MD THESIS

UCL

HUMAN IMMUNODEFICIENCY VIRUS AND OPEN FRACTURES. IS WOUND OR FRACTURE HEALING AFFECTED IN SURGICALLY STABILISED OPEN FRACTURES? A PROSPECTIVE STUDY

BY

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Abbreviations:

MW	Mann Whitney
FE	Fisher exact
X^2	Chi squared
95% CI	95% Confidence interval
RR	Risk ratio
HIV	Human immunodeficiency virus
ART	Anti retroviral therapy
HAART	Highly active anti retroviral therapy
MVA	Motor vehicle accident
PVA	Pedestrian vehicle accident
GSW	Gun shot wound
FU	Follow up

Abstract

Background:

33 million people worldwide are infected with HIV, a complex disease that affects many of the processes involved in wound and fracture healing. There is little evidence available to guide acute management of open fractures in these patients and fears of acute and delayed sepsis often inhibit the use of surgical fixation, which may be the most effective way of achieving union. This study addresses the hypothesis that the presence of either HIV or advanced HIV (CD4 count <=350) leads to an increased risk of complications in patients with open fractures treated with surgical stabilization.

South Africa has one of the highest rates of both HIV and high energy trauma in the world, so was deemed an appropriate place for the study of this interaction.

Methods:

This prospective observational study compared surgical fixation of open fractures in HIV positive and negative patients.

133 patients with 135 open fractures fulfilled the inclusion criteria. 86 fractures were in HIV negative and 33 in HIV positive patients. The remaining 16 patients refused HIV tests. 12 HIV positive patients had advanced disease (CD4 <=350), 14 had early disease (CD4 >350), 7 refused CD4 count testing. This cohort was three times larger (number of HIV positive patients) than any similar previously published study.

There was no randomised allocation; the treatment of these patients was based on locally developed protocols and was dependent on; fracture type, location and the grade of wound. Patients were followed up either till union had been achieved or for 6 months in tibia/femur fractures, and 3 months in other fractures.

The primary outcome was acute wound infection, secondary outcomes tested were fracture union and pin site sepsis.

The analysis of the binary nominal data was done using the Chi squared test. In cases where the expected value was less than 5, then the Fisher's exact test was used. In the assessment of multiple potential risk factors, binary logistic regression was used.

Results:

Analysis of background characteristics showed that HIV positive and negative populations were broadly similar with regard to demographics, injury type/location and grade of wound.

In the analysis of the primary outcome, the risk of wound infection was marginally higher in patients without HIV (22%) as compared to patients with HIV (15%). This difference was small and did not reach statistical significance (n=135, Risk Ratio 0.7, p value 0.40). However, as hypothesized, the infection risk was higher in patients with advanced HIV (26%), compared to patients with early HIV (5%). The numbers, however, were small and this did not reach statistical significance (n=33, Risk Ratio=4.8, P value= 0.12).

Sub group analyses, conceived prior to the study, provided strong evidence that patients with Gustilo Anderson grade 1 injuries had a higher risk of wound infection in patients with advanced HIV than controls (HIV negative and early HIV) (n=46, Risk Ratio=6.3, P value =0.02). Of note, departmental guidelines meant that patients with grade 1 injury were not prioritised for theatre and had, on average, a delay of 3.5 days to surgery. The average delay was similar in both HIV positive and negative groups.

Analysis of the secondary outcome, nonunion, provided strong evidence that the risk of nonunion was higher in HIV positive than HIV negative patients (n=115, Risk Ratio=4.1, P value=0.04). Interestingly, the patients with advanced HIV had a slightly lower nonunion risk (13%) than patients with early HIV (20%). However the numbers were small and the difference was not statistically significant (n=33, Risk Ratio=0.8, P value=1).

The incidence of nonunion was not correlated with the presence of wound infection.

The risk of mild pin site sepsis in fractures treated with external fixation was similar in both HIV positive (60%) and negative (67%) patients (n=31, Risk Ratio=0.9, P value=1). An increased risk of severe pin site sepsis was noted in patients with advanced HIV (50%), compared to controls (25%). Although the difference is large, the numbers are small and the difference was not statistically

significant (n=28, Risk Ratio=2, P value= 0.31). It would require 160 patients to prove a difference of this size.

Conclusions:

Data from this study appears to dispute the conclusion of previous studies that suggest that all patients with HIV are at higher risk of wound infection, and therefore internal fixation should be considered with caution. In this study it was only the patients with advanced HIV that showed a small increase in the risk of wound infection.

Based on this study the author suggests that early HIV should not be a contraindication to either internal or external fixation in open fractures, due to concerns of wound infection. However, advanced HIV should continue to be considered a relative contraindication to internal fixation, until further data becomes available.

Since this finding applied equally to grade 1 (Gustilo Anderson) injuries, the data suggests that any theatre delays in patients with advanced HIV may be detrimental to outcomes. This is contrary to published data that suggests that grade 1 injuries do not need to be prioritised.

The data provides strong evidence that HIV leads to an increased risk of non unions. Interestingly, the risk of non union is less in patients with advanced HIV. This may fit with recently published laboratory studies suggesting that the absence of lymphocytes is beneficial to bone healing.

Based on this evidence the author suggests that in patients with HIV treatment strategies should be aimed at achieving union, rather than on potentially unfounded concerns of preventing infection.

In patients treated with external fixation, the data provides weak evidence of an increased risk of severe pin site sepsis in advanced HIV. This observation may be due to an increased susceptibility to infection, or to problems with bone healing in these patients.

Based on this evidence, and the evidence that patients with HIV may be at increased risk of non union, the author suggests that HIV positive patients being treated with external fixators, should be considered for treatment strategies that will

prolong the life of the pin bone interface. These may include additional pins, wires and/or the use of hydroxyapatite coated half pins.

Chapter 1

Introduction

1 Introduction

1.1 Purpose of the study

The management of open fractures and the prevention of infection remains a challenging and controversial area in orthopaedics. Gustilo et al in 1976 published a paper which has been very influential in guiding the management of these patients[1]. However despite much research over the last 30 years, the optimal management remains uncertain and practice varies. In 2009 the British Orthopaedic Association and the British Association of Plastic, Reconstructive and Aesthetic Surgeons attempted to increase the standard of care by producing guidelines for the management of open fractures of the lower limb. These were based on evidence and where evidence was lacking, on expert opinion[2]. These guidelines have not addressed how patients who are immunosuppressed should be treated.

Clinical studies looking at Human Immunodeficiency Virus (HIV) have shown high rates of wound sepsis in internally fixed open fractures as well as high rates of pin site sepsis in externally fixed fractures[3-6], however these studies have lacked statistical power[7].

Laboratory based studies have shown that HIV infects a variety of cells including lymphocytes and macrophages. Once infected these cells become dysfunctional and may undergo apoptosis[8]. Subgroups of these cells are known to be important for initiating and regulating wound[9] and bone healing[10-12] and in the immune response to bacterial infection[13,14].

The increased availability of Highly Active Anti Retroviral Therapy (HAART) has had a dramatic effect on the life expectancy of patients with HIV[15,16]. HIV remains an incurable disease and this is thought to be due to the presence of reservoirs of the virus within certain cell lineages, which are resistant to treatment[17]. Macrophages have been shown to be one such reservoir, and it is likely that these cells remain chronically dysfunctional even after the initation of HAART[17]. Several studies have also suggested that Protease Inhibitors, a category of drug often used within the HAART regime, may directly affect bone metabolism and fracture healing[18,19]. There is however a paucity of clinical trials addressing these issues.

This study addresses the hypothesis that the presence of either HIV or advanced HIV (CD4 count <=350) leads to an increased risk of complications in patients with open fractures treated with surgical stabilization.

This introduction aims to:

Review the literature regarding wound infection and non union in open fractures.

Review the literature regarding HIV and HAART with respect to surgical procedures, fracture healing and wound infection.

Review the theories postulated for HIV's interaction with wound and bone healing.

The literature search was done using Medline, Old Medline, Pubmed, Google Scholar: search strings used were: HIV, Human immunodeficiency virus, open fracture, prophylactic antibiotics, non union, wound infection, classification, internal fixation and external fixation. All relevant articles were reviewed if in English, and the full text acquired. Non English language article abstracts were reviewed, and their content noted. Experts in the field working with in the field of HIV and fracture healing were contacted and details of current research work was discussed

1.2 Open Fractures

In 1850 a study at St Thomas' Hospital of 362 patients suffering from compound fractures found that there was a risk of mortality of 33% in these patients[20]. The development of differing methods of internal fixation and soft tissue coverage along with improvements in antibiotic protocols has greatly reduced morbidity and mortality and increased rates of union. However with increasing use of internal fixation there have been concerns of increasing risk of deep implant associated infection[21].

Concerns regarding internal fixation in open fractures are predominantly due to the difficulty of treating infections once established. This is commonly due to adherence of bacteria onto the implant and the presence of a biofilm which is impermeable to most antibiotics[22,23]. Over the last 60 years many factors have been identified as possible risk factors for infection through laboratory and clinical trials. The conclusions of these trials have often differed. Clinical differences between the study populations often make the trials incomparable. Fracture patterns and soft tissue injuries and treatments are very variable and difficult to classify accurately. Host factors such as nutrition and smoking which may be extremely relevant have often not been recorded.

In 1946 Nicoll[24], looked at a large series of open tibia fractures from South African miners, he concluded that the rates of non-union were dependant on the character of the initial injury, the most important factors of which were the initial displacement, the comminution, the soft tissue envelope, and the presence of infection. Subsequent papers have backed up Nicoll's astute observations, however it was not until 1976 when Gustilo and Anderson developed a classification system that there was some consensus within the literature on how to grade these injuries [1]. Prior to this paper, clinical trials looking at open fractures are extremely difficult to assess and compare because of the variability of recorded data. A review of these grading systems and subsequent developments is given below.

1.2.1Grading systems

1.2.1.1Nicoll

The classification system developed by Nicoll in 1964 was based on a study of 705 tibia fractures of which 144 were open sustained in both South Africa and the UK[24]. The injuries were classified by wound (**W**), comminution (**C**) and displacement (**D**), and were graded either, nil/slight (**N**), or moderate/severe (**S**). Rates of non union in the cohort were then calculated. These are given in Table 1-1.

Table 1-1

Fracture type	Rates of Delayed/Non union (%)
ND NC NW	9
ND NC SW	12
ND SC NW	15
SD NC NW	27
ND SC SW	39
SD NC SW	55
SD SC NW	31
SD SC SW	39

In developing this classification system Nicoll had identified some of the key aspects of the character of a fracture which have been subsequently elaborated on by other authors

1.2.1.2Gustilo and Anderson

The Gustilo and Anderson classification introduced in 1976 looked at a series of 1025 open tibia fractures. It was amended in 1984 following a series of 75 grade 3 open fractures. It is still the most widely used classification today [1,25]. Table 1-2 summarizes the Gustilo Anderson classification and gives approximate infection rates.

Table 1-2

Grade	Approximate Infection rate	Description
1	0%	Low energy. Small soft tissue defect <1cm
2	3%	Low energy. Skin laceration <10cm with minimal soft tissue damage
3a	4%	Adequate soft tissue coverage of the fractured bone despite extensive soft tissue laceration, flaps, or high energy trauma, irrespective of the size of the wound.
3b	52%	Extensive soft tissue loss with periosteal stripping and bone exposure.
3c	42%	Arterial injury requiring repair

Even this classification system has been shown to have high inter-observer variation[26,27]. Subsequent authors have tried to produce extended classification systems to try to classify more accurately the soft tissue injury and host factors.

1.2.1.3 **Arbeitsgemeinschaft für Osteosynthesefragen (AO) Classification**

In 1983 Tscherne et al[28] described a grading system for soft tissue injury. This system has subsequently been incorporated into the Hanover fracture scale. This is a detailed scoring system taking into account multiple aspects of the injury and is used as part of the AO classification. This classification can be used to describe both open and closed injuries but does not take into account patient co-morbidities.

1.2.1.4 **Host classification**

In 2005 Bowen et al[29] described the Host classification to help predict outcome in patients with significant co-morbidities. This was based on a retrospective study of 174 long-bone fractures. Their grading system is derived from a system looking at adult osteomyelitis which was developed in 1985. This system was then refined for

looking at periprosthetic total hip infection. The patients were divided into three classes based on the presence or absence of fourteen medical and immune-compromising factors. These included: an age of eighty years or more; current nicotine use; diabetes; malignant disease; pulmonary insufficiency; and systemic immunodeficiency (HIV). Patients were categorized as having 0, 1-2 or >3 risk factors. A CD4 count of less than 200 cells/µL, (a mark of advanced HIV disease or Acquired immune deficiency syndrome (AIDS)) automatically places the patient in the final group.

Although this grading system appears to correlate with infection, it takes a lot of variables into account, many of which have not been validated individually.

Of particular relevance to HIV infection is that this classification system may be used to guide clinical decision making. It may lead to patients being inappropriately treated if for example they are deprived of the method of fixation of a fracture that is optimal for achieving union.

1.2.1.5 **Ganga Hospital score**

This scoring system published in 2007, is an extensive scoring system based on the experience of a large trauma and reconstruction unit in India[30]. It takes into account a variety of factors, including soft tissue and skin damage, as well as co-morbidities including diabetes and age greater than 65 years. It has been used as tool to give an indication of outcome, and to guide management as part of a management protocol.

This new scoring system has yet to gain widespread popularity, and there has been some concern as to the emphasis that is placed on the need for debridement within 12 hours which is not felt to be evidence based[31].

1.2.1.6 **Gunshot wounds (GSW)**

GSW, of which there were significant numbers in the series to be reported, pose certain problems in classification.

Gusitllo et al[1] in 1976 classified all GSW as grade 3 injuries. In their paper, it was stated that the management for this type of injury should be debridement, irrigation, and avoidance of internal fixation. Subsequently in 1984 Gustilo et al[25] implied, but did not clearly state, that it is only high energy GSW that should be categorized as grade 3.

Barach et al[32] reviewed the mechanism with which a bullet causes injury and found it has more to do with tissue-bullet interaction than purely bullet velocity. The authors concluded that it is possible for a low energy missile to cause tissue destruction normally characterised by a high energy missile.

Long et al[33] noted that most patients in the civilian setting are unable to describe the type of weapon they were shot with. They have developed a classification system based on the distal femur and looking at the clinical features of the wound to give guidance on management. They also noted that high energy missiles generally result in larger wounds.

Infection following GSW has long been a problem. John Hunter in 1794[34] described how the projectile can carry debris from the surrounding area into a wound. GSW from both low and high energy bullets are not generally sterile. It has been shown that a low energy bullet is not sterile when it penetrates the skin and that the negative pressure associated with the bullet trajectory sucks debris and fragments of clothing into the wound[35].

Management of these injuries has been controversial. John Hunter was an early advocate of conservative management, whilst many of his colleagues were advocating aggressive debridement and early amputation[34]. More recent studies are again suggesting that many GSW can be treated non operatively[36-39] and with oral antibiotics[37].

Some papers are now advocating that patients with small wounds, and fractures not requiring fixation, can be managed as an outpatient [37,39]. An exception to the above management is if there is any possibility of the bullet or fragment of a bullet being within a joint when it would require urgent debridement and removal of that

fragment. This is due to the possibility of lead toxicity[40], and also due to a foreign body interfering with the mechanics of the joint

The above controversy has led to discrepancies over whether a GSW should be classified as a grade 3 wound, as Gustilo et al initially suggested, or according to the size of the wound or the energy of the trajectory.

1.2.1.7 **Conclusions**

Despite the development of different grading systems, the Gustilo Anderson classification remains the most widely used in clinical trials and therefore is the classification utilized in this thesis.

In this study GSW have been graded by the size of the wound in the same way as for the other method of injuries.

1.2.2Debridement in open fractures

The need for urgent debridement in open fractures is currently controversial. Historically priority has been placed on getting patients with open fractures to theatre within six hours for debridement. The evidence base for this originated in 1898 with research done on guinea pigs[41]. Subsequent authors such as Gustilo et al, advocated emergency debridement for optimal results[1]. However in a review on the subject, Crowley et al[42], showed that only one out of nine papers published in the last 30 years showed a reduction in the risk of wound infection in patients who went to theatre in less than 6 hours. In a review of the literature and consensus of expert opinion the British orthopaedic association now recommend that debridement is performed on a day time list, with senior/experienced surgeons operating[43].

Grade 1 injuries are also controversial. Recent evidence suggests that infection rates in Grade 1 injuries remain low with or without debridement, provided early antibiotics are administered[44]. Low energy gun shot wounds which are often classified as Grade 1 injuries also do well without operative debridement. One trial showed that if the fracture did not require operative intervention then these patients could be managed as an outpatient with oral antibiotics[37].

1.2.3Stabilization in open fractures

In a retrospective review of patients from 1955-1968, Gustilo et al[1] looked at infection rates in open femur and tibia fractures treated either conservatively or operatively. In this initial paper the fractures were not graded, and the treatment was dependent on surgeon preference. In a comparison of the 52 fractures treated with internal fixation versus the controls treated with traction or plaster of paris (POP) immobilization they noted infection rates of 19% and 5% respectively. On the basis of this evidence they concluded that internal fixation in open fractures should not be used, except where it is essential due to arterial injury, or where soft tissue damage makes it impossible to manage the fracture by other means.

At about the same time McLaughlin et al[45] hypothesized that the constant small movements seen in conservatively managed fractures may lead to further tissue damage and increase the risk of infection. This was further investigated by Worlock

et al[46] using an experimental animal model looking at the role of stability and infection in open tibia fractures. They found that the rate of osteomyelitis was 50% higher in animals treated with unstable fixation rather than stable fixation, concluding that stable fixation aided revascularization of bone and soft tissue, hence reducing the infection rate.

1.2.4Internal fixation effects on cortical blood supply

Cortical blood supply is thought critical for both bone healing and prevention of wound infection. Cortical blood supply comes from three sources as shown by Rhinlander in Figure 1-1[47].

The Nutrient artery

Periosteal arteries

The Medullary artery (via the Metaphyseal arteries)

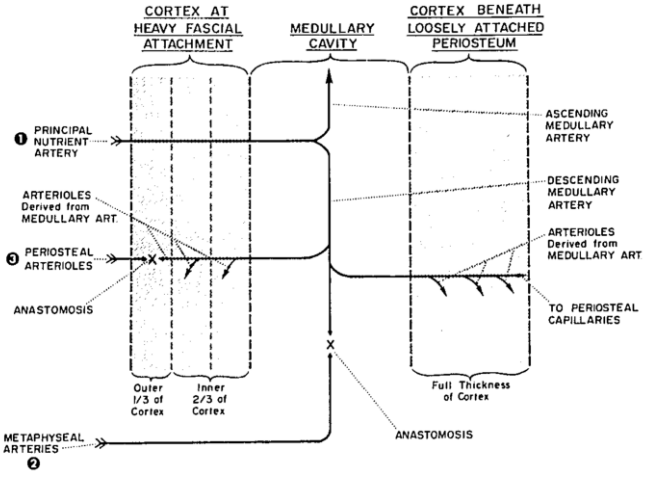


Figure 1-1: Blood supply of a long bone

There has been concern regarding internal fixation of fractures and how it may affect the cortical blood supply. In1968 an animal model showed that dynamic compression plates (DCP) plates lead to devascularisation of up to half of the cortical width in areas with contact with the plate. This is thought to be due to damage to the Periosteal arterioles[48,49]. These findings led to the development of limited contact dynamic compression plates(LCDCP), a 50% reduction in the contact area[50] and more recently to Point Contact locking plates (PCfix). The advantage of the PC fix plates is twofold in that there is reduced metal to bone

contact and its insertion requires less periosteal stripping, as it does not need to be placed directly on the bone, and hence helps preserve the blood supply. [51]. The PC Fix technique appears to increase the inoculum required to cause infection approximately twelve times as compared to conventional DCP plate[52].

With the development of locked intramedullary nails, it became possible to obtain relatively stable fixation using an intramedullary device. There have been concerns over how this may affect cortical blood supply via the medullary artery, especially when reaming is employed. Schemitsch et al[53] looked at blood flow in fractures of sheep's tibiae treated by either reamed or unreamed nails and showed a significant decrease in cortical perfusion in both groups. The reamed group was significantly more affected. Revascularization took up to 12 weeks in the reamed group and 8 weeks in the unreamed group. This loss of medullary circulation is of particular concern in open fractures where soft tissue damage may also have damaged the periosteal blood supply. Damage to both periosteal and medullary blood supply may render the fracture-site ischemic.

In 2000, a meta-analysis[54] looked at stabilization of open tibia shaft fractures with intramedullary nails. This analysis was unable to identify any significant difference in outcome between reamed and unreamed nails.

HIV has been shown to be related to macro and micro vascular disease[55]. How this may affect cortical blood supply has not been studied and maybe a relevant factor related to the devascularisation of the bone cortex by internal fixation.

1.2.5External fixation

External fixation has historically been advocated for obtaining bone stability in severe open fractures of the tibia[25,56-58]. This is because it is thought to affect cortical blood supply in the vicinity of the fracture less than either plate fixation or intramedullary fixation .It avoids placing metal work directly around the fracture site, and it is also simple to remove/resite should infection occur. The presence and position of the fixator can however prove difficult with wound management, both with respect to dressings and flaps. The development of unreamed nails and reduced-contact locking plates have led to the advantages of minimizing disruption to the cortical blood supply and improving access to the soft tissue[59]. External fixators

have the advantage over locking plates and intramedullary devices, in that relative stability can be provided initially, but the fracture can then be manipulate to improve the position or provide stimulation for bone healing. External fixators can also be adjusted postoperatively to provide optimal alignment. Pin tract infection can be a problem in the use of external fixators, however, with careful follow up, the use of occlusive foam dressings, wound hygiene and appropriate antibiotics this can normally be addressed.

1.2.6 Internal or External fixation for Tibia fractures?

The choice between internal or external fixation for open tibia fractures is controversial. A recent meta analysis looking at reamed and unreamed nailing against external fixation of open tibia fractures, shows a significantly reduced reoperation rate in the patients treated with nailing[54]. However the use of nails remains debated in certain situations. Grade 3b tibia fractures requiring free flaps have recently been shown to have higher rates of osteomyelitis and non-union if treated with nailing rather than an external fixator[60]. Many surgeons will use a combination of the evidence available in the literature and their own personal experience and inclination when deciding whether to nail or external fix open tibia fractures.

1.2.7 Bacterial adherence and the formation of the Biofilm

Adherence of bacteria to metallic implants is an important factor in foreign body infection. It is also a prerequisite for the formation of biofilms [22]. Staphylococcal aureus (S.aureus) and Staphylococcal epidermidis (S.epidermidis) are the two most important bacteria involved with foreign body infection. Several studies have shown that S.aureus has an affinity for metallic implants, whereas S.epidermidis is more commonly seen in polymer-based implants[61,62]. The two most commonly used implant materials in fracture fixation are stainless steel and titanium. In studies using animal models titanium showed a lower susceptibility to infection over stainless steel, requiring approximately 10 times the inoculum to cause similar rates of infection.[52,63]

Initially bacteria adhere to the prosthesis. These bacteria then synthesize an extracellular polymeric substance and form into microcolonies[64]. During the formation of a biofilm, attached bacterial cells release antigens and stimulate the production of antibodies thereby attracting phagocytes. In response, phagocytes release enzymes, which are not able to completely destroy the biofilm but instead may damage the surrounding host tissue. As the bacterial colonies mature, surface-associated, non growing (dormant) bacteria on the periphery detach and disperse as suspended, active bacteria that can multiply rapidly.

The dormant bacteria within the biofilm exhibit marked antibiotic resistance compared to the surface (active) bacteria. Several theories for this have been postulated:

Bacteria in biofilms are enclosed within a negatively charged matrix that can physically restrict the diffusion of large molecules, such as some antimicrobial agents, enzymes, and complement components[65].

Nutrient depletion or waste-product accumulation within the biofilm causes some bacteria to enter a dormant state in which they are less susceptible to growth-dependent antimicrobial killing[66]

How HIV affects the biofilm formation, and how increasing immunosupression affects the balance between dormant and active bacteria is as yet unknown but may be a potential source of delayed sepsis.

1.2.8Early primary closure of wound

It is now accepted that the organisms leading to infection in open fractures, are predominantly hospital acquired[21,67] rather than from contamination present in the wound at the time of injury. The traditional teaching has been to leave wounds open for at least 48 hours to allow suppuration before considering closure at that stage if they still appear clean, with prophylactic antibiotic cover[25]. It has been speculated that if an adequate and early debridement has been carried out, it may be possible to close the skin primarily, and hence reduce the access of nosocomial organisms to the fracture site[67]. Rajasekaran et al[68] looked at 557 carefully selected grade 3a open fractures which they closed primarily on initial debridement. They observed

excellent outcomes in 87% of patients with only 5 patients developing deep infections (<1%). This paper may signal a dramatic shift in the management of these fractures towards early wound closure.

1.2.9Risk factors for wound infection/fracture union

Host factors affecting healing have been well studied. There is some evidence to suggest that fracture healing is inhibited in the elderly and that this is exacerbated by difficulty in obtaining solid internal fixation in osteoporotic bone[69,70].

Both animal and clinical trials have shown that diabetes affects fracture healing. In untreated diabetes, it appears that the callus contains significantly less collagen and DNA and that this is associated with decreased strength and stiffness of the fracture repair. Good diabetic control seems to at least partially-alleviate these issues[71,72]. Significantly longer time to union, and increased incidence of non-union is also seen in these patients[73]

Iron deficiency anaemia has been shown to lead to significant deficiencies on bone healing in rats. This is thought to be due to a reduction in the delivery of oxygen to the tissues, but also due to a reduction of iron, which is critical in the electron transport system and the formation of collagen.

It has been shown that nutrition is important in fracture healing. An albumin below 35 grams per litre in trauma patients has been shown to be associated with longer inpatient stay, higher mortality and worse functional outcome[74]. Vitamin and mineral deficiency is also thought to be important. Deficiencies in vitamins C,B6, D, phosphorous and calcium have all been shown to have a negative effect of bone healing[75].

Studies have shown that smoking affects wound and bone healing. The exact mechanism is not understood, but it is thought not to be dependent on nicotine[76]. Clinical trials have shown smokers to have a significantly longer time to union in tibia fractures[77]. Another study showed smokers to be one third more likely to develop non-union and twice as likely to develop infection[78].

Alcohol abuse has been shown to be a significant causative factor for trauma, and is also implicated in fracture healing[79]. The effect of alcohol on bone healing is thought to be a dose-dependent effect on osteoblast activity, this results in a callus

that is not as stiff, has decreased strength and reduced mineral content compared to controls[80]

Several orthopaedic trials into open fractures have used wound infection as an outcome measure[1,3]. The definition of wound infection can be variable from study to study, and may include mild signs of infection which settle rapidly with antibiotics. One study showed that superficial wound infection was not a significant risk factor for subsequent deep infection[81]. Superficial wound infection should perhaps be considered a risk factor rather than an outcome measure in these studies.

1.2.10 Prophylactic Antibiotics

The first account of infection control in open fractures in the literature appears to have been in 1867, when Lister treated open fractures with carbolic acid[82]. This concept evolved out of observing the effect carbonic acid had on Glaswegian sewage! The use of prophylactic antibiotics in open fractures has now been shown to be beneficial however, how they are used remains controversial[83]. The two main trauma societies in the United States have recently updated their guidelines. They both comment on the paucity of recent good quality evidence and as a result there are some differences in their advice[84,85].

The concept of prophylactic antibiotics in open fractures is complicated and is often misunderstood. In contaminated wounds, bacteria that are inoculated into the wound are rarely the bacteria that go on to cause sepsis[86-89]. High numbers of bacteria at the time of initial debridement may reflect localized immunosupression, due to injured or devitalised tissue which may then be vulnerable to nosocomial infection.[11,90,91]

Culture of wound infections in patients following open fractures appears to show higher rates of resistance to prophylactic antibiotics and also higher rates of MRSA infection[86]. There is also concern that these patients are at risk of developing antibiotic resistance to other systemic infections (e.g. Lower respiratory tract infections) secondary to extended prophylactic antibiotic use.

The choice of antibiotic is also debated. In a review of the literature, Hauser et al[85], concluded that an anti staphylococcal agent such as a first generation cephalosporin would be an effective prophylactic choice in open fractures. The use of aminoglycosides for grade 3 open fractures is more controversial. Work by Patzakis et al[83,92], looking into wound infections in open fractures, found that many of the infecting organisms in the grade 3 injuries were Gram negative. He concluded that we should therefore include gram negative cover in our prophylactic regime of these fractures.

There are however many trials, showing no improvement in infection rates by adding Gram negative cover in these injuries[83,86,93-95]. There is also concern that including Gram negative cover at the prophylactic stage will only increase the speed with which antibiotic resistance develops[85]

A Randomized Control Trial (RCT) has shown that a twenty four hour course is as effective as a five day course of prophylactic antibiotics in penetrating abdominal trauma [96] . Dellinger et al looked at prophylactic antibiotic use in open fractures and showed that a one day course of a 2nd generation cephalosporin to be as effective as a five day course in preventing wound sepsis in a double blind RCT[93]. On the basis of this work the American guidelines are now suggesting a 24 hour prophylactic antibiotic protocol in open fractures [85].

The recent British Orthopaedic Association (BOA)/ British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS) guidelines have struggled to incorporate the current evidence on antibiotic usage with the concern over inducing Clostridium difficile infection as a result of their use[43]. They have therefore modified the antibiotic recommendations using microbiological principles, without an evidence base, and are suggesting Co-amoxiclav as an antibiotic which has a similar spectrum of activity, but may have a reduced incidence of Clostridium difficile associated diarrhoea.

1.3 HIV

1.3.1Background

HIV is a member of the retrovirus family that leads to acquired immune deficiency syndrome (AIDS). It is a blood borne virus that is transmitted via blood, semen, vaginal fluid, or breast milk. It was first observed clinically in 1980, but it was not formally identified until 1984[97].

There are 2 main types of the HIV virus. HIV-1 is thought to have jumped species from the common chimpanzee, and HIV-2 which is thought to have come from the Sooty Mangabey monkey. HIV-2 is confined to West Africa, and is not responsible for the HIV pandemic. HIV-1 is further divided into subtypes, which are geographical distributed as in Figure 1-2[98].

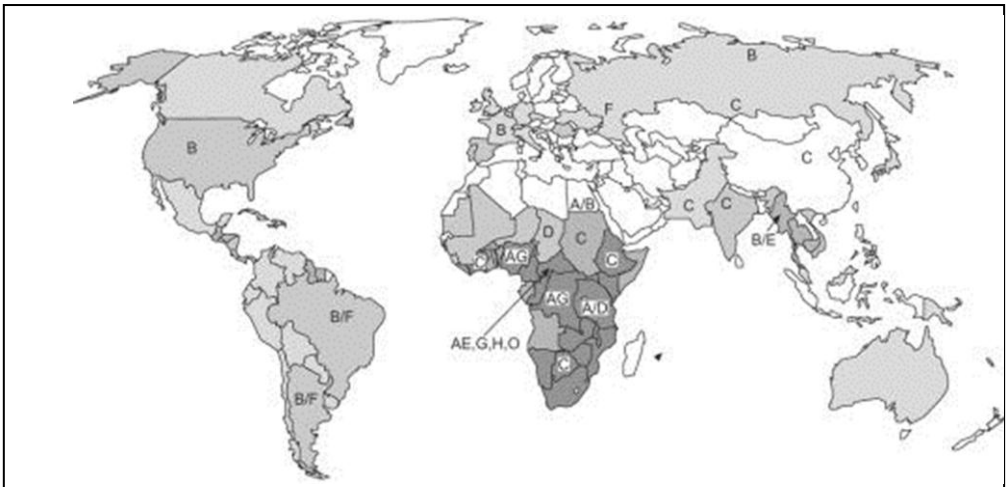


Figure 1-2 . Geographical distribution of HIV-1 sub types. Letters indicate the predominant HIV-1 sub type circulating in selected countries. A/B, B/E, and B/F indicate co-circulation of two dominant sub types; AE and AG indicates circulating recombinant forms.

Sub types although grossly similar do show individual characteristics, especially with regard to response to HAART[98]. The European and North American sub type is different to that found in Southern Africa.

HIV infects 3 main cell lineages, CD4-T cells, macrophages, and dendritic cells. Whilst infected T cells tend to undergo apoptosis, the other cell types tend to

remain chronically infected[99]. As infection progresses the number of CD4 -T cells detected in the blood decreases, this can be used as a marker of disease severity. The number of viral particles in the peripheral blood is a marker of viral replication, and again this can be measured (viral load), and gives an indication of the likely rate of disease progression. Viral load is often used to monitor for treatment failure as controlling the replication of the virus is the mainstay of treatment [100]. Financially CD4 counts are cheaper at approximately $35 a test, whereas viral load costs approximately $60, this means in resource limited settings viral loads are rarely done [101].

1.3.1.1 **Prevalence**

Prevalence of HIV varies from country to country. Exact prevalence rates are difficult to determine because of the lack of universal testing. However estimates are made using specific cohorts of patients such as rates obtained from antenatal care and emergency admissions. Globally 33.2 million people were estimated to be living with HIV in 2007[102]. Prevalence rates vary considerably by communities and regions. South Africa has the highest recorded rates in the world. Recent data from pregnant women in northern Kwazulu-Natal suggests a prevalence of around 38%[103]. In the UK the prevalence was estimated at 0.2 %[104]. Studies looking at patients admitted with major trauma to a Johannesburg Trauma unit showed an HIV point prevalence of 30%.[105]

1.3.2 Highly-Active-Anti-Retroviral Therapy (HAART)

Antiretroviral therapy (ART) for treatment of Human Immunodeficiency Virus type 1 (HIV-1) infection has improved steadily since the advent of combination therapy in 1996. Studies have shown a dramatic decrease in mortality [15,16,106] and in vertical and horizontal transmission[107,108] in patients on ART. Development of the treatment guidelines have shown that patients need to be taking three different drugs in order to get a good response to treatment. This three drug regime has become known as HAART [109] however the individual drugs used in this treatment regime may vary.

Timing of the initiation of HAART has been controversial. Institutions consider starting treatment when the CD4 count is between 350-200 cells/µL [110]. In countries where CD4 count is not available it is possible to start treatment depending on WHO clinical stage and total lymphocyte count.

Once HAART is initiated, CD4 levels typically rise but they do not return to normal. Provided the patient continues the medication, and the virus remains susceptible, CD4 counts should remain high. On discontinuation of HAART CD4 counts will return relatively quickly to their pre HAART levels and will then continue to decline[111].

Governments in Southern Africa have only recently started to distribute HAART freely as national programs, Botswana in 2001[112], South Africa in 2003[113] and Kenya in 2004[114]. In 2006 324,000 patients were taking HAART in South Africa, estimated to be approximately 21% of the patients who required it[102].

Several studies have suggested that HAART may interfere with bone metabolism[19,115-118]. It is however difficult to identify the causative factor, as HAART will always contain at least three drugs, but these vary between and within countries, and all patients will have HIV. Protease Inhibitors, one particular class of ARV's, have been identified in several studies as a possible cause of osteoporosis[19,118]. However most studies have been cross sectional and therefore causation can not be inferred. In the few longitudinal studies conducted, osteoporosis rates are high in patients receiving HAART, but do not appear to progress, once patients have been on treatment for over a year.[119,120].

It is however currently not possible to eradicate completely the virus. The virus continues to infect many cells, particularly macrophages and dendrocytes, which are important mediators of the immune system, and intracellular signalling. It is therefore not possible to assume that once a patient is initiated on HAART, wound and fracture healing will return to normal.

1.3.2.1 **Compliance**

HIV viral suppression, reduced rates of resistance [121,122], and improved survival [123], have been correlated with high rates of adherence to antiretroviral therapy [111].

The development of resistance to ARV's is emerging as a major problem in the treatment of HIV. Poor compliance leads to higher risk of developing resistance.

Most HAART regimes used in developing countries including in South Africa incorporate two Nucleoside Reverse Transcriptase Inhibitors (NRTI) and one Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI). The use of Protease inhibitors (PI) is limited to cases that fail initial therapy. NNRTI regimes are particularly prone to developing resistance in cases of poor compliance. This is predominantly due to the long half-life of the NNRTI[124]. PI have been implicated in osteoporosis[118].

In the series reported in this thesis it was apparent that trauma patients often forget to bring their medication, fail to tell the medical staff that they are taking HAART, or are unable to take because of inability to tolerate oral medication leading to poor compliance whilst in hospital and the consequent risk of developing a resistant virus.

1.3.3HIV and fracture management

In 1987 shortly after HIV was discovered, the first paper linking HIV with orthopaedics was published in the Journal of Bone and Joint Surgery. It described an HIV positive patient presented with diffuse osteomyelitis (Mycrobacterium kansaii) which was resistant to treatment[125]. Most of the subsequent research looking at HIV and surgical complications is based on observational, retrospective reviews, with small sample sizes and unmatched hospital controls[126]. Many of the conclusions from these studies need to be interpreted with caution.

1.3.3.1**Hoekman et al[127]**

In 1991 Hoekman et al published a prospective study case control study looking at internal fixation of closed fractures, comparing wound infection rates of HIV negative patients, HIV positive and asymptomatic and HIV positive and symptomatic. This study utilized the CDC classification, and all grade 4 disease was classified as symptomatic. The study was conducted before the WHO grading system was developed and before CD4 counts were available. The patients received no prophylactic antibiotics and the outcome measure was wound infection with positive microbiology. They followed their patients up for a minimum of 12 months. They had 174 patients who were HIV negative, 26 patients who were HIV positive but asymptomatic, and 17 patients who were HIV positive and symptomatic. There results are summarized in Table 1-3.

Table 1-3.

Group	Infection rate	Risk of developing infection compared to HIV negative cohort
HIV negative	8/174 (5%)	
HIV positive and asymptomatic	0/26 (0%)	RR= 0, p value 0.6[FE]
HIV positive and symptomatic	4/17 (24%)	RR = 1.39, p value 0.01[FE]

All infections in the symptomatic HIV group settled on antibiotics with out the requirement for metal work removal.

This data provided good evidence that the risk of infection was higher in patients with advanced (symptomatic) disease, however the data did not suggest that this should alter the management in this group of patients as they all did well with conservative treatment

1.3.3.2**Obrien et al[128]**

In 1994 O'Brien et al published a study from New York where they looked at 15 open fractures of the tibia, 3 of the 14 patients were HIV positive[128]. They were all managed using the same protocol and HIV did not affect management. All of the HIV positive patients developed wound infection, and two of them developed osteomyelitis. On the basis of this it summarized that HIV adversely affects outcome, and treatment algorithms should account for this.

1.3.3.3**Jellis[4]**

In 1996 Jellis reported his results of a retrospective case series in Zambia as part of a review of HIV and Orthopaedic surgery. His series included a total of 44 HIV positive patients with fractures. His data does not specify how this is broken down into patients with open/closed injuries, or patients treated either operatively or non-operatively and did not include any HIV negative controls.

His results showed that closed fractures, treated conservatively, healed without complications. However of the patients treated with internal fixation 33% developed infection. Open fractures fared even worse with a 72% infection rate and a 28% non union rate, the method of fixation is also not mentioned.

These results need to be treated cautiously due to the lack of controls, and lack of any statistical analysis. It is however an often quoted paper, and has added weight to the concern over treating open fractures with internal fixation.

1.3.3.4**Harrison et al[3]**

In 2002 Harrison et al conducted a single blinded prospective study looking at wound infections in HIV positive patients treated by internal fixation with both open

and closed fractures in Malawi. Patients treated with external fixation were excluded, the management protocols are not described and it is possible that patients with HIV may have been more readily treated with external fixation. This would have lead to a significant selection bias. In their series, open fractures were defined as including fractures with significant abrasions, repeat surgery for non union of a previously open fracture, or patients who had ever had previous surgery at the same site before. All patients received prophylactic antibiotics (1st generation cefalosporin). They used the ASEPSIS scoring system[129] to define infection which attempted to give some objectivity to their findings and had one surgeon scoring all the wounds to give consistency. They had follow up data for three months with only a 6% loss to follow up Their results are summarized in

Table 1-4:

Table 1-4 Summary of results from Harrison et al [3] study looking at wound infection in HIV positive and negative patients following internal fixation of fractures

Closed fractures			
(%)	Infection	No Infection	
HIV positive	1 (4)	27 (96)	P value^{FE} = 0.28
HIV negative	6 (6)	102 (94)	RR = 3.6 (0.5-24.5) $^{95\%CI}$
Open fractures			
(%)	Infection	No Infection	
HIV positive	5 (42)	7 (58)	P value^{FE} = 0.08
HIV negative	3 (11)	24 (89)	RR = 3.75 (1.1-13.2) $^{95\%CI}$

Of the patients who developed infections three of the five HIV positive patients were having operations for previous non-unions, whereas the HIV negative patients were all undergoing primary plating. This suggests the study group and the control may not have been comparable, possibly due to selection bias as a result of higher non union rates in HIV positive patients.

These results suggest there is little evidence of an association between HIV and infection rates in closed injuries. There was a fourfold increase in risk of superficial wound sepsis in open fractures but weak evidence that this finding was not due to chance alone.

1.3.3.5**Harrison et al[130]**

In 2004 Harrison et al published a prospective blinded series of 27 severe open tibia fractures Gustillo Anderson grade 2 and 3 of which 7 were HIV positive, looking at wound infection. Patients were all managed initially with a monolateral exfix till the soft tissues had healed or were at least stable. The frame was then removed and the fracture managed till union in the plaster. HIV did not affect management. The outcomes were the wound scores measured by the ASPESIS scoring system and Checketts pin score and bony union. They showed a significantly higher rate of sepsis in the wounds and a higher rate of non union although this was not significant in the HIV positive group, the data also suggested a slower rate of fracture healing in these patients. They acknowledge that the management of their patients was unusual but it was the method that worked best in the limited resource setting.

1.3.3.6**Harrison et al[131]**

In 2004 Harrison et al also published the 1 year follow up of a group of 36 HIV positive patients who had undergone implant surgery for closed fractures, non unions or joint arthroplasties. At 1 year no implants had developed deep infections to the author's knowledge although 8 patients were lost to follow up and 2 died of causes unrelated to their implant. This study suggests that in clean orthopaedic implant surgery the risk of delayed sepsis at 1 year is minimal.

1.3.3.7**Norrish et al[6]**

In 2007 Norrish et al conducted a single blinded prospective cohort study looking at pin site infections in open fractures treated by external fixation. They had selection criteria of tibia fractures that were grade 2 or more and were suitable for external fixation, with both the surgeon and the wound assessor blinded to HIV status. They attempted to make the scoring system objective using the Checkett's pin site scoring system and they scored pin sites individually, so each patient had six data entries. They did not look at union rates.

They study looked at 15 HIV positive patients and 35 HIV negative patients. In the whole study only 1 HIV positive patient had the external fixator removed due to

infection. Analysis of individual pin sites showed that HIV positive patients were more likely to be treated with antibiotics than HIV negative patients for pin site sepsis, and that the mean pin score was significantly higher in the HIV positive patients. It was also noted that the HIV positive patients had the external fixator on for a longer period of time, although the criteria for removal have not been discussed. They were unable to show any correlation of infection with CD4 count.

External fixation requires maintenance of the mechanical properties of the bone pin interface. Conventional pins are associated with a gradual deterioration of this interface over time, and consequent loosening and or infection[132]. It is possible that the results of the study by Norrish et al[6] is due to delayed fracture healing placing more pressure on the pin/bone interface for longer periods of time and hence the higher rates of infection.

Norrish et al used individual pin sites for statistical analysis, so one patient accounted for 6 pin sites, and therefore 6 data entries. If a pin site becomes infected or loose, it is likely that the other pin sites will also become infected due to proximity and association through the rigid construct. The lack of independence of the data points may invalidate the statistical conclusions that patients with HIV are more likely to develop more severe pin site infections.

1.3.3.1 **Bahebeck et al**

In 2009 Bahebeck published a prospective series of patients who underwent implant surgery in the trauma and orthopaedic department in their hospital in Cameroon they looked at weather starting early ARV treatment and giving prolonged ABx improved outcome. It was a consecutive series, but excluded all patients with open fractures, with non-unions, and all patients who were lost to follow up, had a CD4 count below 500, or any sign of AIDS. The admitted 1145 patients for implant operations over a 36 month period, of whom 500 were excluded. Of those included 74 had HIV. Their study showed that all patients did well regardless of the intervention, with very little difference between the groups, with a 5.4% infection rate in the HIV positive patients and a 6.5% in the HIV negative patients. This study adds weight to the hypothesis that early HIV does not interfere with wound healing in clean procedures.

1.3.3.2**Summary**

The studies that have included closed fractures have been unable to demonstrate any significant difference in the early outcome of asymptomatic HIV positive and HIV negative patients. They generally did not look at rates of delayed sepsis or union rates. Groups that have limited evidence for being at higher risk of developing wound infections in patients with HIV are open fractures, fractures to the tibia and patients with opportunistic infections secondary to HIV.

The studies that included open fractures remain limited. All the trials suggest higher rates of infection in the HIV positive patients, but have only provided weak evidence that this was not due to chance alone. All have bias or confounding factors which may have affected their results, as discussed above. This review of the currently available evidence provides no guidance on how these fractures should be treated, i.e. conservatively or with internal or external fixation. These trials provide no evidence on the rate of delayed sepsis or union rate.

At present there seems to be insufficient evidence in the literature to guide surgeons in the management of these patients. None of the trials on either open or closed fractures included patients on HAART.

1.3.4Surgery and HIV

Many other studies have looked at other surgical procedures, trying to identify whether HIV is a risk factor for infection. Many of these studies are retrospective reviews of medical records. The quality of these trials is variable and there is a huge variety in results. They are summarized in a review article by Rose et al[126], who notes that although the rate of minor complications may be higher in HIV positive patients there is not any obvious difference in outcome. They conclude that HIV should not affect surgical management unless there is a good quality trial for the specific situation.

Two papers are worthy of individual note as they are prospective studies.

Dobson[133] in 1997 undertook a prospective case control study looking at complications following tooth extraction in healthy HIV positive patients in the outpatient setting and compared against age-matched community controls. He noted a slight increase in minor post-operative complication in the HIV positive group but there was insufficient evidence to suggest that this association is real and not just due to chance. All complications were treatable as an outpatient. He concluded that, if the patient was well enough to present as an outpatient then HIV seropositivity should not affect the management of the patient (with regard to tooth extraction).

Louis et al in 2007 published the largest prospective cohort study to date looking at morbidity following caesarean section in HIV positive patients. Caesarean sections are heavily studied, because current guidelines suggest that all HIV positive mothers should have a caesarean section, to reduce the risk of maternal to child transmission of the virus. Although the data was prospectively collected, it was not initially collected for this purpose, and only at a later date was analysis for HIV performed. The series included 378 HIV positive patients and compared them to 54,281 HIV negative patients. Univariate analysis showed that the HIV positive group were more likely to be Afro-American, unmarried, have used tobacco and intravenous drugs during pregnancy, and have decreased education in comparison to HIV negative group. Postoperatively there were increase in endometriosis, maternal sepsis, and maternal death seen in the HIV positive group. CD4 counts or the use of HAART were not recorded. They concluded that the risks of caesarean section needed to be

weighed against the risk of transmission of HIV to the foetus, and that in low risk scenarios caesarean section should be avoided.[134].

This large study supplies the best evidence in the literature that HIV significantly affects the mechanisms involved in wound healing and is therefore of possible relevance to wound healing in open fractures.

1.3.5 Pathophysiology of HIV and wound/fracture healing

Many theories have been postulated as to the mechanism with which HIV and HAART may interact with bone and wound healing.

A summary of postulated interactions is given below:

1.3.5.1 **Host Factors**

1.3.5.1.1 Immunosuppression

Bacterial infections are known to be more common in HIV positive patients[135,136]. Episodes of bacterial septicaemia are also noted more frequently in association with HIV[137-139]. S. aureus infections are also more common in patients with HIV[139,140].

HIV infects CD4 cells, macrophages and dendritic cells. CD4 cells are thought to be involved in cell-mediated immunity, whereas macrophages and dendritic cells are thought to be involved in the innate immune system[8]. CD4, CD8 and macrophages have all been shown to be present in soft tissue bacterial infection. It is thought that the CD4/8 cells are involved in activating the macrophages in the presence of bacterial infection[13].

On initiation of HAART CD4 levels tend to rise, whether these cells regain all their functionality is not known. It is thought that macrophages remain infected, and high levels of virus have been found in macrophages in patients even when on an effective HAART regime [141]. It is thought that these virally infected cells are likely to be functionally impaired[8].

There is also evidence that HIV can affect the functionality of macrophages even if not actually infected with virus, this may be due to a disruption in the inter cellular signalling cascades from other infected cells[142,143].

Recent data have suggested that in contrast to opportunistic infections, the incidence of Gram positive infections may not be reduced by initiation HAART[144-146].

In conclusion there are many ways that HIV may lead to increased susceptibility to bacterial infection, and this susceptibility may or may not be reduced by HAART treatment.

1.3.5.1.2 The immune system and wound healing

The immune system has been shown to be important in wound healing [9]. Macrophages and lymphocytes have been shown to migrate into the surgical wound[147]

Macrophages have been shown to be important in wound healing. Guinea pigs with depleted macrophages have been shown to have impaired wound healing and fibroplasia[148], conversely rats that have had injections dermal of macrophages into scars have shown an increase in collagen synthesis and wound breaking strength[149]. They are thought to be critical by both their antimicrobial/phagocytosis role, but also in the secretion of enzymes (collagenase,elastase), cytokines and growth factors. Macrophages released enzymes and growth factors are listed below[9].

- Tumour necrosis factor–α
- Interleukin-1
- Interleukin-6
- Transforming growth factor–α
- Transforming growth factor–β
- Platelet-derived growth factor
- Insulin like growth factor–1

Many of these processes have been shown to be impaired by infection with HIV[8]. No studies were found looking directly on how this may influence wound healing. In vitro studies would suggest wound healing without functioning macrophages may lead to a wound that is weaker and more prone to infection, however the complexity of wound healing means that this conclusion needs to treated cautiously[9].

Lymphocytes as shown in Figure 1-3, migrate into the wound on day 5 and peak on day 7. Suppressor T cells (which includes CD4 and CD8 cells) have been shown to be important in recruiting cells(macrophages, endothelial cells and fibroblasts) and

also act in a late counter regulatory role, perhaps being responsible for the orderly completion of wound healing [150]

Isolated depletion of CD4 T cells has been shown to lead to an decrease in wound strength, whereas a depletion of CD8 cells leads to an increase in wound strength[151]. It is hypothesized that these T lymphocytes are involved in the regulation of wound healing.

The role of the immune system in wound healing remains poorly understood. Many of the cell lines involved in wound healing are infected in HIV. These cells are involved in recruitment, phagocytosis, and the regulation of wound healing. It is therefore likely that HIV affects wound healing at some level, but further studies are needed to identify these effects.

1.3.5.1.3 Osteoimmunology

The immune system has been shown to be linked to responses wound and fracture healing. Macrophages, lymphocytes and dendritic cells have all been found in early callus formation[152].

Cell mediated immunity is also thought to affect fracture healing. T cells have been implicated in this process. In one study the thymus was removed in mice, this led to an inhibition of natural killer cells, and T cells[153]. The result was a significant delay in fracture healing compared to controls. The authors concluded that T cell immunity was integral in the repair and regeneration of bone tissue. More recently Colburn et al [12] have looked at the role of a small subset of T lymphocytes, γ/δ and their role in fracture healing. These subsets of T lymphocytes are able to recognize products of stressed cells without the need for antigen presenting cells, and are therefore thought to be part of the innate immune system. This study looked at fracture healing in γ/δ T lymphocyte deficient animals and controls. It showed the localization of γ/δ T lymphocytes to the fracture site in controls, and suggested that their presence is detrimental to fracture healing and fracture strength. The exact mechanism for this process is not understood, but the authors speculated that it may be due to the up-regulation of the α/β T lymphocytes, in the γ/δ T lymphocytes deficient animals. The α/β T lymphocytes are thought to be beneficial to bone

healing. CD4 cells are a subtype of α/β T lymphocytes and how HIV infection affects these processes has not been studied.

A recent study by Toben et al[14], showed that mice deficient in both B and T lymphocyte, had enhanced fracture repair, with a stronger and more rapidly occurring healing. A reduction in inflammatory ctyokines and an up regulation of anti inflammatory interleukins was observed in callous compared to that of the controls.

Gaston et al has shown that in long bone fractures in mice, macrophages and T cells are recruited into the fracture site. In the same study nude mice, depleted in T and B cells, had enhanced fracture repair, with a stronger and more rapidly occurring bone healing[154]. Individual cellular lineages responsible have not yet been identified.

The exact role of the macrophage in fracture healing has been poorly studied. It has been observed in the callus, and many of the inflammatory mediators found in the callus are known to be released by macrophages[155]. It is likely that its role is similar to that in wound healing, one of recruitment of other cells, stimulation of cells and phagocytosis of bacteria or foreign bodies.

A literature search, revealed no experimental studies into HIV effects in osteoimmunology in either human or animal studies.

Major trauma is known to lead to a suppression of systemic cell mediated immunity[156,157]; how this occurs is poorly understood. Many theories have been postulated, these are summarised by Ayala et al in a review article[156]. They conclude that changes in the antigen presenting cells at the fracture site, lead to a suppression of the cell mediated immunity and macrophages play a critical role in this process. How an HIV infected individual may react to major trauma has not been studied. It is possible that damage to the macrophages in HIV positive patients may alter their response to major trauma.

1.3.5.1.4 Hypoalbuminaemia

HIV positive patients are known to have increased rates of hypoalbuminaemia [158] In the catabolic phase post trauma, and post-surgery, albumin levels are likely to drop further[159]. Low albumin has been shown to be deleterious to wound healing and fracture healing[74,160].

1.3.5.1.5 Vascular Disease

HIV positive patients are at high risk of developing vascular disease both cardiac[161] and peripheral[162,163] despite lower incidence of traditional risk factors. Periard et al concluded that there is an epidemic of peripheral vascular disease in HIV positive patients which is presenting approximately 20 years earlier than would be expected in the general population[164].

A variety of different mechanisms for this have been proposed. For cardiac disease the hypothesis is that HIV affects visceral fat distribution[165] leading to Metabolic Syndrome. This syndrome occurs in the normal population and is associated with central obesity, diabetes and hypertension. In HIV patients it appears to be more severe, with higher TG levels and lower HDL, despite a lower BMI when compared to similar patients with Metabolic Syndrome without HIV[166],[167]. After starting treatment with HAART, hypercholesterolemia and hypertriglycaemia is often seen. However HDL levels still do not return to normal[168] . The hypercholestrolaemia is thought to be due to HIV infected macrophages being unable to discharge the cholesterol they have mopped up. This makes them redundant for cholesterol transport[169]. HIV and HAART (Protease Inhibitors particularly) are also thought to increase the expression of CD36 markers on macrophages. These markers are critical for the formation of foam cells an essential precursor to atherosclerotic lesions[161].

Other mechanisms:

HIV patients appear to be in a state of sustained acute phase response, as a result of the viral infection: this is shown by a relatively high CRP seen in these patients[170]. Sustained high CRP has been shown to correlate well with cardiac vascular disease[171]

TAT protein is released from HIV infected cells, this interacts with endothelium, and leads through a variety of pathways to endothelial dysfunction[172,173]

Dying CD4 cells, lead to increased production of shed membrane particles, these lead to a reduction in nitric oxide, and prostacyclins, leading to vasoconstriction and endothelial dysfunction[174]

Since vascular supply to both the bone and the wound are critical for healing, damage to the endothelium and the vessels supplying the fracture site/wound, are likely to lead to increased wound infections and decreased union rates.

1.3.5.1.6 Osteoporosis

Several cross sectional studies have shown high rates of osteoporosis and osteopaenia in HIV patients compared to normal controls[115,175,176]. They have hypothesiszed that this might be due to modulation to the TNF alpa superfamily[177] HAART treatment has also been implicated in osteoporosis, although this group of patients also tend to be the ones who have had the longest exposure to HIV[118]. The mechanism by which HIV causes osteopaenia is not known, however a variety of biochemical markers involved in bone metabolism have been shown to be altered in HIV infection.

Osteocalcin – seen in lower levels in HIV positive patients[178-180] -, increasing levels seem to correlate with increased rates of bone formation in fracture healing[181]

TNF alpha – seen in high levels in HIV patients – Can stimulate both osteoblasts and osteoclasts. Its exact role has not been elucidated[182].

Osteoporosis can lead to difficulties in obtaining stable fixation with screws because of poor bone quality, this may then lead to premature failure of the bone fixation.

1.3.5.1.7 Prophylactic antibiotics in HIV positive patients

Studies have suggested that bacterial carriage may differ in HIV positive and negative populations. These studies have suggested that HIV positive patients may have higher rates of S. aureus on nasal swabs, and also higher rates of resistance, particularly MRSA[**183-187**]. Rates of S. aureus carriage and resistance patterns vary significantly between different populations and different institutions. Studies done on healthy volunteers with and without HIV failed to show any variation in colonization rates[188]. Trauma patients (with open fractures) are generally young and otherwise healthy, however on admission, all will have i.v. cannulas inserted, and be started on prophylactic antibiotics. They also are admitted to hospital for anywhere between 2-60 days. These factors all predispose them to becoming carriers of nosocomial bacteria.

In HIV positive patients, the use of prophylactic antibiotics against opportunistic infections such as Co-trimoxazole has been implicated in the high levels of resistant bacteria found on nasal swabs[183,184,189]. In South Africa all patients with HIV and a CD4 count below 500 cells/µL, as well as infants exposed to HIV during child birth are advised to take Co-trimoxazole.

Whether these factors affect the antibiotics that should be used for prophylaxis in open fractures has not been studied, but may be relevant factor in the development of wound sepsis.

1.3.5.2 Environmental Factors

1.3.5.2.1 Multiple Hospital attendance

Patients with HIV are at higher risk of developing many pathologies and therefore are likely to spend a greater proportion of time in hospital than the general population[190]. Once diagnosed with HIV, even patients who are well are likely to spend increased time in hospital, as they are screened for opportunistic infections, base-line bloods are taken, and results given. This period of time spent in hospital may alter the patient's bacterial flora, so increasing the chances of colonization by resistant species such as MRSA[139].

1.3.5.2.2 Decreased social group/education

The social demographics of the HIV positive population are very dependent on the predominant local route of transmission. The demographics of intra-venous-drug (IVD) users, homosexual and heterosexual transmission groups will vary dramatically. Many studies have shown a lower education level in their HIV positive cohort[134]. This may be a relevant confounder when looking at outcomes. High risk behaviour such IVDU, and smoking, may be seen in higher rates in certain cohorts of HIV positive patients[134]. Smoking has been shown to be detrimental to fracture healing[78], and IVD use may lead to increased rates of S. aureus septicaemia compared with the general population. This may lead to delayed sepsis of metal work[191].

1.3.6 Implant infections in HIV

1.3.6.1 Acute Infection – within 6 weeks of operation

Acute implant sepsis occurs in the first 6 weeks postoperatively. Bacteria invade through the open wound, or surgical site, and in the presence of favourable conditions (necrotic bone, mobile fracture or damaged/contaminated tissue) will colonize the implant, create a biofilm, and may lead to chronic sepsis; this process is described in section 1.3.2.

It has been postulated that in cases of contaminated fractures, bacteria may lie dormant, adherent to the implants for many years. If the immune system falters the bacteria multiply and sepsis follows[192]. This may be difficult to differentiate from late infection.

1.3.7Late implant infections in HIV

Late infection is most commonly due haematogenous spread, often from a S. aureus bacteraemia. In this process bacteria enter the blood stream from a site unrelated to the implant. S. aureus can bind to metallic implants and lodge at the implant site. Late infection can occasionally be due to reactivation of bacteria that initially contaminated the wound at the time of the initial injury[193].

Murdoch et[194] al showed that following a S. aureus bacteraemia, 34% of joint prostheses, but only 7% of orthopaedic implants became infected. In a retrospective study looking at joint replacements in HIV infected haemophiliacs, Hicks et al[195] noted high rates of delayed sepsis; these being 18.7% in patients undergoing primary procedures, and 36.3% of patients undergoing revisions, with a mean delay between surgery and sepsis of 47 months. The incidence of S. aureus bacteraemia in the HIV positive population appears to be more common [137-139]. Burkley et al[191], in a study at John Hopkins Hospital (USA), showed a 12% incidence of MRSA bacteraemia per patient year in HIV positive patients. Risk factors for bacteraemia were found to be: intravenous drug use, low CD4 and end stage renal disease.

Harrison et al have published a study looking at delayed sepsis in a cohort of 36 HIV positive patients treated with internal fixation treated for closed fractures, looking at rates of delayed sepsis at 1 year. They had no cases of metal work removal due to infection at 1 year.

In summary there is significant theoretical evidence supporting the removal of implants in HIV positive patients once fracture healing has occurred; however there are few clinical trials addressing this issue. In resource-limited settings such as sub Saharan Africa, the resources are often not available for routine removal of implants. In the development of guidelines for the acute management of these patients in the above setting, it is important to know about the long-term morbidity of internal

fixation. Hence the need for long term follow up studies of internally fixed fractures in HIV positive patients.

1.4 Conclusions

The treatment of open fractures with internal fixation, in certain situations is now considered the optimal method of fixation for obtaining union[54]. The most serious complications associated with this type of management are infection and non-union. There are many factors that influence the development of infection in the presence of metal work. Optimizing these factors appears to have led to a dramatic decrease in the rates of infections. However once infection has occurred, it remains difficult to treat and often requires removal of the metal work and surgical debridement.

The pathophysiology of HIV is complex and affects a huge variety of pathways many of which are related to fracture management and healing. There are few clinical studies looking at outcomes in open fractures in relation to HIV. These studies involved small numbers and most did not attain statistical significance however they all suggested higher rates of infection than in controls. There are no experimental studies on the effect of HIV on fracture healing. However the clinical trials combined with a strong theoretical basis of how HIV and HAART may influence fracture management necessitates a larger prospective study looking at the clinical outcomes.

Aspects felt to be important which have not been adequately investigated are:

Whether HIV is a significant risk factor in the development of wound infections or non union in open fractures?

Whether the rate of wound infections/complications correlates with the CD4 count in HIV positive patients?

Whether external fixation a viable alternative to the treatment of these fractures in this group?

Whether the initiation of HAART affects rates of infection or non union?

What is the incidence of delayed sepsis in HIV positive patients with metal work in situ, and whether we should routinely be removing metal work in these patients?

The Aims of this project are based on the lack of evidence, highlighted above, and the clinical and material resources that were available to us.

1.5 Aims of the study

To investigate the following null hypotheses:

1. That there is no association between HIV (or advanced HIV) and wound infection in open fractures treated with surgical stabilization.
2. That there is no association between HIV (or advanced HIV) and non union in open fractures treated with surgical stabilization.
3. That there is no association between HIV (or advanced HIV) and wound infection in open fractures treated with internal stabilization.
4. That there is no association between HIV (or advanced HIV) and non union in open fractures treated with internal stabilization.
5. That there is no association between HIV (or advanced HIV) and pin site sepsis in open fractures treated with external fixation.
6. That there is no association between HIV (or advanced HIV) and non union in open fractures treated with external fixation.

Chapter 2

Material and Methods

2 Material and Methods

2.1 Location

Because of the high incidence of trauma in an area where HIV is prevalent, providing a rich source of clinical material, the study was carried out on patients admitted to the accident and orthopaedic departments of Ngweelzane Hospital, Empangeni, Kwazulu Natal, South Africa.

Kwazulu Natal is a province in the North East corner of South Africa. Its capital is Durban, it goes north to Mozambique and Swaziland border, and in the west it borders Lesotho. There is considerable health migration from these bordering countries. This leads to a heavy burden being placed on the government funded hospitals in the province. Figure 2-1 shows the location of Ngwelezane Hospital and the area from which it receives referrals.

Figure 2-1 Illustrating the referral area of the study hospital

The hospital takes referrals from 13 peripheral hospitals. It supplies services for orthopaedic and general surgery, internal medicine, paediatrics, casualty, intensive care, ophthalmology, infectious disease, TB and HIV medicine. It has an associated obstetrics and gynaecology hospital nearby. Radiology is available 24 hours a day, with a multi-slice CT scanner, and an MRI scanner. The Orthopaedic Department is staffed by 1 permanent consultant, 1 part time consultant, 5 medical officers/registrars, and 5 interns/house officers.

The hospital provides referral services to an area 36396 sq. km, and a population of 2.3 million, equivalent to that of approximately half the size, and half the population of Scotland. It is a largely rural area, with only 10% of the population currently employed. The average income per capita per annum is 2565 ZAR ($256). The quality of the roads is also poor with three of the peripheral hospitals approximately four hours' drive away. The hospital has access to helicopter and fixed-wing aircraft for emergency transfers from rural hospitals. The helicopter is unable to fly at night. These services are utilized in life and limb threatening scenarios. At night however all forms of transport are dangerous, and if possible transfer will wait for daylight hours.

Mtubatuba, a town approximately 60km north of the hospital, has been shown to have one of the highest rates of HIV in pregnant women in the world, namely 38%.

It is an area of heavy trauma workload, which comes from three sources:

Motor vehicle accidents (MVA)/Pedestrian vehicle accidents (PVA). The roads are of poor quality: the informal buses/pickups are overcrowded and frequently crash. Rural roads often have animals on them, making driving at night dangerous, and there is a high incidence of drink-driving. These factors mean that MVAs and PVAs, with severe trauma, and often open fractures, are commensurately frequent.

Gun-shot injuries, often alcohol-related, predominantly from low energy hand guns and occasional shot guns

Domestic violence/ village justice – often involves multiple chops by a bush knife to the forearm and hands. The blades and the wounds are normally clean but not sterile.

2.2 Trial design

Given the evidence discussed in the introduction which has suggested that HIV infection might lead to higher wound infection through a variety of effects, both direct and indirect. It is postulated that as HIV advances and CD4 count falls the effect on risk of wound infection might rise. The method of fixation is dependent on a variety of factors, as discussed in section 2.7.7, including HIV status. It is not known if the method of fixation leads to a difference in wound infection rates, and whether HIV is a more important risk factor given certain types of fixation. These relationships are summarised in Figure 2-2.

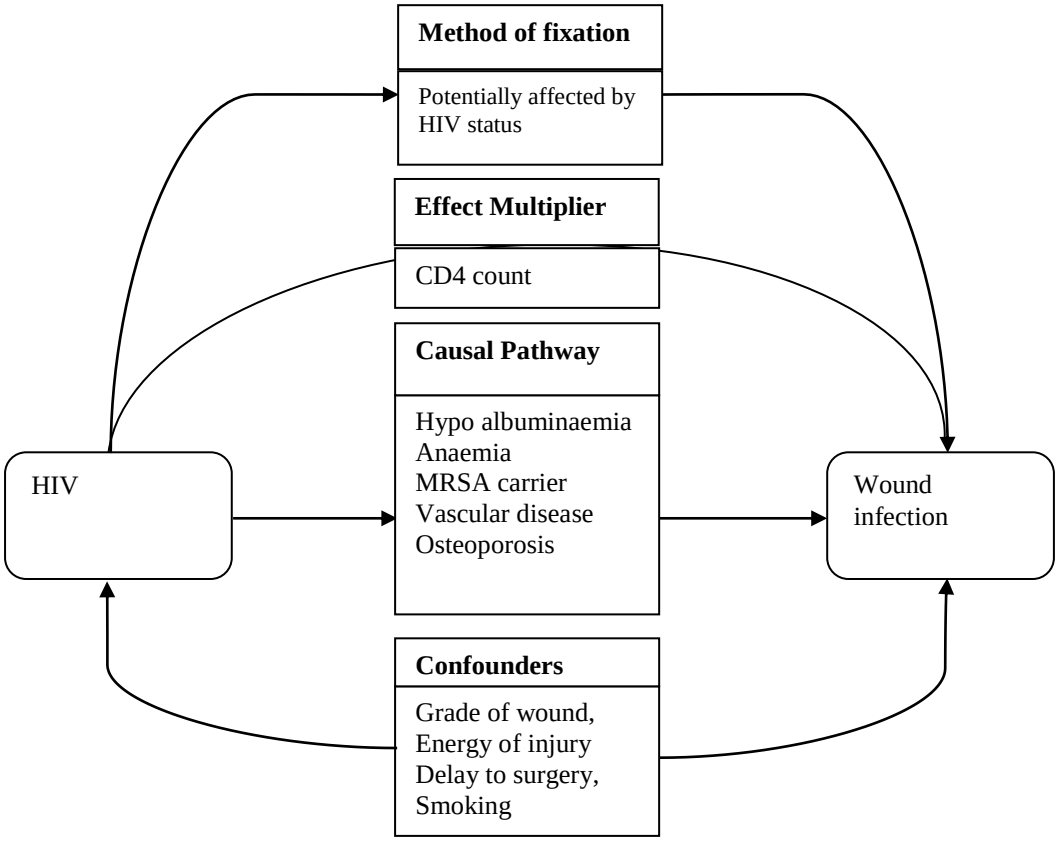


Figure 2-2 Showing the possible routes of association between HIV and wound infection

The scope of this thesis was not to make a direct comparison of internal and externally fixation for open fractures, but to make an assessment of both of their suitability to treat open fractures in patients with HIV, whilst also assessing the effect HIV has on fracture and wound healing .

In assessing the effect of HIV infection on non union and pin site sepsis a similar diagram can be constructed, the only difference being the addition of wound infection as a potential risk factor for non union. These relationships are summarised Figure 2-3 and Figure 2-4

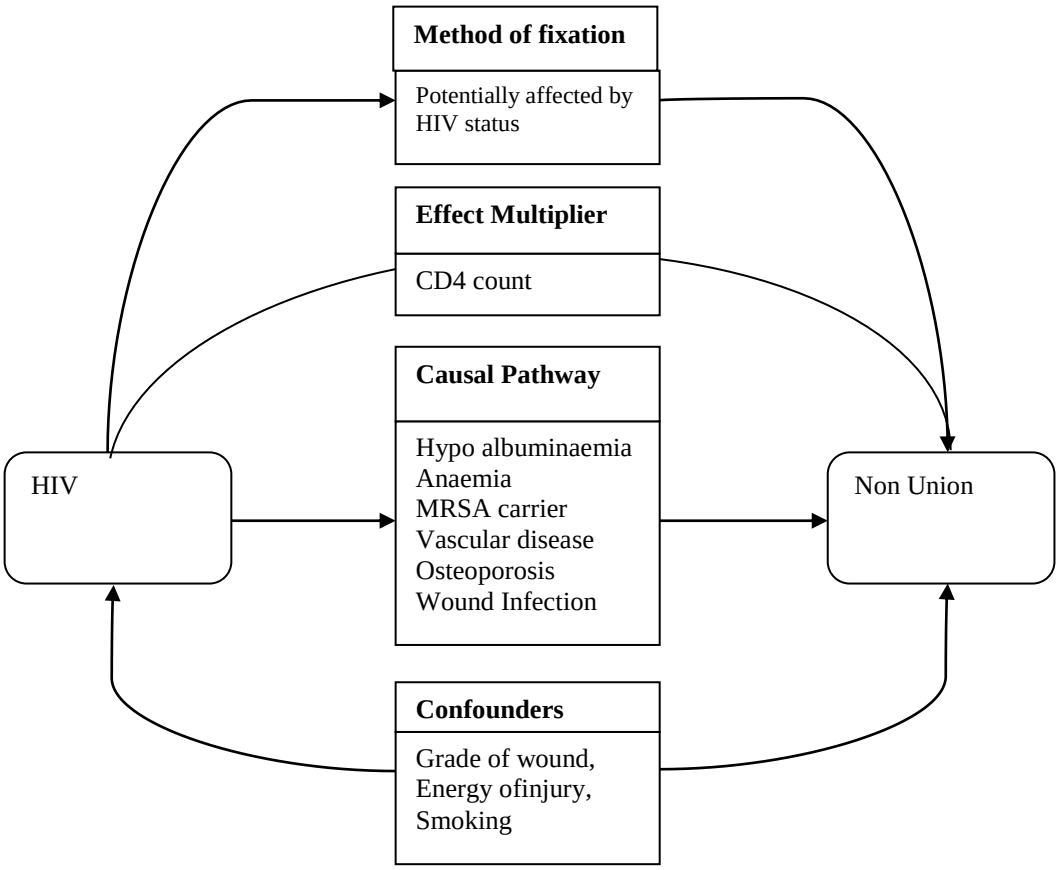


Figure 2-3 Showing possible mechanisms of association between HIV and non union

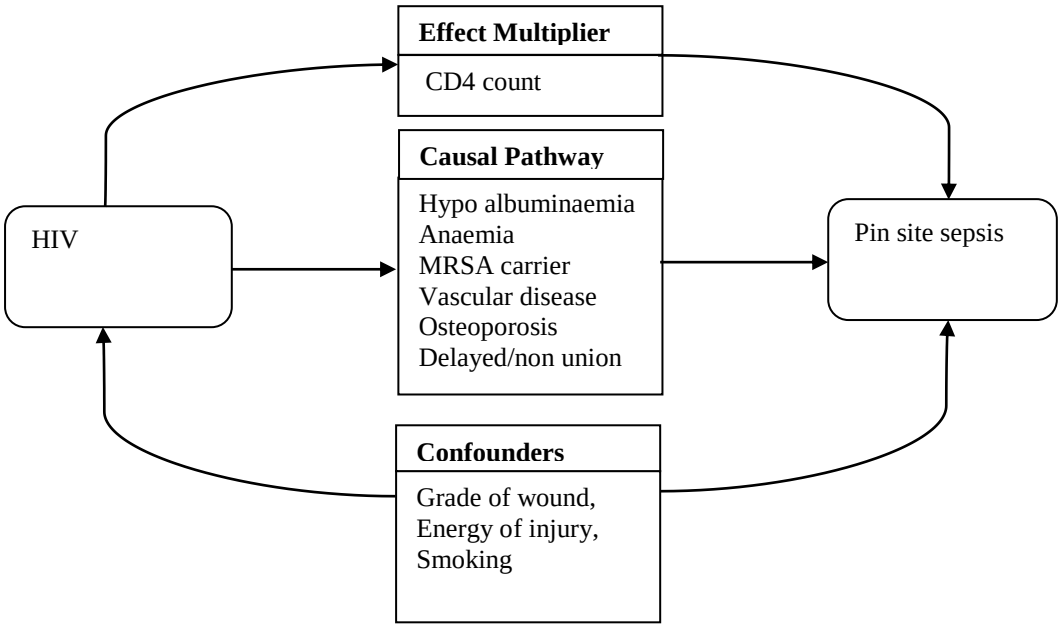


Figure 2-4 Showing possible mechanisms of association between HIV and Pin site sepsis

2.3 Patient Cohorts

In order to address the different aims of the study, 3 distinct cohorts of patients were chosen as shown in Figure 2-5 . The data from the different cohorts is analysed and discussed independently. These cohorts all have distinct inclusion and exclusion criteria:

Figure 2-5 Illustrating the 3 cohorts used in data analysis

2.4 Cohort 1: Open fractures treated by surgical fixation

This cohort will be studied using a prospective-epidemiological-observational study to examine the rates of wound infections and fracture union

2.4.1Inclusion Criteria

All adults (over 16, so able to consent for an HIV test) admitted to Ngwelezane hospital who underwent internal or external fixation for open fractures (including significant abrasions over fracture site) between the between the 27th May 2008 an d the 3rd March 2009 .

Harrison et al[3], looked at patients who had broken skin at the fracture or in the vicinity of the operative site, and placed these patients in infection risk category one. It was using these selection criteria that the authors showed a high rate of infection. It was therefore felt appropriate to include such patients, with significant abrasions, within the open-fracture classification.

2.4.2Exclusion Criteria

Exclusion criteria were designed to try and reduce the heterogenirty of the patients, and to prevent the effect of a loss to follow up bias, by excluding patients who we would have trouble getting to come back to clinic.

Intensive care treatment

Mental illness/Dementia

Patients from outside the hospital catchment area requesting ongoing treatment at their local hospital

Patients who were unable to provide contact information, such as a phone number, in order to chase if missed appointment.

Patients who were discharged to a prison other than the local Empangeni prison, due to difficulty in getting follow-up from these institutions

2.5 Cohort 2: Open fractures fixed by internal fixation

This cohort will be studied using a prospective-epidemiological-observational study to examine the rates of wound sepsis and union rates. It will attempt to identify risk factors, particularly with relation to HIV, for the development of wound infections or non-unions.

2.5.1Inclusion Criteria

All adults (over 16) admitted to Ngwelezane hospital who underwent internal fixation for open fractures between the 27th May 2008 and the 3rd March 2009 .

2.5.2Exclusion Criteria

It was felt inappropriate to include the following categories of patients in the series.

Intensive care treatment

Mental illness/Dementia

Patients from outside the hospital catchment area requesting ongoing treatment at their local hospital

Fixation using K wires, with the intention for removal at approximately 6 weeks (as not regarded as internal fixation)

Patients unable to provide contact information, such as a phone number in order to chase if non-attendees at follow-up

Patients who were discharged to a prison other than the local Empangeni prison, due to difficulty in getting follow-up from these institutions

2.6 Cohort 3: Open fractures treated by external fixation

This cohort will be studied using a prospective-epidemiological-observational study to examine the rates wound and pinsite infections and fracture union.

2.6.1Inclusion Criteria

All adults (over 16) admitted to Ngwelezane hospital who underwent external fixation for open fractures (including abrasions) between the between the 27th May 2008 and the 3rd March 2009 .

2.6.2Exclusion Criteria

Intensive care treatment

Mental illness/Dementia

Patients from outside the hospital catchment area requesting ongoing treatment at their local hospital

Patients unable to provide contact information, such as a phone number in order to chase if non-attendees at follow up.

Patients who were discharged to a prison other than the local Empangeni prison, due to difficulty in getting follow-up from these institutions

2.7 Preparatory work

2.7.1Power studies

2.7.1.1**Wound infection in internally and externally fixed fractures**

The study reported by Harrison et al from Malawi[3], was used as a pilot.

In their small cohort of patients with open or “high risk for infection” fractures treated by internal fixation (n=39) there was a 4.2 times increase in the rate of wound infection in the HIV positive group (n=12) of patients.

However, a power calculation, calculated using Epi Info (ver 3.4.3, see appendix) using 95% confidence, 80% power, and a ratio of uninfected to infected patients of 3:1, shows that the number required to permit statistically valid conclusions were: 21 HIV positive patients and 63 HIV negative patients.

Retrospective data from a review of the operating lists, suggested that at Ngwelezane Hospital on average 5 open fractures a week were internally fixed. With an approximate background rate of HIV of 30%, it was estimated over the nine month period available to complete such a study 180 open fractures would be operated on, 54 of whom would be HIV positive. This would enable statistically valid conclusions to be drawn, for both the combined cohort and the internal fixation cohort.

2.7.1.2 **Non Union in internal and externally fixed fractures**

The paper published by Jellis[4], is the only data available regarding non-union rates in internally fixed HIV positive patients. He quotes a non union rate of 35 % in open fractures treated with internal fixation. The demographics of his population are not commented on. Reports of non union rates in HIV negative patients vary[196] but a rate of 5% in a varied set of injuries, which are predominately grade 1 and 2 was thought to be reasonable by the authors.

A power calculation, calculated using Epi Info (ver 3.4.3) using 95% confidence, 80% power, and a ratio of uninfected to infected patients of 3:1, shows that the number required to permit statistically valid conclusions were: 16 HIV positive patients and 48 HIV negative patients.

The retrospective data discussed in 2.7.1.1 suggests that it would be possible to enrol this number of patients in the time period available to us. This would enable statistically valid conclusions to be drawn, for both the combined cohort and the internal fixation cohort.

2.7.1.3**Pin-site sepsis in externally fixed fractures**

The study by Norrish et al[6] was used as a pilot. In their cohort of patients treated by external fixation for open fractures (n= 50), they showed rates of pin-site sepsis (Checketts grade 2 or greater) of 44% in the HIV positive group (n=15) and 11% in the HIV negative group (n=35).

In a power calculation using Epi Info (ver 3.4.3) with 95% confidence, 80% power, and a ratio of uninfected to infected patients of 3:1, the minimum numbers required would seem to be: 17 HIV positive 40 HIV negative patients. Retrospective data on the number of patients treated to union with external fixators were not available. It was felt that the recruitment of 17 HIV positive, and 40 HIV negative patients should be possible within 9 months.

2.7.1.4**Non union in externally fixed fractures**

Rates of non union in externally fixed fractures are likely to be higher than those treated with internal fixation because the underlying injury is likely to be more severe. Using evidence from the literature, and experience from our unit, the expected non union rate in the HIV negative populations was estimated at 6%. Harrison et al published work on the non-union rates of severe open tibia fractures treated initlly with external fixation, but converted to cast once the soft tissue injury allowed, in the HIV positive patients he had a non-union rate of 42%

In a power calculation using Epi Info (ver. 3.4.3) with 95% confidence, 80% power, and a ratio of uninfected to infected patients of 3:1, the minimum numbers required would be:15 HIV positive 45 HIV negative patients. Retrospective data on

the number of patients treated to union with external fixators were not available. It was felt that the recruitment of 15 HIV positive, and 45 HIV negative patients should be possible within 9 months.

2.7.2Ethics

The approval of the Consultant Staff for this study was generously given, and the protocol of management developed and approved.

The Protocol was submitted to the Regional Health Authority, including the methods of consent for both HIV testing and entry into the trial. A requirement of the ethics panel was that the management of the patients was not altered from the departmental policy at the time. This policy is summarised in section 2.7.7. Regional and National ethical approval was given.

2.7.3Consent

A verbal summary of the trial and the implications of entering it was given to all patients who met the entry criteria, this was given in either Zulu or English. This was reinforced with a patient information leaflet, again in the two languages. A consent form, translated in both English and Zulu was also given, if patients were happy to enter the trial a signature or thumb print was placed on the form.

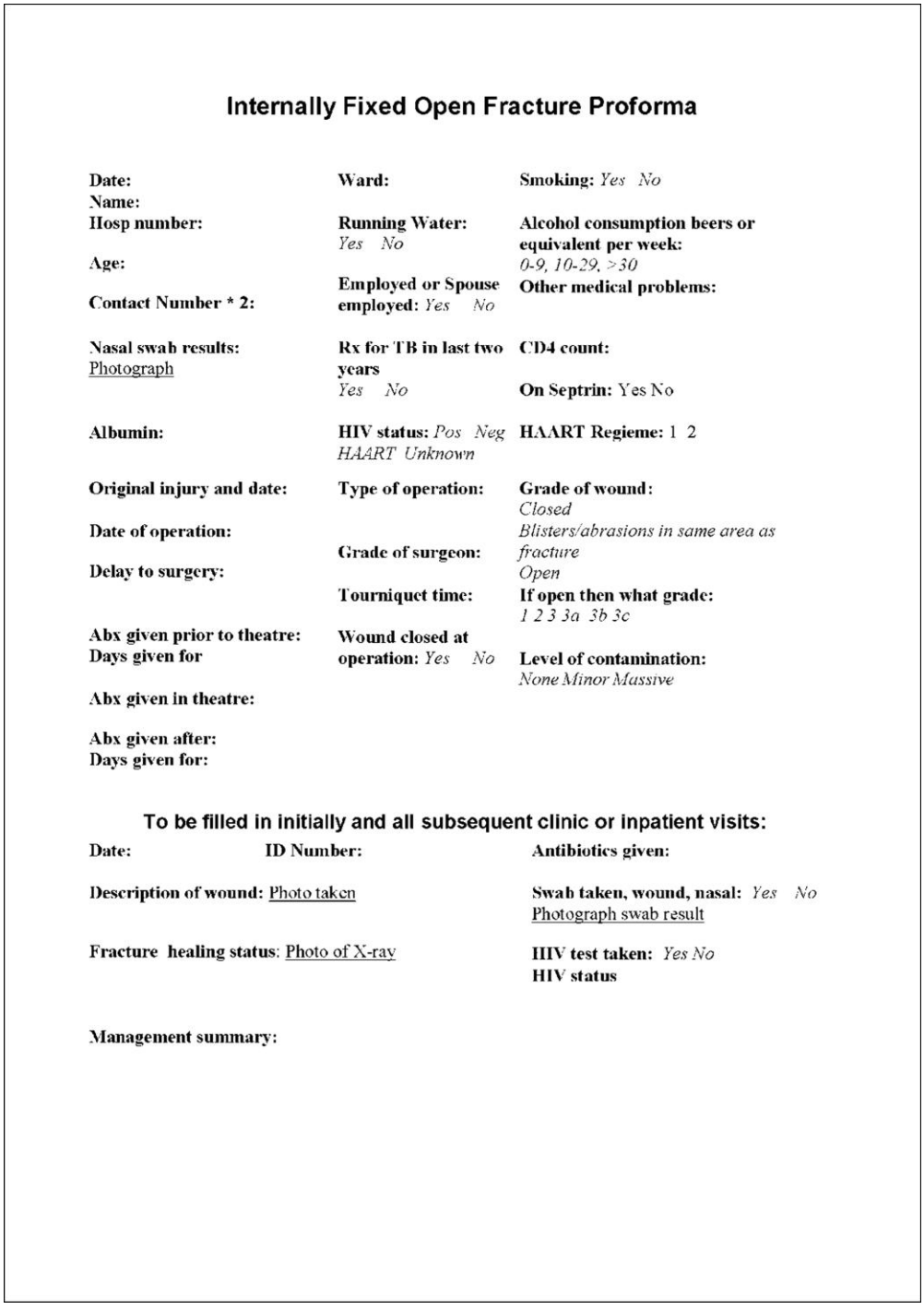
2.7.4Trial Time Line

Preliminary work for the study was started in May 2008, and work was completed by May 2009. Exact timings are shown in Figure 2-6

Figure 2-6. Showing time line for research project

2.7.5Data collection

A proforma was designed to aid data collection from the patients and to ascertain that all relevant risk factors for wound infections, as discussed in the literature review, were recorded. The proforma employed is shown in Figure 2-7 :



Internally Fixed Open Fracture Proforma

Date:
Name:
Hosp number:
Age:
Contact Number * 2:

Nasal swab results:
Photograph

Albumin:

Original injury and date:

Date of operation:

Delay to surgery:

Abx given prior to theatre:
Days given for

Abx given in theatre:

Abx given after:
Days given for:

Ward:

Running Water:
Yes No

Employed or Spouse employed: *Yes No*

Rx for TB in last two years
Yes No

HIV status: *Pos Neg HAART Unknown*

Type of operation:

Grade of surgeon:

Tourniquet time:

Wound closed at operation: *Yes No*

Smoking: *Yes No*

Alcohol consumption beers or equivalent per week:
0-9, 10-29, >30

Other medical problems:

CD4 count:

On Septrin: Yes No

HAART Regieme: 1 2

Grade of wound:
Closed
Blisters/abrasions in same area as fracture
Open

If open then what grade:
1 2 3 3a 3b 3c

Level of contamination:
None Minor Massive

To be filled in initially and all subsequent clinic or inpatient visits:

Date: **ID Number:**

Description of wound: Photo taken

Fracture healing status: Photo of X-ray

Management summary:

Antibiotics given:

Swab taken, wound, nasal: *Yes No*
Photograph swab result

HIV test taken: *Yes No*
HIV status

Figure 2-7

2.7.6Data Management

Data were initially collected on paper proformas. These were stored in the lead consultant's secure office. The data were entered weekly into an electronic database created by Epi Info (ver. 3.4.3). This database was password protected. Patient consent forms for entry into the trial, were collected during primary admission, these were kept by the lead investigator. Consent for HIV testing was made in the patient notes, as per hospital protocol.

2.7.7Patient Management

The system of patient management at Ngwelezane Hospital was based on national and local guidelines. The care pathway from admission to discharge is summarised in figure Figure 2-8 and Figure 2-8

2.7.7.1 **Care pathway from admission till theatre**

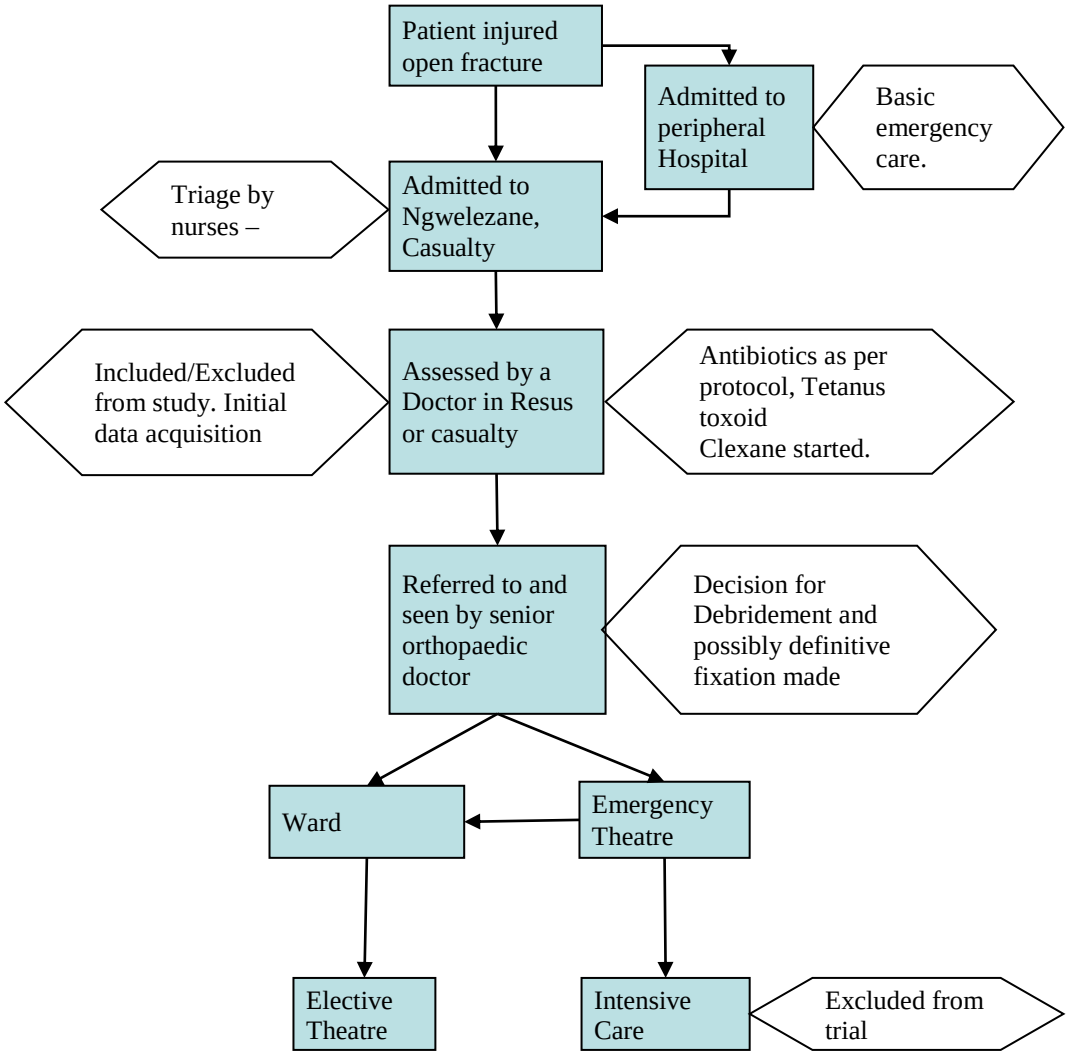


Figure 2-8. Flow chart showing initial patient management

2.7.7.2**Post operative management and follow up**

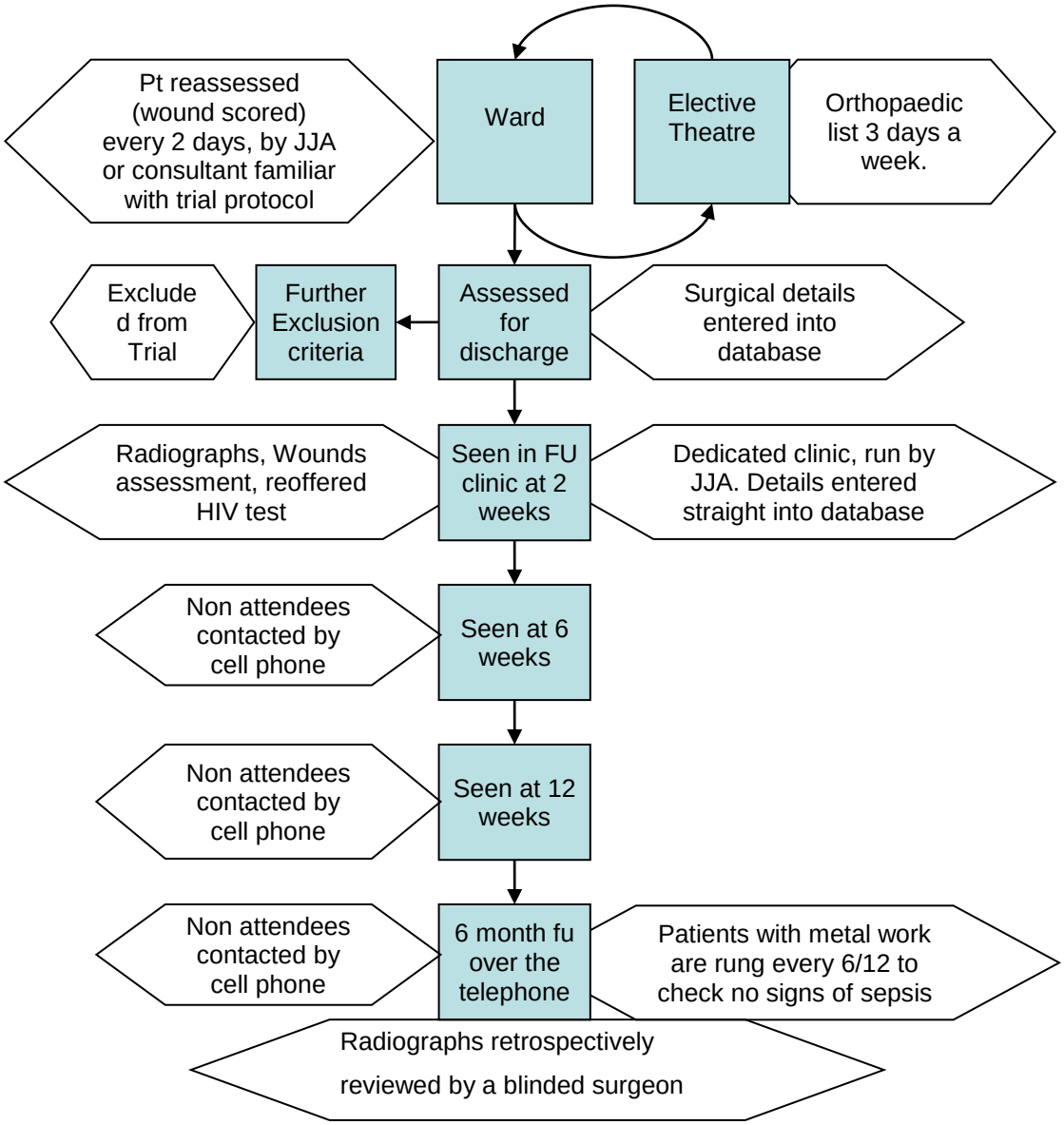


Figure 2-9. Flow chart showing patient management once admitted to ward

2.8 Treatment protocols

The management of open fractures is based on the experience of the lead consultant (PR), the evidence available in the literature, and the resources available. In order to standardize care for the trial, these management principles were summarized in the protocols in sections 2.8.2 to 2.8.5. The lead clinician(PR) assed and graded all the wounds, post debridement and this was then recorded on the patients proforma when they were assessed postoperatively by the lead researcher JA.

2.8.1Method of Fixation

The method of fixation was standardized. All intramedullary nails of the tibia and femur where Trigen nail, using the maximum diameter possible for the bone. Intramedullary devices in the forearm where rush rods, and the humerus were locked Synthes nails. All locking plates were fixed angle synthes plates. External fixators were generally monolateral Ortho-fix Fixators, however if the fracture type required, circular fixators were used.

2.8.2Antibiotic Protocol

This followed regional guidelines, and is based on the Gustilo Anderson classification:

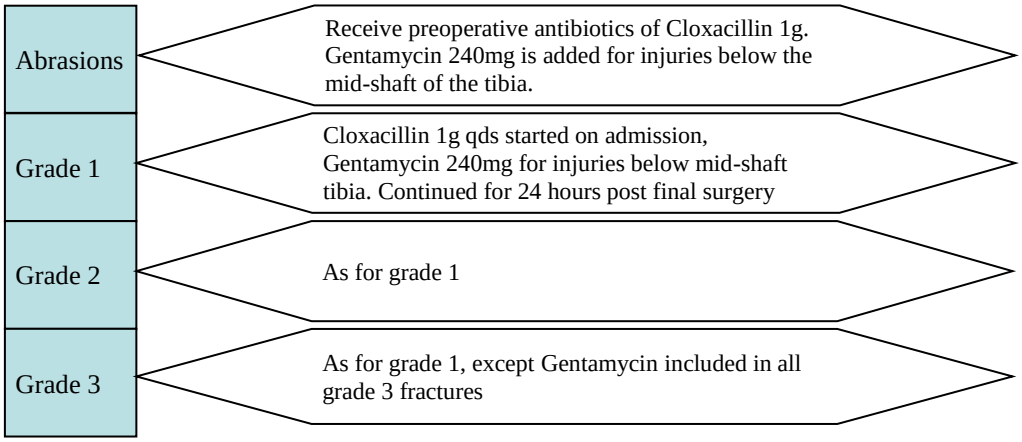


Figure 2-10. Flow chart showing use of prophylactic antibiotics

2.8.3Tibia Fracture Protocol

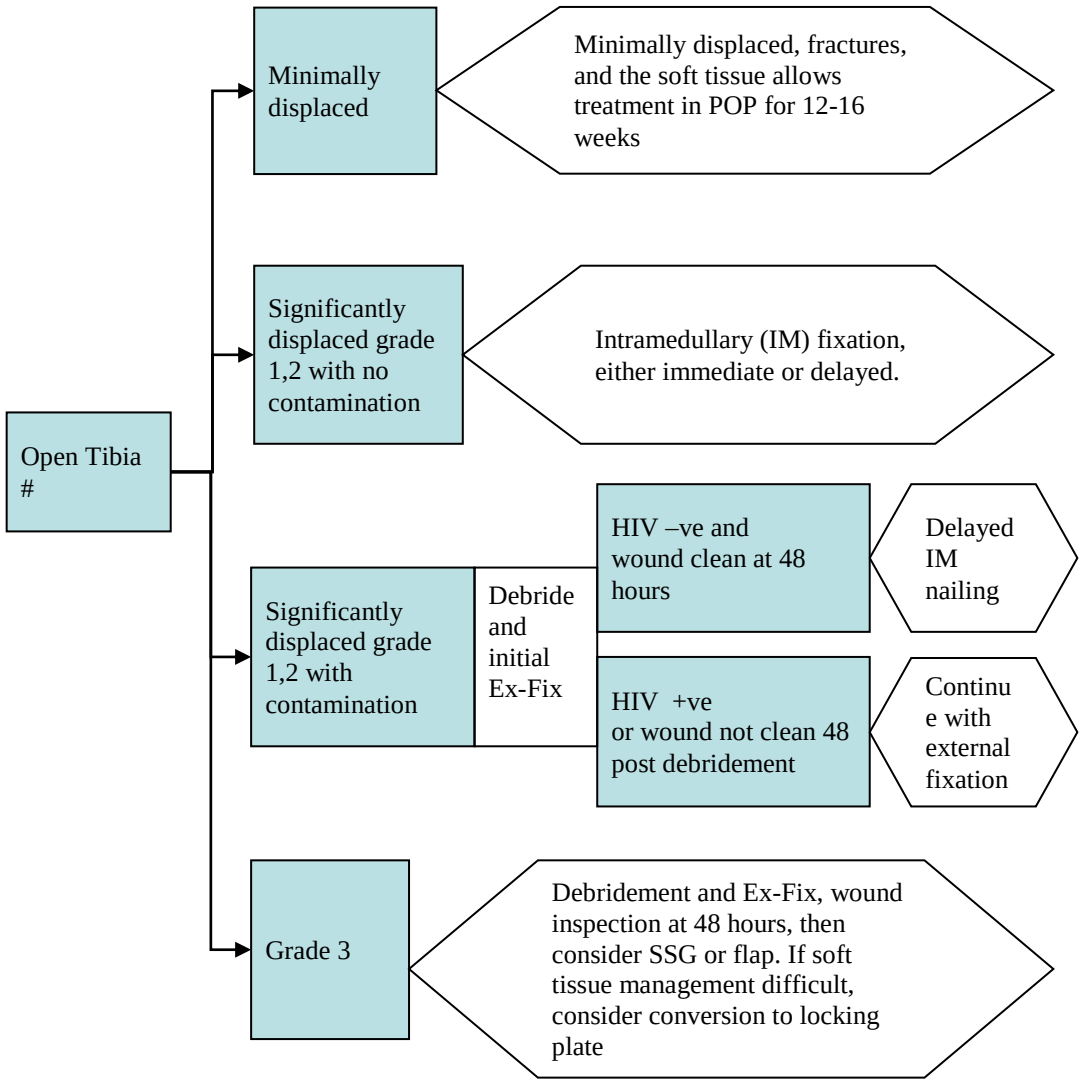


Figure 2-11. Flow chart showing the management protocols for open tibia fractures

2.8.3.1 **Monitoring of patients whose treatment was affected by HIV status**

The surgeon was not blinded to the HIV status. In our setting with limited resources this was not feasible, and it was a condition of the ethics approval that management was not altered from that previously in place at the hospital. It is therefore possible that the choice of fixation was influenced by HIV status. Any case where HIV was thought to have influenced management was noted at the time, any patient who according to the protocol in Figure 2-11 could have had their management affected had their notes reviewed.

2.8.4Management of wound infections

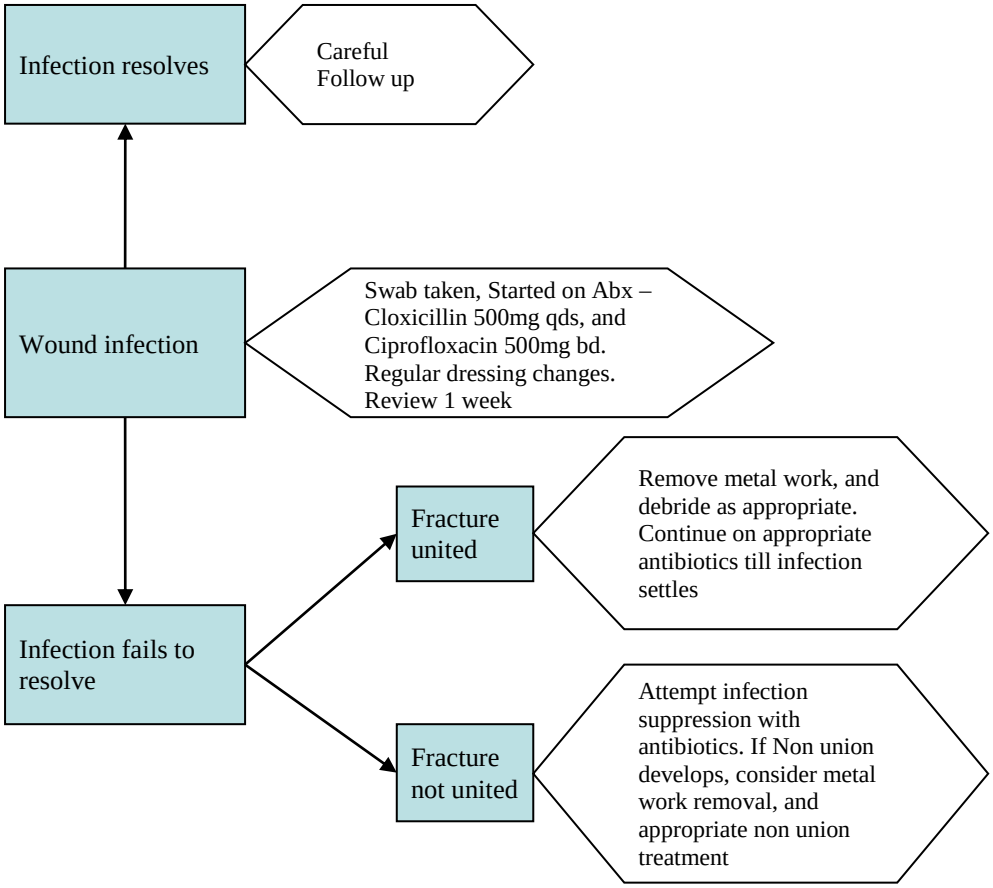


Figure 2-12. Flow chart showing the management of suspected wound infection

2.8.5 Management of patients with suspected pin site infections

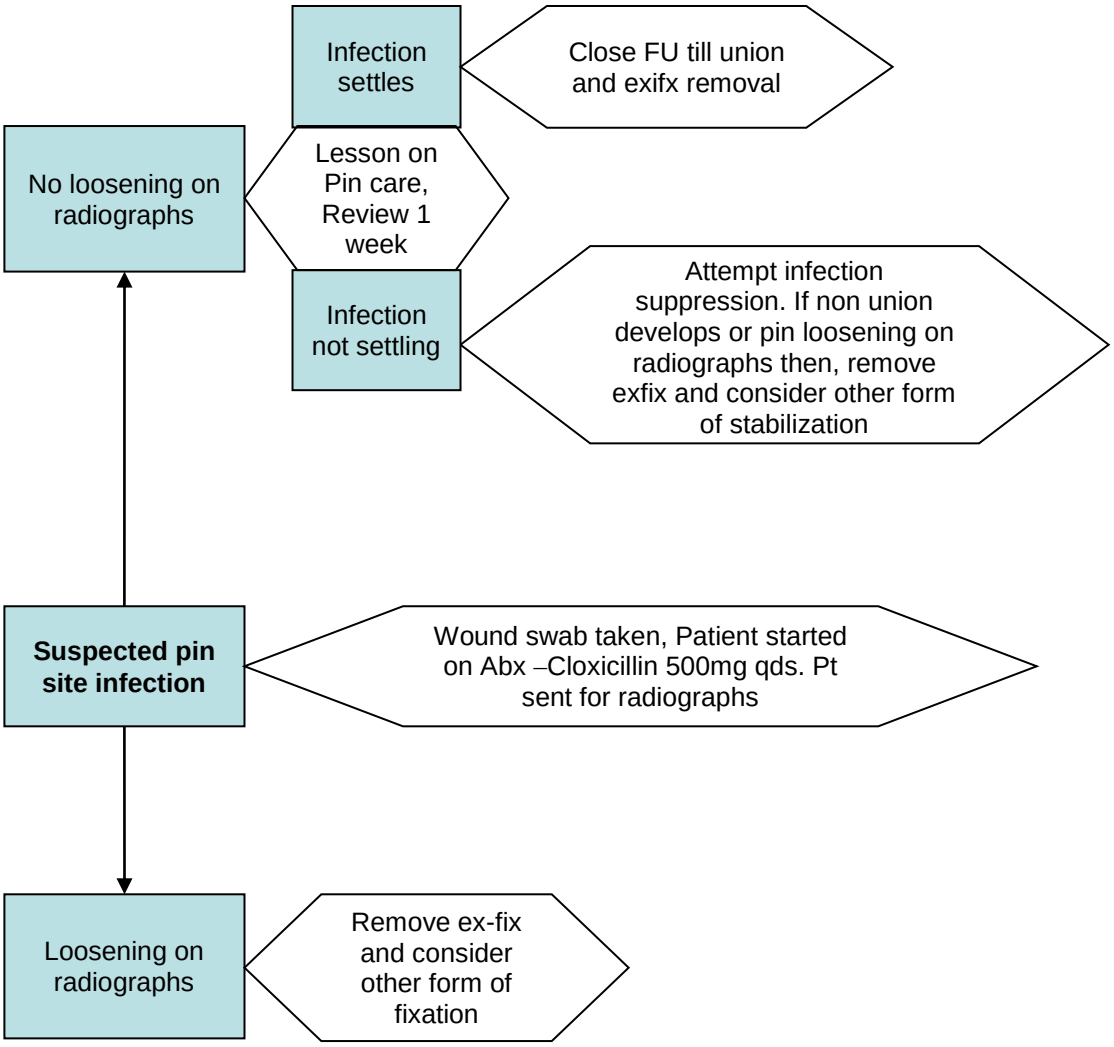


Figure 2-13. Flow chart showing the management of suspected pin site infection.

2.9 General material and methods for all aspects of trial

2.9.1HIV testing

Patients were all counselled prior to testing, and written consent obtained and documented in the notes. Patients, who declined testing, were reoffered testing at follow up. All patients were followed whether or not they agreed to testing.

HIV tests available were:

Abbott Diagnostic OraQuick Rapid HIV-1/2 Diagnostic Test. This has been shown to be a rapid and reliable test in the sub-Saharan African setting[197]. This test was utilized by the walk-in HIV testing centres on site. The sample was taken from a finger prick and the test done in front of the patient. For this trial, these tests were utilized when patients agreed to have a test when coming to follow up. Patients would have the Oraquick test, if positive it would be confirmed with an ELISA.

ELISA: These tests were done using the Abbots Diagnostic IMX platform and the HIV-1/HIV-2 3 plus reagents. This has been shown to be one of the most sensitive immuno assay systems available[198]. This test was utilized for all ward patients, and for confirmatory tests, of patients tested with the Oraquick test.

2.9.2 CD4 Testing

CD4 tests were all done using flow cytometry, tests were all taken at least 3 days post trauma and on average 1 month post trauma. Many patients would not consent to HIV testing at the time of injury but at subsequent follow up, it made it difficult to standardize timing of the test. Patients who were on ARV's were all having regular CD4 counts, therefore if one was available within 3 months of injury this was taken, if not a repeat sample was taken. Due to the current lack of evidence on whether HAART adversely affects wound or fracture healing we have taken the most recent CD4 even if previous CD4 counts prior to starting treatment had been lower.

2.9.3Microbiology technique

Wound swabs were taken in theatre if more than 24 hours had elapsed since the injury. Previous studies suggest that swabs taken in theatre at the time of initial

debridement, are of little clinical use, as swabs grow contaminants that rarely lead to wound infection, if the time since injury is less than 24 hours[86-89].

Wound swabs were taken of wounds or pin sites on the ward or in clinic at any time if there were significant exudate or erythema

Microbiology swabs were collected using sterile dacron swabs placed in an AMIES gel-based medium, manufactured by EUROTUBO. Swabs were subjected to a Gram stain and culture within 24 hours of being taken. Staphylococci and Streptococci were placed on blood agar, and Gram negative organisms were placed on McConkeys Agar. Swabs were read at 3 days and sensitivities obtained using a VITEK 2 automated system. Antibiotic sensitivities tested for were:

Gram positive organisms:

Penicillin, Erythromycin, Oxacillin, Clindamycin, Vancomycin, Cefalothin, Gentamycin

Gram negative organisms:

Ampicillin, Amikacin, Cefuroxime, Cefotaxime, Gentamycin, Ciprofloxacin

2.9.4Time from injury to administration to antibiotics

Many of the patients recruited into the trial were referred from peripheral hospitals, poor documentation regarding time of administration of antibiotics made the accurate assessment of delay to antibiotics impossible to assess.

2.9.5Albumin

Blood for serum albumin levels was taken within the first 48 hours of admission, and was assayed as part of the routine liver function tests. Patients with an albumin <35g/l

2.9.6Methods of Classification used

2.9.6.1 **Open fracture**

We used the modified Gustilo Anderson classification[25] for open fractures. Gun shot wounds as discussed in section 1.2.1 were assumed to be low energy, and the grading was therefore based on the wound characteristics

2.9.6.2 **Classification of union**

Two separate classifications were utilised, based on recent literature review on the subject by Corrales et al [199]: one on radiological grounds and the other on clinical grounds, these systems were

Clinical fracture union was defined as being ability to fully weight bear without pain at the fracture site, or in non–weight-bearing limbs, as being non tender over the fracture site. This was utilized especially in patients who had external fixators removed, so had no internal fixation.

Radiological union was defined as the presence of bridging callus on at least two cortices.

Location specific definitions are taken as the commonly used definitions in the literature as shown in Table 2.1 [199].

Table 2-1 Showing the criteria required to make the assessment of union, taken from commonly used definitions in the literature[199]

	Femur	**Tibia**	**Humerus**	**Forearm**
Proximal fracture				
Radiographic assessment	Bridging of fracture site	Obliteration of fracture line	Obliteration of fracture line or bridging of fracture site	—
Clinical assessment	No pain during weight-bearing	Ability to bear weight	Ability to perform activities of daily living without pain, or no residual pain at fracture site, or full range of motion at adjacent joint	
Shaft fracture				
Radiographic assessment	Bridging of fracture site	Bridging of fracture site	Bridging of fracture site	Bridging of fracture site
Clinical assessment	No pain during weight-bearing	No pain during weight-bearing	No pain on palpation/examination	No pain on palpation/examination
Distal fracture				
Radiographic assessment	Bridging of fracture site	Bridging of fracture site	Bridging of fracture site	Obliteration of fracture line or calcification of callus
Clinical assessment	No pain during weight-bearing	No pain during weight-bearing or ability to bear weight	No pain on palpation/examination	No residual pain at fracture site

A fracture union was classified either on clinical or radiological assessment, or both. A non union was defined on the above grounds at 24 weeks for a tibia/femur fracture; and 12 weeks for all other fractures. Non union was established by these criteria prior to any further surgery taking place to address the non union.

2.9.6.3**Wound infection**

Classification of wound infections is controversial. In this project the ASEPSIS wound scoring system was used as recommended by the Surgical Infection Study Group [200]. This system has been used in similar research projects. Use of the same system should increase the validity of a comparison between our results and those of others. This system scores the appearance of the wound and assesses the necessity for further treatment, such as the administration of antibiotics as shown in

Table 2-2. It is very sensitive and allows objective appraisal of infection, but it may not accurately quantify severity of infection.

The maximum score is 70. For the purpose of our study a score of 0 to 10 was considered to represent normal wound healing, a score of more than 10 to represent an infection. This confers a sensitive, if arbitrary, definition of infection. The author of this thesis personally recorded the scores at 5 days, 2 weeks, 6 weeks and 3 months post definitive wound closure. The use of antibiotics 24h post definitive wound closure, was considered treatment of underlying infection.

Table 2-2 Describing the ASEPSIS wound infection scoring system

ASEPSIS wound score						
	Proportion of wound affected, %					
Wound Characteristic	0	<20	20-39	40-59	60-79	>80
Serous exudate	0	1	2	3	4	5
Erythema	0	1	2	3	4	5
Purulent exudate	0	2	4	6	8	10
Separation of deep tissues	0	2	4	6	8	10
Additional Points for:						
Antibiotic treatment required			10			
Drainage of pus under LA			5			
Drainage of wound under GA			10			
Isolation of bacteria			10			
Stay as an in-patient over 14 days			5			
Total			0-70			
Score >10 suggests wound infection						

2.9.6.4 **Pin-site infection**

Assessment of pin sites is less controversial. The scoring system used in similar trials was the system described by Checkett[201]: it was adopted in this series and is outlined as follows:

0: Normal healthy appearance.
1: Discharge from the pin site.
2: As 1 with antibiotics given.
3: As 2 and pin re-sited.
4: Pin loose and fixator abandoned.
5: Osteomyelitis in pin track.
6: Osteomyelitis requiring surgical treatment.

The highest score of the six pins was taken as the patient's score. This was done in conjunction with the asepsis wound scoring system. Due to the controversy highlighted in section 1.3.3.7 regarding the method of scoring the pins, it was felt most appropriate to take the highest score of the 6 pins on the external fixator, as the "pin score". We used the highest score obtained over the entire use of the fixator

2.10 Statistical analysis

The analysis of the Binary nominal data was done using the Chi squared test. In cases where the expected value was less than 5, then the Fisher's exact test was used. The Mann Whitney test was used to assess differences between the ages of different groups. Tests were all two tailed and a p value was used to gauge significance. This study is a prospective case control study, and therefore risk ratios and corresponding confidence intervals are given where appropriate. When describing the statistics in the text, the strength of the association was dependant on the p value, cut off values are as described: a p value of 1-0.2 was described as no evidence of an association, 0.2-0.05 weak evidence of an association, and <0.05 strong evidence of an association[202].

In the assessment of multiple potential risk factors, binary logistic regression was used.

HIV is a progressive disease and it was felt to be important, in proving causality, to try identify if any effects of HIV were severity dependant. In order to do this a CD4 count of 350 (the point at which HAART treatment is often initiated) was used as a cut off for advanced and early disease. We have compared the four cohorts, HIV negative, HIV positive, Early HIV and Advanced HIV.

2.11 Funding

This project was supported by a research grant from CURE INTERNATIONAL, and the Sir Ratanji Dalal Research Scholarship from the Royal College of Surgeons London.

Chapter 3

Results

3 Results

3.1 Cohort 1, Open Fractures treated with surgical stabilization

This data is being analysed to interrogate the following null hypotheses:

- There are no associations between HIV or advanced HIV and wound infection in open fractures treated with surgical stabilization.
- There are no associations between HIV or advanced HIV and non union in open fractures treated with surgical stabilization.

3.1.1Patients

Over a 9 month period 133 patients with 135 fractures met the inclusion criteria. No patients refused entry into the study.

3.1.2Follow up

97% had follow up of at least 2 months

89% had follow up of at least 3 months

The mean length of follow up was 20 weeks with a range of 4-47 weeks.

4 patients were lost to follow up prior to confirmation of fracture union/non union

Ensuring good follow up was critical to the study. Patients who did not attend their appointments were rung on their cell phone and if necessary a repeat appointment made for the following week. Due to time constraints it was only possible to ensure three month follow up for the final patients recruited. However if union had yet to be confirmed the patient was still followed up.

3.1.3Baseline characteristics

3.1.3.1 **Patient specific characteristics**

Figure 3-1 shows the prevalence of HIV within the cohort.

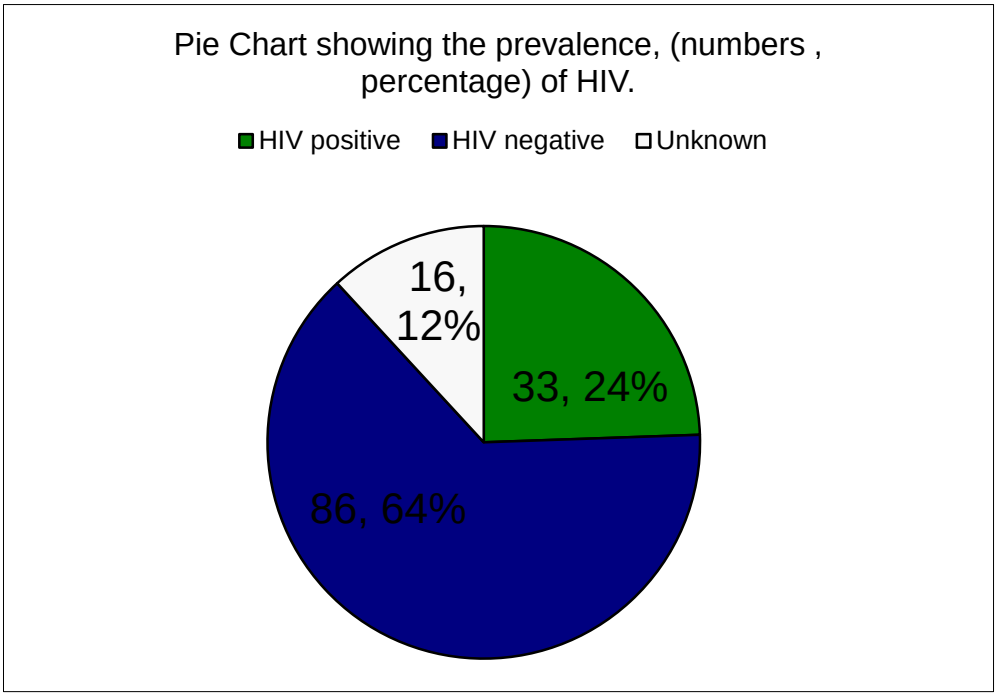


Figure 3-1

Of the 33 HIV positive patients, 6 were taking HAART on admission, of these 4 underwent internal fixation and 2 external fixation. CD4 counts were available on 26 patients. All patients with a positive HIV test were offered CD4 counts. Several patients refused this test despite repeated counselling. Of those who had their CD4 count tested, their distribution is shown in Figure 3-2.

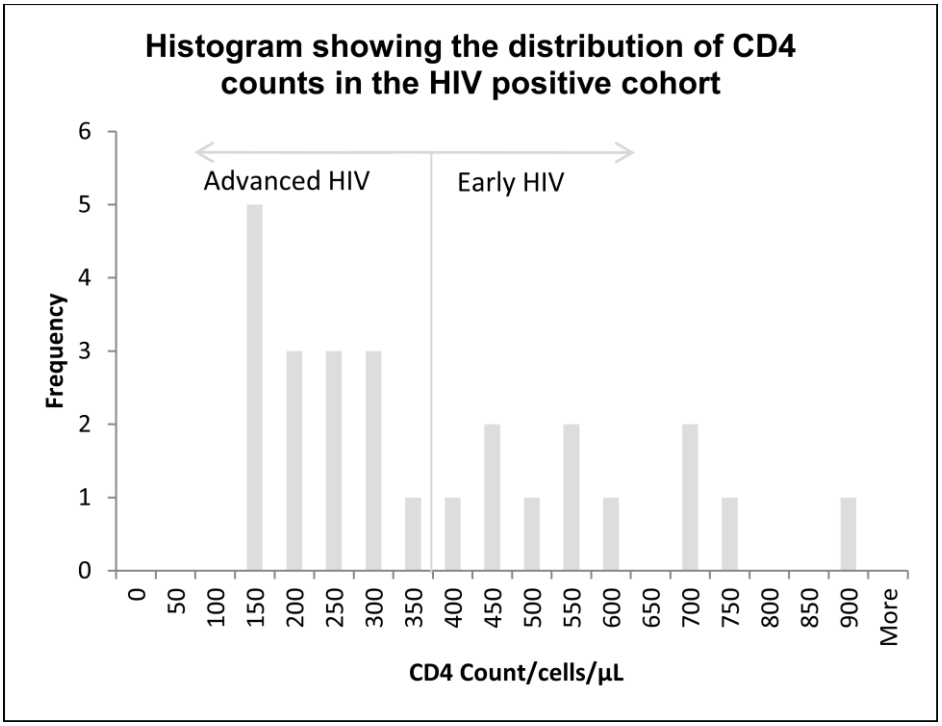


Figure 3-2. The cut off for advanced and early HIV is illustrated

Figure 3-2 shows that amongst the HIV positive patients there were none with a CD4 count result below 100 cells/µL (a sign of very severe disease). Of those who accepted testing approximately half the patients were in the advanced group and half in the early group. Patients who refused CD4 counts were all young males (less than 50), who smoked.. In secondary analyses looking at advanced and early HIV, patients without CD4 counts were excluded. This may introduce some selection bias

Table 3-1 shows demographic comparisons between HIV positive, negative and those who refused HIV testing. Statistical analysis shown below excludes patients whose HIV status was unknown.

Table 3-1. Showing the demographic comparisons in cohort 1

	HIV positive (%)	HIV negative (%)	P value	HIV status unknown	Refused cd4 counts
Number of patients	33	86		16	7
Mean age	32	34	0.86 [MW]	31	27
Male	28/33 (81)	70/86 (81)		13/16 (81)	100%
age<50	31/33 (94)	75/86 (87)	0.51 [FE]	15/16 (94)	100%
albumin<30	16/33 (49)	24/86 (28)	0.03 [X]	6/16 (38)	60%
Smoking	21/33 (64)	35/85 (41)	0.028 [X]	5/15 (33)	100%

[MW] = Mann Whitney, [FE] = Fisher Exact, [X] = Chi Squared

Table 3-1 highlights that the study group is a predominantly a young male population. It also looks at factors which were thought may be important risk factors for wound infection and non union and may vary significantly between the HIV positive and negative populations.

Injury specific characterisitcs

Figure 3-3 to Figure 3-6 shows the features of the injuries within cohort 1.

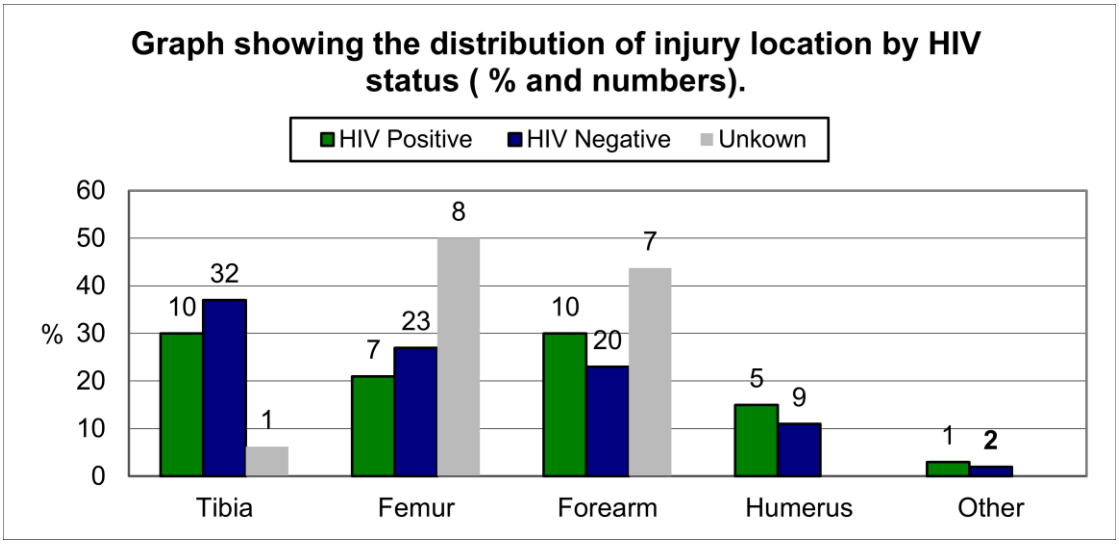


Figure 3-3

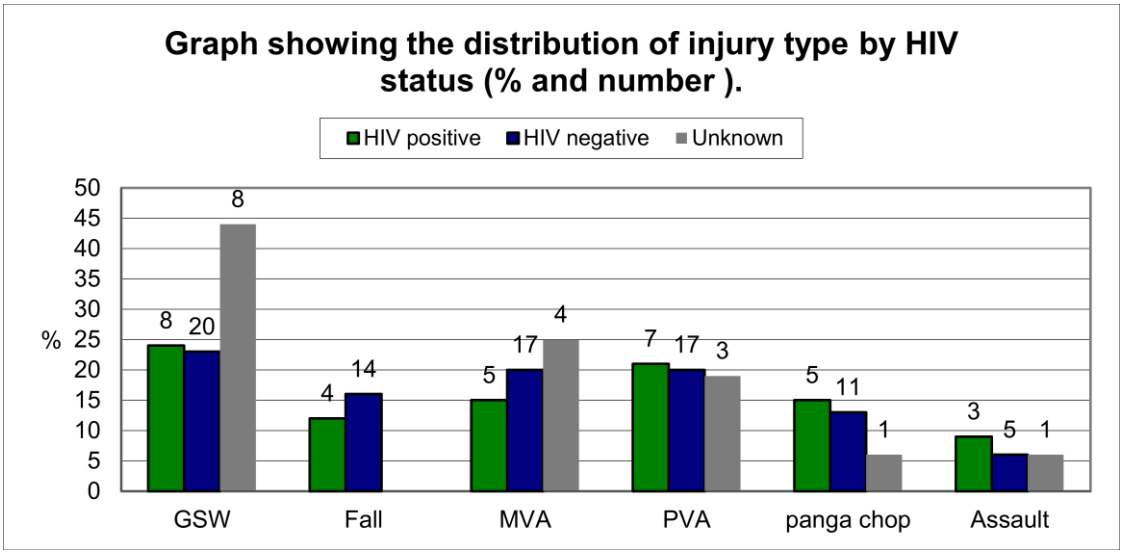


Figure 3-4 GSW- Gun shot wounds, MVA-Motor vehicle accident, PVA – Pedestrian vehicle accident

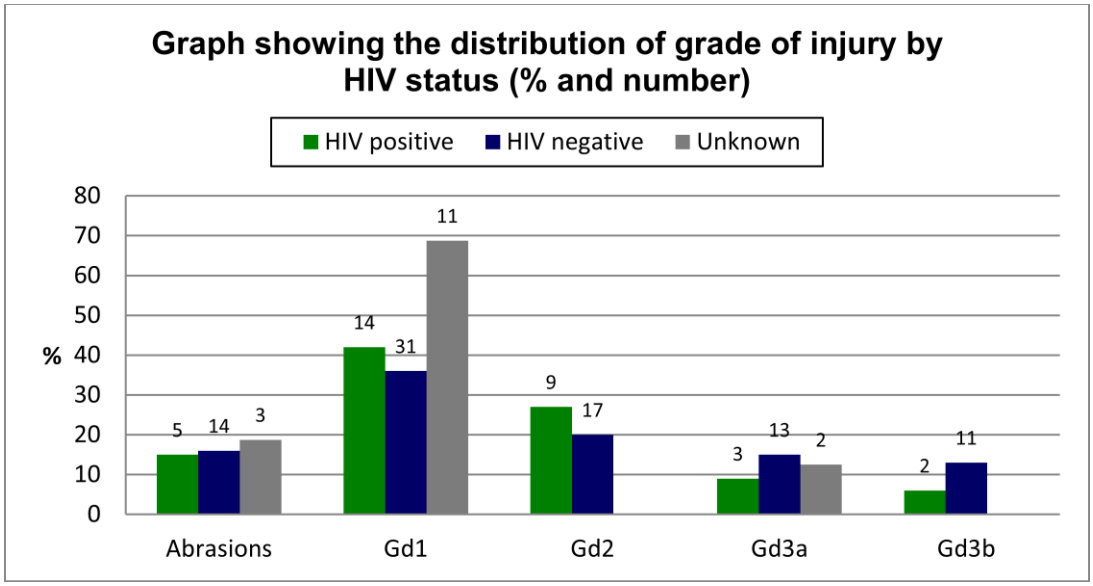


Figure 3-5

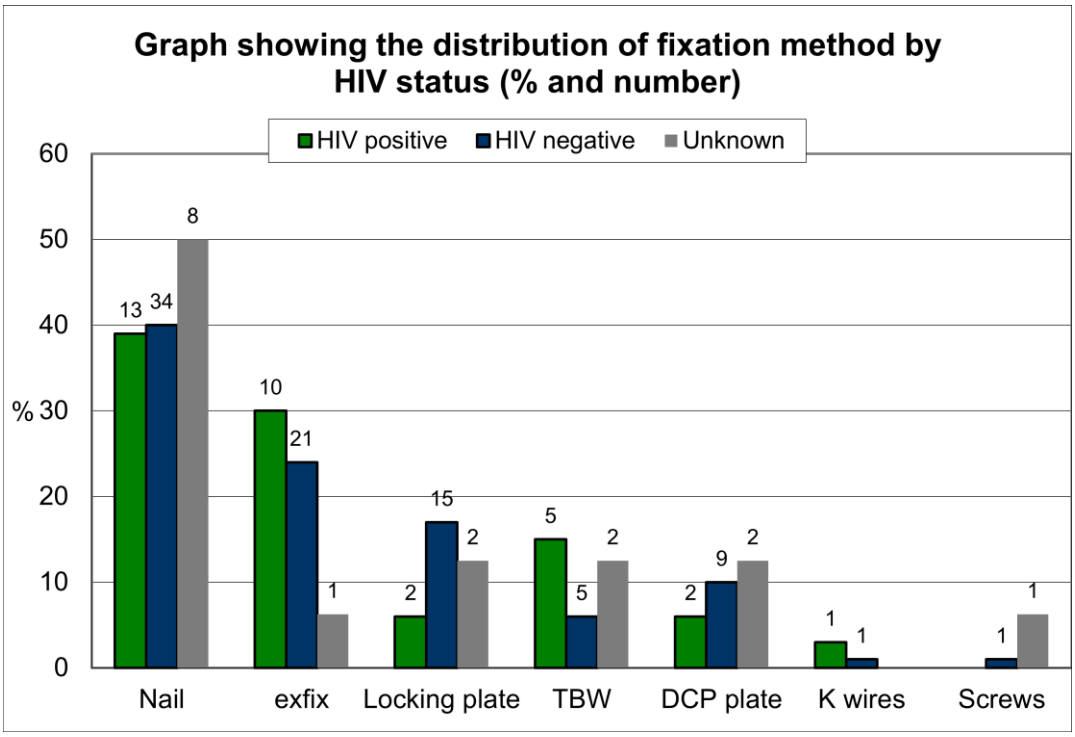


Figure 3-6

3.1.4 Infection rates

This study has looked at the association between wound infections and HIV in cohort 1. The cohort has been further subdivided into grades of wound, and similar analyses have been done on these sub groups.

3.1.4.1 **Overall wound infection rates**

Table 3-2 shows the wound infection rates in the HIV positive and negative groups.

Table 3-2

Wound Infection, Number, (%)			**The risk of developing a wound infection given HIV infection.** Risk Ratio = 0.69 (0.3-1.7) 95% CI P value = 0.4
	Yes	**No**	
HIV positive	5, (15)	28, (85)	
HIV negative	19, (22)	67, (78)	
			The risk of developing a wound infection given advanced HIV infection Risk ratio 1.28 (0.3-5.09)95% CI P value = 0.74
Advanced HIV	4, (27)	11, (73)	
HIV negative	19, (22)	67, (78)	
			The risk of developing a wound infection given advanced HIV infection Risk ratio 0.25 (0.04-1.78)95% CI P value = 0.18
Early HIV	1, (6)	17, (94)	
HIV negative	19, (22)	67, (78)	
			The risk of developing a wound infection given advanced HIV compared to early HIV infection. Risk ratio 4.8(0.6-38.5)95% CI P value = 0.15
Advanced HIV	4, (27)	11, (73)	
Early HIV	1, (6)	17, (94)	

In this cohort, surprisingly patients with HIV have a lower infection risk however there is no evidence that this difference is greater than would be expected by chance alone. As hypothesized patients with advanced HIV have higher infection rates than HIV negative patients although the relative risk is small. There is no evidence that this difference is greater than would be expected by chance alone. The data unexpectedly suggest that patients with early HIV have a 75% reduction in infection rate in comparison to the HIV negative patients, although it only provides weak evidence that this difference is greater than would be expected by chance alone.

reduced rate of infection The data suggest that patients with advanced HIV have high risk of wound infection than patients with early HIV. It provides weak evidence that that this difference is greater than would be expected by chance alone. I

3.1.4.2 **Logistic regression**

Table 3-3 shows logistic regression analysis looking at potential risk factors for wound infection.

Table 3-3. Logistic regression analysis for wound infection

	Adjusted Risk Ratio for wound infection	**P value**
High grade wound (Gd 2 or 3)	2.3	0.12
Delay greater than 24h to debridement	1.7	0.31
Smoking	.5	0.17
HIV Positive	.8	0.64

In this cohort, grade of wound and a prolonged delay to debridement were the biggest risk factors for wound infections. Neither HIV infection nor smoking was associated with increased risk of wound infection.

Table 3-4 shows logistic regression analysis using advanced HIV rather than HIV as a risk factor

Table 3-4 Logistic regression analysis for wound infection

	Adjusted Risk Ratio for wound infection	P value
High grade wound (Gd 2 or 3)	3.0	0.31
Advanced HIV	2.0	0.31
Delay greater than 24h to debridement	1.7	0.37
Smoking	0.6	0.27

In this cohort, grade of wound and advanced HIV had the strongest effect on wound infection.

3.1.4.3 **CD4 counts and wound infections**

CD4 count is a marker of the severity of the immuno-compromise. It is reasonable to expect that as CD4 counts fall the risk of infection may increase. Unfortunately because the ASEPSIS scoring system is threshold scoring system and as the numbers are small, it is not feasible to plot the ASEPSIS score against CD4 count. Figure 3-7 shows patients with HIV, with and without wound infection and their CD4 counts.

Scatter plot showing CD4 counts of the HIV positive patients with and without wound infection

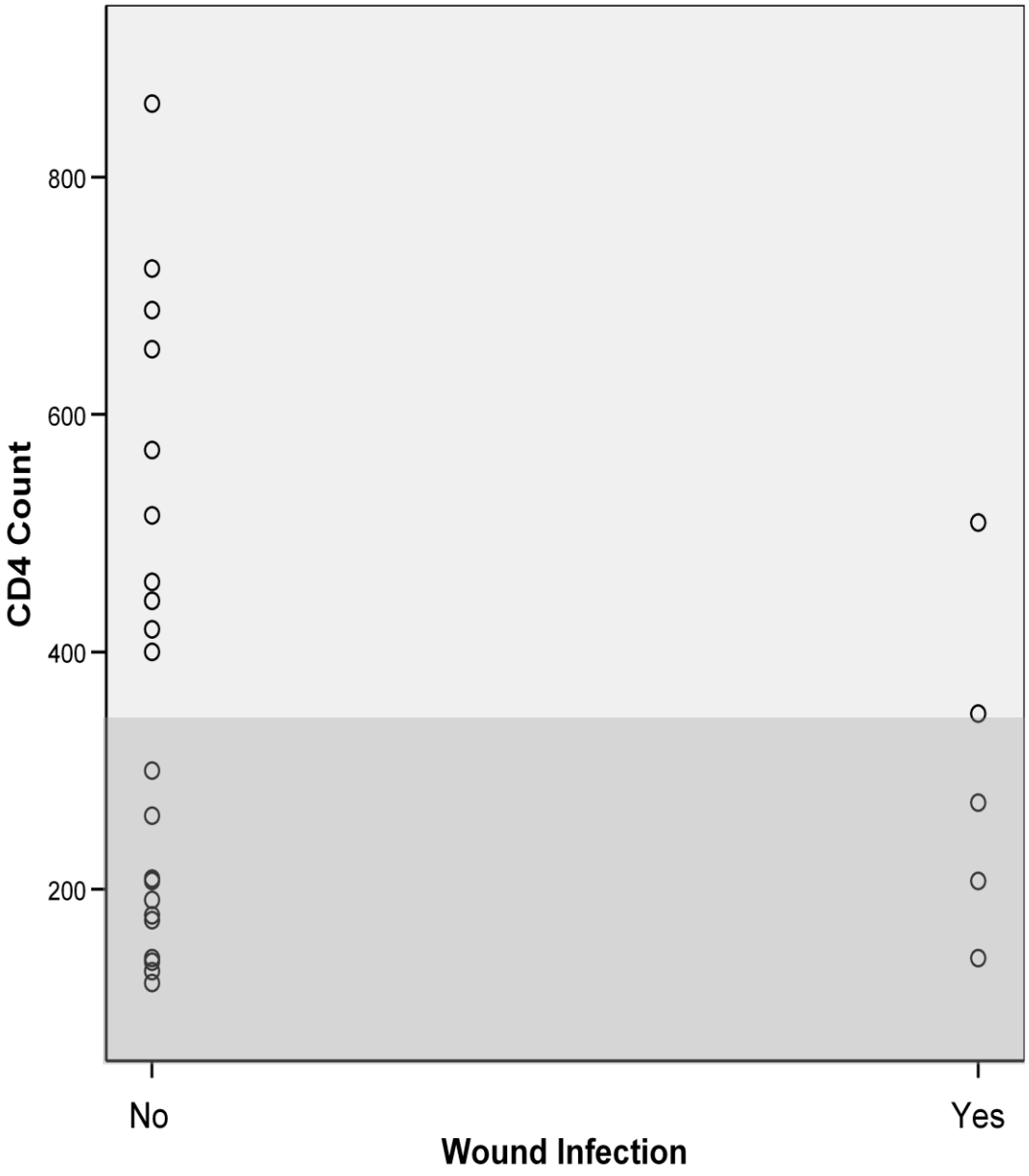


Figure 3-7, The dark shading denotes patients with advanced HIV

Using logistic regression analysis, there is a trend towards increasing infection rates with lower CD4 counts, however the data provides no evidence that this more than would be expected by chance alone, n=26, Risk ratio = 0.998, P value = 0.477.

3.1.4.4 **Subgroup Analysis**

Table 3-5 shows analysis of wound infection by differing grades of HIV and advanced HIV against unexposed.

Table 3-5. Sub group analysis by grade of injury (Gustilo-Anderson) for wound infection given HIV or advanced HIV infection.

	Rates of Wound infection, number of patients (%)				
	Abrasions	**1**	**2**	**3a**	**3b**
HIV positive	0/5 (0)	4/14 (29)	1/9 (11)	0/3 (0)	0/2 (0)
HIV negative	4/14 (29)	3/32 (9)	4/17 (24)	3/12 (25)	4/11 (36)
Risk ratio of wound infection give HIV positivity, (95% CI)	NA	3.1 (0.8-11)	0.47 (0.5-3.6)	NA	NA
P value [FE]	0.53	0.18	0.62	1	1
Advanced HIV	0/2 (0)	4/8 (50)	0/1 (0)	0/2 (0)	0/2 (0)
HIV negative	4/14 (24)	3/32 (9)	4/17 (24)	3/12 (25)	4/11 (36)
Risk ratio of wound infection given advanced HIV, (95% CI)	NA	5.33 (1.48-19.2)	NA	NA	NA
P value [FE]	1	0.02	1	1	1
Advanced HIV	0/2 (0)	4/8 (50)	0/1 (0)	0/2 (0)	0/2 (0)
Early HIV	0/3 (0)	0/6 (0)	1/8 (13)	0/1 (0)	0/0 (-)
Risk ratio of wound infection given advanced HIV, (95% CI)	NA	4.2[X]	NA	NA	NA
P value [FE]	1	0.08	1	1	1

The data shows that in grade 1 injuries rates of wound infection are higher in patients with HIV and particularly in patients with advanced HIV, when compared against both HIV negative and early HIV controls . In grade 1 injuries this data shows weak evidence of an important association between HIV and wound infection, but strong evidence of an important association between advanced HIV and wound infection.

Of note is that in both HIV and advanced HIV infection rates were lower than controls in the more severe grade of injury, however due to small numbers there is no evidence that this difference is greater than would be expected by chance alone.

3.1.5Grade 1 injuries

Due to this isolated increased risk of wound infection in the grade 1 sub group, separate subgroup analysis was performed to investigate any demographic or confounding factor to account for this finding.

Grade 1 injures were the most common injury in the study accounting for 48% of the patients. The management protocol was different to that of the Grade 2 and 3 injuries as discussed on page 66, in that they were not treated with urgent debridement.

3.1.5.1 **Delay to initial surgery and infection rate**

Due to differences in protocol, grade 1 injuries were not taken to theatre for urgent debridement, but were placed on day time lists for urgent fixation (if required), whilst grade 2 and 3 injuries were taken for urgent debridement. Occasionally delay in presentation, or delay in referral from a peripheral hospital meant there was a long delay till debridement. Table 3-6 shows the average time to surgery for the differing grades of wound.

Table 3-6. Median delay to initial surgery for differing grade of wounds

	Delay to surgery/days (Median)		
Grade	**All patients**	**HIV positive**	**HIV negative**
1	3.5	4	3
2	1	1.5	1
3a	0.75	0.5	1
3b	0.5	0.5	0.5

This table shows that grade 1 injuries on average had a longer wait to theatre than the higher grade injuries. Patients with HIV with grade 1 injuries had on average a 24 hour longer delay to theatre than HIV negative patients.

3.1.5.2 **Base line characteristics**

3.1.5.2.1 Patient specific characteristics

Figure 3-8 shows the HIV status of the patients with grade 1 injuries

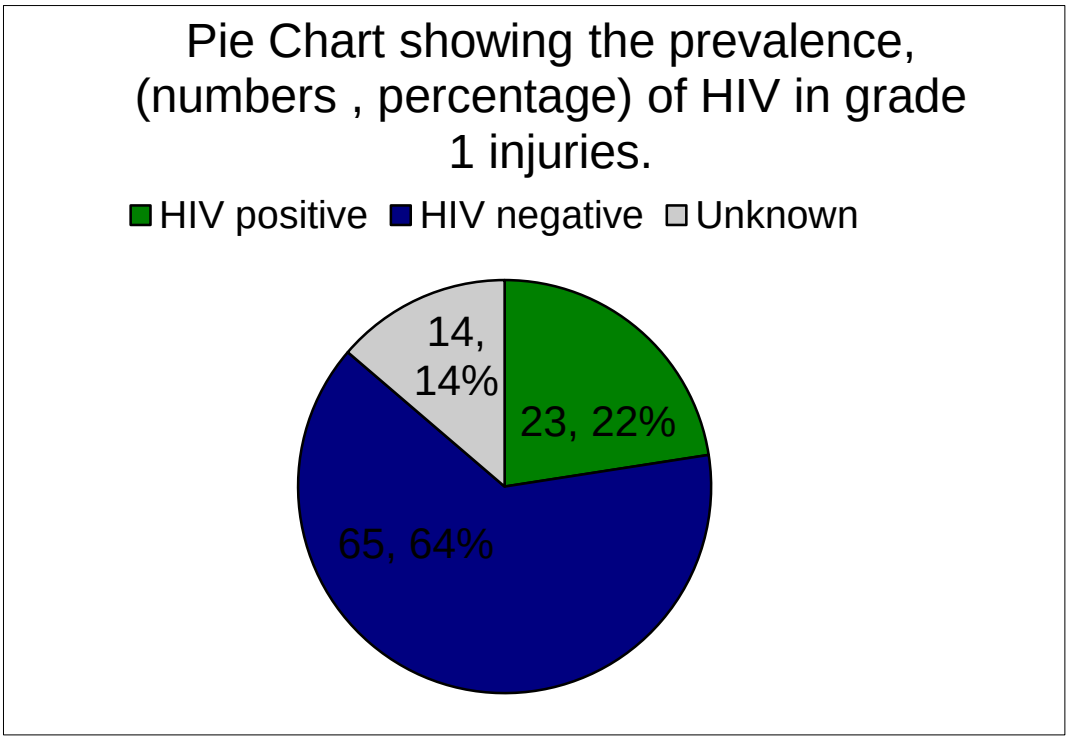


Figure 3-8

Figure 3-9 shows CD4 counts of the HIV positive patients with Grade 1 injuries

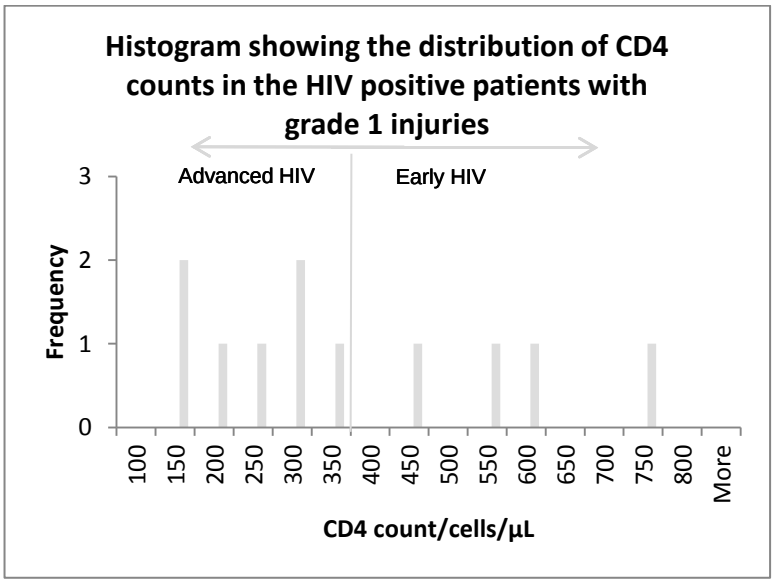


Figure 3-9. The cut off for advanced and early HIV is illustrated

Table 3-7 shows the Demographic comparisons between the HIV positive and negative patients with grade 1 injuries.

Table 3-7

	HIV positive (%)	HIV negative (%)	P value
Number of patients	23	65	
Mean age	32	33	
Male	20/23 (87)	53/65 (81)	FE 0.55
Age<50	21/23 (9)	57/65 (12)	FE 0.49
Albumin<30	9/23 (39)	18/65 (28)	X 0.31
Smoking	14/23 (61)	27/65 (41)	X 0.09

The two groups appear broadly similar, except with regard to smoking. The demographics are similar to that seen in the cohort as a whole.

3.1.5.2.2 Injury specific characteristics

Figure 3-10 to Figure 3-12 show the, cause, method of fixation, and location of the injuries in grade 1 cohort.

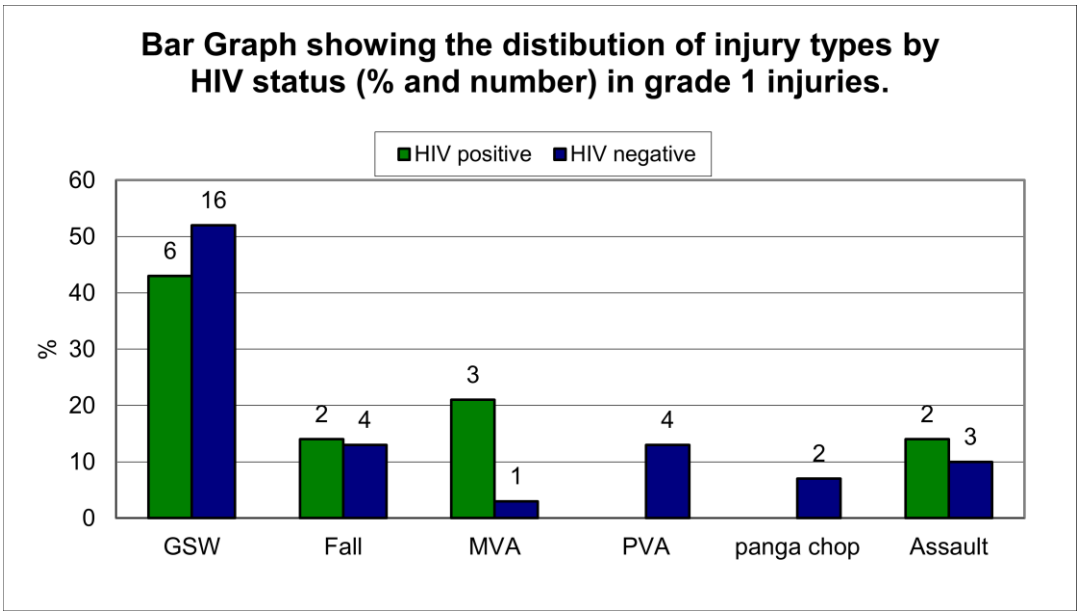


Figure 3-10, GSW – Gun shot wounds, MVA- Motor vehicle accident, PVA-Pedestrian vehicle accident

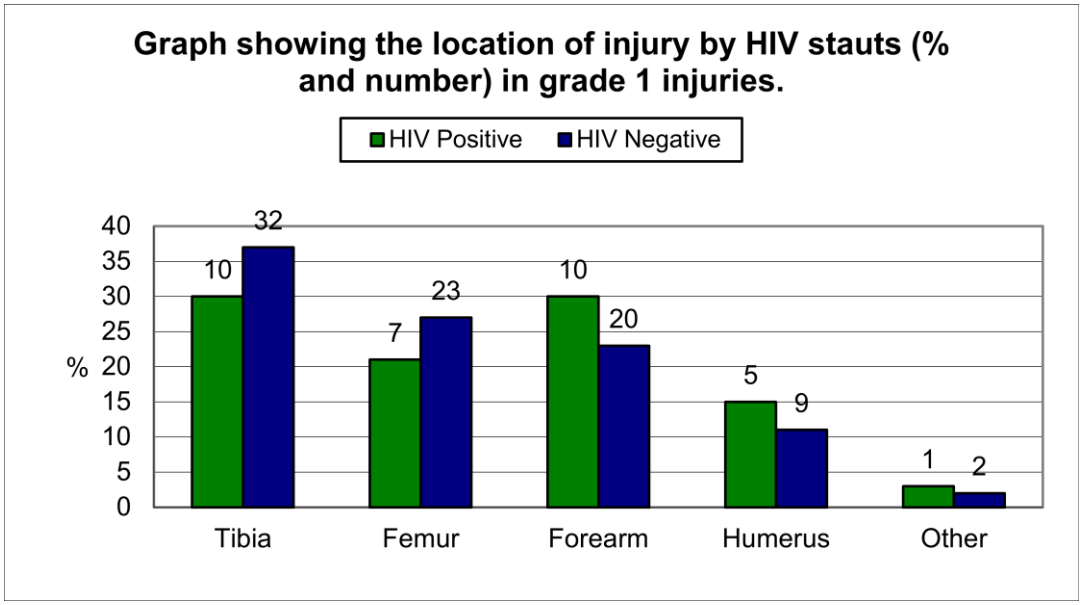


Figure 3-11

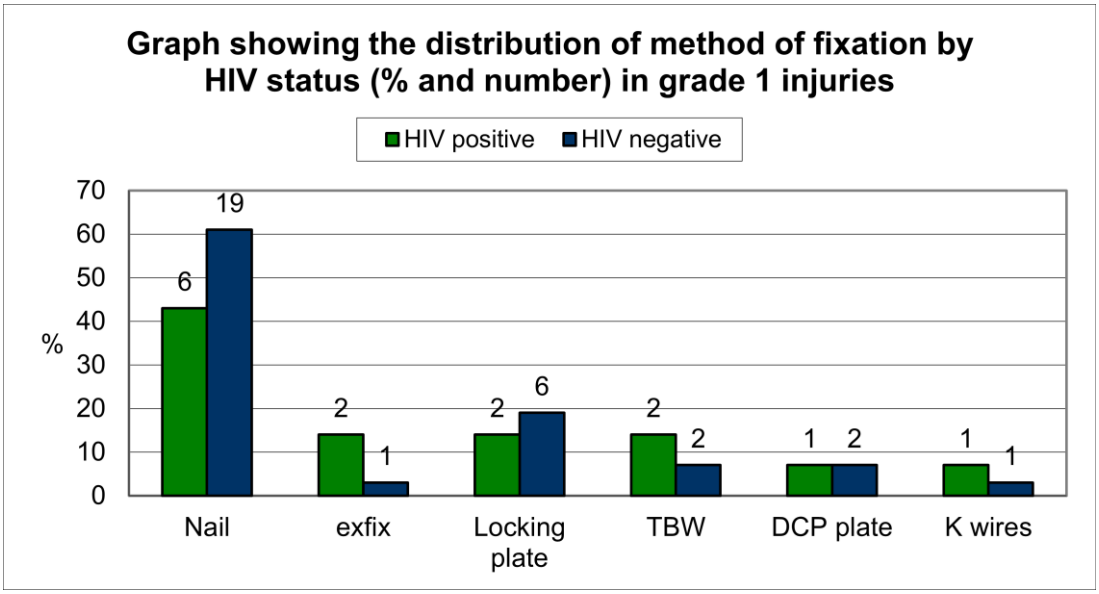


Figure 3-12

3.1.5.3 **Logistic regression analysis**

Table 3-8 and Table 3-9 show logistic regression analysis looking at potential risk factors for wound infection.

Table 3-8, Logistic regression analysis of possible risk factors (including HIV infection) for wound infection in grade 1 injuries.

	Adjusted Risk Ratio for wound infection	P value
HIV Positive	5.0	0.094
Delay greater than 24h to debridement	1.8	0.62
Smoking	0.438	0.35

Table 3-9. Logistic regression analysis of possible risk factors (including advanced HIV infection) for wound infection in grade 1 injuries.

	Adjusted Risk Ratio for wound infection	P value
Advanced HIV	19	0.005
Delay greater than 24h to debridement	1	0.98
Smoking	0.6	0.70

These tables provide suggestive evidence that HIV is associated with wound infections in grade 1 injuries, and strong evidence of an association with advanced HIV. The data suggests that this is a strong association with an adjusted risk ratio of infection given advanced HIV of 19.

3.1.6 Non union rates

3.1.6.1 **Overall non union rate**

Out of the 119 fractures whose HIV status was known, 4 fractures were lost to follow up and were excluded from the following analysis.

Table 3-10 Non union rates in cohort 1

Non union, Number (%)			**The risk of developing a non union given HIV positivity.** Risk ratio = 4.1 (1.1-16.3)$^{95\%CI}$ P value^{x}= 0.04
	Yes	**No**	
HIV positive	5, (15)	28, (85)	
HIV negative	3, (4)	79, (96)	
			The risk of developing a non union given advanced HIV infection compared to HIV negative controls. Risk ratio = 3.64(0.7-20.0)$^{95\%\ CI}$ P value^{FE} = 0.17
Advanced HIV	2, (13)	13, (87)	
HIV negative	3, (4)	79, (96)	
			The risk of developing a non union given advanced as compared to early HIV infection. Risk ratio = 0.8 (0.15-4.18)$^{95\%\ CI}$ P value^{FE} = 1
Advanced HIV	2, (13)	13, (87)	
Early HIV	3, (17)	15, (83)	

Table 3-10 show that there is good evidence of a strong association between HIV infection and the development of non union within the cohort. It also provides weak evidence of a moderate association between advanced HIV and non union when compared against HIV negative controls. In addition, it shows that non union rates are slightly lower in patients with advanced HIV compared to early HIV, however there is no evidence that this difference is greater than would be expected by chance alone.

3.1.6.2**CD4 counts non union**

Figure 3-13 shows the CD4 counts of those patients with and without non union

Scatter plot showing CD4 counts of the HIV positive patients with and without non union

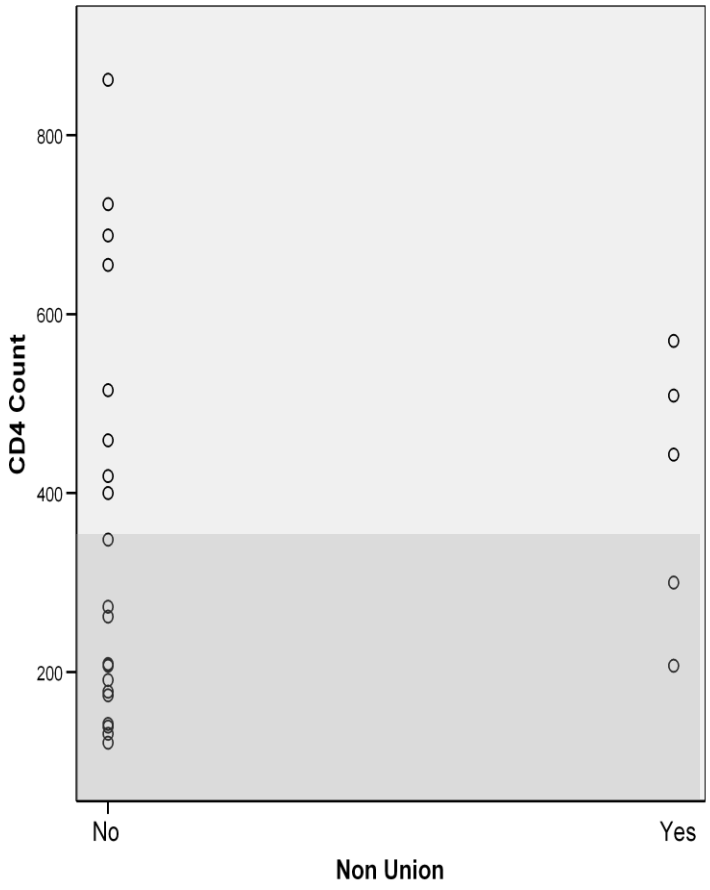


Figure 3-13, The dark shading denotes patients with advanced HIV

Using logistic regression there is a trend of increasing union rates as CD4 counts drop, however the data provides no evidence that this more than would be expected by chance alone, n=26, Risk ratio = 1.001, P value 0.56.

3.1.6.3 **Logistic regression**

Table 3-11 and Table 3-12 show logistic regression analysis with relevant potential risk factors.

Table 3-11, Logistic regression analysis of possible risk factors for non union

	Adjusted Risk Ratio for non union	P value
High Grade wound	15	0.02
HIV Positive	6	0.05
Delay to surgery > 24h	2	0.44
Smoking	1	0.97

Table 3-12, Logistic regression analysis of possible risk factors for non union using advanced HIV as a risk factor

	Adjusted Risk Ratio for non union	P value
High Grade wound	6.1	0.06
Advanced HIV	2.5	0.3
Delay to surgery > 24h	1.2	0.8
Smoking	1.6	0.5

Table 3-11 suggests that in this study HIV was strongly associated with the development of non union. It also provides strong evidence against the null hypothesis that HIV is not associated with non union. Table 3-12 suggests that advanced HIV is associated with an increased risk of non union, although smaller than that seen with HIV infection. It provides weak evidence to reject the null hypothesis that advanced HIV is not associated with non union. This may be due to the small numbers of patients present in this group of patients.

3.2 Cohort 2: Open fractures fixed by internal fixation

This study has examined the rates of wound sepsis and union rates within cohort 2 in order to investigate the following null hypotheses:

- There is no association between HIV or advanced HIV and wound infection in open fractures treated with internal stabilization.
- There is no association between HIV or advanced HIV and non union in open fractures treated with internal stabilization.

3.2.1Patients

Over a 9 month period 102 patients with 103 fractures met the inclusion criteria.

3.2.2 Follow up

96% had follow up of at least 2 months

88% had follow up of at least 3 months

Mean length of follow up 20 weeks, range 4-47 weeks

3.2.3Baseline characteristics

3.2.3.1 **Patient related factors**

Figure 3-14 shows the prevalence of HIV within the cohort.

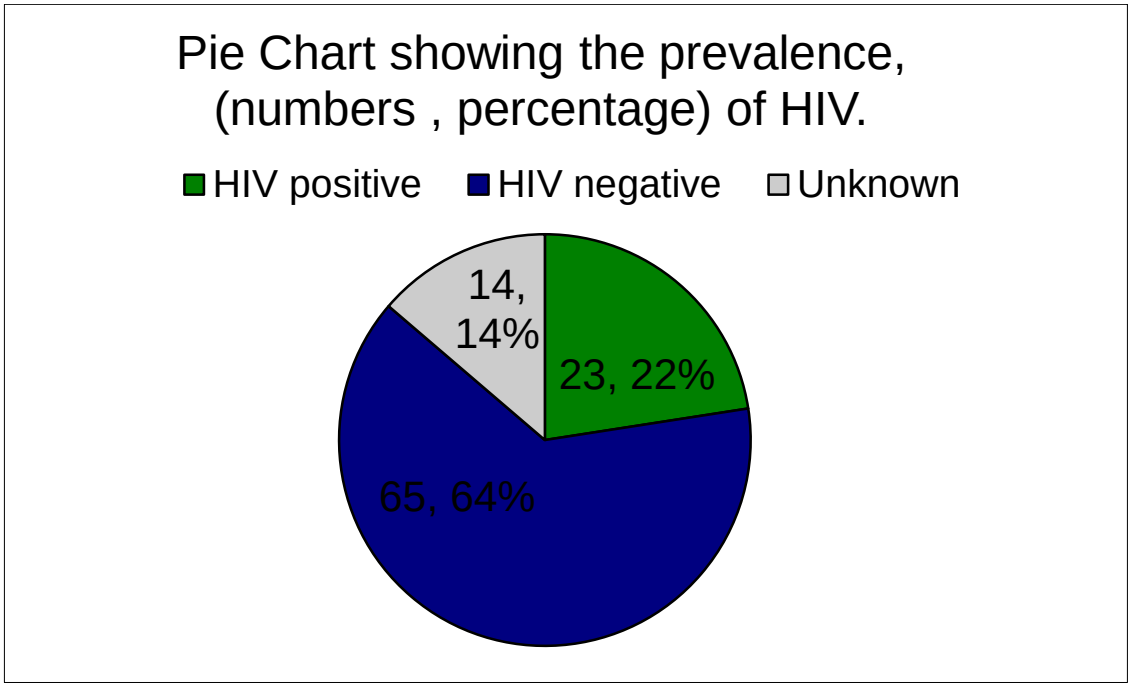


Figure 3-14

4 HIV positive patients were taking HAART, the majority of the remaining HIV positive patients were not aware of their HIV status on admission to hospital.

Of the 23 HIV positive patients CD4 counts were available on 17 patients; their distribution is shown in Figure 3-15 :

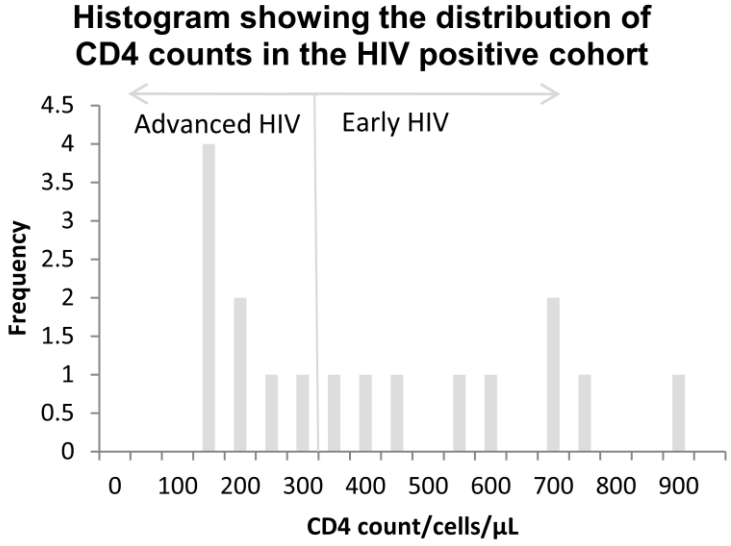


Figure 3-15. The cut off for advanced and early HIV is illustrated

Table 3-13 shows demographic comparisons made only on patients whose HIV status was known

Table 3-13

	HIV positive (%)	HIV negative (%)	P value
Number of patients	23 (27)	65 (73)	
Mean age	32	33	
Male	20/23 (87)	53/65 (81)	0.55 [FE]
Age<50	21/23 (91)	57/65 (88)	0.49 [FE]
albumin<30	9 /23 (39)	18/65 (28)	0.31 [X]
Smoking	14/23 (61)	27/65 (41)	0.094 [X]

3.2.3.2 **Injury related factors**

Figure 3-16 to Figure 3-19 show the cause, method of fixation, grade and location of the injuries.

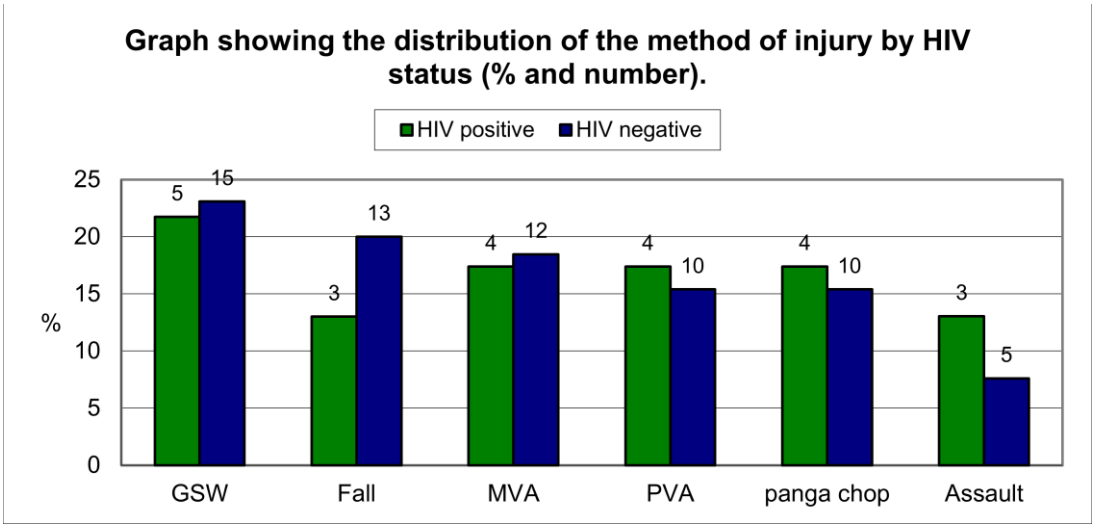


Figure 3-16

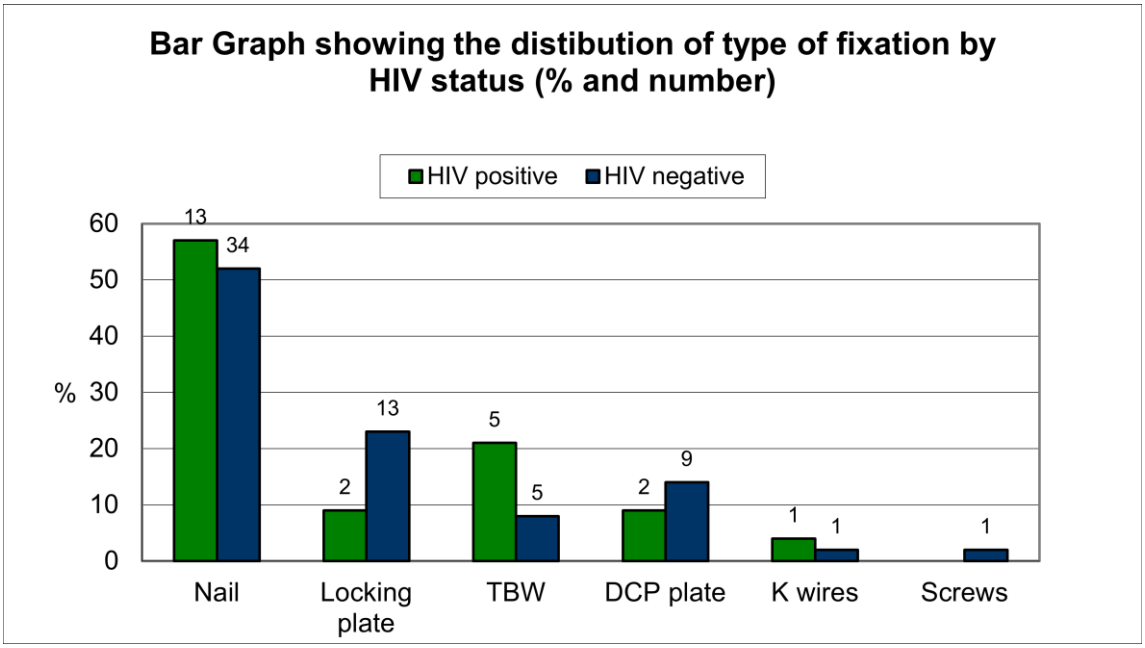


Figure 3-17

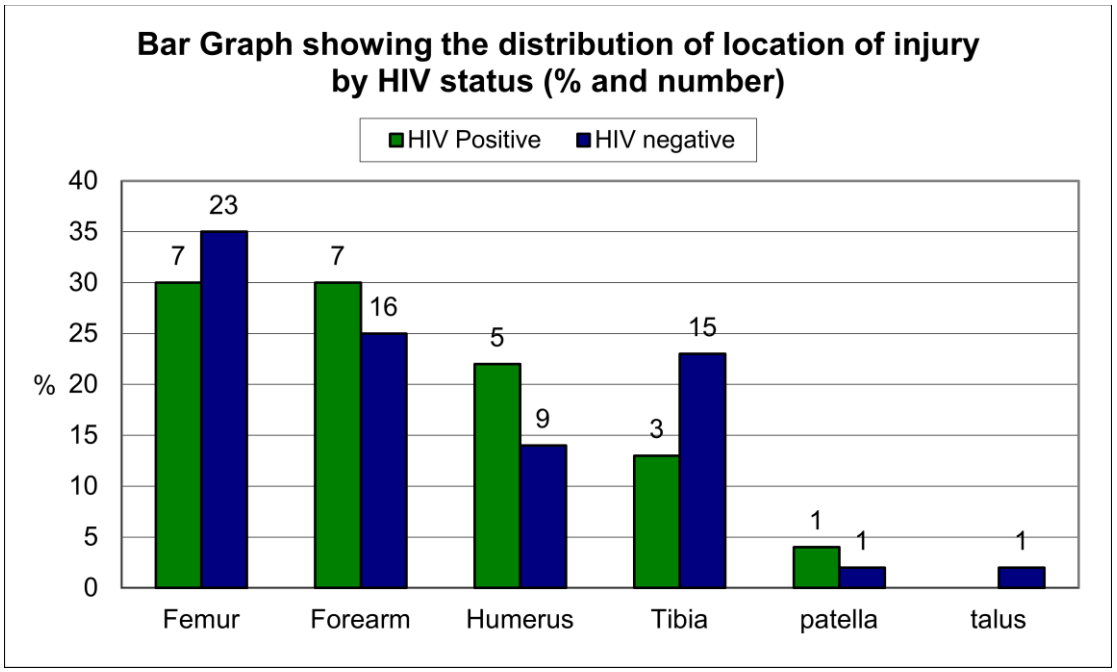


Figure 3-18

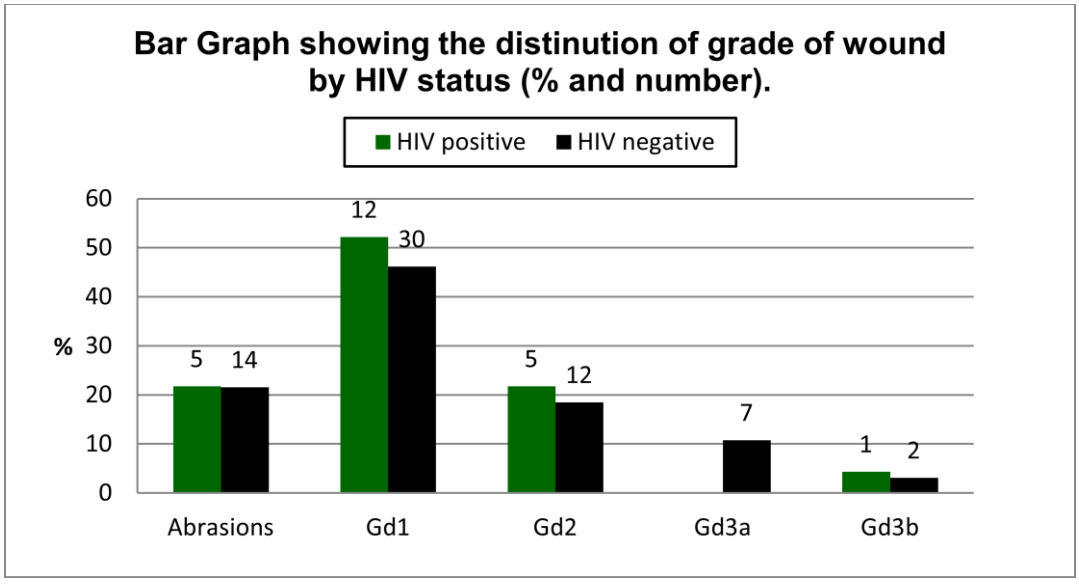


Figure 3-19

3.2.4Infection rates

This study has looked at the association between wound infections and HIV in cohort 2. The cohort has been further subdivided into grades of wound, and similar analyses have been done on these sub groups.

3.2.4.1 **Overall wound infection rate**

Table 3-14 shows the wound infection risk in patients treated with internal fixation.

Table 3-14

Wound Infection, Number, (%)			**The risk of developing a wound infection given HIV infection.** RR= 0.85 (0.3-2.8)$^{95\% CI}$ P value ^{FE} = 1
	Yes	**No**	
HIV positive	3, (13)	20, (87)	
HIV negative	10, (15)	55, (85)	
			The risk of developing a wound infection given advanced HIV infection. RR= 2.17 (0.73-6.42)$^{95\% CI}$ P value ^{FE} = 0.19
Advanced HIV	3, (33)	6, (67)	
HIV negative	10, (15)	55, (85)	
			The risk of developing a wound infection given advanced HIV compared to early HIV infection. Risk ratio =NA P value^{FE} = 0.04
Advanced HIV	3, (33)	6, (67)	
Early HIV	0, (0)	16, (100)	

In patients treated with internal fixation, patients with and without HIV have a similar wound infection risk. As hypothesized patients with advanced HIV have higher infection rates than controls, this data provides weak evidence that that this difference is greater than would be expected by chance alone.

The data suggest that patients with advanced HIV have an increased risk of wound infection than patients with early HIV. It provides strong evidence that that this difference is greater than would be expected by chance alone.

3.2.4.2 **Logistic regression analysis**

Table 3-15 and Table 3-16 shows logistic regression analysis looking at HIV and advanced HIV are associated with wound infection.

Table 3-15. Logistic regression analysis for wound infection, using HIV as a risk factor, within cohort 2

	Adjusted Risk ratio	P value
Delay of greater than 24 hours to debridement	1.6	0.57
High grade wound	1.2	0.57
HIV infection	0.9	0.93
Smoking	0.5	0.27

Table 3-16. Logistic regression analysis for wound infection using advanced HIV infection as a risk factor, within cohort 2

	Adjusted Risk ratio	P value
Advanced HIV	4.1	0.08
High grade wound	1.4	0.67
Delay of greater than 24 hours to debridement	1.2	0.89
Smoking	0.4	0.57

This analysis shows that in this cohort HIV is not associated with wound infection, when adjusted for the above confounders. Advanced HIV is associated with a four times increased risk of wound infection, and the data provides moderate evidence against the null hypothesis that advanced HIV is associated with wound infection in open fractures treated with internal stabilization.

3.2.4.3 **CD4 counts and wound infections**

Table 3-16 suggests that a declining CD4 count might lead to an increased risk of wound infection. Figure 3-20 is a scatter plot showing the CD4 counts of the HIV positive patients and whether or not they developed infections.

Scatter plot showing the CD4 counts of the HIV positive patients and whether or not they developed infections

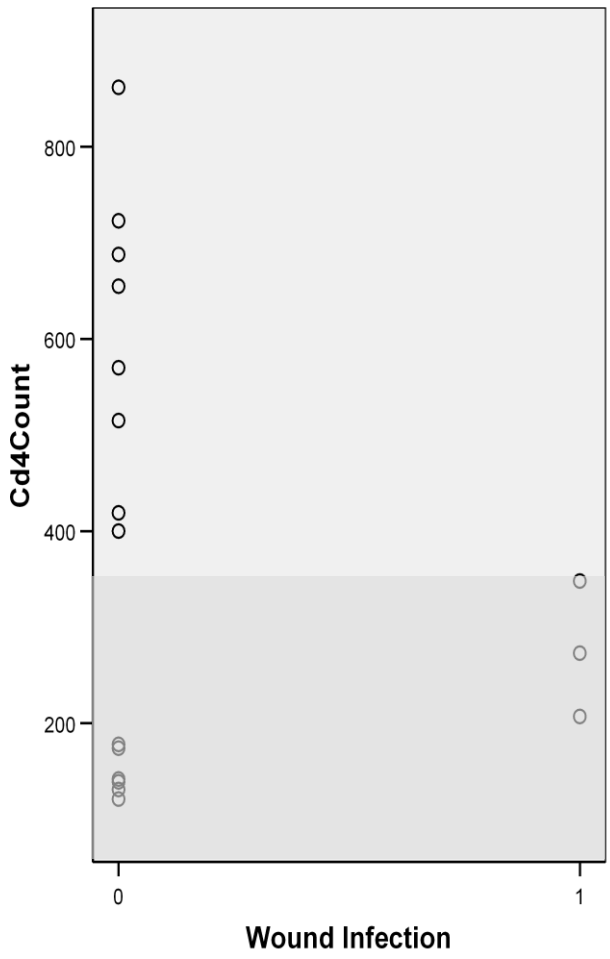


Figure 3-20

Using logistic regression there is a trend of increasing union rates as CD4 counts drop, however the data provides no evidence that this more than would be expected by chance alone, n=13, Risk ratio = 0.99, P value 0.31.

3.2.4.4 **Subgroup Analysis**

Table 3-17 shows analysis of wound infection by differing grades.

Table 3-17. Sub group analysis by grade of injury (Gustilo-Anderson) for wound infection given HIV or advanced HIV infection, within cohort 2.

	Rates of Wound infection, number of patients (%)				
	Abrasions	**1**	**2**	**3a**	**3b**
HIV positive	0/5 (0)	3/12 (25)	0/5 (0)	0/0	0/1 (0)
HIV negative	4/14 (29)	2/30 (7)	1/12 (8)	2/7 (29)	1/2 (50)
Risk ratio of wound infection give HIV positivity, (95% CI)	NA	3.8	NA	NA	NA
P value [FE]	0.53	0.13	1	NA	1
Advanced HIV	0/2 (0)	3/6 (50)	0/0	0/0	0/1 (0)
HIV negative	4/14 (29)	2/30 (7)	1/12 (8)	2/7 (29)	1/2 (50)
Risk ratio of wound infection given advanced HIV, (95% CI)	NA	7.5	NA	NA	NA
P value [FE]	1	0.02	NA	NA	1

These data shows that in HIV positive patients, internally fixed fractures with grade 1 injuries have an increased risk of infection compared to HIV negative controls. This difference is increased in advanced HIV. This data provides strong evidence that advanced HIV is associated with wound infection in grade 1 injuries.

3.2.5Radiological non union

3.2.5.1 **Overall non union risk**

Out of the 88 fractures whose HIV status were known, 3 fractures were not followed to union/non-union and are therefore excluded from the following analysis. Table 3-18 shows the risk of non union within the cohort.

Table 3-18, Risk of non union in cohort 2

Non union, Number (%)			**The risk of developing a non union given HIV positivity.**
	Yes	**No**	
HIV positive	1, (4)	22, (96)	Risk ratio = 2.7 (0.2-41)$^{95\%CI}$
HIV negative	1, (2)	61, (98)	P value ^{FE}= 0.47

There were no significant differences in risk of non union between the HIV positive and negative patients. Due to the low numbers of non-unions both sub group analysis and logistic regression was not possible.

3.3 Cohort 3: Open fractures treated by external fixation

3.3.1Introduction

This data is being analysed to interrogate the following null hypotheses:

- That there is no association between HIV or advanced HIV and pin site sepsis in open fractures treated with external fixation.
- That there is no association between HIV or advanced HIV and non union in open fractures treated with external fixation

3.3.2Patients

Over the 9 month period 32 patients with 32 fractures met the inclusion criteria.

3.3.3Follow up

All patients were followed up until union/non union.

Mean length of follow up 20 weeks, range 10-36 weeks

3.3.4Baseline characteristics

3.3.4.1 **Patients specific**

Figure 3-21 shows prevalence of HIV within the cohort

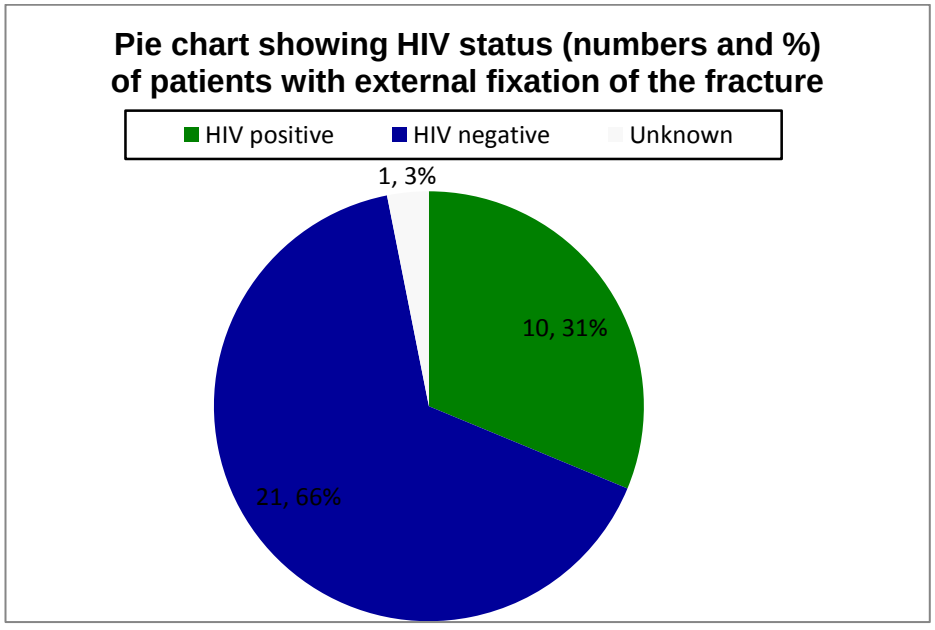


Figure 3-21

CD4 counts were available for 9 out of the 10 patients. Their distribution is shown in Figure 3-22

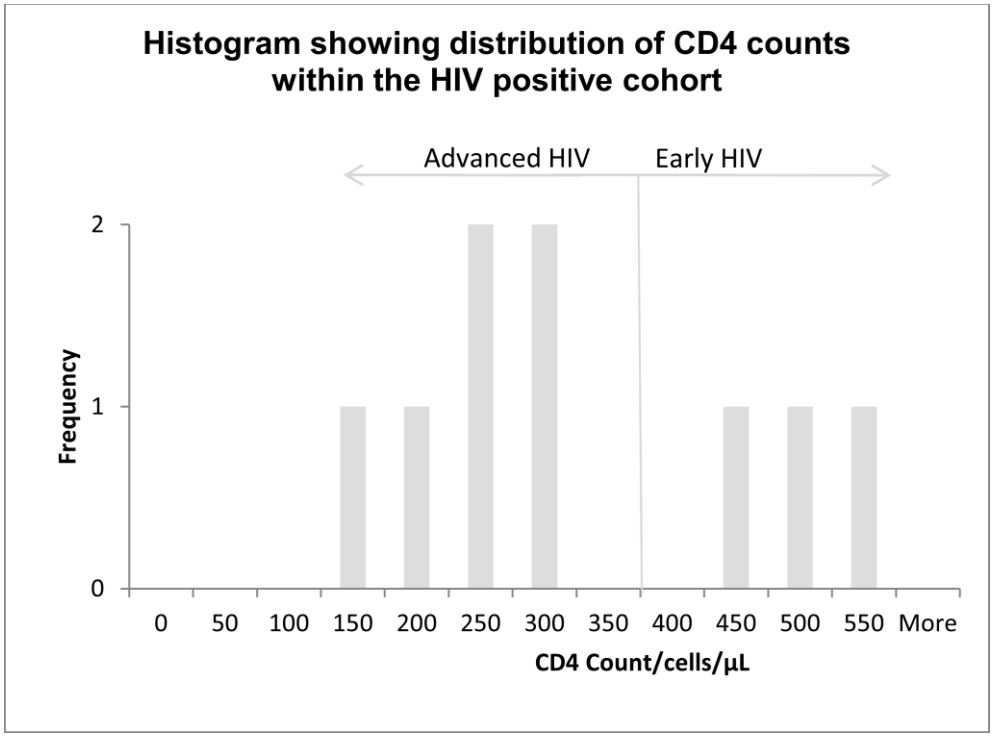


Figure 3-22

Table 3-19 show the demographic comparison in the patients with whom the HIV status was known.

Table 3-19

	HIV positive (%)	HIV negative (%)	P value
Number of patients	10 (32)	21 (68)	
Mean age	31	36	0.77^{MW}
Male %	80	86	0.53^{FE}
Age<50	10/10 (100)	18/21 (86)	0.3^{FE}
Albumin<30	7/10 (70)	6/15 (29)	0.036^{FE}
Smoking	7/10 (70)	9/21 (43)	0.15^{FE}

3.3.4.2 **Injury specific**

Figure 3-23 to Figure 3-25 show the grade, cause and location of the injuries.

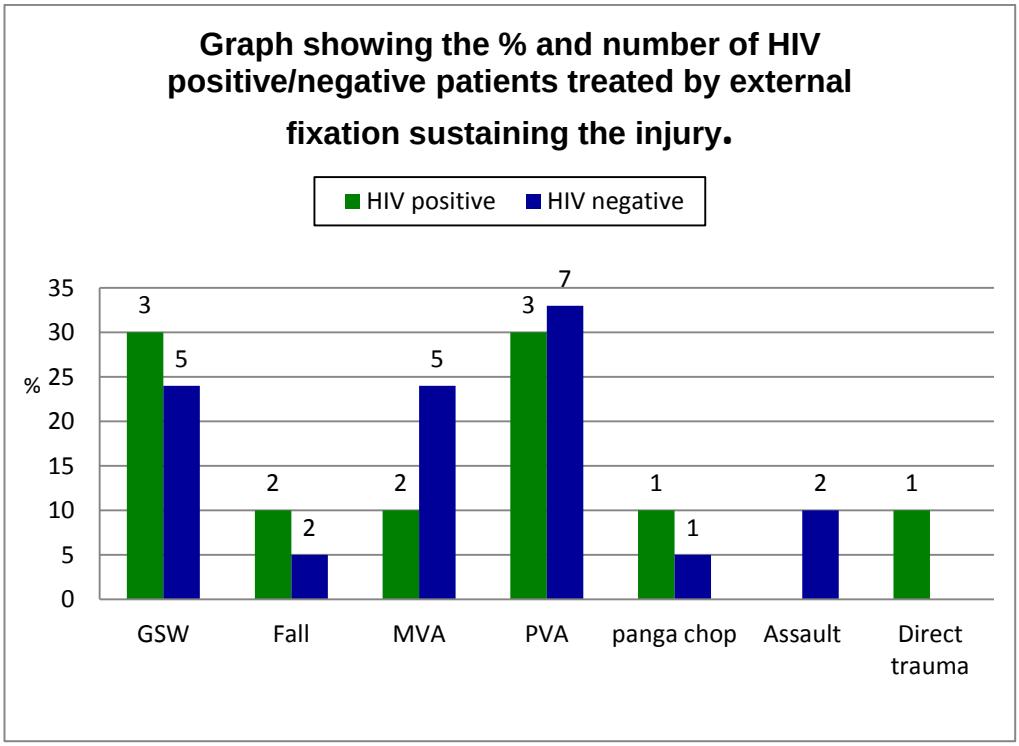


Figure 3-23

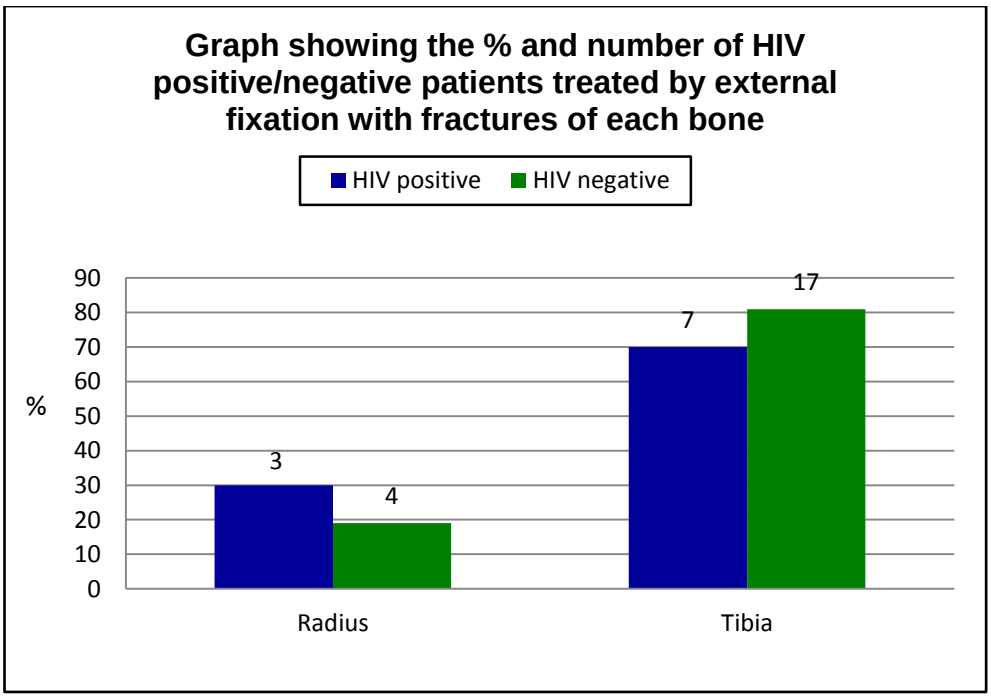


Figure 3-24

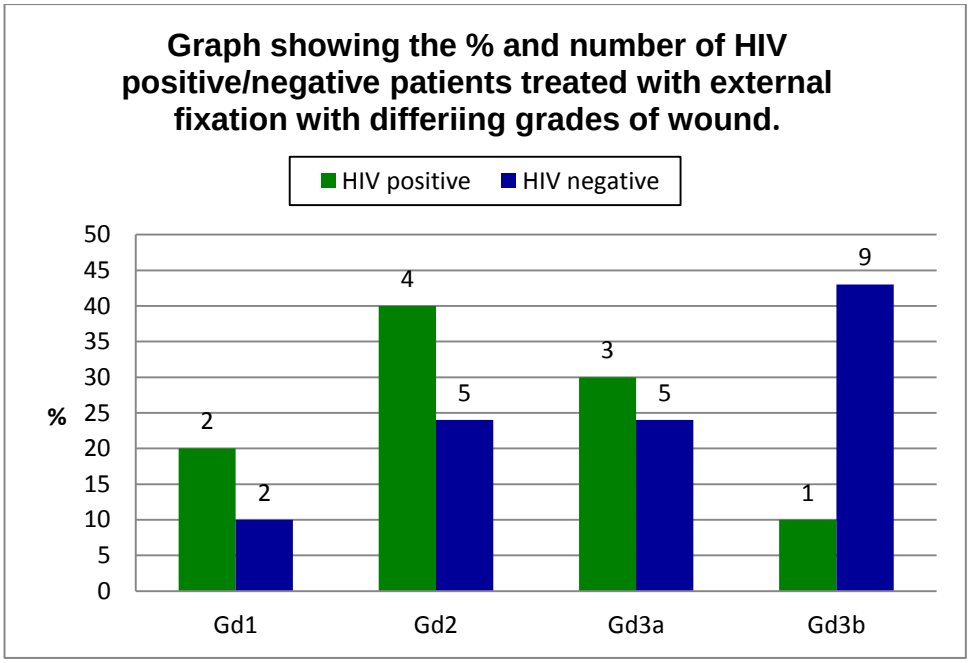


Figure 3-25

Forearm external fixators were all Orthofix Pennig, using a total of 4 pins. Tibia external fixators used were: Orthofix monolateral, AO monolateral and AO hybrid. Monolateral tibia external fixators used 6 pins.

Grade 1 injuries were uncommonly treated by external fixation unless contaminated or the fracture pattern necessitated it.

External fixators remained insitu until the fracture healed, or a deterioration in the pin/wire bone interface, necessitated removal. The mean length of time for the fixator to remain in situ was 13 weeks in the HIV positive group and 12 weeks in the HIV negative group.

3.3.5Pin-site infection

Figure 3-26 shows the maximum Checketts grade obtained from any of the pin sited for the duration of the external fixators application.

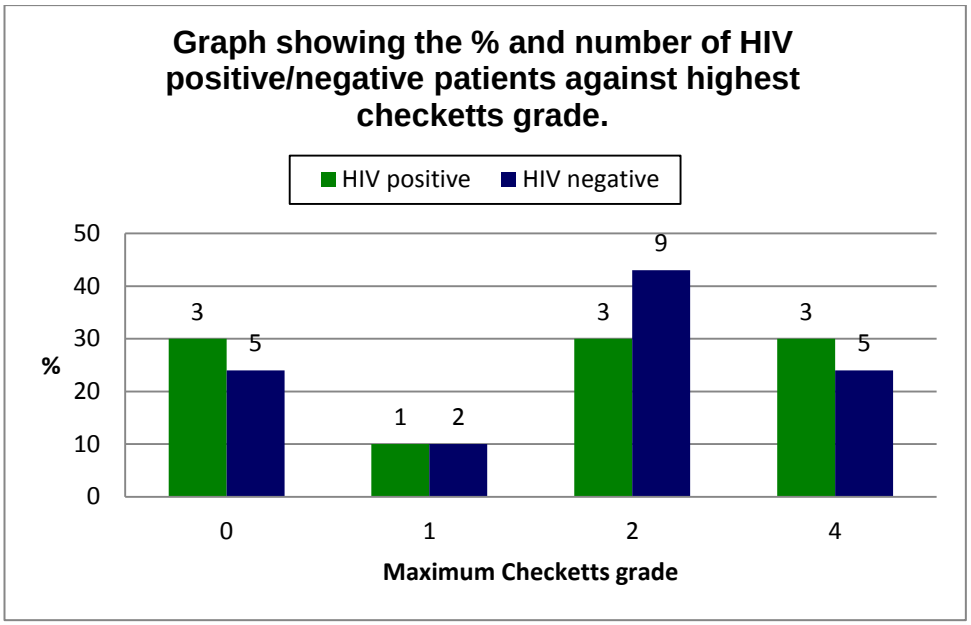


Figure 3-26

Table 3-20 shows statistical analysis on patients who required antibiotics, or removal of their external fixator due to infection (Checketts>=2).

Table 3-20

	Checketts score >=2 (%)		**The risk of a pin site infection (>=2) given HIV infection**
HIV infection	**Yes**	**No**	
HIV positive	6/10 (60)	4/10 (40)	Risk Ratio = 0.9 (0.5-1.6)$^{95\% CI}$ *P value*^{FE}*= 1*
HIV negative	14/21 (67)	7/21 (33)	
Advanced HIV			**The risk of a pin site infection (>=2) given advanced HIV infection**
Advanced HIV	4/6 (67)	2/6 (33)	Risk Ratio = 1.0 (0.1-10.4)$^{95\% CI}$ P value^{FE}=1
HIV negative	14/21 (67)	7/21 (33)	

Table 3-21 shows statistical analysis on patients who required removal of their external fixator due to infection (Checketts=4).

Table 3-21, showing risks of severe pin site sepsis in cohort 3

	Checketts score =4 (%)		**The risk of a pin site infection (=4) given HIV infection**
	Yes	**No**	Risk Ratio = 1.26 (0.4-4.3)$^{95\%CI}$
HIV positive	3/10 (30)	7/10 (70)	*P value^{FE}= 1*
HIV negative	5/21 (24)	16/21 (76)	
Advanced HIV	2/4 (50)	2/4 (50)	**The risk of a pin site infection (=4) given advanced HIV infection**
HIV negative	5/21 (24)	16/21 (76)	Risk Ratio = 2.1 (0.23-47.1) $^{95\%CI}$ P value^{FE} = 0.55

3.3.5.1 **Logistic regression analysis**

Table 3-22 Logistic regression analysis for pin site infection using HIV infection as a risk factor . Table 3-23 Logistic regression analysis for pin site infection using advanced HIV infection as a risk factor

Table 3-22 Logistic regression analysis for pin site infection using HIV infection as a risk factor

	Adjusted Risk ratio for pin site infection	**P value**
HIV infection	0.7	0..9
High grade wound	0.9	1
Delay of greater then 24 hours to debridement	0.1	0.1
Smoking	0.7	0.7

Table 3-23 Logistic regression analysis for pin site infection using advanced HIV infection as a risk factor

	Adjusted Risk ratio for pin site infection	**P value**
Advanced HIV	1.5	0.8
Delay of greater then 24 hours to debridement	0.02	0.09
Smoking	0.7	0.5

3.3.6Non-unions

External fixation is used for patients with severe fractures, with significant soft tissue injury, there being often severe contamination and extensive periosteal stripping. These patients are therefore at high risk of developing non union. The risk of developing non union is shown in Table 3-24.

Non Union, Number, (%)			The risk of developing a wound infection given HIV positivity.
	Yes	No	
HIV positive	3/10 (30)	7/10 (70)	RR= 4 (0.59-16)$^{95\% CI}$
HIV negative	2/20 (14)	18/20 (86)	P value ^{FE} = 0.3

Table 3-24

3.3.6.1Logistic regression analysis

Table 3-25 and

Table 3-26 show logistic regression analysis looking at potential risk factors for non union.

Table 3-25 Logistic regression analysis for non union, using HIV as a risk factor, within cohort 3

	Adjusted Risk ratio	P value
HIV infection	10	0.07
Delay of greater than 24 hours to debridement	2	0.4
Smoking	0.8	0.9
High grade wound	100	1

Table 3-26- Logistic regression analysis for non union, using advanced HIV infection as a risk factor, within cohort 3. High grade wound removed, due to inadequate numbers

	Adjusted Risk ratio	P value
Advanced HIV	5	0.2
Delay of greater than 24 hours to debridement	3.4	0.3
Smoking	2.4	0.4

3.3.6.2Summary of the patients who developed non union with external fixation

Two patients who were HIV negative developed non unions:

1. 60 year old male who sustained a grade 3b tibia fracture following an assault. He was not a smoker but did confess to heavy alcohol

consumption, and was malnourished with an albumin of 21. He was treated with an external fixator but developed sepsis and subsequent non union. This was treated with closed IM nailing and he went on to union.

2. 65 year old male he sustained a close range shot gun injury to his distal forearm. He had a massive soft tissue injury and amputation of the hand was initially considered. He underwent external fixation, with vac dressing. Then delayed skin grafting when the risk of infection was thought to have diminished. He developed a fibrous non-union of the radius, but due to the massive soft tissue and nerve injury this was not painful. He therefore did not undergo further treatment

Three patients in the HIV positive group:

1. 32 year old male, smoker, who suffered multiple high energy gun shot wounds to femur and forearm. His CD4 count was 443 on admission. His femur was nailed and united, his radius was treated with external fixation, at 4 months there was no evidence of callus on radiographs, however he suffered from few symptoms and therefore no further surgery was planned.
2. 33 year old male, non smoker who sustained a grade 2 tibia fracture following a fall from his bike. He was diagnosed with HIV on admission to hospital and was noted to have a CD4 count of 509 cells/µL. He underwent external fixation. He developed worsening pin site and wound sepsis over the next 3 months which did not settle with antibiotics, and the fracture drifted into valgus. He refused to return to the hospital after 4 months when infected non union was diagnosed, and the Ex Fix removed but he was contacted via telephone at six months, and remained unable to put weight through this leg.
3. 26 year old male who was a smoker, who sustained a 3b tibia fracture following a wall falling on to his leg. He was diagnosed with HIV on admission to hospital, and was found to have a CD4 count of 207 cells/µL. He was treated with a fascio-cutaneous flap, and external fixation. Despite no evidence of sepsis he failed to unite, and underwent delayed closed IM nailing at six months post injury. He united following this intervention. He had at this stage been started on HAART

3.3.6.3 **Time to radiological union**

Time to radiological union as defined as the presence of bridging callus visible on radiographs, were similar in both HIV positive and negative patients, 15 and 16 weeks respectively. Small numbers and variable dates of clinic appointments made subgroup analysis, on location and grade of injury impossible.

3.4 Patients whose treatment was affected by HIV

All patients were carefully assessed at the time of treatment and all notes were reviewed to see if there was any indication if HIV status had influenced management. The only patient who would have been managed with intramedullary nailing according to the protocol discussed on page 69, had a fracture that was too low for intramedullary nailing and was therefore treated with a hybrid external fixator.

3.5 Patients with missing data

3.5.1HIV test

There were 16 patients who refused HIV testing, 81% were followed up for at least 3 months. There were no cases of superficial wound infection. 2 patients had problems with bone healing. One patient sustained a GSW to the mid shaft radius and ulna treated with an external fixator, he developed a painless non-union of the radius 6 months later. The other patient sustained a grade 1 injury of the radius and ulna, treated with locking plates. The ulna locking plate pulled off and she required repeat surgery and bone grafting.

3.5.2CD4 result

There were 7 HIV positive patients for whom CD4 counts were not available. These patients all went on to union without any incidence of wound infection.

Chapter 4
Discussion

4 Discussion

4.1 Methods

This study was a prospective observational cohort study. The study design and the environment that the study was conducted in presented many challenges and introduced many potential sources of error.

4.1.1Selection Bias

This was a cohort study that selected all contiguous admissions with open fractures in adults. Exclusion criteria were designed to exclude patients who would be difficult to follow up, or who had major poly-trauma that management was difficult to standardize. It was felt unlikely that any of these criteria would have introduced a significant selection bias.

4.1.2 Lack of blinding altering management

The lead clinician was not blinded to HIV status but review of the notes suggests that no patient management was altered as a result of HIV status, and that management was based on the treatment protocols. Despite this it remains possible that small management changes were made as a result of HIV status, perhaps favouring treatment external fixation over internal fixation. The data suggests that patients who were HIV positive were more likely to be treated by external fixation than HIV negative patients(30% vs 24%). Previous work has suggested that the risk of non union is higher in fractures fixed by external fixation in comparison to IM nailing[54]. This differential bias has occurred it may lead to an increased observed risk of non union in the HIV positive patients.

4.1.3 Lack of Randomization

This study was an observational study and therefore no randomization took place. It is therefore not possible to answer the question, “Is internal or external fixation the best method of fixation for open fractures in HIV positive patients?”. To answer this question would require a separate trial.

4.1.4Measurement Bias

An attempt to limit the subjectivity of the scoring systems of wound infection and pin-site sepsis was made in this study. The outcome measures used still have a degree of subjectivity in their assessment. Due to the lack of blinding in the assessment of wound and pin sties it is possible despite all the precautions taken that this subjectivity could have influenced the results leading to a differential bias.

Local validation of the individual assessment tools was not conducted. It is therefore possible that this may have introduced an additional source of bias.

4.1.5 Application to other populations

The study hospital was a government hospital, in a Zulu rural community and predominantly took patients engaged in unofficial employment and without health insurance. These were generally patients from lower socioeconomic groups. This group of patients may have higher prevalence of HIV and higher rates of trauma than the general population.

The subtype of HIV seen in this study is C, this is different to that seen in population in Europe and North America which is B.

These factors may have implications with applicable of this trials results to other populations.

4.1.6 Patient related factors

The prevalence of HIV was 22%, this is lower than expected, given the prevalence in pre natal checks in women in the area. This may be explained by the male preponderance (81%) of the study population. It has been shown that women have higher prevalence of HIV than men: this is attributed to sexual behaviour, and transmission routes[203].

Comparison of the demographic data of the cohorts revealed several differences. Patients with HIV were more likely to be smokers with a p value=0.028. Smoking is known to be a risk factor for non-union[78]. It is possible that patients with HIV are more likely to smoke, due to an increased risk taking behaviour, and therefore smoking should not be treated as a confounder. Although patients were all counselled about the need to try to stop smoking, very few changed their behaviour. It was not possible to accurately quantify the numbers patients smoked. Financial

constraints meant that most patients bought cigarettes one at a time, smoking more around pay day, and relatively few for the rest of the month. Smoking has been used in the logistic regression analysis however its significance needs to be interpreted with caution.

HIV infection was also associated with higher levels of hypoalbuminaemia. Patients with HIV are known to have an increased rate of hypoalbuminaemia and this is also known to be a risk factor for non union. This is therefore considered to be on the causal pathway and is not used in logistic regression analysis.

In at least one patient it was considered likely that the domestic abuse in which she sustained a grade 3 forearm fracture may have been related to her HIV diagnosis. This effect was not thought to be widespread, but could lead to an alteration of the study population.

On comparison of the grades of wounds between the HIV positive and negative patients, the groups are broadly similar with a slightly increased percentage of grade3 injuries in the HIV negative group. It was therefore not felt that the phenomena noted above significantly altered our outcomes. .

4.1.7 Injury specific factors

The location of the injury is varied, with open tibia fractures being the most common; this is similar to previous studies. The high number of forearm fractures is secondary to the high level of domestic abuse where these injuries are commonly inflicted by bush knives. These injuries most often occur in females, often include tendon and nerve injuries and were in one instance bilateral. This has obvious repercussions with hygiene and rehabilitation.

HIV positive patients proportionally have fewer grade 3 open fractures. This should lead to a slight bias favouring a higher union rate in the HIV positive group.

During the period of the study, no grade 3c fractures were admitted with a viable limb. This is probably a result of the often long time delay between injury and arrival at hospital.

The huge variety of severity and mechanism of injury, being categorised into such broad categories (Gustilo Anderson classification) was a significant limitation of this study. Clinical studies into open fractures and HIV, have to date been unable to compare like for like injuries, because of the relative infrequency of the injuries.

Fractures in this study have been treated through a variety of mechanisms; the methods of internal fixation are varied and may have implications on union rate. It is possible that HIV infection may be more relevant in certain types of fixation such as reamed intramedullary nailing. Due to the small numbers of patients in the study and the variety of types of injury, it is not possible to comment on the effect of type of internal fixation on union rates in these patients.

4.1.8 Short follow up

Due to the time constraints associated with this type of study, we do not have the data available from long term follow up.

The incidence of delayed sepsis is critically important in guiding initial treatment decisions. This data is being collected, in ongoing work set up by the author. It will be several years before this will be ready for analysis.

It is still a widely held belief that HIV positive patients are at much higher risk of delayed sepsis. There is little published evidence to support this, and recently some evidence to dispute it[131], but there is a biologically plausible explanation, and much practice in this area remains based on local expert opinion.

In this study non unions have been classified as a fracture requiring a repeat operation to stimulate bone healing, once a reasonable period of time has elapsed. This was 6 months for a tibia/femur fractures or 3 months for other fractures. It is acknowledged that other studies would only classify a fracture as a non union, after multiple fracture stimulating procedures and a much longer period of time, this study was constrained by time limitations.

4.1.9 Assessment of time union

It was difficult to ensure regular follow up, especially as patients neared union and were beginning to mobilize without pain. Radiographs were also not always available at each visit, due to staffing problems, and long waits. Patients would come back if asked, but at a time that suited them. It was therefore difficult to consistently ascertain a precise time to union. It would be an interesting aspect of a larger study to look at time to union in similarly classified fractures

4.1.10 Small numbers

Patient numbers in this study, although greater than in previous studies, were small, and therefore it is at risk of a type 2 error. The incidence of HIV was lower than expected, and therefore the study did not achieve the numbers required by the power study in the external fixation cohort. Ideally this study should guide further research, to give the result more statistical validity. This could include a metanalysis with previous work done by Harrison et al..

4.1.11 Low CD4 counts:

No patients with a CD4 counts below 100 cells/µL were seen in this study. The results may not accurately represent this group of severely immuno-compromised patients.

4.1.12 Loss to follow up

The follow up rates in this study were high, in the internal fixation group they were 88% at 3 months or radiological union and in the external fixation group they were 100%. Several issues made ensuring follow up more challenging. The catchment area for the hospital was an area half the size of Scotland, and we had very little funding available to assist with transport costs. The increasing availability of mobile phones meant that we were able to maintain contact with the patients, and minimize the need to travel to the hospital. A small proportion of patients were either difficult to contact or refused to come back to the hospital, these patients were from a varied background, and it is not thought that it would have significantly affected the outcome of the study

4.1.13 Missing HIV/CD4 Data

It was not possible to obtain a full data set, with regard to HIV tests and CD4 counts. HIV remains a very controversial disease in South Africa, with many social and economic implications. It carries with it a stigma which affects both staff and patients. Over the last few years there seems to be increasing numbers of patients willing to have the HIV test, this is probably driven by the increasing availability of HAART. Patients were offered HIV testing on admission, on discharge and all subsequent clinic visits.

One logistical problem presented an unexpected problem with regard to obtaining CD4 counts. In the catchment area of the hospitals, it was common for patients not to know their exact date of birth and names were often similar, therefore the hospital number and the patient's national identification (ID) number were both important form of identification. HIV services often required co-ordination between health facilities and a move had been made to use the national identification number for CD4 counts and viral loads. Patients were often unaware of their national ID number and this led to several episodes when the lab refused to process samples, whilst patients obtained their ID number

It has been carefully considered whether the missing data from both the HIV tests or the CD4 counts could have lead to a selection bias. 44% of the patients who refused HIV tests sustained injuries from gun shot wounds. The combination of the social circumstances leading to the injury, and psychological impact of the injury, may make subsequent HIV test refusal more likely. Of those who refused HIV testing there were no cases of wound infection and two patients (13%) had problems with bone healing as shown on page 120. It is possible that this group of patients is less likely to be compliant with rehabilitation advice, and therefore be at higher risk of adverse events.

Patients, for whom a CD4 count was not available, were included in the overall analysis, but excluded when considering the effect of advancing HIV. It is thought that the CD4 counts of these patients is likely to be evenly distributed, and should have little effect on the overall result.

4.1.14Summary

Many of the limitations of this study are limitations that have been seen in other previous studies looking at this type of population. Whilst all results need to be interpreted in view of these significant limitations, this study remains the largest clinical study on this population group.

4.2 Results

4.2.1Summary of key results

Based on the data from this study there is no evidence to reject the null hypothesis that there is no association between HIV (or advanced HIV) and wound infection in open fractures treated with surgical stabilization.

Sub group analysis has highlighted that patients with grade 1 injury have a higher risk of infection if HIV positive. The data provides weak evidence of an association between HIV and wound infection in this group. Stronger evidence is seen with advanced disease.

A similar trend is seen in patients treated with internal fixation.

This study provides strong evidence to reject the null hypothesis that HIV is not associated with non union in fractures treated with surgical stabilization. The evidence is weaker for advanced disease. This trend is also seen in patients treated with external fixation and provides weak evidence to reject the null hypothesis that HIV is not associated with non unions in open fractures treated by external fixation.

This study provides no evidence to reject the null hypothesis that neither HIV nor advanced HIV are associated with pin site sepsis.

4.2.2 Wound Infection

Section 3.1 shows that the risk of infection was similar in both the HIV positive and negative patients. This was unexpected, previous studies had suggested much higher risks of infection in HIV positive patients. The risk of infection increased slightly in the patients with advanced HIV, compared to both HIV negative and patients with early HIV, the difference remains small with a p value of 0.049. Interestingly the wound infection rate was lowest in patients with early HIV, with relative risk of 0.25 when compared to HIV negative patients.

It seem likely that in patients with severe immunosuppression secondary to HIV, infection rate are likely to increase, this would correlate with increases rates of other bacterial infection seen in patients in HIV and discussed in section 1.3.5. It is however unexpected that patients with early HIV should have reduced infection

rates when compared to HIV negative patients. Whilst it is possible this observation has occurred by chance alone, it is interesting to speculate if it could be possible that HIV in its early stages could be beneficial to wound healing. All previous laboratory based studies identified have suggested that HIV would be detrimental to wound healing[9,148-151]. One possibility is that the body's response to trauma is dampened by HIV, leading to a reduction in serous exudate and erythema, in patients with only mild immunosuppression this may not hinder the healing process, in patients with more advanced immuno-suppression it may lead to wound breakdown or bacterial infection. This hypothesis would need to be tested in further research

Subgroup analysis shows a strong association in grade 1 open fractures between HIV and wound infection. This maybe the result of the different management protocols in place for the management of grade 1 fractures. This led to grade 1 injuries having an increased time to debridement, less extensive debridement and a shorter course of antibiotics (due to shorter time to definitive fixation) in comparison to the higher grade injuries. Patients with HIV and grade 1 injuries did on average wait one day longer than patients who were HIV negative. This could introduce a selection bias.

A previous study looking at the optimal management of open fractures in HIV negative patients has suggested that the risk of infection in grade 1 open injuries treated with antibiotics remain low, with and without urgent debridement[44].

One possible mechanism for the above observation would be that in the immuno-competent patient, the relatively small inoculums from a grade 1 injury is adequately controlled by the immune response and antibiotic administration. In the immuno-deficient patient the immune response is inadequate, and if the inoculum is left insitu the bacteria are able to proliferate, leading to a wound infection. This may be mediated as a result of increasing numbers of dysfunctional macrophages and lymphocytes, which have been shown to be important in wound healing as discussed on page 40. Again this hypothesis needs to be tested in further research

Other notable observations were:

- Logisitc regression analysis showed that in this cohort, a high grade wound was the greatest risk factor for infection (3 fold), followed by advanced HIV (2 fold).

- The risk of infection for fractures associated with abrasions was higher in those without HIV than those with HIV. A possible explanation for this is that patients with HIV mount a smaller inflammatory response to trauma. This manifests as reduced exudate forming over the abrasions (a clinical observation), and leads to lower levels of infected exudate, which would qualify as wound infection on the ASEPSIS scoring system.
- Table 3-5 shows how in HIV negative patients the risk of wound infection increase as the grade of wound increased, as would be expected from previous studies[1]. The reverse trend is seen in HIV positive patients. The numbers are very small and this may have occurred by chance. Table 3-2 and Table 3-14 show that patients with early HIV have the lowest infection rates when compared with advanced HIV and HIV negative patients. The question arises; could HIV be beneficial to wound healing in severe soft tissue injuries? This would require further investigation. All previous laboratory based studies identified have suggested that HIV would be detrimental to wound healing. It is possible that the assessment of wound infection may be affected by HIV. One of the assessment criteria is the formation of a serous or purulent exudate and erythema, it is possible that HIV infection may dampen down the expression of exudate or erythema following injury and lead to a reduction in severity of the apparent wound infection.
- Although wound infection was more common in the HIV positive patients with grade 1 injuries, the majority of infections settled with antibiotics. Only four patients, out of the nine who developed non union, had a wound infection as a potential risk factor for non union. It should be noted that a superficial wound infection, whilst undesirable, is often not detrimental to outcome.

4.2.3Non union

Table 3-11 showed that in this study HIV was strongly associated with the development of non union with an adjusted risk ratio of 15. It also provided strong evidence against the null hypothesis that HIV is not associated with non union with a p value of 0.04. Table 3-12 suggests that advanced HIV does not lead to an

increased incidence of non union, so providing little evidence to reject the null hypothesis that advanced HIV is not associated with non union. This maybe due to the small numbers of patients present in this group.

The incidence of non union was low in patients treated with internal fixation. This is likely to be due to selection bias, with the internal fixation cohort recruiting the less severe injuries, and making direct comparison with external fixation impossible.

The incidence of non union in the external fixation cohort followed the same trend as the combined cohort. The numbers are smaller and therefore the power of the statistical tests is reduced.

The variety of injuries seen in this study is huge and the classification of the injuries remains very broad. It is notable that despite this very broad categorization the HIV negative patients did appear to have the more severe injuries, yet still had an improved outcome when compared to the HIV positive patients.

The data from this study suggests the fracture healing is affected by HIV and that it is possible that this may be an important factor even in early HIV infection. In the introduction, a variety of postulated theories for HIV's interaction with bone healing have been proposed, and any combination of these could lead to the effect observed in this trial. It is interesting that some recent research has suggested that loss of functioning lymphocytes might lead to improved fracture healing[14]. There is some evidence to suggest that macrophages play an important role in fracture healing. The loss of functioning macrophages may result in problems in fracture union. Could this interplay between dysfunctional macrophages and lymphocytes account for the observed results in this study?

This study was unable to provide any statistically valid data on time to union. There are many factors that affect time to unions, in order to minimize these factors a case control study, looking at internally fixed lower limb fractures, with assessment of radiological union with monthly radiographs.

4.2.4Pin site sepsis

In this study there is little difference in the risk of pin site infection in the HIV positive and negative patients. The biggest difference noted in this study is the risk of severe pin site sepsis leading to removal of the external fixator in patients with advanced HIV compared to those with either early HIV or without HIV. This study

provides no evidence that this finding could not have been due to chance alone and therefore needs to be interpreted with caution.

It is possible that in this group with advanced HIV and severe open fractures, that delayed healing puts increasing stress through the bone pin interface. This will place the pin sites at risk of infection, in a patient who is also significantly immunocomprimised.

This data does not suggest that external fixation is inappropriate for severe open fractures in patients with advanced HIV, if these fractures were treated with internal fixation it is possible that they may be at higher risk of developing deep infection. It does suggest that if the treatment is to be external fixation then techniques which allow the pins to remain in situ for longer without the breakdown of the bone pin interface may be appropriate. Possible techniques which have been shown to improve the bone pin interface would be the use of hydroxyapatite coated pins or the concurrent administration of alendronate[204,205].

4.3 Comparison with previous published literature

Table 4-1 shows a comparison of the data presented in this thesis and previous studies regarding wound infection in open fractures.

Table 4-1

		Infection	No Infection	The risk of developing a wound infection given HIV infection.
Obrien[128]	**HIV positive**	3 (100)	0 (0)	RR= 15(2.3-99))[95% CI] P value[FE]= 0.04
	HIV negative	1 (7)	14 (93)	
Paiement[5]	HIV positive	4 (31)	9 (69)	RR= 3.04 (1.1-8.5))[95% CI] P value[FE]= 0.06
	HIV negative	9 (10)	80 (90)	
Harrison[3]	HIV positive	5 (42)	7 (58)	RR = 3.75 (1.1-13.2))[95% CI] P value [FE]= 0.08
	HIV negative	3 (11)	24 (89)	
Aird	HIV positive	5 (15)	28 (85)	RR = 0.69 (0.3-1.7))[95% CI] P value[X] = 0.4
	HIV negative	19 (22)	67 (78)	

Our data differs significantly from all other studies, both in numbers of patients and in outcome. This study has three times the number of patients of any of the previous studies, and is the only study not to show an increase in the risk of wound infection. Although all three studies are looking at the same outcome, the inclusion criteria vary significantly, and no study has included a marker of the severity of HIV. Although the work by Harrison et al has included CD4 counts, the small number of patients has prevented statistical analysis being performed. Paiement et al[5] were looking at any procedure that required surgical debridement, whereas Harrison included many patients who were undergoing repeat operations for previously infected non-union's. The grades of injuries have not been described in any of the previous studies. The findings from this study in patients with advanced HIV and those with grade 1 fractures show similarities to the findings of the previous studies. It is possible that the patients seen by departments in Malawi and San Francisco have had more advanced disease, less severe injuries or the patient management may have differed to that conducted in this study.

In summary, this study has shown that in patients who are HIV positive prior to sustaining an open fracture, the infection rate is higher in patients with a low CD4

count. The data suggests a strong effect, but only weak evidence that this observation is not due to chance alone. The data is consistent with previous studies and there is biologically plausible explanation for this finding.

It has also shown that patients who are HIV positive prior to sustaining a Grade 1 open fracture have an increase in risk of infection. This effect is more dramatic in advanced HIV infection. The data suggests a strong association. The findings are compatible with previous similar studies and are biologically plausible.

It is therefore likely that advanced HIV is a risk factor for wound infection, but early HIV does not appear to significantly affect infection rates.

4.3.1Non Union

Harrison et al are the only group to have looked at bony union in HIV positive patients with severe open tibia fractures treated with external fixation (with early removal of frame and then plaster). He noted an increase in the rate of non union in the HIV cohort. A comparison of his results and the results from this study are shown in Table 4-2

Table 4-2

		Non Union	Union	The risk of developing a wound infection given HIV infection.
Harrison[131]	HIV positive	3 (42)	4 (58)	Risk Ratio = 7.3 (0/92-58))[95% CI] P value [FE]= 0.059
	HIV negative	1 (6)	16 (94)	
Aird	HIV positive	5, (15)	28, (85)	Risk ratio = 4.1 (1.1-16.3)[95%CI] P value [x]= 0.04
	HIV negative	3, (4)	79, (96)	
Aird **Gd2 and 3 Tibial Fractures only**	HIV positive	2, (33)	4, (67)	Risk ratio = 3.7 (0.64-20)[95%CI] P value [x]= 0.19
	HIV negative	2, (9)	20 (91)	

Whilst the two studies have similar findings, the higher rate of non union in Harrison's study can be attributed to its selection criteria of only open tibia fractures.

When selecting only severe open tibial fractures treated with surgical stabilization from the data in presented in this thesis, the non-union rates are comparable. Harrison only managed his patients in external fixators until the soft tissues permitted the use of a plaster cast. This method may be sub-optimal for achieving bony union.

In summary, this study has shown that when HIV infection is present at the time of an open fracture there is a strong association with an increased risk of non union. The data has been unable to show a dose related response (in relation to advanced CD4 count). There are a variety of plausible biological mechanisms for how this might occur, but little previous data to compare with.

It is therefore likely that HIV infection is a risk factor for non union.

It is not possible to say whether this risk is increased or reduced in patients with advanced HIV.

4.3.2Pin Site Sepsis

Pin site sepsis has been previously studied by Norrish et al whose work suggested that there was a higher risk of severe pin site sepsis in externally fixed open fractures in HIV positive than in negative patients. Whilst the results for the HIV positive patients are very similar, the rate of pin site sepsis is much lower in the HIV negative patients from this study as summarized in Table 4-3.

Table 4-3

		Checketts score >=2 (%)		Mean lenth of Ex-Fix (weeks)	The risk of a pin site infection (>=2) given HIV infection
Norrish[6]	**HIV infection**	**Yes**	**No**		
	HIV positive	9/15 (60)	6/15 (40)	*8.3*	Risk Ratio = 3.6(1.6-7.95)$^{95\% CI}$ P value^{FE}=0.0023
	HIV negative	7/35 (20)	28/35 (8)	*7.5*	
Aird	**HIV Positive**	6/10 (60)	4/10 (40)	15	Risk Ratio = 0.9 (0.5-1.6)$^{95\% CI}$ *P value^{FE}= 1*
	HIV negative	14/21 (67)	7/21 (33)	16	

The difference in results seen between these two studies may be a result of the difference in recording technique as discussed in section 1.3.3.7 and a difference in the duration of use of the external fixation. The longer the external fixator is insitu for the more likely it is to develop a pin site infection. Doubling the length of time is likely to more than double the rate of infection as there are a variety of factors

predisposing to infection, including bacterial contamination, skin necrosis from dressings and a deterioration in the bone pin/wire interface. It is likely that this factor accounts for the difference seen in the HIV negative patients pin site infection rate. It is surprising therefore that the HIV positive patients pin site infection rate is so similar, when you would expect them to be much higher in our study as the frames had been on for twice as long. It is possible that the difference in recording technique meant our pin site infection was lower, as in patients with multiple pin site infections; we only scored the most severe, whereas Norrish et al used each pin site as a separate data entry point. If patients who were HIV positive were more likely to get multiple pin site infections this would account for the differences.

In summary this study has shown that when advanced HIV infection is present at the time of injury it is associated with a 2 fold increase in severe pin site infection. It has not demonstrated a dose dependant response. The data lacks power and the observation may be due to chance alone. There are a variety of plausible biological mechanisms for this observation, and the findings are compatible with the previous similar trial.

It is therefore not possible to infer a causal relationship between HIV or advanced HIV and pin site sepsis.

4.4 The Significance of the results

The data from this thesis suggest that early HIV is not a significant risk factor for wound infection, and therefore these patients should not have their treatment altered as a result of concerns over increased infection rates. It is possible that these patients have delayed/impaired bone healing and management strategies need provide the best possible environment for bone healing to occur.

Advanced HIV does appear to confer an increased risk of infection in this study population, and it is therefore be wise to treat these patients with some caution when considering internal fixation in a severe open fracture. If the skills and equipment are available, an appropriate external fixation device would appear to be a good alternative in these patients.

The data presented in this thesis suggests that grade 1 injuries in HIV positive patients would fare better with urgent debridement. It may be appropriate that in grade 1 open fractures, HIV positive and negative patients should be treated differently, with grade1 injuries in HIV positive patients being treated as a though they had a more severe grade of injury. This hypothesis needs to be tested further with a RCT.

Many hospitals are currently amending their departmental protocols following the BOA/BAPRAS[43] open fracture guidelines which suggest that open fractures do not require urgent debridement at night. It would be the author's suggestion that patients, who are immuno-compromised due to HIV or other systemic conditions, should be prioritised for urgent debridement as soon as the appropriate skills/personnel are available.

Recent data has also suggested that some open fractures may benefit from early thorough debridement followed by immediate primary wound closure[68]. The author would suggest that primary wound closure, whilst possibly appropriate in patients with early HIV, should be approached with caution in advanced HIV due to the increased rate of wound infection seen.

The length of use of prophylactic antibiotics remains controversial, but guidelines are now suggesting shorter courses. The data presented in this thesis are unable to guide practice on the use of prophylactic antibiotics in HIV positive patients. It is the author's opinion that until further evidence becomes available prophylactic antibiotics should be the same in HIV positive and negative patients

The data from this thesis would suggest that external fixators can be used safely in patients with HIV. Pin site infection may be a problem, but in most cases it settled with antibiotics. It is the author's view that whilst external fixation is a good option for the treatment of severe open fractures, the use of internal fixation should not be excluded.

4.4.1Future work

A meta-analysis of this thesis with previously published work by Harrison et al, might give greater statistical power to the results.

Further clinical studies are required to look at union rates in HIV positive patients with high risk fractures. These studies should attempt to quantify time to radiological

and clinical union, for different types of fractures in the HIV positive and negative patients. To detect a 10% difference in the rate of delayed union, a study would require 80 HIV positive patients and 240 HIV negative patients.

A clinical RCT looking at a direct comparison of externally fixed and internally fixed grade 2 and 3 tibia fractures would also be helpful in planning surgical treatment. A power calculation using 95% confidence and 80% power a non union rate 25-35% and a 10% difference in union rates between the methods would require 681 HIV negative patients and 182 HIV positive patients. Given that this is a relatively uncommon injury, this would require a multicentre trial.

Experimental studies looking at how the fracture environment is altered on both a cellular and molecular level by HIV status, CD4 level, and HAART medication, would help provide insight into how HIV effects wound and fracture healing

4.4.2Concluding remarks

In 1850 open fractures had a mortality of 33%, with a poor functional prognosis even in those who healed. In the1980's the HIV epidemic was spreading across the world and had an expected mortality of 100%. Huge advances have been made in these two devastating conditions. Understanding of how HIV interacts with fracture healing however is still in its infancy. This thesis provides some insight to guide both future research, and operative management in these complex and controversial conditions.

The author hopes that the evidence provided in this thesis may contribute to a significant shift in the management of open fractures in patients with HIV. This will hopefully lead to this already disadvantaged population getting more appropriate treatment in these severe injuries.

On the basis of the evidence presented, the management of open fractures in HIV positive patients should focus on obtaining fracture union, and should not be governed by the unproven fear of sepsis.

5 Appendix

5.1 Statistical analysis

Epi Info is available as freeware from www.cdc.gov/epiinfo

5.2 Diagnostics

Abbott Diagnostic OraQuick Rapid HIV-1/2 Diagnostic Test – This test is available to purchase from a variety of web sites, for institutional purchases please contact

Abbott Diagnostics
Abbott House
Vanwall Business Park
Vanwall Road
Maidenhead, Berkshire
SL6 4XF England
Telephone: 01628 784041
Fax: 01628 644205

EUROTUBO is manufactured by Deltalab, they can be contacted through their web site on http://www.deltalab.es/

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