/ml (Range; 0.045–5503, Mean 574, SD 1290, SEM 263.4), median IL-1A concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 5.426pg/ml (Range; 0.045–23.51, Mean 8.602, SD 10.64, SEM 5.321), Median IL-1A concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 6.585pg/ml (Range; 0.045–54.4, Mean 15.87, SD 19.23, SEM 6.799), Median IL-1A concentration in acute infections with symptom duration less than 6 weeks (n=15) was 8.898pg/ml (Range; 0.045–5503, Mean 573.9, SD 1449, SEM 374.2), Median IL-1A concentration in chronic infections (n=16) was 117.7pg/ml (Range; 0.045–5503, Mean 853, SD 1518, SEM 379.5), Median IL-1A concentration in infections caused by Gram stain positive organisms (n=18) was 40.25pg/ml (Range; 0.045–5503, Mean 740.9, SD 1460, SEM 344.2), Median IL-1A concentration in infections caused by gram stain negative organisms (n=5) was 1.56pg/ml (Range; 0.8507–10.27, Mean 4.748, SD 4.904, SEM 2.193). Statistical analysis was performed by Kruskal Wallis test and Dunn’s test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

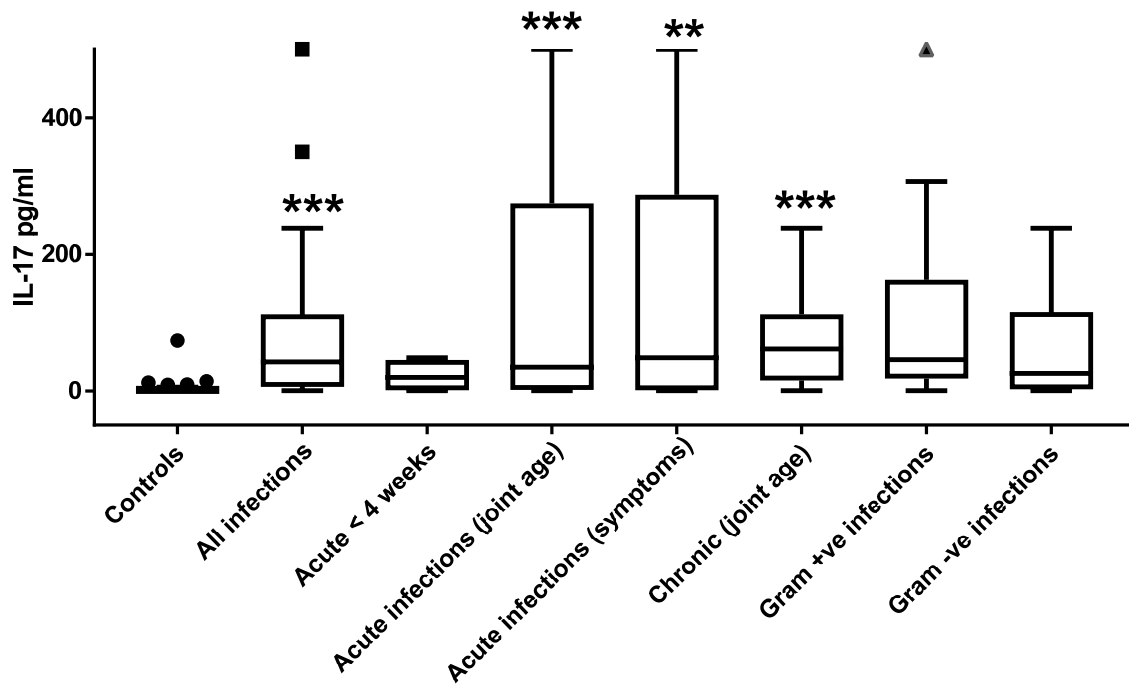


Figure 4-38 Synovial IL-17 concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-17 concentration in controls (n=54) was 1.434pg/ml (Range; 0.2573–1864, Mean 38.09, SD 253.4, SEM 34.49), median IL-17 concentration in all infections (n=24) was 42.52pg/ml (Range; 0.37–2627, Mean 230, SD 582.4, SEM 118.9), median IL-17 concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 19.86pg/ml (Range; 0.37–48.44, Mean 22.13, SD 24.06, SEM 12.03), Median IL-17 concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 34.71pg/ml (Range; 0.37–2627, Mean 515.5, SD 976.1, SEM 345.1), Median IL-17 concentration in acute infections with symptom duration less than 6 weeks (n=15) was 48.44pg/ml (Range; 0.37–2627 Mean 329.2, SD 725.2, SEM 187.2), Median IL-17 concentration in chronic infections (n=16) was 61.66pg/ml (Range; 03.7–306.6, Mean 87.28, SD 101.5, SEM 25.37), Median IL-17 concentration in infections caused by Gram stain positive organisms (n=18) was 45.65pg/ml (Range; 0.37–2627, Mean 286.3, SD 665.6, SEM 156.9), Median IL-17 concentration in infections caused by gram stain negative organisms (n=5) was 3.241pg/ml (Range; 0.37–238.2, Mean 63.9, SD 102.4, SEM 45.78). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

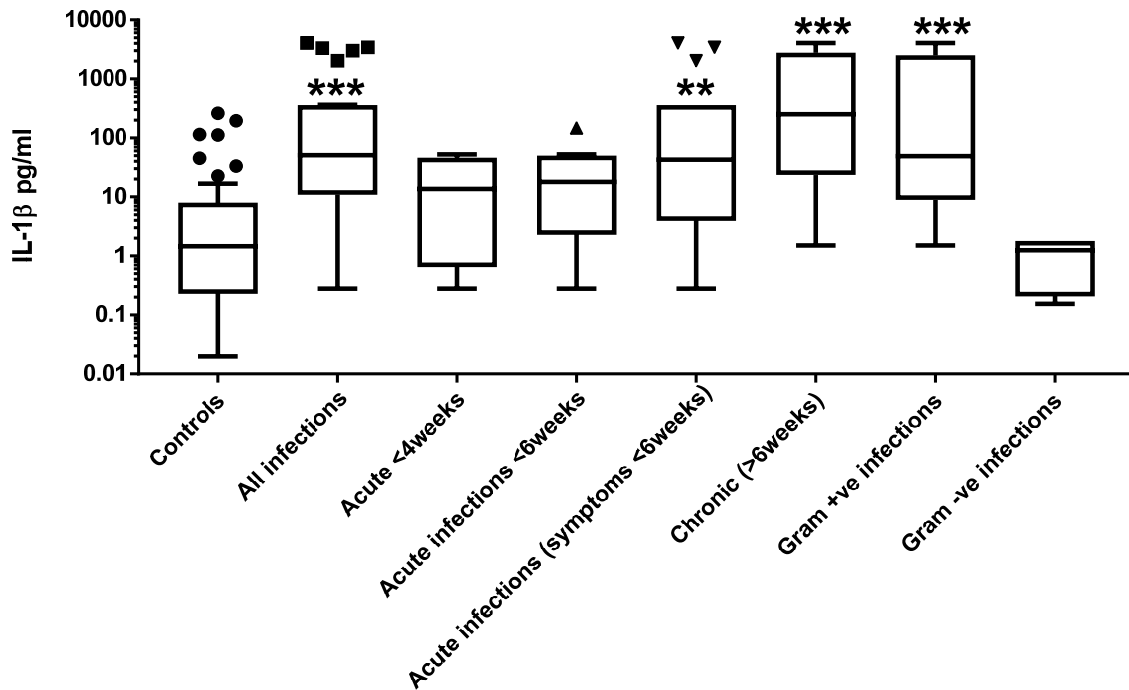
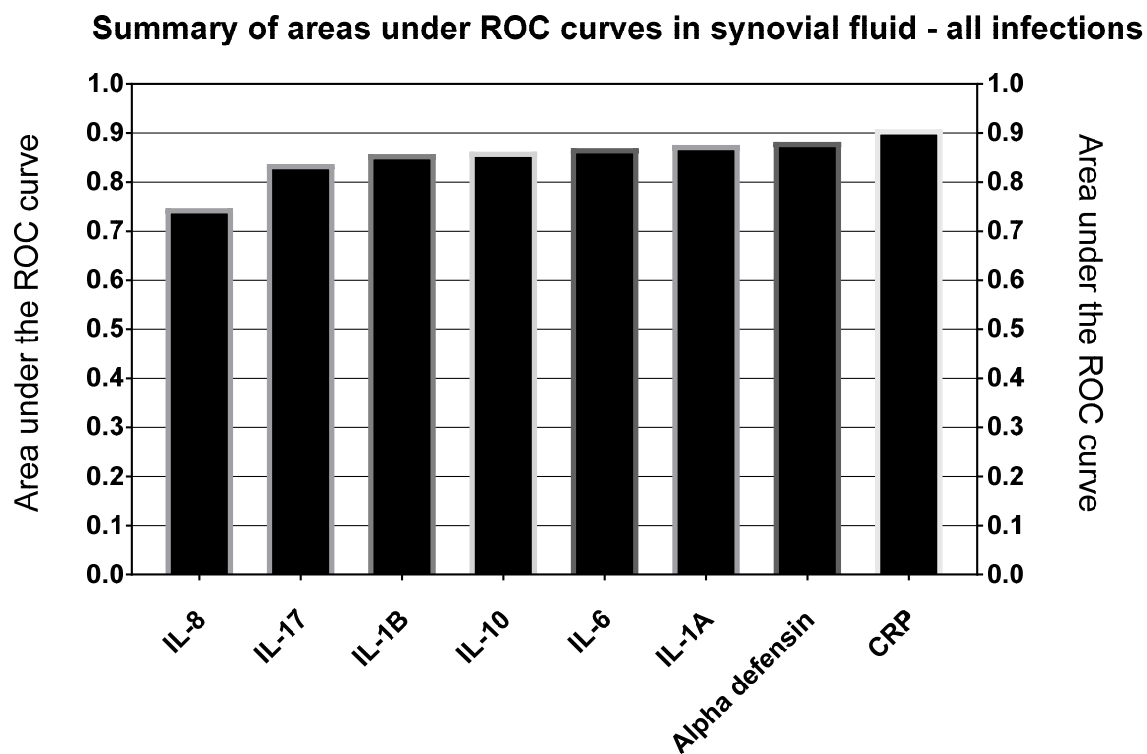


Figure 4-39 Synovial IL-1B concentration in infected patients (n=24) compared to controls (n=54). Data shown as Box and whisker plots. Median IL-1B concentration in controls (n=54) was 1.47pg/ml (Range; 0.02-262.7, Mean 17.26, SD 47.99, SEM 6.53), median IL-1B concentration in all infections (n=24) was 51.08pg/ml (Range; 0.2796-4067, Mean 734.5, SD 1324, SEM 270.3), median IL-1B concentration in acute infections occurring less than 4 weeks from surgery (n=4) was 13.66pg/ml (Range; 0.2796-52.96, Mean 20.14, SD 24.76, SEM 12.38), Median IL-1B concentration in acute infections occurring in less than 6 weeks after surgery (n=8) was 18.12pg/ml (Range; 0.2796-147.9, Mean 35.73, SD 49.4, SEM 17.47), Median IL-1B concentration in acute infections with symptom duration less than 6 weeks (n=15) was 42.78pg/ml (Range; 0.2796-4067 Mean 685.1, SD 1355, SEM 349.8), Median IL-1B concentration in chronic infections (n=16) was 252.8pg/ml (Range; 1.508-4067, Mean 1084, SD 1515, SEM 378.8), Median IL-1B concentration in infections caused by Gram stain positive organisms (n=18) was 49.2pg/ml (Range; 1.508-4067, Mean 989.7, SD 1508, SEM 365.8), Median IL-1B concentration in infections caused by gram stain negative organisms (n=5) was 1.248pg/ml (Range; 0.1539-1.801, Mean 1.051, SD 0.8036, SEM 0.3594). Statistical analysis was performed by Kruskal Wallis test and Dunn's test for multiple comparison where statistical significance $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$

4.6.1 ROC curve analysis synovial fluid markers of joint replacement infection

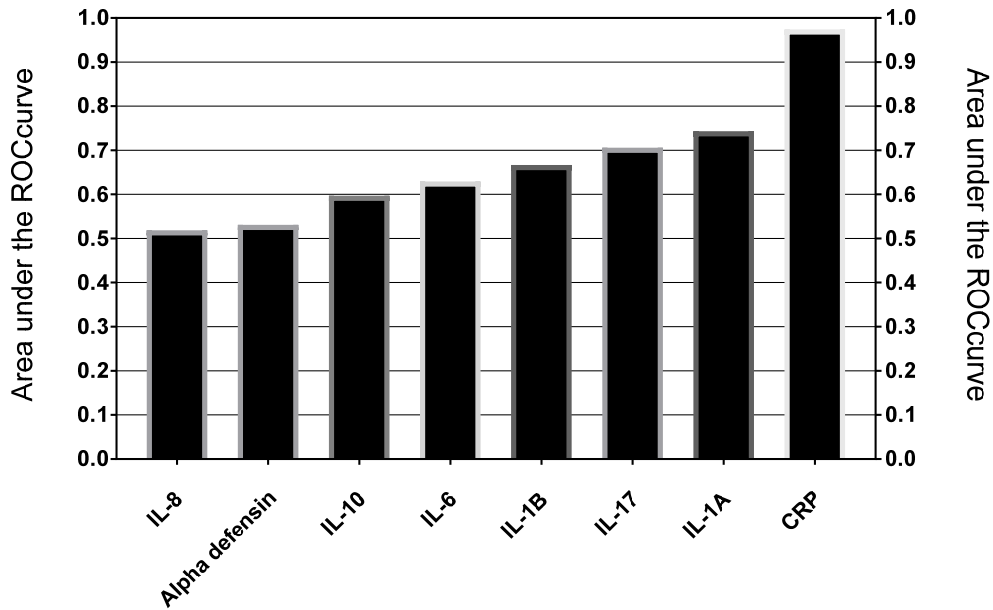
ROC curve areas for synovial fluid infection markers are summarized below for each category of infection.



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-8	0.75	0.63 - 0.87	4863.00	75.00	75.93
IL-17	0.84	0.72 - 0.95	14.16	75.00	96.30
IL-1B	0.86	0.77 - 0.95	6.65	83.33	74.07
IL-10	0.86	0.76 - 0.96	6.44	83.33	83.33
IL-6	0.87	0.78 - 0.96	1633.00	87.50	79.63
IL-1A	0.88	0.79 - 0.96	1.71	87.50	81.48
Alpha defensin	0.88	0.78 - 0.95	6.82	83.33	81.25
CRP	0.91	0.84 - 0.98	8.13	87.50	81.25

Figure 4-40 Summary of synovial infection markers – all infections. Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden’s j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid for all infections (n=24).

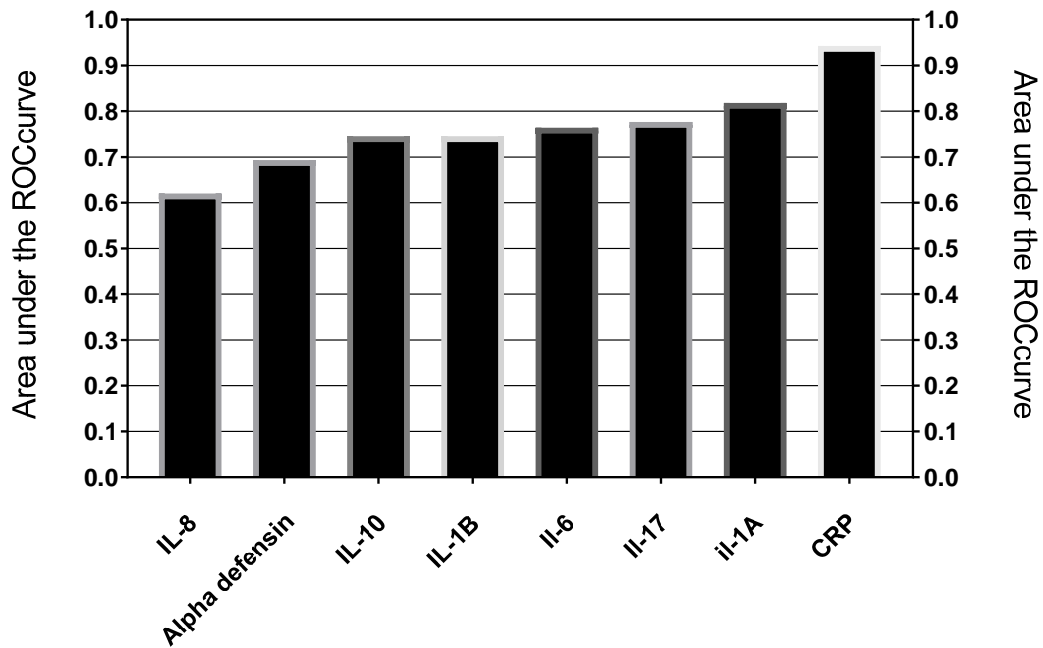
Summary of areas under ROC curves in synovial fluid - acute infections < 4weeks



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-8	0.52	0.161 – 0.876	8420	50	71.63
Alpha defensin	0.53	0.388 - 0.671	2.43	50.00	72.92
IL-10	0.60	0.191 - 1.001	6.44	50.00	83.33
IL-6	0.63	0.238 - 1.021	1108.41	75.00	72.22
IL-1B	0.67	0.353 - 0.979	22.57	50.00	88.89
IL-17	0.71	0.320 - 1.092	2.84	75.00	75.93
IL-1A	0.74	0.478 - 1.001	1.71	75.00	81.48
CRP	0.97	0.931 - 1.017	18.36	100.00	95.83

Figure 4-41 Summary of synovial infection markers – acute infections (<4weeks) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden’s j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid from acute infections less than 4 weeks from surgery (n=4).

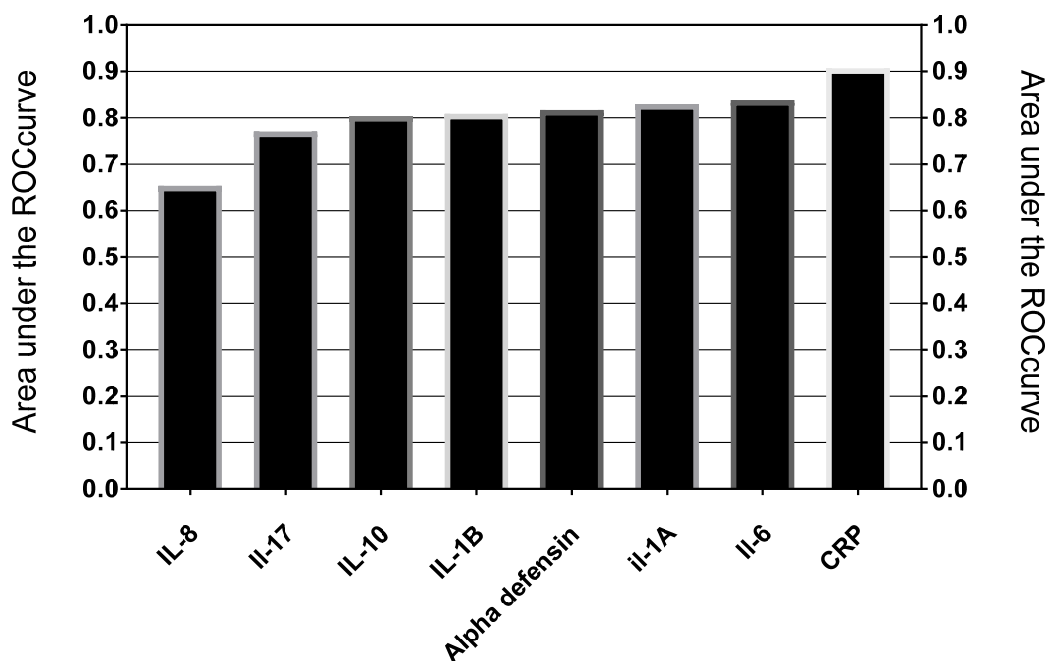
Summary of areas under ROC curves in synovial fluid - acute infections < 6weeks



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-8	0.62037037	0.414 - 0.826	4863.549075	62.5	75.93
Alpha Defensin	0.693	0.555 - 0.809	0.625	75	66.67
IL-10	0.74537037	0.515 - 0.974	6.444168827	62.5	83.33
IL-1 β	0.74537037	0.565 - 0.925	1.723000218	87.5	59.26
IL-6	0.763888889	0.543 - 0.984	936.6345872	87.5	70.37
IL-17	0.77662037	0.551 - 1.00	14.15817662	62.5	96.3
IL-1 α	0.818287037	0.667 - 0.969	1.713776825	87.5	81.48
CRP	0.942708333	0.8640 - 1.021	18.36255451	87.5	95.83

Figure 4-42 Summary of synovial infection markers – acute infections (<6weeks) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden’s j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid from acute infections less than 6 weeks from surgery (n=8).

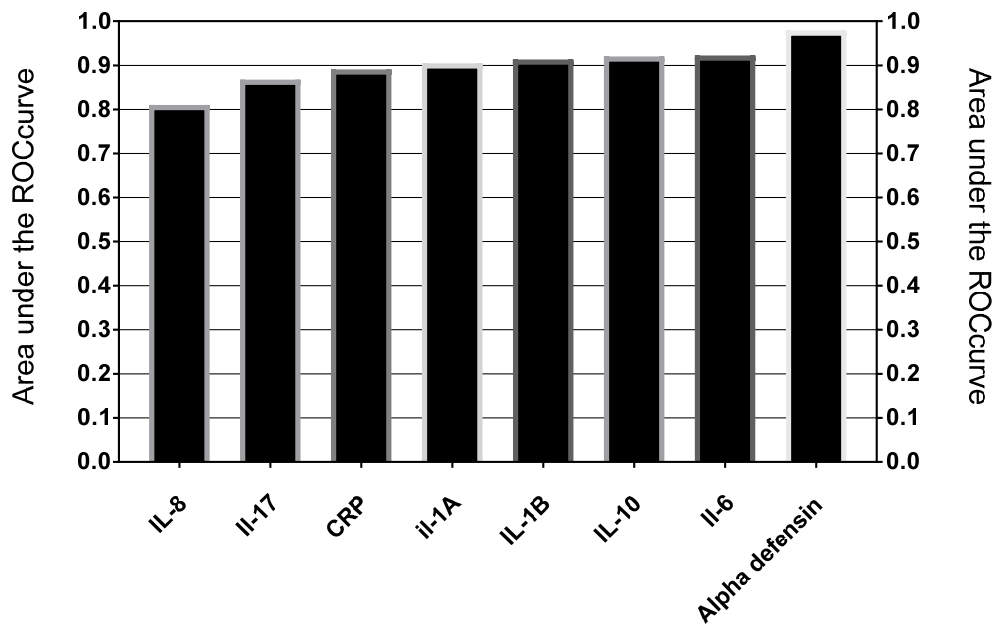
Summary of areas under ROC curves in synovial fluid - symptoms <6 weeks



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested Cut-off	Sensitivity	Specificity
IL-8	0.65	0.502 - 0.803	4863.55	60.00	75.93
IL-17	0.77	0.598 - 0.942	14.16	66.67	96.30
IL-10	0.80	0.656 - 0.951	6.44	73.33	83.33
IL-1B	0.81	0.683 - 0.933	22.57	60.00	88.89
Alpha defensin	0.82	0.699 - 0.903	6.82	73.33	81.25
IL-1A	0.83	0.711 - 0.947	1.01	86.67	75.93
IL-6	0.84	0.706 - 0.969	936.63	93.33	70.37
CRP	0.91	0.823 - 0.991	15.66	80.00	89.58

Figure 4-43 Summary of synovial infection markers – symptoms (<6weeks) Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden’s j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid from infections with symptom duration less than 6 weeks (n=15)

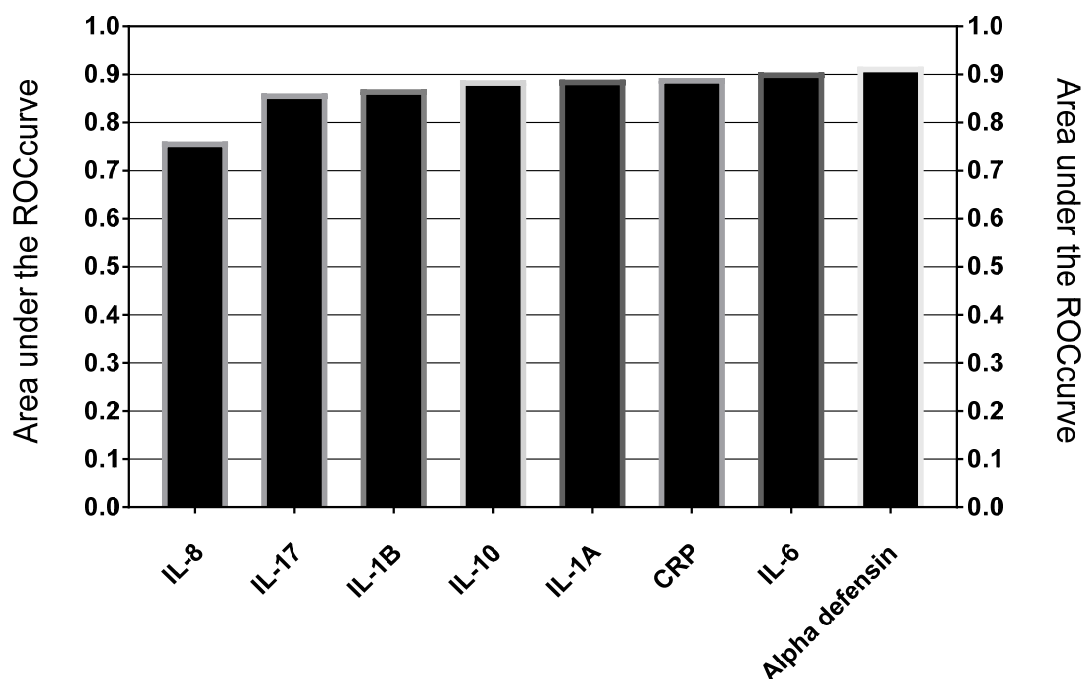
Summary of areas under ROC curves in synovial fluid - chronic infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut-off	Sensitivity	Specificity
IL-8	0.81	0.695 - 0.925	5969.24	81.25	77.78
IL-17	0.87	0.735 - 0.999	14.16	81.25	96.30
CRP	0.89	0.809 - 0.972	8.13	87.50	81.25
IL-1A	0.90	0.816 - 0.992	1.01	93.75	75.93
IL-1B	0.91	0.836 - 0.989	6.65	93.75	74.07
IL-10	0.92	0.838 - 1.012	6.44	93.75	83.33
IL-6	0.92	0.860 - 0.984	1632.94	100.00	79.63
Alpha defensin	0.98	0.912 - 0.999	7.85	100.00	85.19

Figure 4-44 Summary of synovial infection markers – chronic infections > 6 weeks since surgery. Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid from chronic infections > 6 weeks since surgery (n=16)

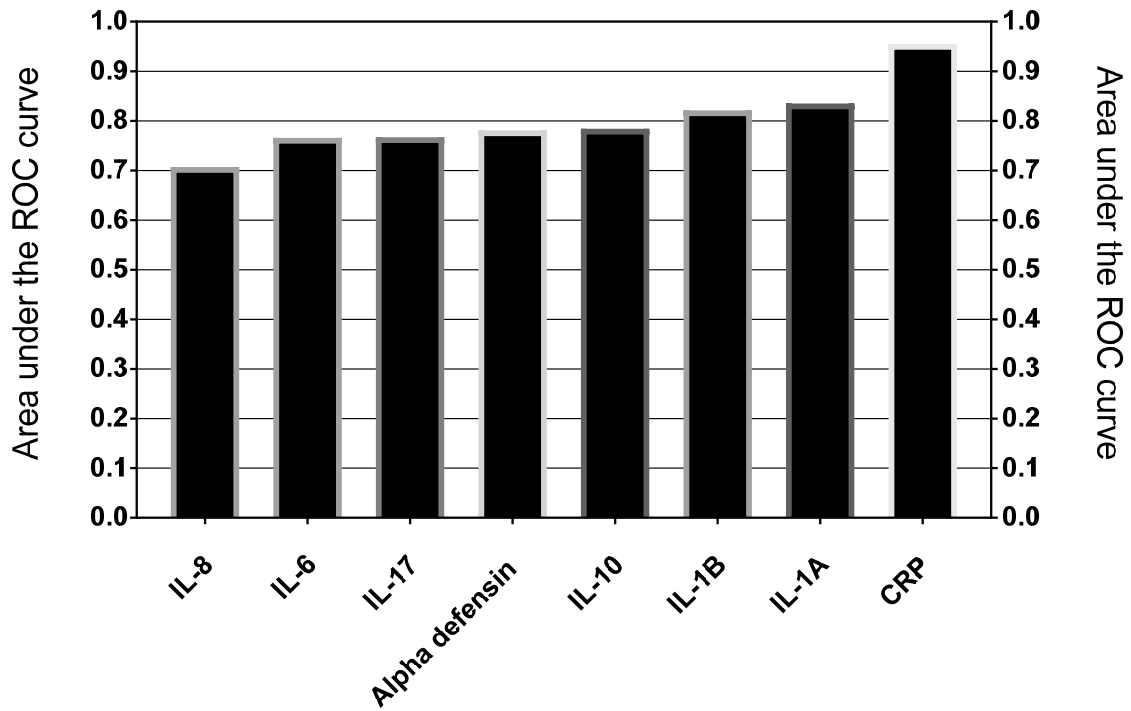
Summary of areas under ROC curves in synovial fluid - Gram +ve infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested Cut-off	Sensitivity	Specificity
IL-8	0.76	0.639 - 0.881	4863.55	72.22	75.93
IL-17	0.86	0.732 - 0.988	14.16	83.33	96.30
IL-1B	0.87	0.781 - 0.957	6.65	83.33	74.07
IL-10	0.89	0.795 - 0.981	6.44	83.33	83.33
IL-1A	0.89	0.804 - 0.974	1.01	94.44	75.93
CRP	0.89	0.812 - 0.971	8.13	83.33	81.25
IL-6	0.90	0.835 - 0.973	936.63	100.00	70.37
Alpha defensin	0.92	0.821 - 0.971	7.85	88.89	83.33

Figure 4-45 Summary of synovial infection markers – infections caused by Gram +ve organisms. Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden's j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid from infections caused by gram +ve organisms (n=18)

Summary of areas under ROC curves in synovial fluid - Gram -ve infections



Test Variables	ROC Curve Area	95% Confidence Interval	Suggested cut off	Sensitivity	Specificity
IL-8	0.69	0.271 - 0.812	4863.55	80.00	77.08
IL-6	0.72	0.316 - 0.791	1632.94	80.00	77.08
IL-17	0.73	0.208 - 0.791	2.84	80.00	77.08
IL-10	0.73	0.316 - 0.833	6.44	80.00	81.25
Alpha defensin	0.78	0.266 - 0.800	20.54	60.00	100.00
IL-1B	0.80	0.287 - 0.875	15.60	80.00	83.33
IL-1A	0.82	0.133 - 0.895	9.92	80.00	87.50
CRP	0.95	0.896 - 1.013	10.76	100	85.42

Figure 4-46 Summary of synovial infection markers – infections caused by Gram -ve organisms. Bar chart and table summarizing the area under the ROC curve with 95% confidence intervals, the suggested cut-off from youden’s j statistic and sensitivity and specificity values at this cut off for alpha defensin, CRP, IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-17 measured in synovial fluid from infections caused by gram -ve organisms (n=5)

4.6.2 Discussion synovial fluid markers of joint replacement infection

To date several studies have evaluated synovial fluid biomarkers for the diagnosis of PJI^{71,106,132,136}. These have focused on patients having revision surgery and not acute infections although none of these studies have described their patient populations in detail in terms of the chronicity of the presentations of infection, duration of symptoms or indeed organism types. Of note the first publication evaluating alpha defensin in synovial fluid did however exclude patients within the first 4 weeks after their index procedure⁷³ and indeed the latest recommendations from the Zimmer Biomet advisory group do not cover the use of this test within the first 3 months following joint replacement. Furthermore in the largest evaluation of alpha defensin in synovial fluid from the CD diagnostics reference laboratory in the United States¹³⁶ the authors declare that the test is yet to be validated in “immediate post-operative patients”.

In this series CRP and alpha defensin are the best performing synovial biomarkers overall with AUCs of 0.91 and 0.88 respectively when considering all infections (n=24). In the case of CRP this AUC is comparable to previous reports in the literature evaluating synovial CRP and a recent meta-analysis and systematic review on the use of synovial fluid CRP which pooled the results from six eligible studies demonstrating a combined AUC of 0.96. In the case of alpha defensin most studies have shown better diagnostic performance in terms of sensitivity and specificity which might be explained by the exclusion of acute cases as described above.

On closer inspection of the results of this study there are clear differences in the performance of synovial CRP and alpha defensin in acute and chronic scenarios. Synovial CRP appears to have a clear diagnostic advantage in early post-operative infections. When using a cut off of 4 weeks the performance of CRP was exceptional demonstrating an AUC of 0.97 with a sensitivity of 100% and specificity of 95.83%. Using a cut off of 6 weeks post op CRP was also the best performing biomarker this time with a slightly lower AUC of 0.94 and reduced sensitivity of 87.5% and specificity of 95.83%. Of note alpha defensin performed poorly at these time points with AUCs of 0.53 at 4 weeks and 0.693 at 6 weeks.

In contrast when considering chronic infections as defined by a period of greater than six weeks from surgery alpha defensin shows a higher diagnostic accuracy than CRP with an AUC of 0.98 conferring a sensitivity of 100% and specificity of 85.19 % which is comparable to other published series ¹³⁶.

When considering assay performance the median levels of all markers measured in synovial fluid from infected patients were above the LLOQ of their respective assays but median levels of IL-1 alpha, IL-1 beta and IL-17 alpha in control samples were below the LLOQ meaning these control values are subject to potential error in their values.

The finding that different synovial fluid markers perform differently according to the clinical classification as demonstrated in this study is a novel concept. As mentioned previously, studies to date have focused on patients having revision surgery and therefore presumed chronic infection and in some cases studies have actively excluded acute infections. Although this finding is certainly exciting it must be treated with caution given the small numbers of acute cases (n=4 less than 4 weeks and n=8 less than 6 weeks, n=12 >6 weeks). Further work is clearly required particularly in order to validate the observations made here in acute infection.

Chapter 5 The effect of operating lights on laminar flow

5.1 INTRODUCTION

Deep infection in hip and knee arthroplasty is a significant complication associated with morbidity, prolonged hospital stays, high healthcare costs and increased mortality.^{116,137,138} Infection is the 3rd commonest cause for revision arthroplasty surgery in the US.¹³⁹ As the rate of implantation of primary joints increases, infection is likely to become an even greater problem in the future.¹⁴⁰

The source of bacteria resulting in intraoperative surgical site colonisation, in the absence of glove perforation, are those remaining following skin preparation or contamination by airborne bacteria.⁴⁴ Several methods are currently employed to reduce both of these sources including use of ultraclean air to reduce airborne bacterial content. Laminar flow was initially introduced by Charnley in the 1970s but became widespread following the work of Lidwell et al. who demonstrated reduced deep infections following use of ultraclean air theatres for hip replacement surgery.^{44,56} Air is initially filtered through a High Efficiency Particulate Air (HEPA) filter removing 99.97% of all particles larger than 0.3 micrometres in size⁵⁷ The system then streams this ultraclean air over the operating field. This has been shown to reduce the number of airborne particles, airborne bacteria and wound bacteria counts significantly.^{44,58,59} For these reasons the use of laminar flow theatres for lower limb arthroplasty is well established and widely used.

Recently several authors have challenged the use of laminar flow, demonstrating similar or increased infection and revision rates with laminar flow compared to conventional theatres.^{45,61,62,63} Thus whilst it currently remains the gold-standard in theatres undertaking joint replacement procedures there is now some uncertainty as to its true benefits.⁶⁰

Surgeons considering whether or not to use laminar flow must consider if effective laminar flow is being achieved over the operative field. Several factors have been demonstrated to profoundly disrupt the effect of laminar flow; interposition of a surgeon between the clean air source and the patient has been shown to increase operative field

bacterial counts 27 fold.¹⁴¹ Whilst the position of the surgeon is not easily altered other factors can be. These include avoiding the use of forced air warming and limiting circulating staff movement.^{142,143,144} An additional factor may be the positioning of theatre lights in the laminar flow. This is not a new concept; and this may be the reason that banks of lights built outside the laminar flow canopy were used to illuminate the operative field in early Charnley / Howorth theatre designs. Despite this there is a paucity of data examining the influence of lights on laminar flow. Computational fluid dynamic modelling in the engineering literature suggests lights may have a significant disruptive effect on laminar flow.^{142,145} In one such study Brohus et al. concluded operative lights “may be the single worst factor causing unintended bacterial transport in close proximity to the patient”.¹⁴²

Based on current NJR data 98% of orthopaedic implant surgery in England and Wales is performed in laminar air flow theatres¹. In contrast in the United States a survey of over 400 hospitals across 4 separate US states found that only 30% of hospitals used laminar flow in greater than 75% of cases.¹⁴⁶

5.2 Aim

To investigate the effect of contemporary surgical lighting on laminar flow using neutrally buoyant bubbles to simulate movement of bacteria

5.3 MATERIALS AND METHODS

An experiment was designed to represent the typical setup in a knee arthroplasty procedure. A mannequin knee, flexed at 90 degrees, was draped on an operating table raised 85 cm above the floor in a laminar flow theatre. The mannequin was placed in the centre of the partially walled laminar flow hood (Exflow 90, Howorth, Bolton, UK). Neutrally buoyant bubbles were produced using a bubble generator (SAI Model 5, Sage Action, Ithaca, New York). The generator combines detergent, helium and air to produce neutrally buoyant bubbles that remain suspended in still air, enabling the visualisation of air currents and serving as a visible surrogate for microscopic particles. Bubbles were introduced to the area of the operative field for 1 minute with the bubble generator outlet placed at a consistent point on the knee. This 'filling' phase allowed any bubble accumulation to occur. After 1 minute the bubble outlet was removed. High intensity lighting was positioned outside the laminar flow zone and used to illuminate the bubbles. A digital still camera (Canon 500D, Reigate, United Kingdom) was used to record the bubble clearance. Photographs were taken every 2 seconds for 40 seconds controlled by a timing controller (Giga T Pro II Hähnel, Cork, Ireland). Bubbles were counted in a predetermined area of the frame in each image using ImageJ software (National Institute of Health, Bethesda, Maryland USA). Example photographs are given in Figure 1. This method has been used previously to study laminar flow systems.^{143,144}

Experiments were performed to examine the effect of operating lights. Maquet (Rastatt, Germany) X'ten lights were used. These lights are cross shaped with a 62.5 cm widest diameter and were suspended from the ceiling of the laminar flow hood.

5.3.1 Experimental design:

1. No lights
2. One light directly above surgical field
3. Two lights touching, directly above surgical field
4. Two lights 50 cm apart
5. Two lights 160 cm apart

Configurations 1 and 2 enabled us to compare the laminar flow system's operation with and without obstruction. Configurations 3, 4 and 5 allowed us to assess different arrangements of 2 lights. The distances were chosen pragmatically; 50 cm apart was found to be the distance when a single surgeon positions the lights above the surgical site with arms outstretched; 160 cm was the furthest apart the lights could be positioned under the laminar flow hood.

In all set ups the lights were positioned in the midline sagittal plane of the mannequin and focussed on the operative field.

Each experiment was repeated 5 times and the order of experimental runs was randomised using Microsoft Excel (RANDBETWEEN function). Measurements were taken of light positions to ensure consistency between readings. All investigators remained motionless outside the laminar flow boundary throughout experiment runs and the operating theatre doors were locked to prevent inadvertent external airflow disturbances.

5.3.2 Statistical analysis

As the rate of bubble output from the bubble generator is not constant the percentage of bubbles remaining in the frame area compared to $t=0$, over time was analysed. For each lighting configuration we fit, as a model, the following exponential function:

$$P(t) = \alpha e^{-\beta t}$$

where P is the percentage of bubbles remaining; t is the time (seconds); α is a constant corresponding to the value of the function at $t=0$, i.e. $\alpha = 100$ (%); and β is the decay constant. The function, with β as the only free parameter, was fitted to data for each lighting configuration using the function *nlinfit* from Matlab (The MathWorks Inc., Natick, MA) via nonlinear least-squares regression using the Levenberg-Marquardt algorithm. The fit of each model was assessed using the coefficient of determination R^2 .

The estimated value for the decay parameter (β) [$\pm 95\%$ confidence intervals] of the model allows each lighting configuration to be compared statistically and the percentage of bubbles cleared per second calculated.

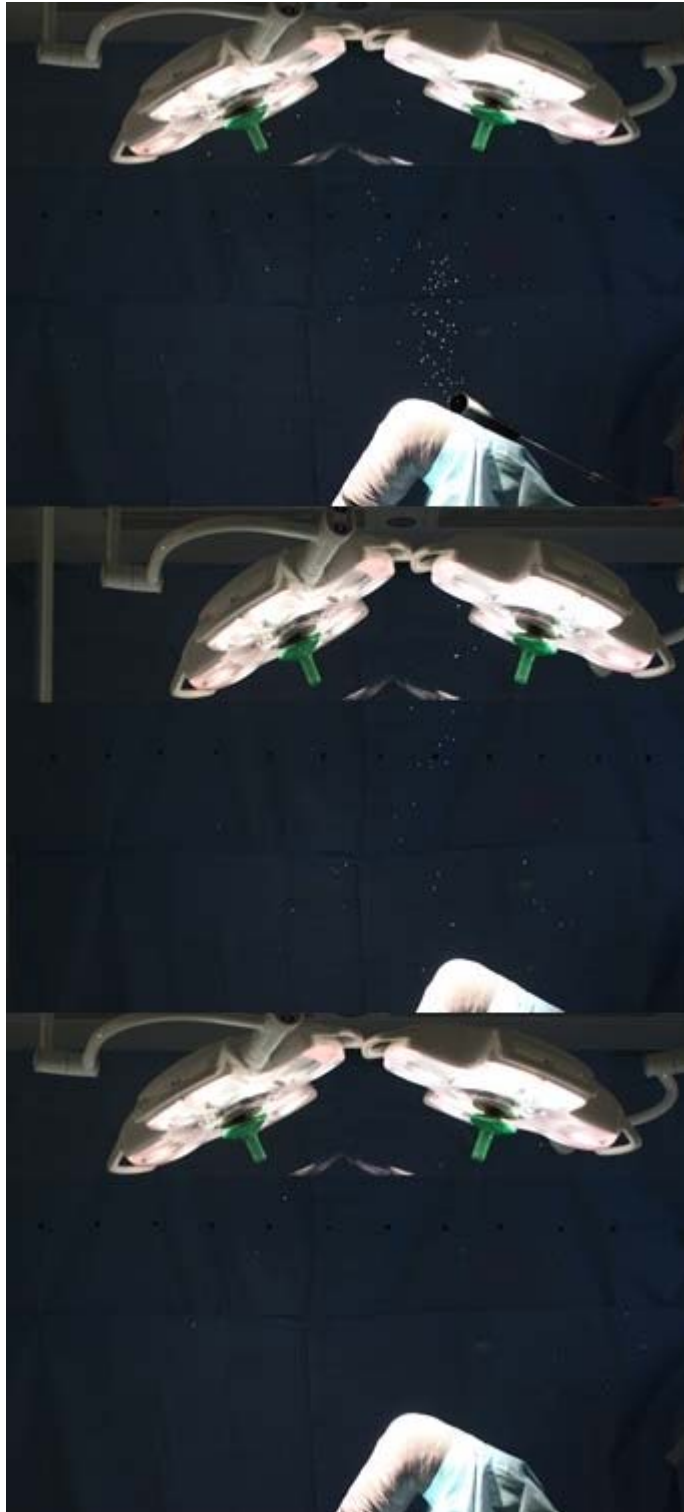


Figure 5-1 Three representative photographs from a run of 'two lights touching' taken at $t=0$ (top), $t=6$ (middle) and $t=18s$ (bottom). Bubbles can be seen to travel upwards from the bubble outlet towards the lights and bubbles are cleared slowly.

5.4 RESULTS

Results are displayed graphically in Figure 5-2 and the nonlinear regression analysis is summarised in Figure 5-3.

In the absence of overhead lights laminar flow was effective at removing the neutrally buoyant bubbles from the surgical field and bubbles were unable to accumulate. A light above the surgical field had a striking effect on the laminar flow system's ability to clear the air of bubbles. Bubbles were readily able to accumulate in the surgical field throughout the 'filling' phase. On removal of the bubble outlet the bubbles were not cleared rapidly. Furthermore the movement of bubbles in the area of the surgical field indicated that airflow was not laminar, but turbulent. Bubbles could be seen rising from the surgical field towards the non-sterile lights, then circulating back to the surgical field. This continued for some time until the bubbles were eventually cleared at around 35 seconds. The decay parameters of the two setups differ significantly; no light $\beta=1.591$ (95% CI 1.581 - 1.60) vs. one light $\beta=0.1633$ (95% CI 0.156 - 0.169). This is visually obvious without statistical analysis in figure 1.2.

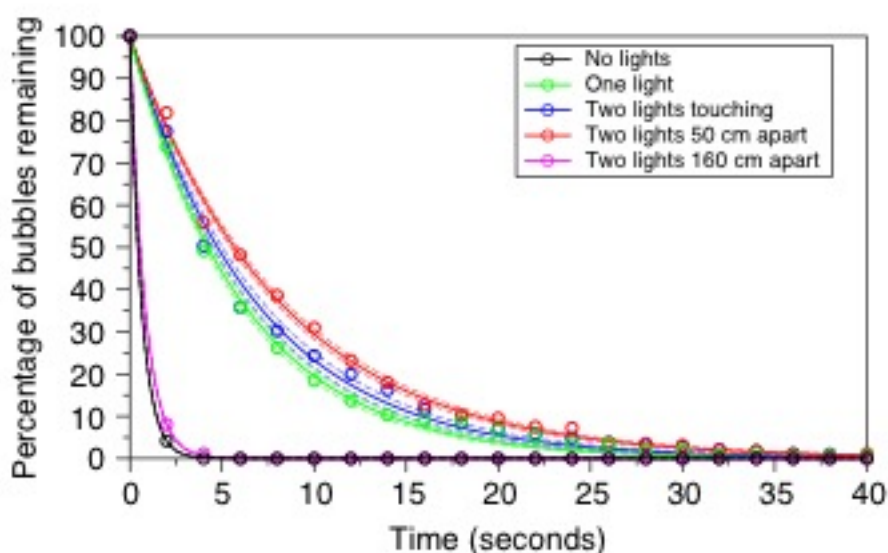


Figure 5-2 Plot of percentage of bubbles remaining over time for the five experimental conditions. Circles represent the percentage of bubbles remaining as a function of time. Lines represent the exponential functions fitted to the data. Dashed lines represent the 95% confidence interval for the nonlinear regression models.

With two lights touching or 50 cm apart above the surgical field, again bubbles accumulated during the filling phase and were cleared very slowly from the surgical field. Airflow could be seen to take the bubbles from the knee upwards to the lights and back to the surgical field. With lights 160 cm apart, laminar airflow appeared restored with bubbles readily removed from the surgical field.

Two lights touching had a significantly smaller decay than for a single light. The value of the decay parameter for two lights 50 cm apart was significantly smaller than for two lights touching and the lowest rate of clearance of bubbles of the 5 setups. With lights 160 cm apart, despite visualising laminar airflow, the value of the decay parameter was significantly lower than with no lights present, corresponding to an 8% reduction in the proportion of bubbles removed per second.

Light configuration	R ²	Decay parameter (β)	95% CI for decay parameter (β)	Reduction of bubbles per second
No lights	0.999	1.591	1.581 - 1.60	79.63%
Two lights 160 cm apart	0.999	1.255	1.241 – 1.269	71.49%
One light	0.996	0.163	0.156 - 0.169	15.06%
Two lights touching	0.992	0.146	0.137 - 0.153	13.54%
Two lights 50 cm apart	0.996	0.122	0.118 - 0.126	11.48%

Figure 5-3 Results from nonlinear of regression fitting for each light configuration.

5.5 DISCUSSION

Laminar airflow is regularly employed to reduce surgical site infection (SSI) in orthopaedic surgery. The use of moveable lights suspended within the laminar flow canopy to illuminate the surgical field is common practice. We visualised the interaction between the two and have quantified this effect using neutrally buoyant helium bubbles. This study demonstrates that surgical light use has a significant negative effect on laminar airflow which may be reduced with altering light positions.

The bubbles in this study are used to visualise air currents. As they are neutrally buoyant, they are suspended stationary in still air. Therefore, their movement allows us to see where air is flowing, and infer that microscopic airborne particles will follow a similar path. Airborne skin squames have been shown to be colonised with viable pathogenic bacteria, particularly staphylococci.^{147,148} Additionally the number of airborne particles has been shown to correlate with airborne bacterial counts.^{59,149} An experiment phage typing specific staphylococci strains demonstrated that the air in the immediate vicinity of a surgical wound was the greatest contributor to the bacteria found in the wound at the end of an operation.¹⁵⁰ Given this background, we consider the methods used in this experimental study to be of use to the practising orthopaedic surgeon.

The experiment clearly demonstrated that unimpeded laminar flow rapidly cleared bubbles, and by inference potentially infective particles, from the area surrounding the surgical field. The positioning of one or two operating lights above the operative field significantly affects this ability. Perhaps the most striking observation was that with operating lights above the surgical field, laminar airflow is lost. Bubbles were seen to circulate, rising in upward currents from the surgical area, coming into contact with the unsterile lights, before returning to the surgical site. Such airflow patterns are not only contrary to what laminar flow aims to achieve but could feasibly lead to increased contamination of the surgical field. It should be noted, as discussed, that the bubbles used in this study are not markers of infection per se and this study do not indicate if clinical infection rates would be higher in any particular airflow pattern. Notwithstanding this limitation this study suggests it is feasible that the combination of laminar flow and

poorly managed theatre lights could lead to increased numbers of contaminated airborne particles in the surgical wound, which may increase infection rates.

We examined three setups of two lights, designed to reflect the practicalities of the operating theatre environment and assess strategies to limit the negative effect of lights on laminar flow. The separation of two lights positioned by a single surgeon with arms outstretched, as is commonly seen clinically, did not lead to return of laminar airflow, rather was found to have the greatest negative effect on laminar flow of the setups studied. This demonstrates the complexity of the interaction of lights and laminar flow, with lights influencing a larger area than themselves. Surgeons should not be falsely reassured that if the lights are not directly above the surgical field the wound will be receiving adequate laminar airflow. With the lights more widely separated, 160 cm apart, laminar airflow was observed yet the rate of bubble clearance was still significantly slower than with no lights present. Thus it appears that there is no 'safe' distance for surgical lighting above which the lights have no influence on the airflow over the surgical field. We would recommend, to maximise the effect of laminar flow, surgeons place lights as far from the operative field as practicable or ideally avoid suspended lighting within the laminar flow hood altogether. Whilst anecdotally we found even with the lights far apart the modern surgical lighting satisfactorily illuminated the operative field some surgeons may choose to use headlamps, or the standard lighting available in the room – as used by the rest of the surgical team. This has been the practice of the senior author since being alerted to the potential effect by a colleague in 2013.

Concerns have been raised that laminar flow systems do not reduce infection rates, and may even be associated with increased infection rates^{45,61,62,63}. However, initial research with laminar flow did demonstrate a benefit.^{44,56} When evaluating this clinical literature, the current study suggests that is not merely the presence or absence of a laminar flow theatre that should be considered but whether true laminar air flow was achieved. It may be that the arrangement of theatres, including the lights, differed in these early studies to what is routine today. Other disruptive factors such as forced air warming have also been introduced since the initial clinical studies.^{143,144} Any future clinical trials examining the role of laminar flow must control for these factors.

The interactions between lights, surgeons, patients and the laminar flow environment are complex. Though this topic has received little attention in the orthopaedic literature engineers have undertaken a small number of studies. Brohus et al. attempted to assess the influence movement of the surgeon and lights have on the laminar airflow field using smoke visualisation and computational fluid dynamics. In agreement with the current study they demonstrated that lights influence the airflow substantially and identified air flow from the surgical field to the lights. Furthermore, they demonstrated that movements within a laminar flow system by the surgeon create areas of turbulence possibly transporting bacteria from the floor to the operating table from where the turbulent flow under the lights distributed the bacteria throughout surgical field. Chow et al. used complex computational modelling to assess the influence of surgical lighting positions on laminar flow. They also demonstrated that airflow over the surgical field was lowest when a light was directly over the patient. In agreement with the current study their computer modelling also suggested that lights create an area of turbulent flow over a wider area than themselves and laminar flow is not re-established in small gaps between lights.¹⁴⁵

There are limitations to this study in addition to those already discussed. Our experiments do not simulate a true operative situation; we have only examined the interaction between the operative lights and the laminar airflow field. This does however allow us to avoid other confounding factors such as surgical team members and warming devices.^{143,144} Additionally the surgical lights were placed in midline sagittal plane of the mannequin at height that was convenient when positioned by the hand of the surgeon. This was to simplify the setups and increase reproducibility. In reality lights are commonly positioned in a variety of sites and at variable heights by different surgeons. These factors are likely to affect the influence of lights on laminar flow and require further study.

We have quantified and visualised for the first time the interaction between operative lights and the laminar airflow field. It is clear that operative lights have a significantly negative effect on laminar airflow. The use of neutrally buoyant bubbles as a surrogate for contaminated air particles, shows that using surgical lights close to the operative field has the potential to increase particulate contamination. Although no direct link has been established between particulate debris and infection we suggest that an increased

number of circulating particles around the surgical field is likely to increase initial bacterial load in a wound and with it the risk of surgical site infection. Based on this study we recommend increased awareness of the potential interaction of lights with the laminar airflow field and positioning of lights as far from the operative field as is practically possible to reduce this effect.

Further studies considering not only the effect of different light designs but also the mechanisms underpinning these effects are necessary to fully understand the consequences of light positions around the operative field. In addition the role of laminar flow itself should be further explored to ascertain whether or not it is truly beneficial in light of more recent studies showing that it is associated with increased infection rates.

Chapter 6 General discussion and future directions

The focus of this PhD has been investigating and validating novel or alternative approaches to PJI. In the first instance we have demonstrated that in our population and by following an evidence based protocol for DAIR that this offered an effective treatment for early joint replacement infection. This is not a new finding but nonetheless given the uncertainty surrounding this treatment in the wider literature, our work confirms that this approach works in our local population. Building on this we looked into the outcomes of these patients and here came perhaps the most interesting finding, which was that in functional terms patients following DAIR for hip replacement PJI appear to do as well as in terms of a change in OHS compared to patients having routine hip replacement surgery without infection. This finding was not replicated in knee replacement patients treated for infection who appear to fare worse than knee replacement patients having routine surgery without infection. There are a number of limitations to this work in particular the small sample size and also the fact that although statistical differences did not exist between hip replacement patients treated for infection that does not necessarily mean that clinically significant differences were not present. Nonetheless this is the first investigation of its kind and should be followed up by further investigation. In order to achieve the kind of numbers required for high powered analysis in this patient group a multi-centre design will be key.

Having investigated the use of DAIR the next step was to evaluate new potential biomarkers for PJI looking both at peripheral blood and synovial fluid. The key findings from this were that CRP, which is often perceived to have limitations in relation to its prolonged elevation in the immediate post-operative period, did not seem to affect its ability to predict infection even in the early post-operative phase. CD64, which was the focus of the cohort study, showed its best performance in the early post-operative phase but demonstrated limited utility in chronic infections. These findings confirmed that CRP, as recommended by all international PJI guidelines, is still of great utility. Once again these findings require further validation in larger cohorts, in particular in patients who present even earlier after joint replacement. Recruiting such a cohort prospectively is however challenging due to the low incidence of PJI in general and therefore any further

studies should be conducted across multiple centres. When examining synovial fluid we also found CRP to show excellent discriminatory characteristics in early infections but in chronic cases alpha defensin showed the best diagnostic performance. Reading between the lines of the published literature on alpha defensin which has been largely driven by the group who have commercialised this test this finding fits with their comments about further validation being needed in early post-operative patients and the fact that the initial work on alpha defensin excluded patients in the early post-operative phase. These findings once again require validation in larger cohorts.

In order to build on previous work carried out at Northumbria Healthcare the final series of experiments looked at a laminar flow operating theatre and the impact that operating theatre lights can have on how effective laminar flow is at clearing particles from the operative field. Although a logical conclusion is that placing a light between the stream of laminar air flow and the operative field would counteract the laminar flow currents this has previously not been demonstrated. This adds important information to the on-going debate about the use of laminar flow and highlights the fact that careful light placement can maintain the effect of laminar flow. These experiments of course have limitations and do not fully recreate the setup during an operation as in particular surgical personnel were absent from the experimental setup. Nonetheless this is the first demonstration of this important yet intuitive phenomenon.

Appendix A – Favourable ethical opinion



Health Research Authority

NRES Committee North East - Sunderland

Room 002
TEDCO Business Centre
Viking Business Park
Jarrow
Tyne & Wear
NE32 3DT

Telephone: 0191 4283563

16 October 2013

Dr Ramsay Refaie
11 Auburn Gardens
Fenham
Newcastle upon Tyne
NE49XP

Dear Dr Refaie

Study title: Is CD64 level an effective test for the early diagnosis and management of joint replacement infection.
REC reference: 13/NE/0270
IRAS project ID: 133171

Thank you for your letter of 14 October 2013, responding to the Committee's request for further information on the above research [and submitting revised documentation].

The further information has been considered on behalf of the Committee by myself as Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager, Mrs Helen M Wilson, nrescommittee.northeast-sunderland@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **favourable ethical opinion** for the above research on the basis described in the application form, protocol and supporting documentation [as revised], subject to the conditions specified below.

Ethical review of research sites

NHS sites

A Research Ethics Committee established by the Health Research Authority

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

- 1 On point 5 on the consent form, ""I give permission for these individuals to have access to my records" has been deleted but this will need to remain in the form.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

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

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering Letter	Email from Ramsay Refaie	14 October 2013
GP/Consultant Information Sheets	Version 1.0	02 September 2013
Investigator CV	Mr Mike Reed	19 November 2012
Letter of invitation to participant	Version 1.0	02 September 2013
Other: CV	Ramsay Refaie	
Other: CV	Kenneth Rankin	
Other: Email confirmation of grant award		14 February 2013
Participant Consent Form	Version 1.1 (Clean and Tracked Changes)	10 October 2013
Participant Information Sheet	Version 1.1 (Clean and Tracked Changes)	10 October 2013
Protocol	Version 1.0	02 September 2013
REC application	Version 3.5	06 September 2013
Response to Request for Further Information		14 October 2013

Statement of compliance

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

After ethical review

Reporting requirements

The attached document "*After ethical review – guidance for researchers*" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.


Further information is available at National Research Ethics Service website > After Review

13/NE/0270	Please quote this number on all correspondence
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We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

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

Yours sincerely

pp 

Mr Paddy Stevenson
Chair

Email: nrescommittee.northeast-sunderland@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Mr Mike Reed, Northumbria Healthcare NHS Trust

Ms Caroline Potts, Northumbria Healthcare NHS Foundation Trust

Appendix B – Oxford hip and knee scores

Problems with your hip

During the past 4 weeks..

✓tick one box
for every question.

1.	<p>During the past 4 weeks.....</p> <p>How would you describe the pain you <u>usually</u> had from your hip?</p> <p>None Very mild Mild Moderate Severe</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>
2.	<p>During the past 4 weeks.....</p> <p>Have you had any trouble with washing and drying yourself (all over) <u>because of your hip?</u></p> <p>No trouble at all Very little trouble Moderate trouble Extreme difficulty Impossible to do</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>
3.	<p>During the past 4 weeks.....</p> <p>Have you had any trouble getting in and out of a car or using public transport <u>because of your hip?</u> (<i>whichever you tend to use</i>)</p> <p>No trouble at all Very little trouble Moderate trouble Extreme difficulty Impossible to do</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>
4.	<p>During the past 4 weeks.....</p> <p>Have you been able to put on a pair of socks, stockings or tights?</p> <p>Yes, Easily With little difficulty With moderate difficulty With extreme difficulty No, Impossible</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>
5.	<p>During the past 4 weeks.....</p> <p>Could you do the household shopping <u>on your own?</u></p> <p>Yes, Easily With little difficulty With moderate difficulty With extreme difficulty No, Impossible</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>
6.	<p>During the past 4 weeks.....</p> <p>For how long have you been able to walk before <u>pain from your hip</u> becomes severe? (<i>with or without a stick</i>)</p> <p>No pain/ More than 30 minutes 16 to 30 minutes 5 to 15 minutes Around the house <u>only</u> Not at all -pain severe on walking</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>

During the past 4 weeks...

✓tick one box
for every question

7	<p>During the past 4 weeks.....</p> <p>Have you been able to climb a flight of stairs?</p> <p>Yes, Easily <input type="checkbox"/> With little difficulty <input type="checkbox"/> With moderate difficulty <input type="checkbox"/> With extreme difficulty <input type="checkbox"/> No, Impossible <input type="checkbox"/></p>
8	<p>During the past 4 weeks.....</p> <p>After a meal (sat at a table), how painful has it been for you to stand up from a chair <u>because of your hip</u>?</p> <p>Not at all painful <input type="checkbox"/> Slightly painful <input type="checkbox"/> Moderately painful <input type="checkbox"/> Very painful <input type="checkbox"/> Unbearable <input type="checkbox"/></p>
9	<p>During the past 4 weeks.....</p> <p>Have you been limping when walking, <u>because of your hip</u>?</p> <p>Rarely/never <input type="checkbox"/> Sometimes, or just at first <input type="checkbox"/> Often, not just at first <input type="checkbox"/> Most of the time <input type="checkbox"/> All of the time <input type="checkbox"/></p>
10	<p>During the past 4 weeks.....</p> <p>Have you had any sudden, <u>severe</u> pain - 'shooting', 'stabbing' or 'spasms' - <u>from the affected hip</u>?</p> <p>No days <input type="checkbox"/> Only 1 or 2 days <input type="checkbox"/> Some days <input type="checkbox"/> Most days <input type="checkbox"/> Every day <input type="checkbox"/></p>
11	<p>During the past 4 weeks.....</p> <p>How much has <u>pain from your hip</u> interfered with your usual work (including housework)?</p> <p>Not at all <input type="checkbox"/> A little bit <input type="checkbox"/> Moderately <input type="checkbox"/> Greatly <input type="checkbox"/> Totally <input type="checkbox"/></p>
12	<p>During the past 4 weeks.....</p> <p>Have you been troubled by <u>pain from your hip</u> in bed at night?</p> <p>No nights <input type="checkbox"/> Only 1 or 2 nights <input type="checkbox"/> Some nights <input type="checkbox"/> Most nights <input type="checkbox"/> Every night <input type="checkbox"/></p>

PROBLEMS WITH YOUR KNEE

During the past 4 weeks..

✓ tick one box
for every question

1	<p><i>During the past 4 weeks.....</i></p> <p>How would you describe the pain you <u>usually</u> have from your knee?</p> <p style="text-align: center;"> None Very mild Mild Moderate Severe <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </p>
2	<p><i>During the past 4 weeks.....</i></p> <p>Have you had any trouble with washing and drying yourself (all over) <u>because of your knee</u>?</p> <p style="text-align: center;"> No trouble at all Very little trouble Moderate trouble Extreme difficulty Impossible to do <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </p>
3	<p><i>During the past 4 weeks.....</i></p> <p>Have you had any trouble getting in and out of a car or using public transport <u>because of your knee</u>? (whichever you would tend to use)</p> <p style="text-align: center;"> No trouble at all Very little trouble Moderate trouble Extreme difficulty Impossible to do <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </p>
4	<p><i>During the past 4 weeks.....</i></p> <p>For how long have you been able to walk before <u>pain from your knee</u> becomes severe? (<i>with or without a stick</i>)</p> <p style="text-align: center;"> No pain/ More than 30 minutes 16 to 30 minutes 5 to 15 minutes Around the house <u>only</u> Not at all - pain severe when walking <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </p>
5	<p><i>During the past 4 weeks.....</i></p> <p>After a meal (sat at a table), how painful has it been for you to stand up from a chair <u>because of your knee</u>?</p> <p style="text-align: center;"> Not at all painful Slightly painful Moderately painful Very painful Unbearable <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </p>
6	<p><i>During the past 4 weeks.....</i></p> <p>Have you been limping when walking, <u>because of your knee</u>?</p> <p style="text-align: center;"> Rarely/ never Sometimes, or just at first Often, not just at first Most of the time All of the time <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </p>

During the past 4 weeks... ✓tick one box for every question

7	<p><i>During the past 4 weeks.....</i></p> <p>Could you kneel down and get up again afterwards?</p> <p>Yes, Easily <input type="checkbox"/> With little difficulty <input type="checkbox"/> With moderate difficulty <input type="checkbox"/> With extreme difficulty <input type="checkbox"/> No, Impossible <input type="checkbox"/></p>
8	<p><i>During the past 4 weeks.....</i></p> <p>Have you been troubled by <u>pain from your knee</u> in bed at night?</p> <p>No nights <input type="checkbox"/> Only 1 or 2 nights <input type="checkbox"/> Some nights <input type="checkbox"/> Most nights <input type="checkbox"/> Every night <input type="checkbox"/></p>
9	<p><i>During the past 4 weeks.....</i></p> <p>How much has <u>pain from your knee</u> interfered with your usual work (including housework)?</p> <p>Not at all <input type="checkbox"/> A little bit <input type="checkbox"/> Moderately <input type="checkbox"/> Greatly <input type="checkbox"/> Totally <input type="checkbox"/></p>
10	<p><i>During the past 4 weeks.....</i></p> <p>Have you felt that your knee might suddenly 'give way' or let you down?</p> <p>Rarely/never <input type="checkbox"/> Sometimes, or just at first <input type="checkbox"/> Often, not just at first <input type="checkbox"/> Most of the time <input type="checkbox"/> All of the time <input type="checkbox"/></p>
11	<p><i>During the past 4 weeks.....</i></p> <p>Could you do the household shopping <u>on your own</u>?</p> <p>Yes, Easily <input type="checkbox"/> With little difficulty <input type="checkbox"/> With moderate difficulty <input type="checkbox"/> With extreme difficulty <input type="checkbox"/> No, Impossible <input type="checkbox"/></p>
12	<p><i>During the past 4 weeks.....</i></p> <p>Could you walk down one flight of stairs?</p> <p>Yes, Easily <input type="checkbox"/> With little difficulty <input type="checkbox"/> With moderate difficulty <input type="checkbox"/> With extreme difficulty <input type="checkbox"/> No, Impossible <input type="checkbox"/></p>

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