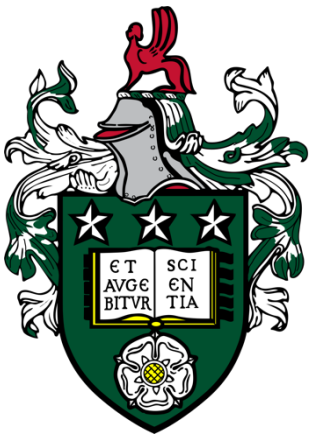
Subtrochanteric fracture non-unions: development of a risk scoring system for predicting non-unions and biological characterisation of the non-union tissue



Michalis Panteli

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

List of publications

1. **Bone Healing: The Diamond Concept (Chapter 2)**
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List of presentations

1. **Risk factors for progression to non-union following subtrochanteric femoral fractures**
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2. **Subtrochanteric femoral fractures: issues, challenges, solutions**
   Michalis Panteli
   Instructional lecture: 40th Cyprus Orthopaedic Conference, Nicosia, Cyprus

3. **Subtrochanteric fractures: does open reduction increase the risk of infection and fracture healing complications?**
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Abstract

The aim of this thesis was three-fold. Firstly, to investigate the characteristics of patients presenting with subtrochanteric fractures treated with intra-medullary fixation and describe their outcomes, with a special reference to the development of non-union; secondly, to identify risk factors predisposing to non-union and to develop a scoring system that could predict the development of this complication; thirdly, to analyse the biological and molecular profile of fracture non-union tissue.

Between January 2009 and December 2016 (eight years), 545 consecutive patients (561 fractures) were treated for proximal femoral fractures extending into the subtrochanteric area, fulfilling the inclusion criteria. Their management and outcomes were reported, with subgroup analysis identifying associations for developing common complications (including non-union; infection; effect of open reduction / osteoporosis / bisphosphonates; atypical fractures; type of nail used; transfusion requirements; presence of a 'weekend effect', medical complications and mortality).

Regression analysis identified several factors associated with an increased risk non-union (deep infection; self-dynamisation; presence of an atypical fracture; diabetes; and malreduction, as demonstrated by a lateral cortex fracture gap size and varus malalignment), with another factor (moderate comminution) being associated with a decreased risk. Based on these findings, a risk scoring system for predicting non-unions was developed. Additionally, open reduction of subtrochanteric fractures was not associated with an increased risk of deep infection and non-union. The use of cerclage wiring however was found to be associated with a decreased incidence of non-union without an increase in complications.

Regarding the laboratory part, following ethics committee approval, ten patients undergoing revision surgery as part of their treatment for atrophic non-union were recruited. Functional assays and gene analysis were performed on the isolated osteoprogenitor cells to elucidate their biological and molecular profile. Comparing the samples' gene expression at baseline, three genes were found to be over-expressed in non-union tissue mesenchymal stem cells (MSCs) (ICAM1, MMP10 and GLI1), whilst another four genes were under-expressed (EGF, IGF2, MMP8 and COL14A1). Comparing non-union versus bone MSCs following osteogenic stimulation (i.e. osteoblastic differentiation), only IGF2 and EGF were significantly under-expressed in non-union MSCs.

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List of Abbreviations

25(OH) Vitamin D	25-hydroxyvitamin D
AFF	Atypical Femoral Fracture
AKI	Acute Kidney Injury
ALP	ALkaline Phosphatase
AO	Arbeitsgemeinschaft für Osteosynthesefragen
AP	AnteroPosterior
ASA	American Society of Anaesthesiologists
ASBMR	American Society for Bone and Mineral Research
ATF4	Activating Transcription Factor 4
ATLS	Advanced Trauma Life Support
BM	Bone Marrow
BMD	Bone Mineral Density
BMP	Bone Morphogenic Protein
BMSC	Bone Marrow Stromal Cells
BMU	Basic Multicellular Unit
BOAST	British Orthopaedic Association Standards for Trauma
^{o}C	degrees Celsius
CAP	Community Acquired Pneumonia
CCS	Charlson Comorbidity Score
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
CFU-F	Colony Forming Units – Fibroblast
CI	Confidence Interval
CKD	Chronic Kidney Disease
CRP	C-Reactive Protein
CT	Computed Tomography
CVA	CerebroVascular Accident
DBM	Demineralised Bone Matrix
DCN	Decorin
DEXA	Dual-Energy X-ray Absorptiometry
DHS	Dynamic Hip Screw
Dkk-1	Dickkopf 1
DM	Diabetes Mellitus
DMEM	Dulbecco's Modified Eagle Medium

DMSO	DiMethylSulfOxide
DNA	DeoxyRibonucleic Acid
DOB	Date Of Birth
DVT	Deep Venous Thrombosis
EDTA	EthyleneDiamineTetraacetic Acid
e.g.	exempli gratia
eGFR	estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked ImmunoSorbent Assay
ESWT	Extracorporeal Shock Wave Therapy
etc.	et cetera
FBS	Foetal Bovine Serum
FDA	Food and Drug Administration
FFP	Fresh Frozen Plasma
FGF	Fibroblast Growth Factor
FISH	Fluorescence in-situ Hybridisation
FWB	Full Weight Bearing
GDF	Growth Differentiation Factor
GF	Growth Factor
GH	Growth Hormone
GMP	Good Manufacturing Practice
h	hour
HAP	Hospital Acquired Pneumonia
Hb	Haemoglobin
HDU	High Dependency Unit
HRT	Hormone Replacement Therapy
ICU	Intensive Care Unit
IDDM	Insulin Dependent Diabetes Mellitus
IGF	Insulin-like Growth Factor
IL	InterLeukin
IM	Intra-Medullary
ISS	Injury Severity Score
LAT	Lateral
LFA-1	Lymphocyte Function-associated Antigen - 1
LIPUS	Low Intensity Pulsed UltraSound
LOS	Length of Stay
LTH	Leeds Teaching Hospitals
LWMH	Low Weight Molecular Heparin

M-CSF	Macrophage Colony-Stimulating Factor
MI	Myocardial Infarction
MMP	Matrix MetalloProteinase
MNC	MonoNuclear Cell
mRNA	messenger RiboNucleic Acid
mRUS	modified Radiographic Union Score
MSC	Mesenchymal Stem Cell
NFATc1	Nuclear Factor of Activated T-cells, cytoplasmic 1
NHFD	National Hip Fracture Database
NICE	National Institute for Health and Care Excellence
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NRES	National Research Ethics Service
NSAIDs	Non-Steroidal Anti-Inflammatories
NUSC	Non-union Stromal Cells
NWB	Non Weight Bearing
OCT	Optimal Cutting Temperature compound
OPG	OsteoProteGerin
OR	Odds Ratio
ORIF	Open Reduction and Internal Fixation
P	Passage
PACS	Picture Archiving and Communication System
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PDGF	Platelet Derived Growth Factor
PE	Pulmonary Embolism
PEFS	Pulsed Electromagnetic Field Stimulation
PRF	Platelet Rich Fibrin
PRP	Platelet Rich Plasma
PSI	PolySpermine Imidazole-4,5-imine
PTH	ParaThyroid Hormone
Pts	Patients
PWB	Partial Weight Bearing
®	Registered trademark
RANK	Receptor Activator of Nuclear factor κB
RANKL	Receptor Activator of Nuclear factor κB Ligand
RBC	Red Blood Cells
RCT	Randomised Control Trial

RIA	Reamer-Irrigator-Aspirator
RNA	RiboNucleic Acid
ROC	Receiver-Operator characteristic
rpm	revolutions per minute
RTC	Road Traffic Collision
RT-PCR	Reverse transcription Polymerase Chain Reaction
SD	Standard Deviation
SERM	Selective Estrogen Receptor Modulators
SNP	Single-Nucleotide Polymorphism
SOP	Standard Operating Procedure
SSI	Surgical Site Infection
T4	Thyroxine
TACR-1	TAChykinin Receptor-1
TAD	Tip-Apex Distance
TGF	Transforming Growth Factor
THA	Total Hip Arthroplasty
TNF	Tumour necrosis factor
TRAP	Tartrate-Resistant Acid Phosphatase
TSH	Thyroid Stimulating Hormone
TTWB	Toe Touch Weight Bearing
Tx	Transfusion
UTI	Urinary Tract Infection
VEGF	Vascular Endothelial Growth Factor
Vs	Versus
VTE	Venous ThromboEmbolism
WCC	White Cell Count
WHO	World Health Organization
XTT	2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide
y.o.	years old

Chapter 1
General Introduction

1.1 Trauma

Trauma remains a major health problem worldwide; according to WHO, unintentional trauma is the sixth commonest cause of death (1). Fractures, a potential sequelae of trauma, are very common and represent a major social and financial burden in every healthcare system. The age distribution of fractures in the male population is bimodal, in contrast to the female population where the age distribution is unimodal with a significant increase in the incidence of fractures post menopause (2, 3).

With a rapidly growing aging population, the incidence of traumatic injury, especially from low energy injuries, is expected to increase (4). Not only this, but these individuals have healthier lifestyles and are more active (5). It has also been reported that the severity of comorbidities of patients sustaining traumatic injuries, is associated with a higher hospital mortality rate; in the same group of patients, mortality is reported to be associated with less severe injuries (ISS<25 and particularly ISS<16) (6). Polytrauma from simple falls is now a common occurrence in Level 1 Trauma Centres, and their outcomes are far worse from the younger population (7). Additionally, mental impairment which is more common in the elderly, not only contributes to the increased risk of trauma, but also complicates the recovery and inadvertently affects the outcomes of the these patients (8).

1.2 Hip fractures

Hip fractures are those occurring in the region of the proximal femur and can be the result of high energy trauma or the consequence of deprived bone quality, so-called ‘fragility fractures’. These are defined as fractures that result from low energy trauma, such as falling from a standing height (9, 10). Fragility fractures occur on the background of osteoporosis, a pathological metabolic disease characterised by low bone mass (11). In the UK alone, it has been previously reported that as many as 70,000 patients a year suffer from a hip fracture (12). Overall, these injuries often lead to devastating consequences for the patients, their families and the society

thereafter, posing a huge burden to every healthcare system (over £2.2 billion per year in the UK) (12). The importance of their optimal management has been recognised by a number of national and international organisations, who have published guidelines and recommendations to improve their standards of care (12-14).

Most importantly, hip fractures in the elderly are associated with high mortality (approximately 10% at 30 days and 30% at one year post injury) and morbidity (15-17). The overall mortality has been linked to an increasing age, male gender, increased number of co-morbidities, mental impairment on admission, low haemoglobin (Hb) concentration on admission, living in an institution and presence of malignant disease (18, 19). Those patients surviving such an injury are often left with a significant disability and reduced quality of life, with only 10% of the patients being able to return to their previous level of mobility within four months post-injury (20-22). When compared to other types of fragility fractures, hip fractures account for the greatest loss of quality-adjusted life years (23).

1.2.1 Types of hip fractures

According to their anatomical features, hip fractures are divided into intracapsular (the region from the femoral head to the attachment of the hip joint capsule at the base of the femoral neck) and extracapsular fractures (occurring distal to the joint capsule) (**Figure 1.1**) (24, 25). Extracapsular fractures are further subdivided into pertrochanteric (intertrochanteric) fractures involving the region extending from the extracapsular basilar neck region distally to the lesser trochanter before the development of the medullary canal, and subtrochanteric fractures that occur in the region extending from the lesser trochanter to 5 cm (centimetre) distally in the femoral diaphysis (26, 27).

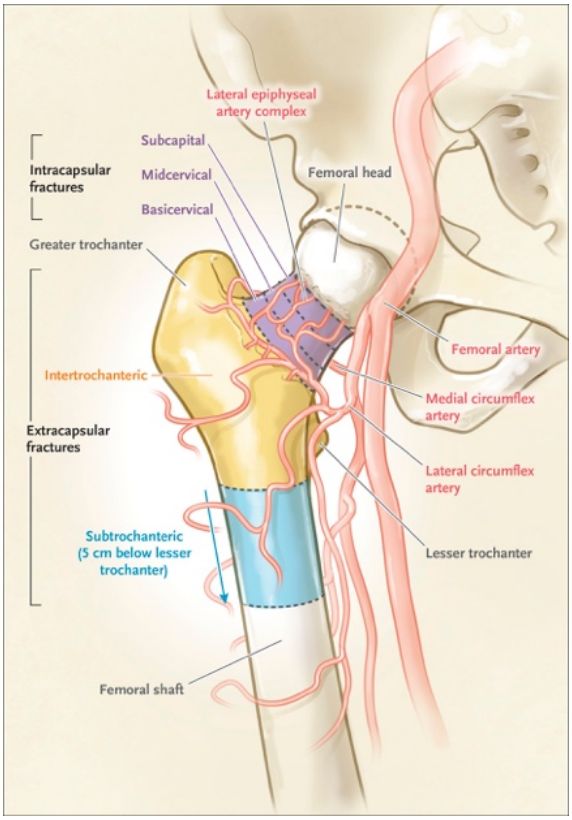


Figure 1.1 Classification of proximal femoral fractures

A. Intracapsular fractures: located in the region from the femoral head to the attachment of the hip joint capsule at the base of the femoral neck
   Further subdivided to:
   - Femoral head fractures
   - Femoral neck fractures

B. Extracapsular fractures: located distal to the joint capsule
   Further subdivided to:
   - Intertrochanteric fractures
   - Subtrochanteric fractures

   Figure adapted from Management of Acute Hip Fracture (28)

1.3 Osteoporosis

Osteoporosis is the most common bone metabolic disease and is characterised by low bone mineral density (BMD) and micro-architectural deterioration of bone tissue; this pathological process can lead to bone fragility and a consequent increase in fracture risk (29). The most common manifestation of osteoporosis are vertebral wedge fractures, distal radius fractures and hip fractures (30). The prevalence of osteoporosis is strongly associated with the advancement of age in both genders, but it is more prevalent in post-menopausal women (2% of 50-year-old women, compared to 25% in women over the age of 80 years; 16% in 80-year-old men) (13,

31-33). Furthermore, the risk of osteoporosis increases in patients suffering from systemic diseases (such as thyrotoxicosis, hyperparathyroidism etc.), patients receiving drug therapy (such as corticosteroids, thyroxine etc.), excessive alcohol intake (> 3 units / day), smokers, low body weight (BMI < 19 kg / m^2) and genetic factors (13, 30, 34). There is also evidence that mechanical loading of the bone increases BMD, in contrast to immobility that leads to bone loss (35).

The diagnosis of osteoporosis relies on the calculation of BMD at the spine and hip, using dual-energy X-ray absorptiometry (DEXA). Osteoporosis has been defined as a BMD with a T-score of -2.5 standard deviations or less, compared to the average seen in young healthy bone (29, 36), or in the presence of a fragility fracture (30, 37). T-scores between –1.0 and –2.5 are classified as osteopenia, whilst T-scores between –1.0 and +2.5 reflect to normal bone density. On the other hand, T-scores above +2.5 suggest high bone density (29, 36).

1.3.1 Pathophysiology of bone modelling and remodelling

Bone modelling describes the process by which bones are shaped or reshaped by the action of osteoblasts and osteoclasts, which act independently. It is responsible for skeletal development and growth, as well as the shaping of bones and their movement through space (38). Remodelling on the other hand is the process in which the osteoblasts and osteoclasts are coupled, working sequentially in the same bone remodelling unit (38). It has been estimated that remodelling proceeds at about 10% of the adult skeleton per year (39, 40). These two processes are balanced after the peak bone mass is reached, resulting to a ‘stable’ bone mass for one or two decades; following this, the onset of age-related bone loss becomes evident (38). This complex but well-co-ordinated process is generally controlled by cellular and molecular events, generating a number of signalling pathways and control mechanisms. The main cell populations involved are osteoclasts, osteoblasts and osteocytes, but immune cells also play a very important role (39).

Subsequent to appropriate signalling, bone remodelling commences with the attraction of osteoclast precursors to the remodelling site, which then differentiate to clusters of bone-resorbing osteoclasts (40). These, along with bone-forming osteoblasts, become arranged within temporary anatomical structures known as basic multicellular units (BMUs) (39, 41). The resulting active BMU consists of a leading front of bone-resorbing osteoclasts, reversal cells which are responsible for covering and preparing the newly exposed bone, whereas the tail portion of the BMU

is covered by osteoblasts which then secrete and deposit unmineralised bone matrix (osteoid) and direct its formation and mineralisation into mature lamellar bone (39, 41, 42). BMUs function can be divided to five distinct and sequential phases: activation, resorption, reversal, formation, and termination (39, 41).

The exact mechanisms involved in both bone modelling and remodelling are poorly understood and the majority of our current understanding is based on animal models. It is well accepted however that any uncoupling or imbalance between bone formation and destruction leads to metabolic bone disease, such as osteoporosis (43, 44).

1.3.2 Prevention / management of osteoporosis

The optimal management of osteoporosis and thus the prevention of fragility fractures relies on a combination of lifestyle modifications (diet, exercising, reducing alcohol intake and smoking cessation), treating and reversing of the secondary causes of osteoporosis, evaluating and addressing falls risk, and finally commencing medication targeting different areas of the osteoporosis pathway. Therefore, several organisations and other committees have issued guidelines for both the prevention and management of osteoporosis and its sequelae (13, 30).

1.3.3 Drug therapies for osteoporosis

Several drug treatments are available for the management of osteoporosis. According to their mechanism of action, they can broadly divided into: antiresorptive agents (those that suppress bone resorption); anabolic agents (those that increase bone turnover, stimulating bone formation more than bone resorption); and vitamins and minerals (normally available through a balanced diet).

1.3.3.1 Antiresorptive agents

With the signalling pathways regulating bone turnover becoming better understood (45), several agents targeting bone resorption, mainly by inhibiting the osteoclastic activity, have been developed and used in clinical practice. Bisphosphonates are the commonest agents used and represent the first line of treatment for osteoporosis, whilst hormone replacement therapy (HRT) and calcitonin are also commonly used.

- Bisphosphonates

Bisphosphonates are inorganic, stable analogues of pyrophosphate, a naturally occurring inhibitor of mineralisation, that have a high affinity to bind to hydroxyapatite crystals found in bone mineral (46). Their mechanism of action is by strongly binding to calcium ions and becoming incorporated within hydroxyapatite, with their concentration being higher at sites of increased bone remodelling. Following osteoclastic resorption of the bone containing the bisphosphonate, a high concentration of the drug is released within the cell, disrupting key enzymes causing inhibition of bone resorption, inhibiting recruitment of osteoclasts, promoting osteoclast apoptosis as well as indirectly stimulating osteoblastic activity (47-49).

The anti-resorptive properties of bisphosphonates were first demonstrated in animal models in the 1960s (50, 51); the first generation bisphosphonate etidronate was then developed and was first used to treat conditions such as Myositis Ossificans Progressiva (52) and Paget's disease (53-55). It was later discovered that the potency with which bisphosphonates inhibit bone resorption could be increased with the incorporation of nitrogen, leading to the introduction of the second generation of bisphosphonates in the 1980s (46, 56). Since then, a number of studies investigating the effects of bisphosphonates have demonstrated that their regular administration significantly reduces the rates of fragility fractures (57, 58). More specifically, alendronate has been suggested to reduce vertebral fractures by 50%, nonvertebral fractures by about 17% and post-menopausal hip fracture by up to 40% (58-60). Other studies have also reported similar beneficial effects of risedronate (61-63), ibandronate (64), etidronate (65), and zoledronic acid (66, 67).

Regardless of the positive outcomes of bisphosphonates use, an expanding body of evidence has linked their use with distinct adverse events, such as oesophagitis, oesophageal cancer (68), osteonecrosis of the jaw (69, 70) and importantly atypical femoral fractures (AFFs) (70, 71).

- Calcitonin

Calcitonin is a hormone produced by the thyroid gland that binds to osteoclasts, inhibiting bone resorption (72). Higher potency salmon calcitonin has been used in the treatment of osteoporosis (73-75), but it has not been shown to reduce non-vertebral fragility fractures, relegating it to an adjunct role (57).

- Denosumab

Denosumab on the other hand, a monoclonal antibody that inhibits osteoclast formation and function (43, 76), has been shown to significantly decrease the risk of fractures (up to 40% decrease in hip fractures) (77). Though it is a promising new drug, its long-term effects and safety are yet to be established.

1.3.3.2 Hormone Replacement Therapy

Post-menopausal decrease with oestrogen levels decline has been strongly associated with osteoporosis, as oestrogens suppress pro-osteoclast cytokines and promote osteoclast apoptosis (43, 44). HRT and selective estrogen receptor modulators (SERMs) have therefore being used in the treatment of post-menopausal osteoporosis, even though they are generally prescribed to women with premature menopause due to reported risks associated with their use, such development of breast cancer, cardiovascular disease and risk of thromboembolism (78-80).

1.3.3.3 Anabolic agents

Anabolic agents have recently been introduced in the treatment of osteoporosis. Their anabolic activity is based on the stimulation of new bone formation on the quiescent bone surface, which is not remodelled at the same time (81). They result in an increase of BMD being able eventually to restore it back to normal, therefore reducing the risk of osteoporotic fractures (81). The most commonly used anabolic agent is teriparatide, whist other newer agents have been recently introduced into clinical practice.

Teriparatide, a synthetic analogue of parathyroid hormone (PTH), acts mainly on the periosteal surface of bone, also causing endosteal resorption thus increasing the diameter of the bone (34). It has an anabolic effect on bone formation (82) and has been reported to reduce the risk of fragility fractures both by improving the microarchitecture of bone and by increasing BMD (83). Treatment duration should last 24 months and not be repeated over a patient's lifetime because of the risk of osteosarcoma (34). Nevertheless, its use is still limited because of the prohibitive costs and lack of strong evidence to support its effectiveness.

1.3.3.4 Vitamins and minerals

A balanced diet with adequate protein and energy levels is advisable for the prevention of osteoporosis. Additionally, adequate amounts of minerals and especially calcium is crucial for optimal bone health (84, 85). The current recommended dose of daily calcium intake in the UK is 700 mg, compared to 1,000 mg in pre-menopausal and 1,500 mg in post-menopausal advised in the United States. Vitamin D on the other hand, which is vital for the optimal absorption of calcium, has a limited availability in foods, whereas the amount of vitamin D synthesised by the skin can be very limited especially in northern latitudes. It has been therefore suggested in the UK that the recommended daily intake of 400 IU vitamin D is adequate to maintain a serum 25(OH) Vitamin D (25-hydroxyvitamin D) level above 50 nmol/L (86). In the United States, a daily recommended dose of 600 IU in adults up to the age of 70 years and 800 IU above the age of 70 years is advised (87).

Chapter 2
Bone Healing

2.1 Introduction

Bone healing is a complex but well-orchestrated physiological process which recapitulates aspects of the embryonic skeletal development in combination with the normal response to acute tissue injury (88, 89). It encompasses multiple biological phenomena and is margined by the combination of osteoconduction (extracellular matrix formation); osteoinduction (timed cellular recruitment, proliferation and differentiation of mesenchymal stem cells (MSCs) into osteoblasts and chondroblasts, controlled by multiple signalling molecules); and osteogenesis (new bone formation) (89-93). In contrast to scar formation, which occurs in the majority of other tissue types in adults, bone has the innate capability to repair and regenerate, regaining its former biomechanical and biochemical properties (94-96).

2.2 Bone healing process

Bone has a high capacity for regeneration and healing, through a process of biologically distinct, but overlapping phases: that of inflammation, granulation tissue formation, soft callus formation (hyaline cartilage), hard callus formation (woven bone), and remodelling (26, 94, 97-100). Following an injury the bone architecture is disrupted, as is the surrounding soft tissue continuity. Consequently, the local blood vessels are disrupted, a haematoma is formed and the coagulation cascade is activated (100). This fracture haematoma contains cells that originate from the peripheral and intra-medullary (IM) blood circulation, as well as from the bone marrow (BM) (101). Inflammatory immune cells, neutrophils, monocytes and macrophages are activated by the coagulation process, whilst fibroblasts and MSCs are also recruited to the site of injury (94, 100). Prostaglandins, cytokines and other proteins are also abundant in this environment (94). These mediators are known to increase cellular migration, proliferation, enhance osteogenesis, collagen synthesis and angiogenesis (94).

Subsequently, the necrotic or damaged bone is removed and the fracture haematoma is gradually replaced by a fibrin-rich granulation tissue (102). The osteoprogenitor cells then proliferate and differentiate, leading to deposition of collagen and formation of soft callus (102). An increased vascularity and intense cell

proliferation in the cambium layer of the periosteum is evident in this stage (102, 103). Bone formation then occurs by endochondral or intramembranous ossification (hard callus). Initially, immature woven bone characterised by coarse collagen fibres arranged in a haphazard fashion is formed, but is then transformed to mature lamellar bone (remodelling) in a slow process (102, 103). During remodelling that could last several months to years after fracture, both osteoblast and osteoclast activity is intense, with bone resorption followed by appositional production of new bone by osteoblasts (102).

2.3 Types of bone healing

During the bone-healing process, a well-regulated series of overlapping processes take place in the cortical bone, the periosteum, the bone marrow and the undifferentiated fascial tissue surrounding the fracture (103, 104). According to the histological appearance, two basic types of bone healing have been identified (26, 94, 95). The primary (direct) healing pattern occurs when anatomical reduction in achieved, along with almost absolute stability (90, 101). The disrupted continuity of the bone in this type of healing is re-established with regeneration of the Harvesian system and the lamellar bone, with therefore no need of any remodelling (101, 104). On the contrary, the secondary (indirect) healing pattern that occurs in the vast majority of clinical cases, depends on the formation of fibrocartilaginous callus that matures to mineralised cartilage and finally bone (90, 94).

2.4 Biologic prerequisites for successful union

As mentioned, the bone-healing process is very complex, with multiple biological phenomena taking place at the same time. Generally, the components involved in bone healing can be broken down into cellular and molecular elements, even though the mechanisms behind their actions and interactions are not well understood.

2.4.1 Cellular elements

Inflammatory cells

In the initial phase following a fracture, several inflammatory cells migrate to, proliferate and differentiate at the site on injury. These include monocytes, granulocytes, lymphocytes, neutrophils, macrophages and haemopoietic stem cells (94, 100, 105, 106). This is followed by the activation of T- and B- cell subpopulations,

leading to the production of several signalling molecules that play a significant role in fracture healing (105, 106).

Progenitor cells

Progenitor cells are those arising mainly from the periosteum, endosteum and peripheral blood, and which are involved in the formation of the fracture callus. These cells respond to the fracture by upregulating specific genes promoting proliferation and releasing a number of cytokines and chemokines promoting chondrogenic and osteogenic differentiation (99, 107). Regarding the progenitor cells arising from the periosteum, these originate from the innermost layer, and can rapidly proliferate as early as 24-48 h (hours) post-injury (99). Additionally, osteoblasts and quiescent osteoblast-lineage cells located in the endosteum can be activated and contribute to the healing process (108).

Chondrocytes

Chondrocytes, the cells that produce and maintain the cartilaginous matrix, play a very important role in bone healing. During the soft callus phase, they play a crucial role in endochondral ossification and also supply the extracellular matrix with many components (108).

Osteoblasts

Osteoblasts derive from MSCs and function in groups of cells called osteons, which are responsible for synthesising bone. They originate from the periosteum, endosteum and progenitor cells. Osteoblasts synthesise dense, crosslinked type I collagen and specialised proteins, such as osteocalcin, bone sialoprotein, osteopontin and other proteins, which compose the organic matrix of the bone (108).

Osteoclasts

Osteoclasts are the primary cells responsible for bone resorption and their role is especially important during bone remodelling, as well as primary bone healing through the formation of ‘cutting cones’ (109). They derive from the haematopoietic cell linage, and their function is partially regulated by osteoblasts through the expression of RANKL (receptor activator of nuclear factor κB ligand) (activation) and osteoprotegrin (inhibition) (110).

2.4.2 Molecular elements

Several signalling pathways are involved in the complex coordination and regulation of bone healing and can be found within the fracture haematoma. These are categorised into three groups: the pro-inflammatory cytokines; the TGF-β superfamily (transforming growth factor-β) and other growth factors (GF); and the angiogenic factors (95, 108).

In more detail, the major signalling molecules include: TGF-β that stimulates the undifferentiated MSCs (100, 103); bone morphogenic proteins (BMPs) that promote the differentiation of MSCs into chondrocytes and osteoblasts, and osteoprogenitor cells into osteoblasts (100, 103, 104); fibroblast growth factor (FGF) that enhances mitogenesis for MSCs, chondrocytes and osteoblasts (100, 103); insulin-like growth factor (IGF) that promotes proliferation and differentiation of osteoprogenitor cells (100, 103); platelet derived growth factor (PDGF) that is mitogenic for MSCs; and osteoblasts and responsible for macrophage chemotaxis (100, 103). Vascular endothelial growth factor (VEGF) is responsible for the blood vessel invasion of hyaline cartilage, growth plate morphogenesis, and cartilage remodelling, by regulating recruitment, survival and activity of endothelial cells, osteoblasts and osteoclasts (100). An increase to the secretion of factors promoting the recruitment of inflammation cells and angiogenesis is also evident (TNF-α, IL-1, IL-6, IL-11 and IL-18) (101, 103).

Many of these molecules have been extensively studied to evaluate their clinical effectiveness in enhancing fracture healing. BMPs represent the sole clinically approved agent for applications related to fracture repair (BMP-2 has gained FDA (Food and Drug Administration) approval for the treatment of open tibial fractures and spinal fusion surgery) (89). The clinical data on their safety and efficacy appears to be positive (111-114), whereas their application for off-label indications is also promising (111-120). Nevertheless, several studies report that large amounts of BMPs are required to achieve their clinical benefits, whilst their long term effect is questionable because of their rapid clearance locally and their short half-life (121, 122). Additionally, BMPs antagonist, Noggin is induced in their presence, having a further negative impact on their action (123, 124).

PDGF also acquires promising results in the enhancement of fracture healing when used in animal studies (125, 126). Other GF that are currently under investigation

include GDF-5 (growth differentiation factor – 5) (127), IGF-1 (128, 129), GH (growth hormone) (130) and PRP (platelet rich plasma) (131-133).

2.4.3 Mechanical environment

The process of inflammation and angiogenesis depend largely upon the mechanical conditions (94) and should therefore be taken under consideration for optimising fracture healing. Mechanical stability is essential for the formation of callus and its progressive maturation from woven to lamellar bone (90), in contrast to the case of rigid fixation where no callus is produced (primary bone healing). According to Wollf's law, both the distribution and mass of bone tissue are determined by the forces acting to that area (134). Kenwright et al. also suggested that in an experimental animal model, healing was affected by the frame stiffness and the characteristics of a short duration mechanical stimulus to the construct (134).

The degree of mechanical stability at the fracture site is determined by the selected type of fixation and can be achieved using open reduction and internal fixation (ORIF), locking plating systems, IM and external fixation systems (135). Thereafter, any surgical intervention (external or internal fixation systems) that improves fracture stability enhances healing, even though too rigid fixation can inhibit fracture healing (92).

2.4.4 Vascularity

Blood supply and revascularisation are essential elements for a successful fracture healing, including the final stage of remodelling (101). The process of revascularisation involves not only neo-angiogenesis, but also the apoptosis of chondrocyte cells, the cartilaginous degradation and the removal of cells and extracellular matrices for blood vessel in-growth (101). During uncomplicated bone repair, the medullary, periosteal and osseous blood supply can be enhanced according to the physiological needs through the regulation of GF and cytokines (100).

Two molecular pathways mainly regulate the vascularisation process: the angiopoietin-dependent pathway; and the VEGF-dependent pathway, which is considered the key regulator of vascular regeneration (101, 136). VEGF is an osteogenic, pro-resorptive, oxygen-sensitive, signalling molecule that can regulate osteoblasts, osteoclasts and osteocytes function (137). Evidence of the importance

of this molecule has been reported with the inhibition of VEGF activity, by neutralising VEGF receptor (138). On the contrary, exogenous administration of VEGF enhanced blood vessel formation, ossification, and new bone (callus) maturation (138). Evidence is now emerging that VEGF can be used to promote angiogenesis and osteogenesis, therefore improving bone repair (139-141).

2.5 Mesenchymal stem cells

The key cells responsible for fracture healing are the osteoprogenitor cells, the so-called MSCs. MSCs in the human body are viewed as reservoirs of reparative cells having tissue specific characteristics. They are ready, under different signals, to migrate and differentiate into cells of connective tissue lineages. Such signals include damage in the tissues like trauma, fracture, inflammation, necrosis and tumours (142). The fate and chemotaxis of MSCs can be influenced by interactions with the extracellular matrix through transmembrane proteins like integrins (143, 144). MSCs subsequently differentiate under the influence of the local microenvironmental cues towards the local dominant mesenchymal lineage cell population (94, 143, 145). An example includes the ability of MSCs to migrate and colonise the injured site after intravenous injection (146, 147). Myocardial infarction, fracture, ischemic cerebral disease and spinal cord injury are conditions where these beneficial properties are demonstrated in animal models (146-152). Similarly, in some studies suspended MSCs injected intra-articularly into the knee joint following injury appeared to engraft and regenerate damaged meniscus and cartilage (153). These issues however are still poorly understood, as MSCs' basic science research, in relation to fracture repair, is largely carried out in animal models or by using culture expanded human MSCs in animal systems which may differ significantly from MSCs resident *in vivo*.

For the characterisation of the MSCs, the International Society of Cellular Therapy suggested that these should meet the following criteria: the cells should be adherent to plastic in standard culture conditions; they must express CD105, CD73 and CD90, whilst being negative for CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules; and they must be able to demonstrate *in vitro* differentiation to osteoblasts, adipocytes and chondroblasts (154).

2.6 Non-union

The physiological sequence of fracture healing depends on numerous endogenous and exogenous factors (155, 156). If this sensitive balance is altered in any way, complications such as delayed union or non-union may arise. The criteria for defining a non-union are not yet standardised (157). FDA defines a non-union as the incomplete fracture healing within nine months following injury, along with absence of progressive signs of healing on serial radiographs over the course of three consecutive months (158). In the United States alone it is estimated that 5% to 10% of all fractures are complicated by non-union or delayed union (159), posing an enormous economic burden to every healthcare system (160, 161). The tibia and the femur are reported to be the most common long bones associated with the development of non-union (162, 163). Moreover, in a large epidemiological study by Simpson et al., the relative incidence of non-union was 20 per 100,000 population, peaking in the fourth decade of life (164), whilst in another study by the same group the overall risk of non-union per fracture was reported as low as 1.9%, but this was as high as 9% for some fractures / age groups (165).

According to the radiologic and histologic appearance, non-unions are characterised as: hypertrophic, usually resulting from insufficient fracture stabilisation (extensive callus formation) (166); and atrophic, where the fracture stabilisation is adequate but there is localised dysfunction in biological activity (little callus formation and presence of a fibrous tissue-filled fracture gap) (166, 167). Ilizarov on the other hand, classified non-unions to stiff (hypertrophic non-unions with presence of fibrous tissue) and mobile (atrophic non-unions with very little fibrous tissue present) (168). Synovial pseudarthrosis is considered as a different pathological entity, caused by inadequate immobilisation with or without the presence of infection (169). Moreover, non-unions can be characterised according to the presence of bacteria at the fracture site, as septic or aseptic non-unions (170).

2.7 Risk factors for impaired bone healing

It is generally accepted that the progression to a non-union in most cases represents a multifactorial process. Various risk factors have been implicated with compromised fracture healing, generally divided in two main categories: patient dependent and patient independent factors. Nonetheless, in the majority of the cases no obvious cause can be found, while an important factor that is often underestimated is the

body's ability to heal a fracture. At a cellular level, this can be sub-classified as defective MSC proliferation or inability of the MSCs to undergo differentiation towards bone or cartilage lineage (171). Between these two factors, proliferation is deemed to be an absolute essential stage as differentiation can be induced from the local microenvironment (172).

2.7.1 Patient dependent factors

A number of patient dependent factors have been identified by the literature to be associated with a compromised fracture healing. These include age at the time of the injury, gender, presence of medical comorbidities (i.e. anaemia, diabetes, hormone disorders), smoking, administration of pharmacological agents (i.e. steroids, NSAIDs etc.) and poor bone stock (155, 158, 173-176).

2.7.2 Patient independent factors

Patient independent factors generally include the factors which are not related to or cannot be altered by the patient. These include amongst others the 'personality' of the fracture, the presence of infection and the adequacy of surgical technique (i.e. adequacy of reduction, residual gap in the medial surface of the femur in the region of the lesser trochanter, need for open reduction, varus malalignment (defined as angulation of more than 10° at the fracture site in the femoral shaft), the tip-apex distance (TAD) distance, and the entry point to the femoral canal) (26, 155, 158, 173, 177-183).

2.8 Biological enhancement of fracture healing

Even though the regenerative capabilities of bone are truly remarkable, when the balance of the bone-healing process is disturbed, it is not uncommon for the fracture to progress to a non-union (26). In those cases, local and / or systemic biological enhancement, along with optimisation of the mechanical stability and local vascularity, can be utilised to increase the chances of a successful outcome.

2.8.1 Bone grafts and bone graft substitutes

During the natural process of indirect fracture healing, a fibrin-rich granulation tissue derives from the fracture haematoma (101). This extra-cellular matrix provides a natural scaffold (osteoconductive properties) where all the cellular events and

interactions take place, including cell adhesion, migration, proliferation and differentiation (89, 90, 135). In the clinical setting, the ideal material to be used should mimic the native characteristics of the tissue, provide a source of cells capable of promoting proliferation and differentiation, as well as act as a scaffold for angiogenesis, cell migration and attachment (184). Various materials simulating some of the properties of this extra-cellular matrix have been clinically used. According to their origin, these can be: autologous bone graft (autograft); allograft; and bone graft substitutes (185).

Autologous bone graft (cortical or cancellous) harvested from the iliac crest remains the 'gold standard' for bone augmentation in non-unions, retaining all the required properties of osteoinduction, osteoconduction and osteogenesis (26, 186). The reamer-irrigator-aspirator (RIA) technique has also been used for obtaining autologous bone graft but avoiding some of the complications related to the iliac crest harvesting, whilst the acquired volume can be significantly higher (187). Limitations of the use of autologous bone graft however include its limited availability, potential donor site complications, and in the case of cancellous bone, the lack of structural integrity (185, 188).

Allograft is the traditionally freeze-dried or irradiated cancellous or cortical bone, or the specially prepared DBM (demineralised bone matrix; the inorganic portion is removed, with some of the proteins and GF being retained), originating from cadaveric tissue. Its advantages include its near unlimited availability and osteoconductive properties (and in the case of DBM, some osteoinductive properties), but its use is limited by its lack of osteoinductive and osteogenic properties, and the potential of disease transmission (185). When used in combination with autograft however, its properties can be enhanced and the outcomes improved (92).

Bone graft substitutes on the other hand are synthetic materials which generally act as a scaffold and lack osteogenic or osteoinductive properties. These include amongst others hydroxyapatite, polylactic or polyglycolic acid, bioactive glasses and calcium-based ceramics, and can be used as bone-void fillers (90, 189). In the recent years, their combination with antibiotics has made them an attractive option, especially on the background of infection. Additionally, modern scaffolds recently introduced involve osteoconductive synthetic metallic materials (Porous Tantalum,

Trabecular Titanium etc.), offering a three dimensional reticular frame where osteoblasts and osteoclasts proliferate producing bone (189-191).

2.8.2 Cell-based therapies

With the ever increasing use of allografts and bone graft substitutes throughout the last few years, the fundamental importance of osteoinduction and osteogenesis was soon recognised. Therefore, the isolation, enhancement and local delivery of the desired cell populations became an attractive therapeutic approach. These 'cell therapies' can range from simple autologous transplantations of cells (minimally manipulated), to *in vitro* expanding of selected cell populations (especially MSCs) and tissue engineering (185).

BM aspirates or concentrates on the other hand, contain the essential MSCs, but also a number of other cells (stromal cell populations, haematopoietic stem cells, erythrocytes, leucocytes, platelets and plasma), as well as several GFs which reportedly enhance healing (192, 193). Even though there are no randomised control trials (RCTs) assessing their efficacy, several case series support their clinical effectiveness, especially following further concentration (192-196).

2.8.3 Platelet concentrates therapies

Additionally, platelet concentrates (PRP and PRF), are a relatively simple and cost-effective therapeutic option in impaired fracture healing (185). The pro-inflammatory cytokines (TGF-β, PDGF, VEGF, FGF and IGF) originating from monocytes, lymphocytes and granulocytes reportedly enhance tissue regeneration (197, 198). Even though there is an emerging body of evidence supporting the use of PRP, there is still no consensus on the dosage, frequency of treatment and type of PRP used in each clinical scenario (185). Most importantly, PRP preparations are often not reported and are also not standardised between trials; this leads to the large heterogeneity in the reported outcomes and the resulting uncertainty observed in the literature (199).

2.8.4 Systemic enhancement of fracture healing

Several molecules have been used in an attempt to enhance fracture healing. Most of them are part of the normal osteogenic pathway and even though animal studies and pre-clinical data are promising, their clinical effectiveness is yet to be verified.

These include amongst others BMPs, VEGF, PDFG, PTH, GH, IGF, TGF-β, members of the Wnt pathway and arachidonic acid metabolites (138, 200-207).

2.8.5 Physical enhancement of fracture healing

Physical enhancement of fracture healing includes non-invasive or minimally invasive methods of accelerating fracture healing, including low intensity pulsed ultrasound (LIPUS), electrical stimulation and extracorporeal shock wave therapy (ESTW). Their use has been steadily increasing over the last few years, even though there is conflicting evidence regarding their effectiveness.

LIPUS exploits low intensity and pulsed mechanical waves, reportedly inducing regenerative and anti-inflammatory effects in bone and other tissues (208). In animal models, a significant increase of the osteoprogenitor cells in the zone of treatment has been demonstrated (209), whilst in non-unions an 82% rate of healing was reported, being higher in hypertrophic non-unions (208, 210).

Similarly, electric stimulation utilises electric potential (direct current, capacitive coupling and pulsed electromagnetic field stimulation – PEFS), directing its action to the calcium–calmodulin pathway regulation (211). A recent meta-analysis reported a reduction in pain, a 35% relative risk reduction and 15% absolute risk reduction of non-union, even though there was no improvement in functional outcomes (212).

ESWT employs a sonic pulse generating a high pressure in the area of treatment, for a short period of time. The reported effect is stimulating osteogenesis in the bone marrow stromal cells (BMSC) (213). Several studies support its safety and effectiveness, but generally, there is lack of high quality evidence to support its routine use (213, 214).

2.8.6 Gene therapy

Apart from the previously described biological variation of the host, genetic predisposition is believed to be yet another important element of fracture healing (215-217). Gene therapy is an emerging but rapidly developing, highly promising approach to the treatment of non-unions (218, 219). Gene therapy utilises viral (transfection) or non-viral (transduction) vectors to transfer genetic material into the target cell genome, either using an *in vivo* or *ex vivo* gene-transfer strategy (220). Several animal studies successfully attempted delivery of several osteogenic genes

and GF (such as BMPs), scaffolds and cells, but it is challenging to identify the most promising ones that could be translated into clinical trials (221).

Chapter 3
Subtrochanteric Fractures

3.1 Introduction

Subtrochanteric fractures are fractures involving the proximal femur and generally account for 10–34% of hip fractures (26). There is no consensus in the literature regarding the definition of these injuries and therefore their description remains inconsistent; thus the wide reported range. The anatomical region they involve is also not clearly defined, with most authors considering them as those occurring distally to the lesser trochanter and no more than 5 cm distal to it, at their most proximal point (**Figure 3.1**) (27).

Typically, their incidence has a bimodal age distribution, with one peak in young age and a second in the elderly. In young and healthy individuals they frequently occur as a result of high-energy trauma (commonly road traffic collisions – RTCs), leading to complex fracture patterns (26, 27, 222). In the elderly population they commonly present as a result of low energy trauma, relating to osteoporosis or pathological lesions, usually being associated with spiral fracture configurations (26, 27, 222-224). In the recent years, in patients exposed to bisphosphonate therapy, a new group of subtrochanteric fractures has emerged, that of the ‘atypical’ fractures. Regarding the age distribution of the atypical fractures however, there is an overlap with the elderly population peak detected in the ‘typical’ subtrochanteric fractures, but the former are usually characterised by prodromal thigh pain (225).

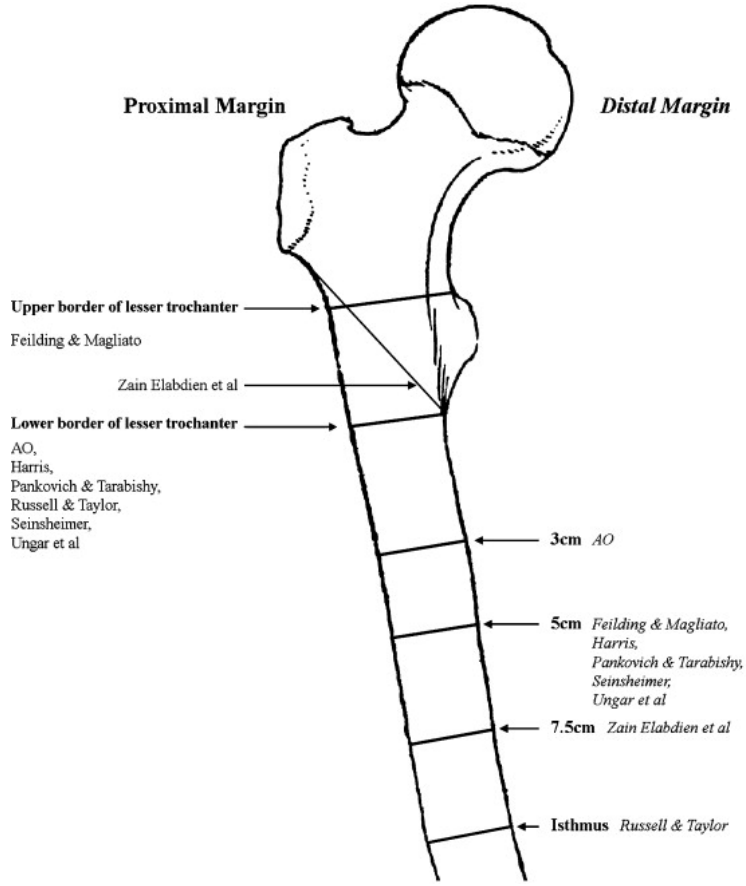


Figure 3.1 Definition of subtrochanteric fractures according to the different classification systems
Figure adapted from Loizou et al. (27)

3.2 Classification of subtrochanteric fractures

Subtrochanteric fractures give rise to an abundant array of fracture configurations and several fracture classification systems have been developed to describe them. Most of these take into account the number of different fragments involved and especially the integrity of the proximal fragment, the geometry (transverse, oblique or spiral) and topography of the fracture line and the level of displacement.

The most widely used are the Seinsheimer classification (**Figure 3.2**) and the AO (Arbeitsgemeinschaft für Osteosynthesefragen) classification (**Figure 3.3**). The Russell Taylor Classification system (**Figure 3.4**) is also frequently used (27, 226-228). Nonetheless, these classification systems are not always reliable or reproducible and have significant interobserver variations (27, 228). Additionally, none of the current classification systems can successfully determine treatment or predict outcomes (27).

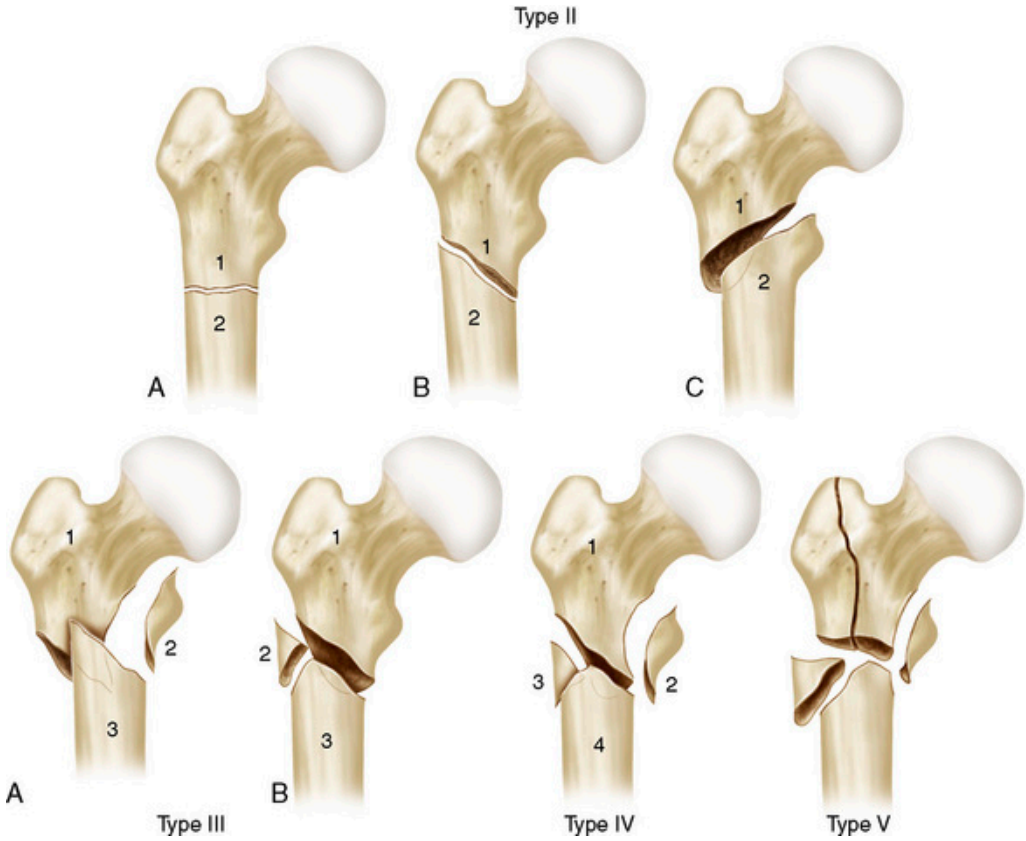


Figure 3.2 Seinsheimer classification of subtrochanteric fractures

Type I: Undisplaced fractures
Type II: Two-part fractures
- A. Transverse
- B. Spiral with lesser trochanter attached to proximal fragment
- C. Spiral with lesser trochanter attached to the distal fragment

Type III: Three-part fractures
- A. Spiral with lesser trochanter part of the third fragment
- B. Spiral with the third part a butterfly fragment

Type IV: Four or more parts fractures
Type V: Subtrochanteric–intertrochanteric fractures

Figure adapted from https://musculoskeletalkey.com (229)

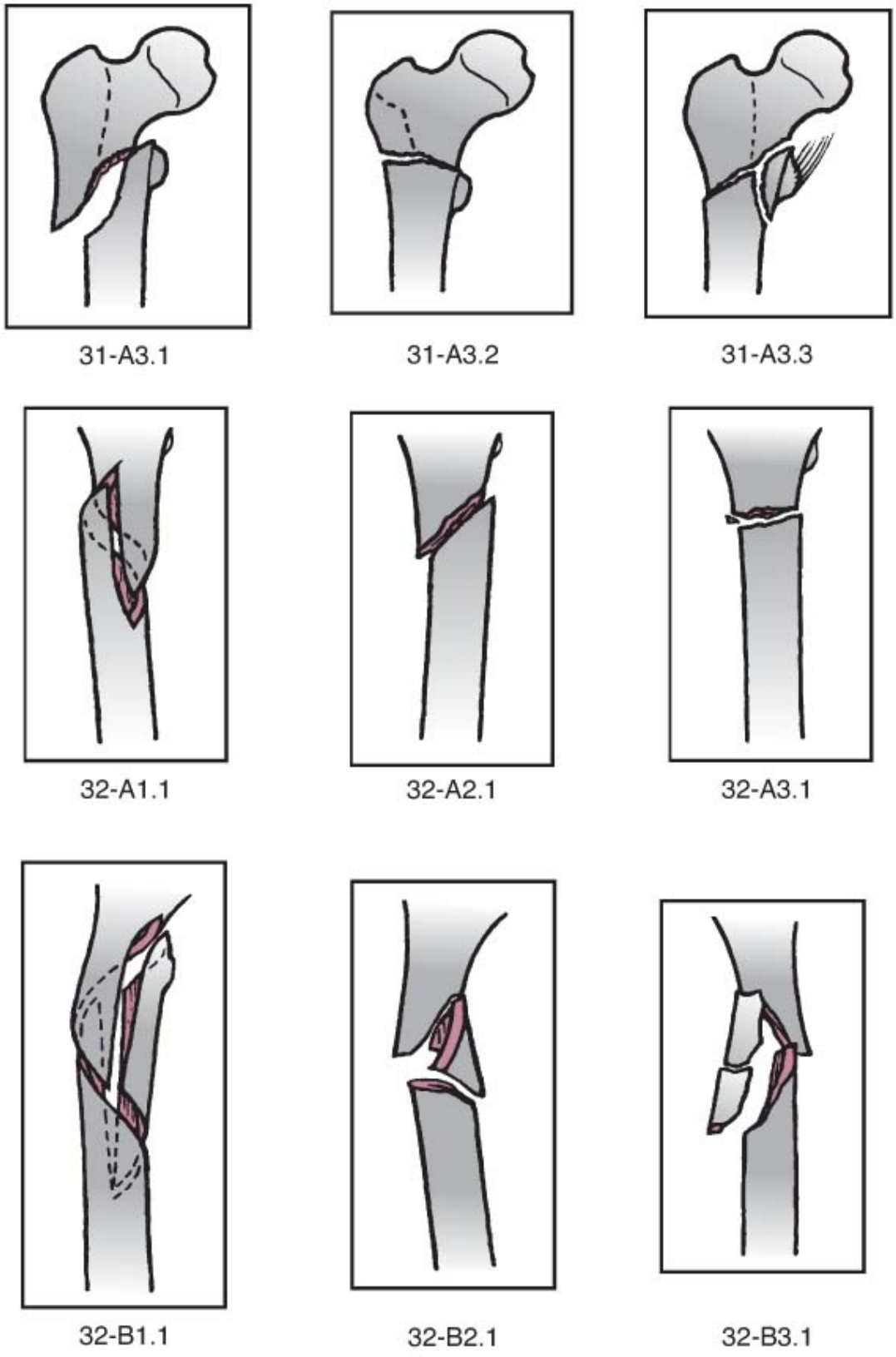


Figure 3.3 AO classification of subtrochanteric fractures

Fracture location: Femur (3), Diaphysis (2), Subtrochanteric region (0.1)
Fracture pattern: Simple (A), Wedge (B), Complex (C)

Figure adapted from Rockwood and Green's Fractures in Adults: Subtrochanteric Femur Fractures (230)

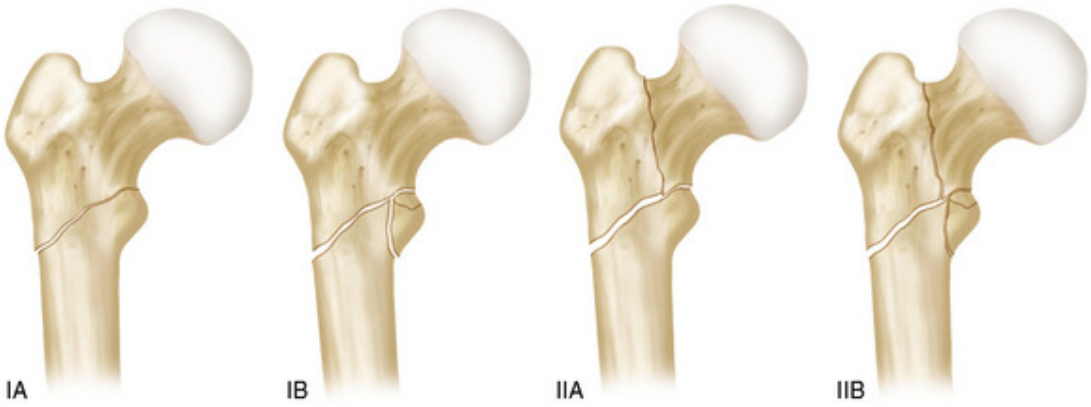


Figure 3.4 Russell Taylor Classification system
Type I: Fracture does not extend into the trochanteric fossa
IA: without extension to the lesser trochanter
IB: with extension to the lesser trochanter
Type II: Fracture extends into the trochanteric fossa
IIA: without comminution of the lesser trochanter
IIB: with comminution of the lesser trochanter

Figure adapted from https://musculoskeletalkey.com (231)

3.3 Special considerations for subtrochanteric fractures

The management of subtrochanteric fractures presents a challenge for the treating surgeon, because of the unique anatomical and biomechanical features of the subtrochanteric region.

The blood supply to the femoral diaphysis is provided by three main systems : (i) the nutrient artery which supplies the bone marrow and inner 2/3rds of the diaphyseal cortex, (ii) the metaphyseal-epiphyseal system which forms anastomoses with the nutrient system, providing blood supply to the distal ends of the long bone, and (iii) the periosteal system which supplies the outer 1/3rd of the cortical bone, and anastomoses with the nutrient system (232). Being the principal blood supply, the nutrient artery derives itself from one of the three perforating branches of the deep femoral artery, and enters the bone through the nutrient foramen, located most commonly in the middle segment of the femur. As the main entry point of the nutrient artery system, the middle 1/3rd of the femoral shaft has been described as zone of ‘high’ vascularity (232). In contrast, blood supply to the subtrochanteric area arise in majority of the cases from (i) the most superior of the three perforating branches of deep femoral artery and (ii) the periosteal system. Therefore, blood supply to the

subtrochanteric region is much more vulnerable to injury and has been described as a zone of 'moderate' vascularity (232).

From a biomechanical perspective, the subtrochanteric region represents an anatomical region which is subjected to constant lateral tensile and medial compressive stresses, reaching several multiples of body weight, due to weight bearing and the act of powerful muscles (27, 233). Koch et al. in a milestone manuscript published in 1917 was the first to calculate these forces. He suggested that in a 200-lb person, the compressive forces in the medial cortex can lead to pressures exceeding 1200 lbs/in^2, whereas the tensile forces in the lateral cortex can lead to pressures exceeding 900 lbs/in^2 (234, 235). Additionally, the presence of torsional forces that subsequently lead to significant rotational shear forces around the hip should also be considered (183).

Following a fracture in this region, the unbalanced pull of muscles acting around the proximal femur lead to significant deforming forces, resulting to fragment displacement. The inherently short length of the proximal fragment makes the deformity even more difficult to control. More specifically, the pull of Iliopsoas causes flexion and external rotation of the proximal fragment, reinforced by the action of the short external rotators. Gluteus medius on the other hand causes abduction of the proximal end, whereas the quadriceps mechanism, hamstrings and adductors lead to adduction and shortening of the femoral shaft (distal fragment). Furthermore, the subtrochanteric region represents a transition zone between high concentration of cancellous bone in the trochanteric region to thick cortical bone distally to it, leading to a more vulnerable blood supply (236).

All of the above should be considered in the pre-operative planning because of their important implications both for the anatomical reduction of the fracture, as well as for implant choices. Failure to do so will lead to an increased risk of complications, such as impaired fracture healing and / or implant failure.

3.4 Management of subtrochanteric fractures

The cornerstone of management of subtrochanteric fractures is prompt anatomical reduction and surgical fixation, in an attempt to reduce the deformity, improve healing rates and avoid prolonged immobilisation which is linked to an increased risk of thromboembolic events and decubitus ulcers. Nonoperative management is only

reserved for the most infirm patients with prohibitive medical co-morbidities or patients who refuse surgery. Even in non-ambulatory patients, surgical fixation would allow easier hygiene and transfers of the patient, also reducing the risk of pulmonary complications.

Regarding to the timing of the fixation, this should be as soon as possible. Factors such as patient's co-morbidities however, anticoagulant intake, associated injuries that need emergency management, head injuries, open fractures, and others, can alter the management plan (237). More specifically, in the setting of polytrauma, patients should be managed by the multidisciplinary team according to the ATLS® (Advanced Trauma Life Support®) guidelines, with the 'damage control concept' still having its role restoring the physiology and addressing life threatening injuries (238, 239). In the elderly population where subtrochanteric fractures are usually the result of low energy injury, these should be managed according to the NICE (National Institute for Health and Care Excellence) guidelines which suggest fixation on the day of or the day after admission (12, 240).

3.4.1 Implant choices

An abundance of implants have been used for the management of subtrochanteric fractures. These can either be extra- or intra- medullary devices (241). Use of external fixators is reserved as a temporary measure in polytraumatised patients and is generally not used for definitive treatment. In cases of extensive neoplastic lesions of the proximal femur, non-unions and neglected cases, total hip arthroplasty (THA) (proximal femoral replacement) also remains a valid option.

The aim of operative management is to achieve a stable internal fixation, restoring the anatomy of the proximal femur. This would enhance fracture healing whilst maintaining the function of the joints and soft tissues, aiding to return to the pre-injury level of function. This however usually requires extensive approaches which further compromise the already affected soft tissue envelope and contribute to an increased necrosis at the fracture site (242, 243). Newer techniques utilising the 'biological' internal fixation avoid the need of precise reduction, but only aim to the alignment of the fracture fragments through indirect reduction manoeuvres, hence reducing the surgical insult to the zone of injury and therefore the risk of complications (242, 244). This principle equally applies to extra-medullary (bridge plating and internal fixator-like devices) and IM devices (locked nails) (242).

3.4.1.1 Extra-medullary devices

The use of plating techniques (ORIF) still has a role in the primary management of subtrochanteric fractures, but strict patient selection is the key to a successful outcome. Short proximal fragments, simple fracture patterns and cases of secondary procedures following complications of nailing, such as mal-reduction, delayed union and non-union, are the commonest indications. Relative indications also include narrow IM canal, genetic or acquired deformities of the femur that would prevent the passage of the nail and significant pulmonary trauma in which case reaming of the canal could cause further damage (230).

Dynamic hip screw (DHS) has been widely used in the past, but because of high risk of complications including medialisation of the femoral shaft and generally unfavourable outcomes in up to 70% of the cases, this is now rarely used (236, 245). Blade plates and locking plates have been used with good results, especially when used according to the 'biological' fixation principles as previously described (246-249).

3.4.1.2 Intra-medullary devices

Intra-medullary fixation of subtrochanteric fractures is the 'gold standard' treatment, having several advantages over alternative methods of fixation (230, 250, 251). It combines the principles of minimally invasive surgery, with biomechanical advantages including a shorter lever arm of the fixation, a better load sharing and less bending movement across the fracture site and the implant (25, 26, 252, 253). Several authors support that the operative time, transfusion rate, length of hospital stay and risk of failure of IM implants is reduced compared to extra-medullary implants (254-256).

When using antegrade femoral nails, there are currently many options for implants with different characteristics and entry points. For the trochanteric entry point, the tip of the trochanter leads to the most neutral alignment regardless of the make of nail used, causing less soft tissue injury and reducing operative time, while at the same time the tip of the trochanter is generally easier to identify and more accessible (257-259). Nevertheless, lateral entry points of these nails, can cause varus malalignment and opening of the lateral cortex of the fracture, with subsequent high position of the lag screw that predisposes to cut-out, whilst medial points can cause varus malalignment in some design of nails (257, 260). On the other hand, previous

anatomical studies of the 'ideal' entry point for a straight nail place it over the tendinous insertion of the piriformis muscle (261, 262). A piriformis entry point however is more technically demanding and has been associated with advancement of the fracture line and comminution of the proximal fragment, as well as increased soft tissue injury and neurovascular complications (258, 261). Finally, a lateral entry point nail can avoid the soft tissues around the greater trochanter, but it is known to impinge to the medial cortex causing comminution of the proximal fragment (261). Regardless of the type and make of nail used, the surgeon should be aware of the characteristics of the nail used and pre-operatively plan correctly to ensure anatomical variations are taken into consideration.

With regard to the number of screws locking the nail proximally, some authors suggested that two smaller diameter screws can overcome the potential weakness at the place of the insertion aperture of the nail, compared to a single larger diameter screw (263). In a 2-screw configuration, the stresses transferred to the nail and screws are considerably reduced; nonetheless, the stresses transferred to the cancellous bone around the screws are increased, theoretically increasing the risk of cut-out (263, 264). Other authors however reported no significant difference in biomechanical testing of different screw configurations in stable fractures, but in unstable fracture patterns, they identified that larger diameter screws allow for less motion at the fracture site (241). Grisell et al. on the other hand reported that cross-screw proximal locking configuration is associated with higher failure loads (265). Finally, Fissel et al. suggested that there is an increased stiffness of 3-screw construct compared to 2-screw constructs for reconstruction nails (266). Because of all the above, a number of surgeons use 2-screw configurations in young patients, whereas in older patients with osteoporotic bone they use one large central lag screw (235).

3.4.2 Reduction challenges

Positioning of the patient can have a great impact on one's ability to reduce subtrochanteric fractures. First and foremost, the use of fracture tables can prevent adequate entry point of a cephalomedullary nail. In fact, if the affected extremity is adducted to allow for an appropriate trochanteric entry point then the post will cause a varus deformity. If the leg is abducted to prevent a varus deformity, the entry point will be too lateral. This is especially true for obese patients. This conundrum can be solved by placing the patient in a sloppy lateral position. This allows flexion of the hip, which helps reduce the deforming forces causing flexion of the proximal fragment.

This position also allows adduction of the affected lower extremity to gain adequate nail entry point without causing a varus deformity. Nevertheless, supine position on a fracture table requires less assistance for limb manoeuvres, facilitates fracture reduction and retainment of this position, and allows easy fluoroscopic access; it is therefore the positioning of choice of most surgeons.

If closed reduction is possible, the fracture haematoma and local vascularity of the fragments is preserved and therefore the healing cascade is not interrupted. Nevertheless, because of the unbalanced pull of the muscles around the greater trochanter as previously described, closed reduction can be challenging and difficult to achieve (267). Some authors have therefore suggested open reduction with direct visualisation of the fragments. Open reduction of subtrochanteric fracture is however not without risks, with infection, non-union, prolonged surgical time and blood loss requiring blood transfusion being the commonest concerns (268-271). Published evidence remains conflicting, with some studies reporting an increased risk of non-union and infection (268); whilst other studies report no difference with regards to non-union, infection, transfusion rates and fracture vascularity (269, 270). Furthermore, the majority of these studies only report on outcomes and experience with open reduction of subtrochanteric fractures (174, 272-278). Thereafter, there has been a trend of 'limited' open reduction, with the use of adjuncts such as reduction clamps, bone-holding forceps, Schanz pins and bone hooks (267, 278, 279). Supplementary cerclage wires / cables have also been used by many authors advocating a biomechanical benefit (273), but concerns of additional stripping of soft tissues and compromise of the periosteal blood supply reduce their use (268).

3.5 Complications

Prompt anatomical reduction, stable fixation, respect of soft tissues and early post-operative mobilisation are key factors for a successful outcome (233). Nevertheless, complications following the management of subtrochanteric fractures remain relatively common. These can be related to the mechanism of injury and fracture geometry, the operation itself and the patient's co-morbidities and medication.

3.5.1 Inadequate reduction / mal-union

Varus malreduction, femoral malrotation and leg length discrepancy are all consequences of inadequate fracture reduction. Their incidence is probably higher

than the one reported in the literature, especially in the elderly. The need for reoperation however is rare, apart from cases where this leads to failure of the nail or progression to a non-union and those where the deformity is extreme causing posture or gait problems. Generally, the amount of deformity required for this to be symptomatic is undetermined and patient dependent.

Varus malreduction is the commonest and is usually caused by a lateral entry point, and the unopposed pull of iliopsoas and of the adductors. Moreover, the high density bone around piriformis fossa, especially in young patients, can push the entry reamer more laterally if not careful. Biomechanically, varus malalignment increases the medial cortex compression stresses at the fracture site because of the alteration of the trajectory of weight-bearing forces across the neck of the femur, therefore increasing the risk of implant failure. At the same time, the efficiency of the abductors decreases because of the shorter lever arm.

3.5.2 Bleeding

The blood supply to the subtrochanteric region originates mainly from the trochanteric and cruciate arterial anastomoses (280). Bleeding can occur both as a result of the injury and because of the subsequent surgical insult. Bleeding secondary to the original trauma is usually the result of the fractured bony surfaces and the disrupted IM vascular network, particularly where cancellous bone is involved. The nutrient artery is also often involved, along with the damaged periosteal blood vessels. Additionally, the surrounding soft tissues are often damaged and larger diameter arteries such as the perforating branches of profunda femoris can shear off or transected by the sharp bony fragments. Previous reports calculate the blood loss between 400ml – 2200ml, even though there is a wide variation between the different studies (280). Intra-operatively, meticulous surgical technique and protection of local blood supply does not just reduce bleeding, but also retains the vascularity at the fracture site and therefore enhances healing (92).

3.5.3 Infection

Infection is one of the most difficult complications to manage, often being associated with impaired healing. Early post-operative superficial infections can be managed with retention of metalwork if adequate stability, and systemic antibiotics (oral or intravenous). Deep infections on the other hand should be managed with extensive debridement and if the fracture is healed, removal of metalwork, debridement,

irrigation and administration of local or systemic antibiotics (281). In infected non-unions, extensive IM debridement using the RIA technique, delivery of local antibiotics with cement nails of spacers (Masquelet technique) and subsequent exchange nailing have been used with promising results (281).

3.5.4 Impaired fracture healing

The risk of non-union following subtrochanteric fractures is high because of the unique anatomical and biomechanical features of the subtrochanteric region. Several patient characteristics such as poor bone stock, diabetes, smoking and steroids have been identifying as contributing factors (174-176). Moreover, characteristics from the primary surgery such as adequacy of reduction, residual medial cortex gap, open reduction, lateral entry point, varus malalignment and increased TAD have also been reported as potential risk factors (26, 177-183). The management of established non-unions is usually complicated and needs to be individualised to the patient's needs and expectations. In general terms, the aim of management should be restoration of the normal anatomy, ensuring presence of adequate stability at the fracture site and adherence to the 'Diamond Concept' in terms of biological enhancement (92).

3.6 Atypical femoral fractures

Atypical femoral fractures represent a relatively new entity in the orthopaedic literature. It emerged with the increasing use of bisphosphonates and it is a rather rare event. Following the increasing interest and inconsistencies in its definition, the ASBMR (American Society for Bone and Mineral Research) task force convened releasing two documents defining the characteristics of AFFs, their aetiology and management, as well as setting the groundwork for future research in this field (282). According to ASBMR, AFFs are the fractures located between the lesser trochanter and the supracondylar flare of a femur (282). Additionally, at least four out of five 'Major Features' must be evident, most of which represent radiological features, whilst another four 'Minor Features' were listed, that may be associated with AFFs but do not need to be present for the diagnosis to be established (**Table 3.1**).

Table 3.1 ASBMR Task Force 2013 revised case definition of AFF

The Fracture is located between the lesser trochanter and the supracondylar flare of the femur (282).
The fracture satisfies at least four out of the following five Major Features: 1. It is a pathological or low energy injury, i.e. not associated with trauma, or minimal trauma such as a fall from a standing height. 2. The fracture line starts at the lateral cortex and is mainly transverse in orientation, or may become oblique as it progresses medially. 3. There is no or minimal comminution. 4. Complete fractures go through both cortices and may produce a medial spike; incomplete fractures involve only the lateral cortex. 5. Beaking or flaring is present at the lateral cortex of the fracture site, indicating local periosteal thickening.
The fracture may satisfy any of the following Minor Features: 1. Generalised increase in thickness of the cortices of the femoral diaphysis. 2. Prodromal symptoms such as thigh or groin pain. 3. Bilateral incomplete or complete fractures of the diaphysis. 4. Delayed fracture healing.

3.6.1 Pathogenesis

Since the histological studies from Odvina, et al., who performed bone biopsies in patients suffering from spontaneous fractures after prolonged bisphosphonate therapy, the pathophysiology underlying AFFs understanding has been steadily increasing (283). More specifically, the authors reported ‘severely suppressed bone turnover’ in these patients, evidenced by reduced osteoblastic and osteoclastic active surfaces and a reduced or absent uptake of tetracycline labelling which acts as a marker of bone growth (283). Similarly, other human studies reported several structural and bone composition changes that could potentially be related to AFFs (284). Further animal models studies, demonstrate that bisphosphonates inhibit the normal repair of micro-fractures, leading to accumulation of microdamage with prolonged stresses, hence increasing the risk of pathological fractures (285). Another consequence of inhibiting osteoclastic bone resorption is unregulated mineralisation of bone, leading to undirected over-mineralisation that makes bone more brittle (286), contributing to the increased risk of atypical fractures (285). While these changes secondary to bisphosphonate therapy occur throughout the skeleton, the femur

undergoes unique stresses through its weightbearing role, a factor that further predisposes it to atypical fractures (287). It is important however to state that not only bisphosphonates give rise to AFF, but also glucocorticoids, HRT, proton-pump inhibitors (288) and the antiresorptive RANKL blocker denosumab (287-291).

3.6.2 Epidemiology

It is very difficult if not impossible to estimate the true incidence of AFFs. Many times, prodromal symptoms are overlooked and when they become complete fractures, they can be misdiagnosed as 'typical' fractures, i.e. osteoporotic. Moreover, even if a patient is on bisphosphonates, it is very difficult to check the compliance to the medication, as well as the true absorption of the oral agents by the gastrointestinal tract. Nevertheless, since the introduction of the ASBMR criteria, their reporting accuracy has improved. Their overall incidence has therefore been reported between 1.6 to 23 per 100,000 person-years, even though some other reports place them as high as 61 to 113 person-years, based on the length of bisphosphonates use (34). The risk of developing AFFs on the background of long term bisphosphonates on the other hand has been reported 1.7 to 2.71 times greater than that of the general population (292, 293).

As mentioned, the incidence of AFFs is increasing along with the increased use of bisphosphonates (34). Other epidemiological characteristics of AFFs include the higher incidence of subtrochanteric fractures compared to those of the femoral shaft (294), the higher incidence in women (295), the younger age of the affected patients (296, 297), and the higher incidence in Asian populations (296, 298).

Most important however is the relation between AFFs and the duration of bisphosphonates use (299-301). A large epidemiological study in the United States reported the rate of AFF as 1.78 per 100,000 per year for those who had been using bisphosphonates for less than two years, compared to 38.9 per 100,000 per year AFFs in those taking bisphosphonates for six to eight years, and 107.5 per 100,000 per year AFFs in those on bisphosphonates for greater than ten years (299). The increasing incidence with increased duration of bisphosphonates was also reported by other studies (300-302). Moreover, alendronate is the bisphosphonate most consistently linked to AFFs, whist risendronate and newer generations of bisphosphonates have been reported to have a lower risk (303, 304). In addition, the link between bisphosphonates and AFFs remains unproven, and the pathophysiological changes observed in AFFs in patients taking bisphosphonates

can be seen in patients with no history of bisphosphonate intake, whilst patients presenting with AFFs can have a normal BMD and no evidence of hypermineralisation on bone biopsy (305, 306).

3.6.3 Diagnosis

A high index of suspicion is of paramount importance for the prompt diagnosis of AFFs, in any patient complaining of thigh or hip pain in the context of prolonged bisphosphonate use. Common features in the clinical history typically include prodromal thigh / groin / knee pain which can be as common as in 86% of the patients (307), and can also be bilateral in case of both limb involvement. Some authors suggest that patients presenting with an ipsilateral AFF should be monitored for contralateral changes for up to six years after the original episode (297, 308). Because these symptoms are non-specific they can be often missed or misdiagnosed.

Plain radiographs are the most readily available investigation for the diagnosis of AFFs and orthogonal radiographs should be obtained for every patient presenting with any of the associated symptoms. In case of complete fractures, the diagnosis can be simple, as long as the fracture satisfies the ASBMR criteria. The most specific feature though is a transverse fracture line, which has been reported to have a specificity of 0.93 (282, 304). In incomplete or impending fractures however, the diagnosis can be more challenging. Features include transverse fracture lines, lateral focal thickening and a medial beak (282, 309). Nevertheless, AFFs are often not immediately recognised and reported as such by radiologists (310).

Other imaging techniques frequently used in clinical practice include CT, having the advantage of outlining bony structures in more detail; MRI, demonstrating the bone oedema which helps in the diagnosis of occult fractures but also as a follow-up examination to confirm the response to medical treatment; bone scintigraphy, that demonstrates hotspots of increased activity in the area of lateral thickening; and DEXA scans which can either diagnose or monitor AFFs (311-313).

3.6.4 Management

Patients presenting with AFFs represent a subset of the population that suffers fragility fractures. Their associated comorbidities and complex needs necessitate a specialised multidisciplinary approach by orthopaedic surgeons, radiologists,

endocrinologists and physiotherapists. Following the diagnosis of an AFF, the priorities should be to manage pain, restore mobility and prevent complications such as non-unions, as well as to prevent future fractures, especially of the contralateral femur. Incomplete fractures are at risk of progressing to complete fractures, which could result in increased morbidity from pain, bleeding at the fracture side and inability to mobilise. Moreover, displacement of previously undisplaced incomplete fractures could potentially lead to a technically more complex operative intervention.

The choice of management depends on several factors, including the fracture characteristics, whether this is complete or incomplete, any history of impaired healing, as well as patient factors such as medical co-morbidities and patient's wishes and expectations. Management could therefore be operative (surgical), or non-operative (medical). Especially in complete AFFs of the subtrochanteric region, non-operative management has a very limited role, reserved in the most infirm patients who are not suitable for anaesthetic.

3.6.4.1 Non-operative management

According to ASBMR recommendations, conservative treatment should be offered in patients with incomplete AFFs and with minimal or absent radiographic and clinical findings. Where there is no pain at all and the diagnosis was incidental, reducing activity levels can be adequate. On the other hand, weight bearing can be protected in patients complaining of groin, thigh or knee pain (282). It is very important however, before offering non-operative treatment, to ensure that the patient will be compliant with the instructions and the follow-up. In cases where symptoms persist for more than three months from initiation of treatment, along with persistent imaging findings, prophylactic nailing should be considered (282).

The outcomes of non-operative management of incomplete AFFs are generally poor, especially when the subtrochanteric area is involved (314-316). Symptoms include impaired healing, persistent pain, progression to complete fracture and requirement of operative management (317, 318).

3.6.4.2 Operative management

All complete / displaced AFFs should be offered operative management, unless the patient is not fit for an anaesthetic. In case of incomplete fractures, those failing conservative management following 2-3 months of treatment, those located in the

subtrochanteric area, those with a previous history of contralateral AFF and those with an increased varus femoral bow should also be offered an operation (319). A recent meta-analysis of 733 patients (834 AFFs) suggested that surgical fixation with IM nailing of complete and incomplete AFFs is associated with the best outcomes (320). The same study reported failure of non-operative management in 47% of the patients, compared to 97% of union rate with prophylactic nailing, within an average time to union of seven months (320). Regarding the choice of implant, antegrade IM nails offer smaller revision rates and implant failure compared to plating techniques, and are therefore considered the 'gold standard' (320).

3.6.5 Complications

The types of complications of AFFs are comparable to those of 'typical' fractures. AFFs however are commonly associated with higher rates of implant failure and impaired healing, as well as higher rates of intra-operative fractures (321). Mortality remains lower in AFF patients compared to 'typical' fractures (322).

3.6.5.1 Impaired bone healing

Several authors suggest that bisphosphonates are associated with impaired bone healing following AFFs (323-326). Nevertheless, there is no strong evidence to suggest a delay in healing if the bisphosphonates are commenced following a typical osteoporotic fracture, making them ideal for secondary prevention of additional osteoporotic fractures (323). In fact, a recent RCT reported that early administration of alendronate (within two weeks from injury), did not adversely affect union or clinical outcomes, following distal radius fractures (327). When primary healing is intended though, Savaridas et al. demonstrated that bisphosphonate treatment can have an inhibitory effect on healing (328).

Regarding healing times in AFFs, there is a wide reported range in the literature, ranging from 5 to 10.6 months (34, 325, 326, 329, 330). The retrospective nature of most of these studies however, as well as the differences in follow-up and definition of union, may account for some of these differences. Impaired healing (delayed union and non-union) has also been reported in as high as 40% of the patients (308, 325, 326, 331), whilst the incidence of revision for any cause as high as 46% (34, 331, 332).

3.6.5.2 Management of impaired bone healing

Once an AFF is diagnosed, it is very important to consider the special pathoanatomical features of these injuries, including the already compromised local and systemic biological functions. Some authors advocate that bisphosphonate therapy should be discontinued in order to reduce the risk of further AFFs (333-335), a risk that has previously been reported as high as 50% compared to 20% when bisphosphonates are discontinued (334).

Calcium and vitamin D supplementation is commonly recommended in patients following AFFs, whilst a referral to an endocrinologist is advocated (311, 320, 333). Besides, most patients on long-term bisphosphonates are often on concurrent calcium and vitamin D supplements, which they continue even after discontinuing bisphosphonates following an AFF (336). Nevertheless, there is no clear evidence supporting a beneficial effect of calcium and vitamin D in treating AFFs (337).

Teriparatide, a PTH analog, works by activating osteoblasts more than it does osteoclasts and can therefore promote and accelerate healing (338). Its use in osteoporotic fractures has demonstrated promising results, with shorter fracture healing time and improved functional outcomes, especially in the lower limb (316, 338). Specifically in AFFs, it has been reported that histologically it leads to an increased bone formation (339), whilst clinically it promotes healing (340). Its use however is limited, because of the lack of evidence on its effectiveness, the lack of agreement regarding the dosing and duration of treatment and finally its contra-indications (history of radiotherapy, bone forming tumours, Paget's etc) (34, 340-343).

3.6.6 Prevention of AFFs

With an ever increasing number of patients on bisphosphonates and an increasing pool of evidence linking prolonged bisphosphonate use to AFFs, the concept of a 'Drug Holiday' is gaining popularity. This concept suggests discontinuing bisphosphonate therapy at certain intervals in patients whose risk of AFF becomes greater than their risk of 'typical' osteoporotic fractures.

In the UK, the National Osteoporosis Guideline Group has incorporated the concept of drug holidays, advocating that alendronate, risedronate, and ibandronate regimes should be reviewed after five years of use, whilst zoledronic acid regimes should be reviewed after three years (344). If bisphosphonates are stopped, the patient should

be reviewed again after two or three years and recommencement of bisphosphonates should be considered (344). Nevertheless, patients who are found to be at high risk for osteoporotic fractures should not receive a drug holiday, as the risks and morbidities of osteoporotic fractures outweigh those of AFFs, which are still rare in absolute numbers. Patients identified as high risk include patients over the age of 75, patients who sustained fractures despite being on bisphosphonates and patients on glucocorticoids (344, 345).

Additionally, partly due to the evidence of increasing risk of AFFs with duration of bisphosphonate use, FDA published a systematic review of three long-term trials and found no clear benefit of bisphosphonate therapy beyond five years in further reducing the rates of typical osteoporotic fractures (346). They therefore recommended reviewing bisphosphonate treatment guidelines in order to consider the increased risk of AFFs, compared to the minimal benefit of bisphosphonate use beyond five years (346). Similar recommendations have been incorporated in the UK NICE Guidelines as they now advise reviewing the need for continuing bisphosphonate treatment every 3-5 years, and specifically suggest for identifying symptoms of AFFs, in which case treatment should be discontinued (37).

3.7 Aims, hypothesis and objectives

Subtrochanteric fractures pose a challenge for the treating physician. In addition, the development of post-operative complications, such as the case of a non-union, is common, carrying devastating consequences. The presence of a scoring system able to predict the development of non-unions accurately will aid the physician into avoiding patient-dependent and surgeon-controlled factors that influence the outcome of a successful healing, thus preventing the development of a non-union or acting as a guide for early intervention in high risk cases. Additionally, a description of the related complications of subtrochanteric fractures and their associations, can help in the early identification and in some cases prevention of some of these complications.

Examining the literature to identify studies investigating subtrochanteric fractures and their potential associations with non-unions or other complications, only a few studies reported on such outcomes (250, 272, 347-349). However, most studies only contained a small numbers of patients and lacked suitable statistical modelling, only limited into descriptive statistics and simple comparisons between the groups (272,

347-349). Only one study by Krappinger et al. examined the risk factors of non-unions, assessing the accuracy of each parameter used, but the number of patients was still very small to support their findings (250). Another study by Johnson et al. performed a logistic regression to identify risk factors for nail breakage in proximal femoral fractures, but the numbers were similarly too low to support use of regression (350). It was therefore apparent that there was a paucity of evidence and such a study could benefit clinicians in their decisions.

Hypothesis:

Risk factors / associations of common complications of subtrochanteric fractures can be identified.

In order to address these hypotheses, the project has the following aims:

1. To identify all consecutive subtrochanteric fractures treated with IM fixation in Leeds Teaching Hospitals (LTH) trauma service and describe their outcome, with a special reference to the development of non-union.
2. To identify risk factors for progressing to non-union and develop a scoring system that will predict the development of this complication.
3. To analyse the characteristics and outcomes of treatment of subtrochanteric fractures with IM nails.

Objectives:

- To define the incidence and associations of non-unions, and to develop a risk-scoring system for predicting non-unions.
- To define the incidence and associations of infections.
- To define the incidence and associations of open reduction of subtrochanteric fractures and investigate whether this is associated with an increased risk of deep infections and non-unions.
- To define the incidence and associations of osteoporosis and its effect on complications.
- To define the incidence of bisphosphonate intake and its effect on fracture healing and other complications.
- To define the incidence of atypical fractures and their effect on fracture healing and other complications.
- To compare the two commonest types of cephalomedullary nails used and investigate if any of their design features offers any advantage.

- To define the incidence of transfusion and investigate the presence of risk factors / associations with an increased risk.
- To investigate if there is any evidence of a 'weekend effect'.
- To define the incidence of medical complications (HAP, MI / CVA, post-operative delirium, VTE) and their associations.
- To define the mortality rates and to investigate any associations related to an increased mortality.

3.8 Materials and methods

3.8.1 Patients

Following institutional board approval (LTH Institutional Review Board; #2591; **Appendix A**), we performed a retrospective review of all consecutive patients treated for a subtrochanteric femoral fracture in LTH (Level I Trauma Centre), between 01 January 2009 and 31 December 2016 (eight years). Patients were managed according to a standardised protocol (**Appendix B**). Potential patients were identified from: daily trauma lists, theatre records, the NHFD (National Hip Fracture Database) and coding records. Patients were then screened for eligibility according to the inclusion / exclusion criteria. The medical records of all eligible patients were then comprehensively reviewed and all the information was inserted in an electronic database.

Subtrochanteric fractures were defined as injuries occurring distally to the lesser trochanter and no more than 5 cm distal to it at their most proximal point (26, 27).

3.8.1.1 Inclusion and exclusion criteria

The inclusion criteria were the following:

i. Patients presenting to LTH with a subtrochanteric fracture that was subsequently treated with an IM device, regardless of cause / mechanism of injury. Only fractures treated with IM nails were included as this has been considered as the 'gold standard' of their treatment (230, 250, 251).
ii. Patients treated in other institutions and later transferred to LTH, for which all required data was available.

The exclusion criteria were the following:

i. Skeletally immature patients.
ii. Prophylactic nailing for tumours (without a fracture) or incomplete fractures.

3.8.1.2 Clinical parameters collected

Parameters collected and evaluated included:

1. Patients' demographics: date of birth, gender and age at the time of injury.
2. Injury characteristics including mechanism of injury and associated injuries.
3. Past medical history: ASA (American Society of Anaesthesiologists) Score, CCS (Charlson Comorbidity Score), co-morbidities, medication (steroids, bisphosphonates etc.), smoking habits, alcohol intake, presence of malignancy, dementia, osteoporosis, social status, mobility status and history of frequent falls.
4. Fracture characteristics: side, fracture classification (AO, Russell-Taylor and Seinsheimer Classifications).
5. Primary operation details: characteristics of the implant used, surgical technique used (open / close reduction, distal locking, use of anti-rotation screw, set screw, cerclage wires, reaming of the medullary canal), time to operation, time of operation, level of operating surgeon.
6. Radiographic measurements for assessing adequacy of fixation: details on reduction achieved, gap size at the fracture site post-operatively, involvement of medial calcar, need for open reduction and characteristics of implant placement.
7. Details of any additional procedures required until union is achieved.
8. Length of hospital stay, mortality, cause of death.
9. Time to radiographic / clinical union.
10. Complications (implant related complications, fracture related complications, medical complications, infection, VTE etc.).
11. Blood parameters including Hb, urea, creatinine, bone profile etc., transfusion requirements, microbiology and histology investigations.

3.8.1.3 Data collection

Data was collected from:

1. Daily trauma lists.

2. Patients' medical records: printed or electronic records (PACS, ICE®, PPM®, EPRO®, WinDIP®).
3. Theatre records.

3.8.1.4 Definitions

1. Non-unions

Atrophic non-unions: these were defined in accordance to FDA, i.e. incomplete fracture healing within nine months following injury, along with absence of progressive signs of healing (callus) on serial radiographs over the course of three consecutive months (158). Cases however where it was clear that there was no biological activity at the fracture site on serial radiographs and early intervention was deemed appropriate before the nine month milestone, were also considered as non-unions.

Hypertrophic non-unions: these were defined as incomplete fracture healing within nine months following injury, with excessive callus formation and a visible fracture line on serial radiographs, associated with pain at the fracture site.

Septic non-unions: these were defined as non-unions associated with an infection at the fracture site. The diagnosis of infection was based on positive microbiology cultures from tissue around the non-union site obtained during revision surgery, along with increased inflammatory markers (CRP and WCC).

2. Surgical site infections:

Superficial infections: these were defined as erythema, pain, swelling, discharge and delayed wound healing, along with raised inflammatory markers in the early post-operative period. Superficial infections were generally treated with a short course of oral antibiotics.

Deep infections: these were defined as infection surrounding the metalwork necessitating further surgical interventions and were treated with a combination of intravenous (IV) antibiotics, wound washouts and removal / change of implants, as deemed appropriate.

3. Medical complications:

Community-acquired pneumonia (CAP): this was defined as a chest infection contracted by a patient before admission to hospital, or a chest infection exhibiting symptoms within a maximum of 48 hours after hospital admission.

Hospital acquired pneumonia (HAP): this was defined as a nosocomial chest infection contracted by a patient at least 48–72 hours after hospital admission.

Urinary tract infection (UTI): this was defined as a symptomatic infection of the urinary tract requiring treatment with oral or IV antibiotics.

Myocardial Infarction (MI): this was defined as the symptomatic myocardial damage because of sudden deprivation of circulating blood, demonstrated by elevated troponin I (>50 ng/L).

Cerebrovascular accident (CVA): this was defined as the acute onset of focal neurological findings in a vascular territory as a result of underlying cerebrovascular disease, confirmed by acute changes on a subsequent CT scan.

Post-operative delirium: this was defined as the acute and fluctuating disturbance of consciousness with reduced ability to focus, maintain, or shift attention, accompanied by change in cognition and perceptual disturbances following surgery.

Venous thromboembolism (VTE): this was defined as the acute symptomatic occlusion of a deep vein (deep venous thrombosis – DVT) or dislodgment of a clot distally and occlusion of the lung vasculature (pulmonary embolism – PE).

3.8.1.5 Radiographic measurements

The radiographic measurements performed included the following: femoral neck shaft angle; gap size at the fracture site (lateral, medial, anterior and posterior cortices); reduction angle; tip-apex distance (TAD); nail / canal ratio; and distance of the tip of the nail from the centre of the knee on AP and lateral views.

Additional information obtained from the radiographs included: number of fragments; isolated subtrochanteric extension; presence of atypical features; pathological fractures; periprosthetic fractures; distal extension of the fracture; involvement of the trochanters (greater and lesser trochanter); presence of comminution at the calcar;

classification of the fracture (as per AO / OTA (230), Seinsheimer (229) and Russel Taylor (231) classification systems); mode of the set screw; use of a de-rotation screw; number of distal locking screws and method of locking; evidence of the tip of the nail touching the anterior cortex of the femur; assessment of healing; and presence of nail related complications (failure of the nail at the lag screw junction or failure of the distal locking screws).

For the assessment of healing, the modified radiological union score (mRUS) was used (351). According to this, each cortex was scored according to the presence of callus and a visible fracture line (1 point: absent callus and visible fracture line; 2 points: present callus but visible fracture line; 3 points: present callus and invisible fracture line). For a fracture to be considered as 'healed', a total score of the four cortices of nine or more was required.

For the calculation of all the radiographic measurements on the pre- and post-operative radiographs, a standardised protocol was used. In more detail, all measurements were performed on PACS, using the known dimensions of the nail (nail diameter proximally; nail diameter distally; or lag screw diameter) for calibration. Each measurement calibration was repeated using the nail dimensions as close to the measurement in question as possible.

To increase the accuracy of the radiographic measurements and following training on the method, a second Orthopaedic surgeon individually assessed all radiographs. The inter- and intra- observer variability was also assessed and was within satisfactory limits (less than 5% differences in all values). Any disagreements between the two assessors were resolved by consensus, whereas in any cases of ongoing disagreement, this was solved by the senior author (PVG; this was the case in three cases).

3.8.2 Subgroup analysis

A further subgroup analysis was performed to further investigate the characteristics and associations of the following factors:

- Non-unions
- Infections
- Effect of open fracture reduction
- Effect of osteoporosis
- Effect of bisphosphonates

- Atypical fractures
- Type of nail used
- Transfusion requirements
- Weekend effect
- Medical complications (HAP, MI / CVA, post-operative delirium, VTE)
- Mortality.

3.8.3 Development of a risk scoring system for predicting non-unions

In a recent paper by Simpson et al., it was well demonstrated that assessing healing, especially in the early stages, is very difficult if not impossible (352). Therefore, any tools that could help predict the high risk patients would transform the care of these fracture patients (352).

A number of patient characteristics such as poor bone stock (174); presence of diabetes (175, 176); smoking (175, 176); and steroid intake (175, 176) have been identified by expert clinical opinion, and background literature, as potential risk factors for non-union. Moreover, characteristics from the primary surgery such as adequacy of reduction (177); residual gap in the medial surface of the femur in the region of the lesser trochanter (177); need for open reduction (177); varus malalignment (defined as angulation of more than 10° at the fracture site in the femoral shaft (178)) (26); TAD distance (179); and the entry point to the femoral canal (180-183), have also been reported as potential risk factors.

The above factors along with the outcomes of the analysis were taken into consideration for the development of the scoring system.

3.8.3.1 Estimation of sample size and power

When occurrence of characteristics is close to 50%, the study can detect with 80% power (α=0.05) a difference in prevalence of 12.1%, whereas when occurrence of characteristics is close to 10%, the study can detect with 80% power (α=0.05) a difference in prevalence of 8.8%. Therefore differences in the occurrence of characteristics which are more than 10% are identified.

3.8.4 Statistical analysis

Statistical analysis was performed using the computing environment R (R version 3.6.0) (353). Basic demographic data were presented as count (percentage) or as mean ± SD. Parametric data were analysed using an unpaired independent t-test, whilst count data were analysed using a Chi square test. A p-value of <0.05 was considered as significant. Normality of variables was assessed to determine the further use of parametric or non-parametric tests. Mortality and implant survival findings were graphically presented using Kaplan-Meier survival curves. A p-value < 0.05 was considered significant.

Following the initial analysis and for obtaining further information on each comparison, all statistically significant values were included into a revised adjusted model of logistic regression analysis. For the final model, only those variables having a p-value of less than 0.05 were retained and coefficients and odds ratio (OR) were reported.

For the development of the non-union scoring system, all factors identified by the logistic regression analysis were considered. The weight of each variable was then used to create a scoring system with a maximum score of 100, using the coefficients. Receiver-operator characteristic (ROC) analysis on the scoring system was then used to define utility in predicting outcome and set cut offs with different sensitivity / specificity, whilst repeated 5-fold cross validation was then performed to test for internal validation of the scoring system.

3.9 Results

3.9.1 Basic cohort information

During the investigated period (01 January 2009 and 31 December 2016; 8 years), a total of 545 consecutive patients (561 fractures; 206 male) were treated for proximal femoral fractures extending into the subtrochanteric area, fulfilling the inclusion criteria (**Table 3.2**). Their average age was 73.1 y.o. (median 79.3 y.o.; SD 19.1 y.o.).

Reporting on the medical comorbidities, the median ASA was 3 (**Figure 3.5**), whilst median CCS was 5 (**Figure 3.6**). Dementia was evident in 125 patients (AMTS<8), and 153 patients had a history of recurrent falls. A total of 92 patients had a previous diagnosis of Diabetes Mellitus (DM) and 137 patients had a history of malignancy

(regardless whether the patients at the time of injury were free of disease or not). Smoking was reported in 113 patients, whilst 105 patients reported alcohol intake of more than 10 units / week. Eighty-five patients required high level of support (residential / nursing home or home carers), and 293 patients were mobilising independently (no waking aids). Regarding bone-healing altering medication intake, 29 patients were on long term steroids (at least six month duration) and 94 patients were on bisphosphonates pre-admission, whilst another 158 patients were on calcium / vitamin D pre-admission.

Table 3.2 Basic demographic information of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

General demographics	
N	561 fractures; 549 pts
Age	73.1 ± 19.1 y.o. (79.3 y.o.; 18.5 to 102.1 y.o.)
Gender	Male: 215 (38.4%)
Co-morbidities	
ASA	2.7 ± 0.8 (3; 1 to 5)
Diabetes	Type I – IDDM: 6 pts Type II - Diet controlled: 15 pts Type II – NIDDM: 51 pts Type II – IDDM: 20 pts
Malignancy	137 pts (Lung: 33 pts; Breast: 26 pts; Bowel: 18 pts; Other: 60 pts)
Dementia	125 pts AMTS: 8.1 ± 3 (10; 0 to 10)
Frequent falls	153 pts
CCS	5.3 ± 3.1 (5; 0 to14)
Social history	
Smoking	< 10 / day: 22 pts 11 -20 / day: 51 pts > 21 /day: 40 pts Ex-smokers: 101 pts
Alcohol	< 10 units / week: 85 pts 11 - 20 units / week: 28 pts > 21 units / week: 77 pts
Residence	Carers: 46 pts Residential Home: 34 pts Nursing home: 5 pts
Pre-injury Mobility	Independent: 193 pts Stick(s) / Crutch(s): 146 pts Frame: 95 pts Wheelchair: 19 pts Hoisted: 8 pts
Medications altering bone healing	
Steroids	Inhalers: 48 pts Tablets (long term): 29 pts Tablets (short term): 18 pts

Medications altering bone healing		
Bisphosphonates	Pre-admission: On discharge:	94 pts 136 pts
Calcium / Vitamin D	Pre-admission: On discharge: Vitamin D loading:	158 pts 251 pts 87 pts

Results are presented as: Mean ± SD (Median; Range)
pts: patients

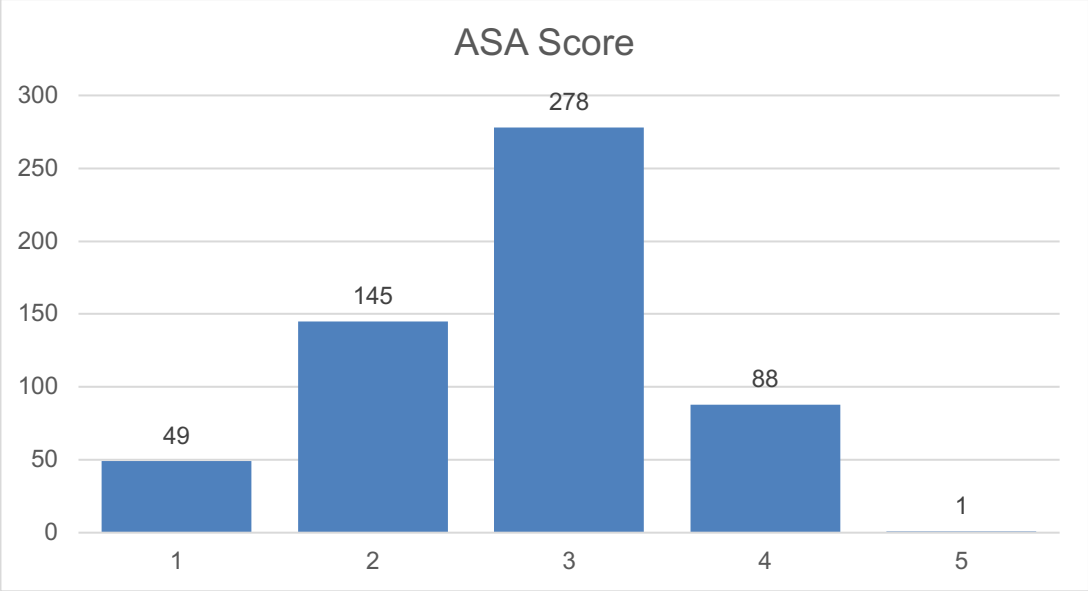


Figure 3.5 ASA distribution of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

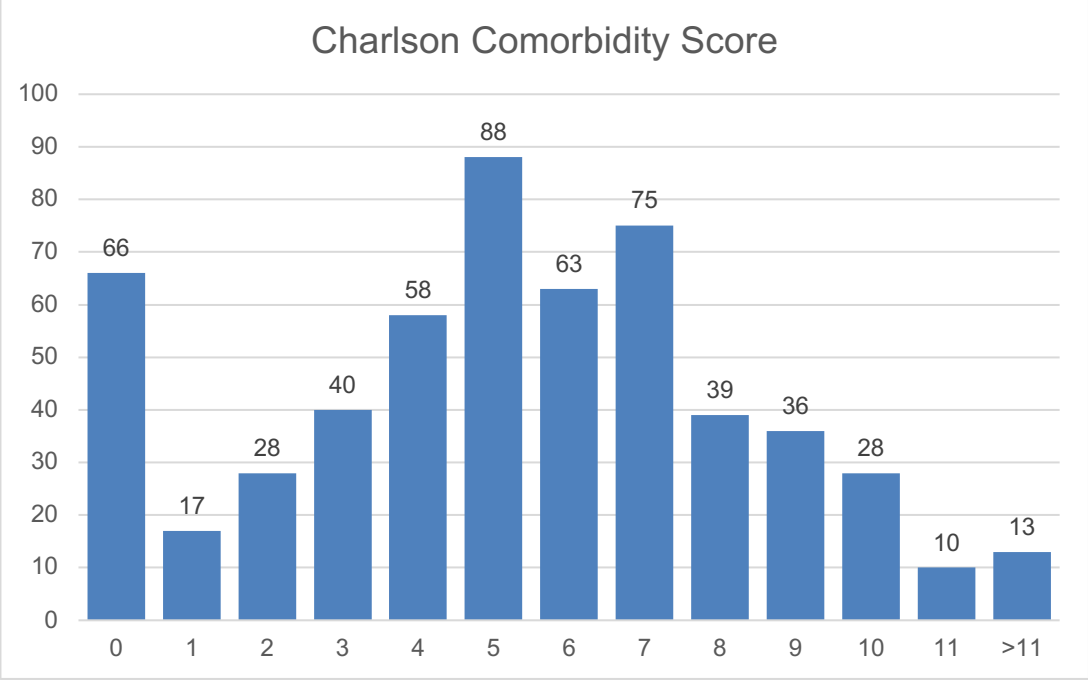


Figure 3.6 CCS distribution of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Of note is that the incidence of fractures extending into the subtrochanteric region was different in males and females. In males that was bimodal, with a peak during the 4th decade of life, mainly associated with RTCs, whilst a second peak was evident during the 9th decade of life that was in turn associated with low energy injuries (fragility fractures). In the female population, it was unimodal, increasing with age and similarly to the second peak in males, associated with low energy injuries (**Figure 3.7**). Overall, the commonest mechanism of injury was 'fall from standing height' (60%), followed by 'unwitnessed falls' (12%), 'RTCs' (9%) and 'pathological fractures' (7%) (**Figure 3.8**). 'Low energy' injuries were therefore reported in 77% of the patients.

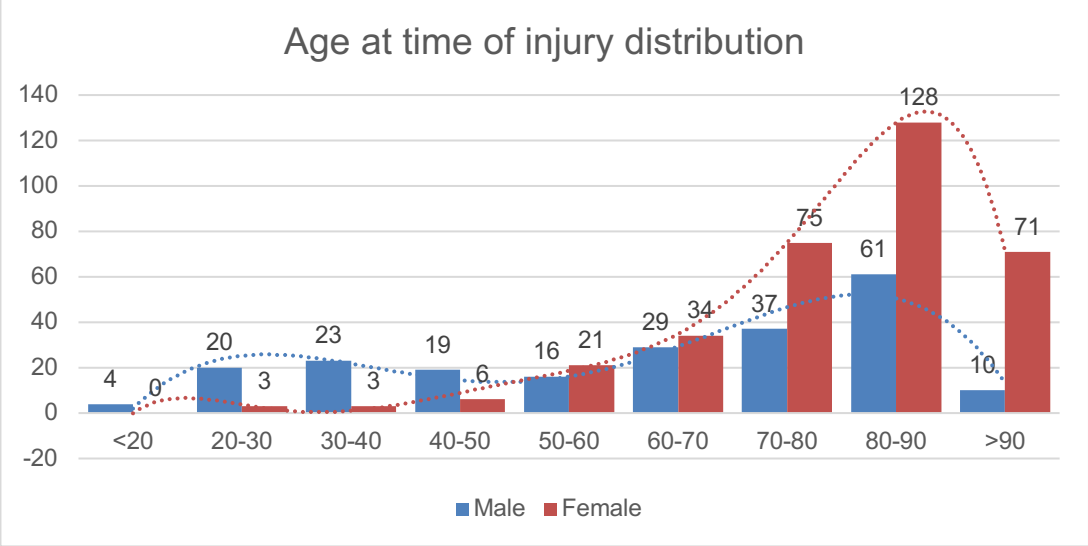


Figure 3.7 Age distribution of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

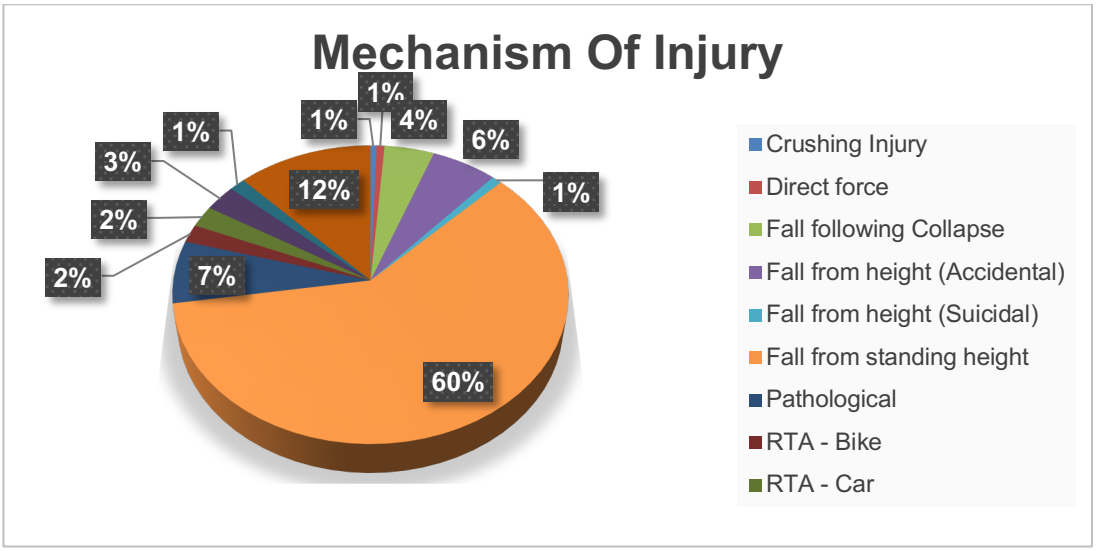


Figure 3.8 Pie chart demonstrating the mechanism of injury

Investigating the injury characteristics, the right side was involved in 47.4% of the cases (**Table 3.3**). The injury severity score (ISS) was greater than 16 (i.e. polytrauma) in 33 patients; noteworthy, one patient sustained his polytrauma injuries following a simple fall. An open fracture was reported in seven patients, while 83 patients had associated injuries during the same episode (one patient had bilateral subtrochanteric fractures) and 37 patients had additional operations on the same setting with the IM nailing. The average time from admission to operation was 2.1 days, with a median of 1 day (range: 0 to 27 days) and 79% of the patients being operated within 48 hours from admission.

Table 3.3 Fracture characteristics of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Fracture characteristics	
Side	Right: 266 fractures (47.4%) Left: 295 fractures (52.6%)
Open fracture	7 pts
Operation details	
Simultaneous procedures	37 pts
Time from injury to operation	2.1 ± 2.5 days (1 day; 0 to 27 days) 444 fractures (79%) operated within 48 hours from injury
Type of nail	Long Affixus Nail: 319 fractures Long Gamma Nail: 198 fractures Versanail: 18 fractures Long PFNA: 13 fractures T2 Recon Nail: 11 fractures Expert LFN: 1 fracture Short Gamma Nail: 1 fracture
Lag screw angle	120 degrees: 7 fractures 125 degrees: 314 fractures 130 degrees: 215 fractures 135 degrees: 1 fracture
End Cup	Used in 404 nails
Augmentation	Only used in 6 fractures / pts (Hydroset: 3 pts, Vitoss: 2 pts; BMP-2 sponge: 1 pt)
Open reduction	265 fractures
Cerclage wires	65 fractures
Post-operative mobilisation	FWB: 307 fractures PWB: 122 fractures TTWB: 73 fractures NWB: 59 fractures

Results are presented as: Mean ± SD (Median; Range)
FWB: full weight bearing
PWB: partial weight bearing
TTWB: toe touch weight bearing
NWB: no weight bearing

The commonest type of nail used was a 'long Affixus nail' followed by 'long Gamma nail' (both are cephalomedulary nails), whilst the commonest angle for the lag screw was 125 degrees. Regarding the dimensions of the nail, 380mm (milimeter) was the commonest length in women compared to 400mm in men, and the commonest diameter used was 11mm (**Figure 3.9**, **Figure 3.10**, **Figure 3.11**). Moreover, a total of 265 fractures (47%) required an open reduction to achieve good reduction, with 65 fractures having at least one cerclage wire / cable to hold reduction. Augmentation was only used in six fractures, as this was deemed appropriate by the operating surgeon. Following the operation, only 55% of the patients were allowed to FWB with the remaining patients advised to protect their WB.

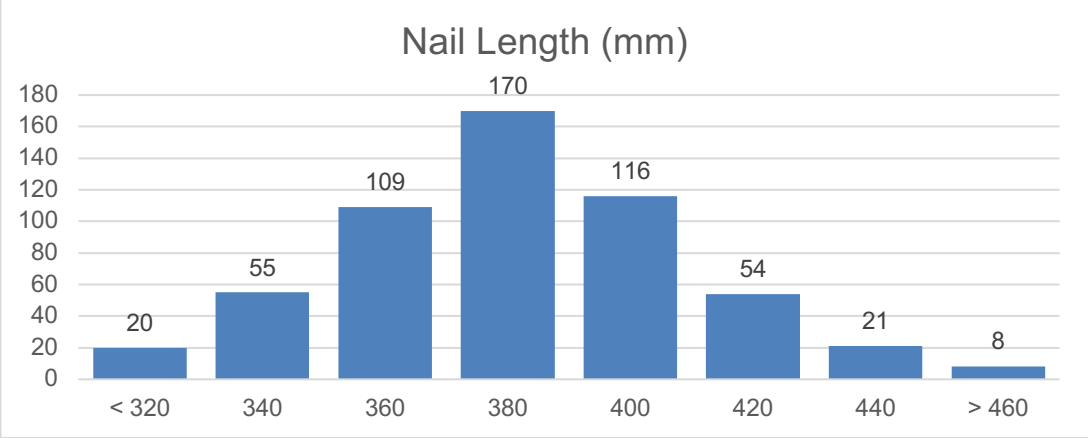


Figure 3.9 Nail length distribution of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region (short nails were not included)

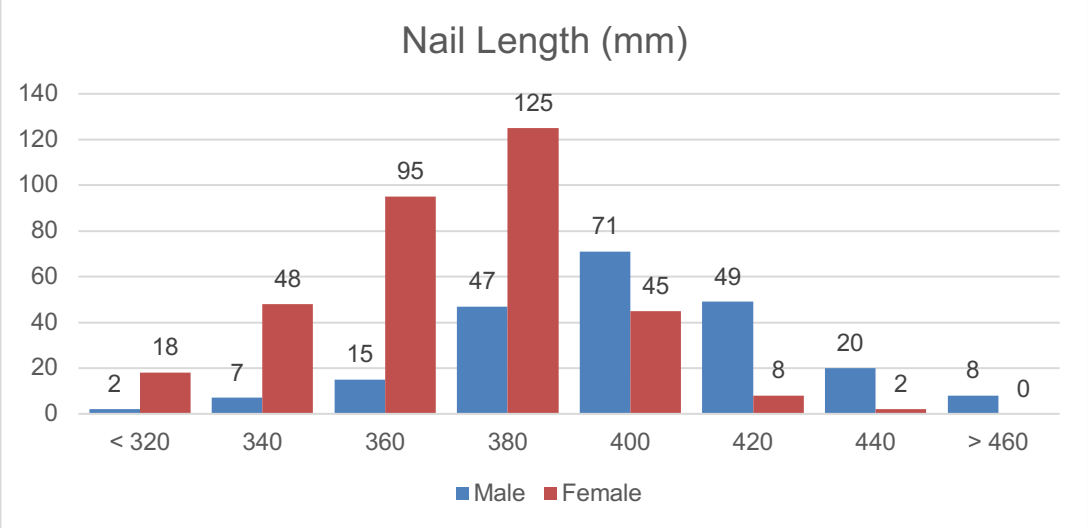


Figure 3.10 Nail length distribution (short nails not included) of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, according to gender

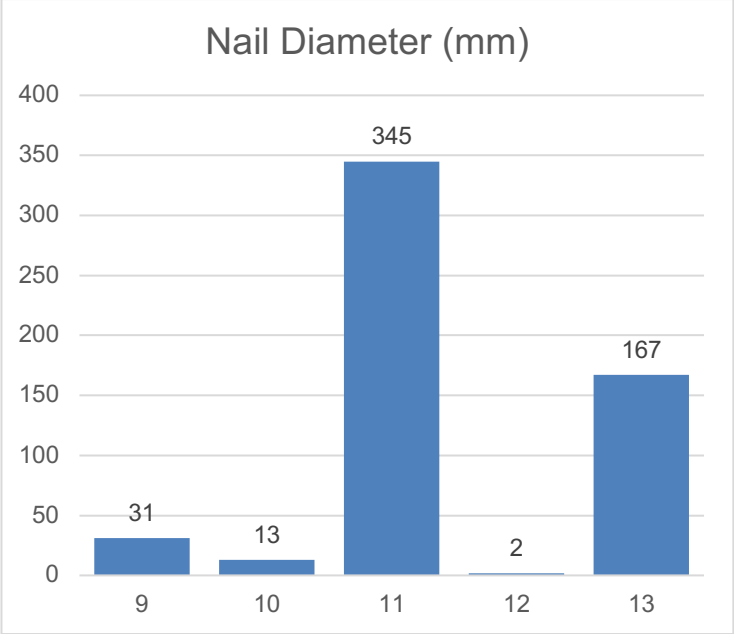


Figure 3.11 Nail diameter of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Surgical time was on average 111.6 minutes, with an additional 48.5 minutes anaesthetic time (**Table 3.4**). A consultant was the operating surgeon in 41% of the cases, whilst a consultant was present in theatres in 45% of the cases.

Table 3.4 Operation characteristics of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Operation characteristics	
Surgical Time (skin to skin)	111.6 ± 45.0 min (102.7 min; 19.9 to 338.9 min)
Anaesthetic Time	48.5 ± 21.6 min (46.8 min; 10.0 to 250.0 min)
Total time (Induction to recovery)	178.8 ± 49.9 min (171.8 min; 75.0 to 405.4 min)
Grade of operating surgeon	Consultant: 226 patients Registrar: 332 patients
Grade of senior surgeon present	Consultant: 252 patients Registrar: 306 patients

Results are presented as: Mean ± SD (Median; Range)

The radiographic characteristics of the cohort before and after the operation are demonstrated in **Table 3.5**. Interestingly, there was a wide variation in fracture classifications with all the classification systems used.

Table 3.5 Radiographic investigations of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Femoral neck angle (contralateral site)	
All patients	135.0 ± 5.9 degrees (135 degrees; 120 to 150 degrees)
	Coxa Valga: 146 patients (26.5%) Normal: 366 patients (66.5%) Coxa Vara: 38 patients (6.9%)
Male	135.3 ± 5.9 degrees (135 degrees; 120 to 150 degrees)
Female	134.8 ± 5.9 degrees (135 degrees; 121 to 150 degrees)
Fracture configuration	
No of fragments	2 fragments: 163 patients 3 fragments: 271 patients 4 fragments: 99 patients >4 fragments: 24 patients
Comminution	No comminution: 163 patients Moderate comminution: 273 patients Severe comminution: 123 patients
Main fracture line	Proximal-medial to distal-lateral: 151 Spiral: 125 Proximal-lateral to distal-medial: 94 Intertrochanteric: 86 Transverse: 47 Proximal-anterior to distal-posterior: 26 Proximal-posterior to distal-anterior: 25
Fracture configuration	
Special features*	Only subtrochanteric involvement: 99 Atypical: 24 Pathological: 30 Periprosthetic: 5 Distal extension: 186 Greater trochanter involvement: 59 Lesser trochanter involvement: 359 Medial Calcar comminution: 31
Fracture classification	
AO / OTA classification*	31-A1.1: 2 patients 31-A1.2: 2 patients 31-A1.3: 12 patients 31-A2.2: 58 patients 31-A2.3: 14 patients 31-A3.1: 26 patients 31-A3.2: 7 patients 31-A3.3: 47 patients 32-A1a: 46 patients 32-A2a: 66 patients 32-A2b: 1 patient 32-A3a: 55 patients 32-A3b: 1 patient 32-B2a: 165 patients 32-B3a: 20 patients 32-B3b: 1 patient 32-C2a: 23 patients 32-C3a: 13 patients

Fracture classification	
Seinsheimer Classification*	IIA: 43 patients IIB: 84 patients IIC: 51 patients IIIA: 199 patients IIIB: 3 patients IV: 44 patients V: 135 patients
Russel Taylor Classification*	IA 174 patients IB 168 patients IIA 27 patients IIB 190 patients
Radiographic measurements	
Lateral Cortex Gap Size (mm)	≤4 343 patients 5-9 142 patients ≥10 73 patients
Medial Cortex Gap Size (mm)	≤4 371 patients 5-9 134 patients ≥10 53 patients
Anterior Cortex Gap Size (mm)	≤4 355 patients 5-9 123 patients ≥10 81 patients
Posterior Cortex Gap Size (mm)	0 427 patients 5-9 100 patients ≥10 32 patients
Distraction / Shortening (mm)	<0 74 patients 0-4 380 patients 5-9 46 patients ≥10 58 patients
Reduction Angle (degrees)	Valgus ≥6 38 patients Valgus 0-5 287 patients Varus 1-5 117 patients Varus 5-10 88 patients Varus >10 28 patients
Antirotation screw	213 patients (versus 337 patients)
TAD (mm)	5-9 23 patients 10-14 132 patients 15-19 204 patients 20-24 115 patients 25-29 50 patients ≥30 22 patients
No of distal locking screws	1 18 patients 2 542 patients
Method of distal locking	Dynamic locking: 3 Secondary dynamisation: 196 Static locking: 356
Distance of tip of the nail from centre (AP) (mm)	Lateral ≥5 93 patients -4 to 4 355 patients Medial ≥5 105 patients
Distance of tip of the nail from centre (LAT) (mm)	Anterior ≥5 106 patients -4 to 4 434 patients Posterior ≥5 15 patients

Radiographic measurements	
Touching Anterior Cortex?	Yes 137 patients No 417 patients
Nail /canal ratio (AP)	<0.70 29 patients 0.70-0.79 69 patients 0.80-0.89 202 patients ≥0.90 178 patients
Nail /canal ratio (LAT)	<0.70 72 patients 0.70-0.79 138 patients 0.80-0.89 190 patients ≥0.90 69 patients
Nail /canal ratio (average)	<0.70 33 patients 0.70-0.79 108 patients 0.80-0.89 256 patients ≥0.90 85 patients

*X-Rays not available in two patients
Results are presented as: Mean ± SD (Median; Range)

The biochemistry investigations are presented in **Table 3.6**. Additionally, in 75 patients (13.4%), histology samples were sent as a malignancy was suspected (**Table 3.7**). Of those patient, in only 22 (29.3%) a malignancy was confirmed; all lesions were metastatic.

Table 3.6 Biochemistry investigations of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Biochemistry investigations	
Pre-operative Urea	7.6 ± 4.1 mmol/L (6.8 mmol/L; 1.0 to 41.2 mmol/L)
Post-operative Urea	7.6 ± 4.3 mmol/L (6.5 mmol/L; 1.3 to 30.5 mmol/L)
Change in Urea (Post-operative - Pre-operative)	0.0 ± 3.5 mmol/L (-0.2 mmol/L; -26.0 to 13.5 mmol/L)
Pre-operative Creatinine	86.6 ± 48.8 umol/L (78.0 umol/L; 24.0 to 569.0 umol/L)
Post-operative Creatinine	84.9 ± 47.8 umol/L (73.0 umol/L; 24.0 to 472.0 umol/L)
Change in Creatinine (Post-operative - Pre-operative)	-1.8 ± 33.2 umol/L (-3.0 umol/L; -309.0 to 224.0 umol/L)
eGFR (Pre-operative)	84.0 ± 40.9 mL/min/1.73m^2 (76.5 mL/min/1.73m^2; 9.8 to 354.0 mL/min/1.73m^2)
eGFR (Post-operative)	87.4 ± 43.0 mL/min/1.73m^2 (80.9 mL/min/1.73m^2; 8.2 to 371.1 mL/min/1.73m^2)
Adjusted Calcium	High: 7 (1.5%) Normal: 341 (74.5%) Low: 110 (24.0%)

Biochemistry investigations		
Albumin	High:	1 (0.2%)
	Normal:	157 (31.3%)
	Low:	344 (68.5%)
Alkaline Phosphatase	High:	96 (19.2%)
	Normal:	358 (71.7%)
	Low:	45 (9.0%)
Phosphate	High:	25 (5.4%)
	Normal:	348 (75.7%)
	Low:	87 (18.9%)
TSH	High:	31 (11.0%)
	Normal:	245 (87.2%)
	Low:	5 (1.8%)
Free T4	High:	39 (14.2%)
	Normal:	229 (83.6%)
	Low:	6 (2.2%)
PTH	High:	130 (51.4%)
	Normal:	123 (48.6%)
25OH Vitamin D	Normal:	35 (12.6%)
	Low:	242 (87.4%)

Results are presented as: Mean ± SD (Median; Range)
eGFR: estimated Glomerular Filtration Rate
T4: thyroxine
TSH: thyroid stimulating hormone

Table 3.7 Histological results confirming presence of metastatic bone lesions in patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Commonest types of malignancy confirmed by histology		
N	22 patients	
Primary	Breast carcinoma:	8 patients
	Lung carcinoma:	7 patients
	Multiple myeloma:	3 patients
	Prostate carcinoma:	2 patients
	Oesophageal carcinoma:	1 patient
	Renal carcinoma:	1 patient

A total of 100 fractures were associated with nail related complications (some patients presented with more than one complication), where self-dynamisation and failure at lag screw junction were the commonest, and both of which were associated with an impaired healing (**Table 3.8**). Cut-out was evident in 13 patients and peri-implant fractures in 14 patients, of which only one happened intra-operatively.

Systemic infection was a quite common complication during hospitalisation (33%), with pneumonia being the commonest, followed-up by UTI. The incidence of wound infections was 6%, but in only 3% (15 patients) the infection involved the deep tissues and the metalwork. One or more washout was performed in 14 of those patients, whilst seven underwent revision procedures.

Analysing the medical complications, it was observed that the commonest complication was delirium (10%), AKI (acute kidney injury – 8%) and venous thromboembolism – VTE (MI / CVA: 4%; DVT / PE: 4%) (**Table 3.8**, **Table 3.9**). Pre- and post-operative renal function is presented in **Figure 3.12**.

Table 3.8 Complications of primary procedure of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

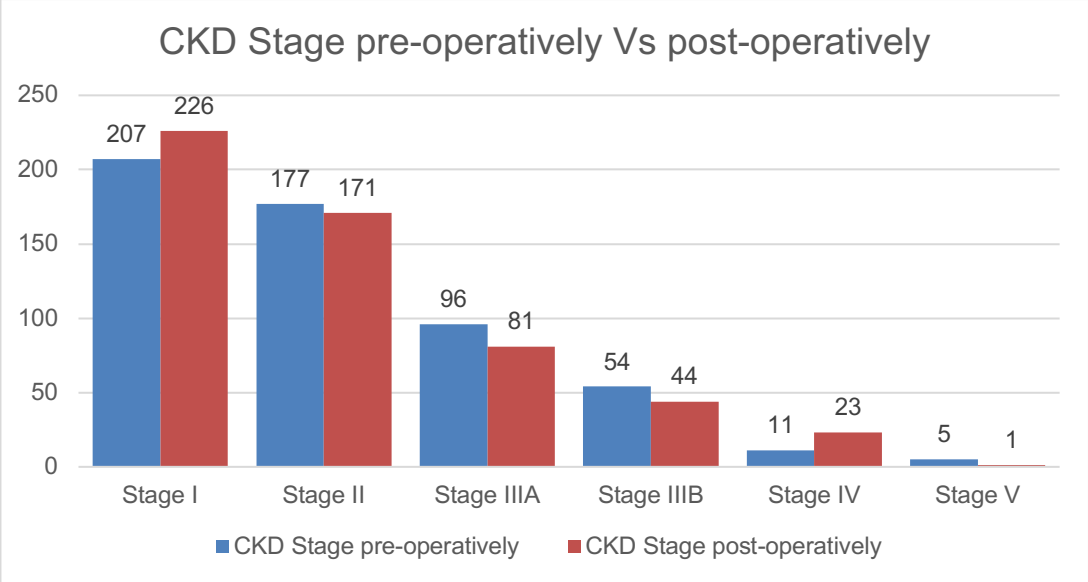
Nail complications	
Number	100 fractures
Failure at lag-screw junction	24 fractures
Self-dynamisation	25 fractures
Cut-out	13 pts
Nail infection	5 pts
Peri-implant fractures	14 pts (one intra-operatively)
Infections	
Systemic infections	187 pts
HAP / CAP	106 pts
UTI	78 pts
Wound infection	Superficial: 21 pts Deep: 15 pts (14 had one or more washouts; 7 were revised for infection)
Medical complications	
CVA / MI	23 pts
AKI	45 pts
Post-operative delirium	56 pts
Bleeding complications	30 pts

CAP: Community Acquired Pneumonia
HAP: hospital acquired pneumonia

Table 3.9 Investigations and confirmed diagnosis of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Thromboembolic events	
DVT	12 patients
PE	10 patients
Negative screen	107 patients

DVT: deep venous thrombosis
PE: pulmonary embolism



CKD: Chronic Kidney Disease

Figure 3.12 Presence of pre- and post- operative CKD in patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Overall, the average length of hospital stay was 22.5 days (median: 18 days; SD: 18.6 days), with 48 patients transferred to the high dependency unit (HDU) for a median of 4 days and 25 patients transferred to the intensive care unit (ICU) for a median of 3 days (**Table 3.10**). The length of stay increased with age, whilst in the younger population increased length of stay was associated with polytrauma (ISS > 16) and a higher incidence of ICU / HDU stay. Of the patients with prolonged hospital stay (over 28 days), 16 patients were aged less than 65 y.o. while 53 patients aged greater than 85 y.o..

To investigate the effect of age to hospital length of stay further (**Figure 3.13**), the following cohorts were used:

- the young (younger than 65 years of age);
- the young-old (65 to 74 years of age),
- the middle-old (75 to 84 years of age),
- the old-old group (older than 85 years of age) (354).

Table 3.10 Length of hospital stay of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Length of hospital stay	
Total length of stay	22.5 ± 18.6 days (18 days; 1 to 193 days)
ICU stay*	8.0 ± 22.3 days (3 days; 0 to 114 days)
HDU stay*	3.8 ± 2.9 days (4 days; 0 to 16 days)

*0 days = less than 24 hours of stay
Results are presented as: Mean ± SD (Median; Range)

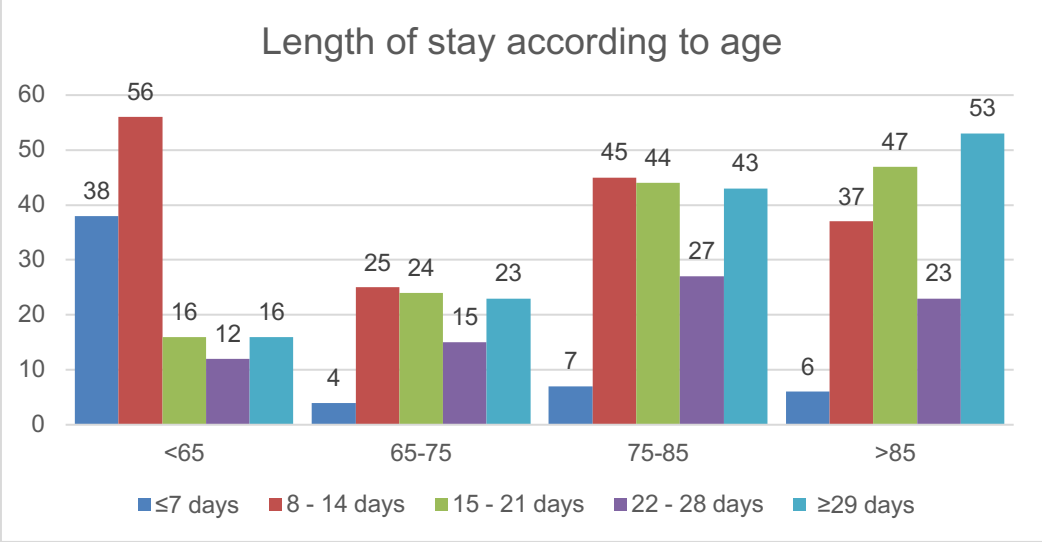


Figure 3.13 Length of hospital stay of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, stratified according to age

With regards to the outcome of the primary procedure, 110 fractures did not have any follow-up, 47 fractures had incomplete follow-up and 19 fractures were followed-up in other institutions (living in other geographic areas) (**Table 3.11**). Of the remaining 382 fractures, 39 patients died before the fracture was fully healed. Therefore, a total of 342 fractures had a complete follow-up (until radiological healing or at least 9 months post injury). Non-union was evident in 84 patients (24.6%). Of the 63 patients that underwent a revision operation, 41 progressed to union, 11 eventually ended with a THR, six failed to unite after revision surgery and four died (**Table 3.12**). The median follow-up of the revised cases was 38 months.

Table 3.11 Outcome of primary procedure of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Outcome of primary procedure	
Incomplete follow-up	No follow-up: 110 fractures Incomplete follow-up: 47 fractures Follow-up in other Hospitals: 19 pts
Patient deceased	As an inpatient: 35 pts Before completion of follow-up: 4 pts
Cut-out	4 fractures (revised)
Peri-implant fracture	1 fracture (revised)
Union	257 / 341 (75.4%) fractures
Non-union	84 / 341 (24.6%) fractures

Table 3.12 Final outcome of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, undergoing revision surgery

Final outcome of revised cases	
Number	63 fractures
Inadequate follow-up	1 fracture
Deceased before union	4 pts
Non-union	6 fractures
Union	41 fractures (nail removed in 2 fractures)
THR	11 fractures
Length of follow-up (revised patients)	45.9 ± 33.3 months (38.1 months; 1.4 to 116.8 months)
Length of follow-up (all patients)	24.4 ± 26.8 months (13.1 months; 0.3 to142.6 months)

Results are presented as: Mean ± SD (Median; Range)

Before the index injury, 123 patients sustained a fragility fracture, with most common location being the contralateral hip (33%) and the wrist (33%) (**Figure 3.14**). After the index injury, 85 patients sustained a fragility fracture, with the most common location being the contralateral hip (25%), the wrist (22%) and the shoulder (22%) (**Figure 3.15**). The quality of the bone density and therefore presence of osteoporosis was assessed with the Singh index (**Table 3.13**). Out of the 501 patients who had adequate radiographs and did not have an implant to the contralateral side, 263 (52%) had a score of 3 or less, and therefore osteoporosis. Additionally, 50 patients had DEXA scans within 12 months from the injury (pre- and post- injury), with 13 patients being diagnosed with osteopenia and 27 with osteoporosis.

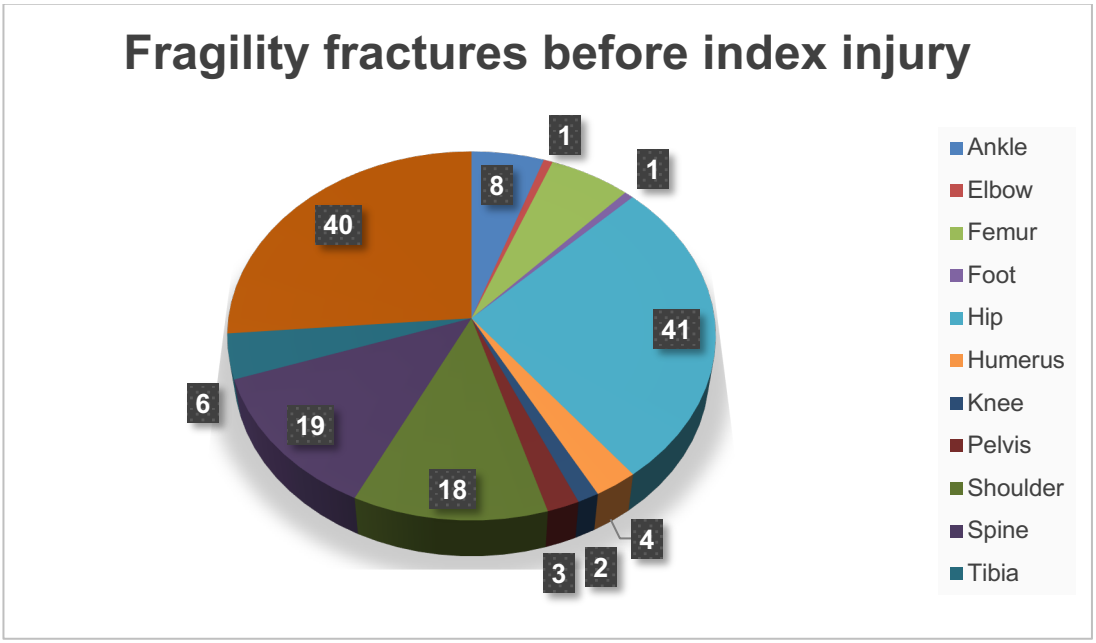


Figure 3.14 Location of fragility fractures before the index injury

(123 patients in total; 19 patients sustained more than one fracture)

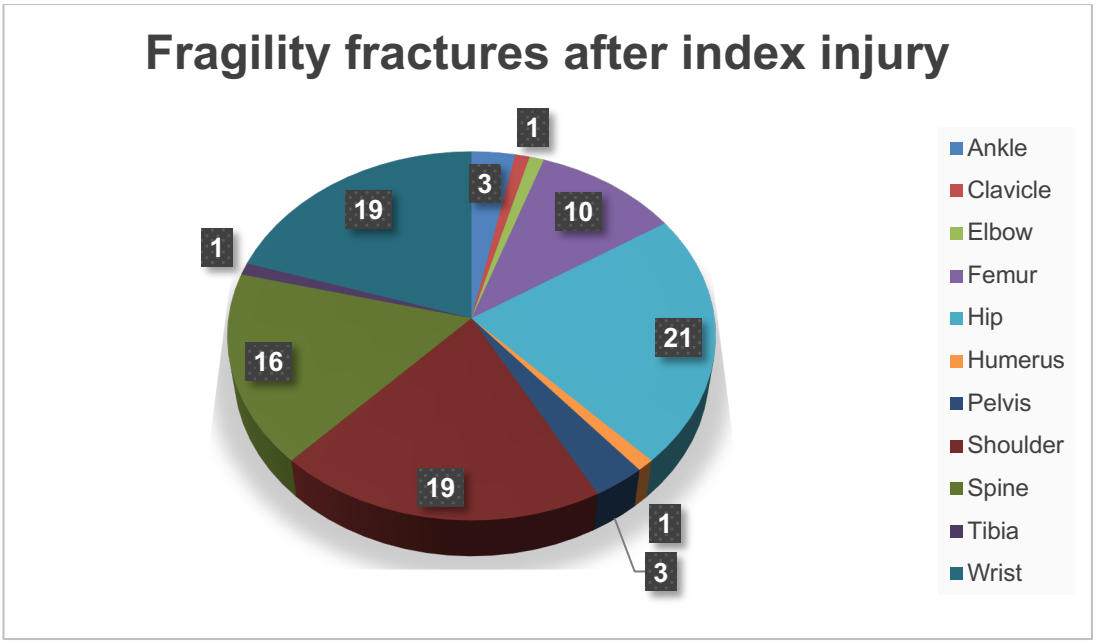


Figure 3.15 Location of fragility fractures before the index injury

(85 patients in total; nine patients sustained more than one fracture)

Table 3.13 Investigations for osteoporosis of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Singh index	
N	501 patients
All patients	3.4 ± 1.5 (3.0; 1.0 to 6.0)
Patients who had DEXA scans	2.6 ± 1.2 (2.0; 1.0 to 6.0)
DEXA scan	
N	97 patients
Outcome	Normal: 8 patients Osteopenia: 13 patients Osteoporosis: 27 patients Out of range*: 47 patients
BMD Hip	0.7 ± 0.2 (0.7; 0.4 to 1.1)
T-Score Hip	-2.4 ± 1.3 (-2.6;-6.5 to 0.5)
Z-Score Hip	-1.1 ± 1.3 (-1.1; -5.2 to 1.5)
BMD Spine	1.0 ± 0.2 (1.0; 0.6 to 1.5)
T-Score Spine	-1.8 ± 1.6 (-1.9; -4.8 to 2.1)
Z-Score Spine	-0.6 ± 1.6 (-0.9; -3.4 to 4.0)
Time of surgery to DEXA	2.5 ± 5.0 months (2.6 months; -10.2 to 10.4 months)

*Out of range: patients had their DEXA scan more than 12 months before or after the index injury

Results are presented as: Mean ± SD (Median; Range)

3.9.2 Non-unions

As mentioned in the previous section, 341 fractures had a complete follow-up (until radiological union or diagnosis of non-union), with 84 fractures (24.6%) failing to unite (**Table 3.14**). Atrophic non-unions were the commonest (66 fractures; 78.6%), followed by hypertrophic non-unions (12 fractures; 14.3%), and septic non-unions (6 fractures; 7.1%) (**Table 3.15**). Even though some fracture classifications as per AO had a relatively higher incidence of non-union, there was no significant difference between the different fracture patterns (**Figure 3.16**). Thirty-two patients did not have any additional procedures to achieve union, either because they declined further treatment, deemed unfit to have further operations or died before the revision operation. The remaining 52 patients were revised, with union achieved with only one procedure in 67.3% of the patients, two procedures in 19.2% of the patients and three or more procedures in 7.7% of the patients (**Table 3.14**). The remaining 5.8% of the patients had a THR.

Table 3.14 Non-union characteristics of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Non-union characteristics	
Number	84 / 341 fractures (24.6%)
Atrophic non-unions	66 fractures (78.6%)
Additional procedures to achieve union	1 procedure: 35 fractures 2 procedures: 10 fractures 3 or more procedures: 4 fractures THR: 3 fractures
Revision for non-union	52 fractures

Table 3.15 mRUS values of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

mRUS	
Unions	10.3 ± 1.2 (10; 8 to 12)
Non-unions	Atrophic: 7.7 ± 2.6 (8; 4 to 12) Hypertrophic: 7.9 ± 2.0 (8; 4 to 12)

Results are presented as: Mean ± SD (Median; Range)
mRUS: modified Radiographic Union Score

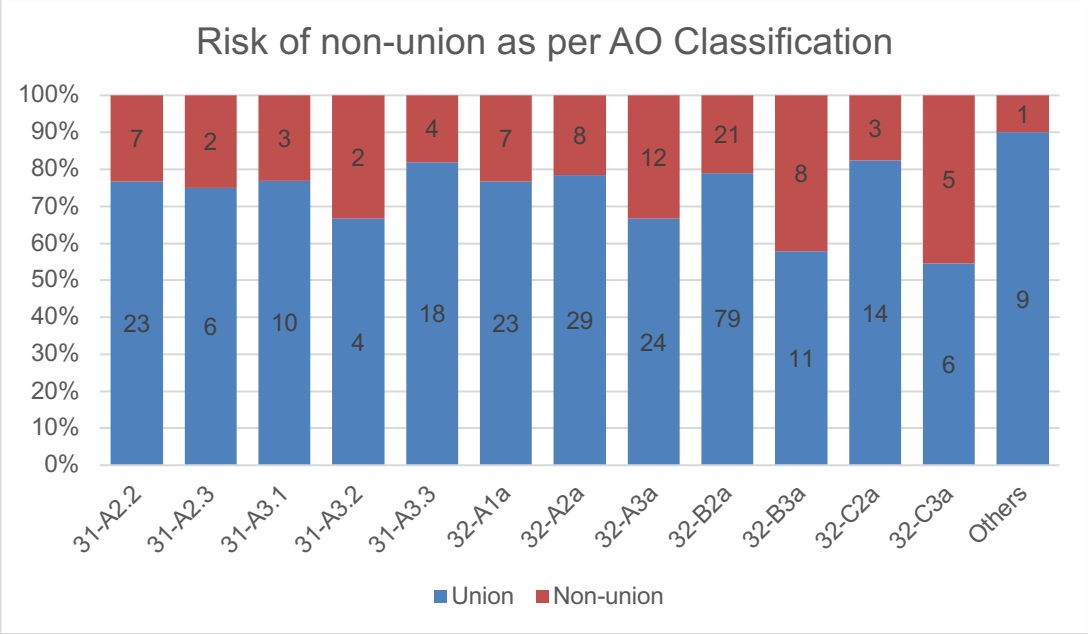


Figure 3.16 Incidence of non-union of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, according to the AO classification

Comparing the patients according to progression to union or not, it was observed that demographics and injury characteristics did not significantly affect healing (**Appendix C; Table C.1**). Of note is that from all medical comorbidities examined, only dementia was found to have a 'protective' effect (p=0.038), whilst there was a trend for impaired healing in patients with a history of diabetes (p=0.073). Pre-admission and post-discharge medication (bisphosphonates, calcium and vitamin D) did not seem to contribute to better healing. From the social history of the patients, smoking and high alcohol intake did not affect outcomes. Operation characteristics including timing of the operation, type of implant used, open reduction and use of cerclage wires / cables, grade of operating surgeon and length of procedure also did not seem to affect outcomes. As one would expect, patients with non-unions had a higher risk of complications including nail complications (p<0.001), failure at the lag screw junction (p<0.001), self-dynamisation (p<0.001), cut-out (p=0.036) and wound related infections (p<0.001). With regards to the fracture characteristics, presence of comminution (p<0.001), atypical fractures (p=0.002) and malreduction as demonstrated by an increased medial (p=0.004), lateral (p<0.001) and posterior (p<0.001) fracture gap, as well as neck-shaft malreduction (p=0.001), seemed to be associated with a higher risk of non-union. Finally, patients with a non-union had a higher risk of requiring a higher level of care (p=0.040), even though length of stay was not different.

Regression analysis identified a total of six factors contributing to the development of a non-union, with another factor having a protective role (**Table 3.16**). Deep infection was the most important factor (OR 17.512), followed by self-dynamisation (failure of the nail distal locking screws) (OR 13.894). Presence of an atypical fracture (OR 3.374) and diabetes (OR 1.992) was also significant in the development of a non-union. Finally, malreduction, as demonstrated by a lateral cortex fracture gap size (OR 2.998) and varus malalignment (Varus 5 – 10 degrees: OR 1.865; Varus >10 degrees: OR 3.471) was also strongly associated with the development of a non-union. On the other hand, moderate comminution (as opposed to single 2-part fracture or multi-segmented fracture) seemed to have a protective effect (OR 0.353).

As self-dynamisation was generally observed at a later stage and in order to be able to utilise the scoring system at the early post-operative phase (i.e. within four weeks from operation), a second regression analysis was performed excluding this factor (**Table 3.17)**. According to this analysis, the effect of diabetes was not significant anymore. Deep infection remained the most important factor (OR 13.044), followed by the presence of an atypical fracture (OR 3.153). Lateral cortex fracture gap size (OR 2.578) and varus malalignment (Varus 5 – 10 degrees: OR 1.946; Varus >10 degrees: OR 3.096) remained strongly associated with the development of a non-union. Finally, moderate comminution (as opposed to single 2-part fracture or multi-segmented fracture) continued having a protective effect (OR 0.377).

Table 3.16 Table A presenting the coefficients and table B presenting the odds ratio estimates of factors associated with progression to non-union, including self-dynamisation

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.1681	-0.6571	-0.4801	-0.2905	2.5244

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.103	0.290	-7.251	<0.001 ***
Diabetes	0.689	0.389	1.771	0.077 .
Self-dynamisation	2.632	0.535	4.915	<0.001 ***
Wound infection (Deep)	2.863	0.735	3.893	<0.001 ***
Degree of comminution (Moderate)	-1.042	0.329	-3.165	0.002 **
Atypical	1.216	0.478	2.543	0.011 *
Lateral Cortex Gap Size (≥5 mm)	1.098	0.317	3.459	<0.001 ***
Reduction Angle (Varus 5 – 10 degrees)	0.623	0.371	1.682	0.093 .
Reduction Angle (Varus >10 degrees)	1.245	0.541	2.299	0.022 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 375.10 on 338 degrees of freedom

Residual deviance: 284.59 on 330 degrees of freedom

(2 observations deleted due to missingness)

AIC: 302.59

Number of Fisher Scoring iterations: 5

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.122	0.069	0.216	<0.001
Diabetes	1.992	0.929	4.272	0.077
Self-dynamisation	13.894	4.866	39.677	<0.001
Wound infection (Deep)	17.512	4.144	74.002	<0.001
Degree of comminution (Moderate)	0.353	0.185	0.673	0.002
Atypical	3.374	1.321	8.616	0.011
Lateral Cortex Gap Size (≥5 mm)	2.998	1.609	5.584	0.001
Reduction Angle (Varus 5 – 10 degrees)	1.865	0.902	3.855	0.093
Reduction Angle (Varus >10 degrees)	3.471	1.201	10.029	0.022

Table 3.17 Table A presenting the coefficients and table B presenting the odds ratio estimates of factors associated with progression to non-union, excluding self-dynamisation

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.1681	-0.6571	-0.4801	-0.2905	2.5244

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.671	0.250	-6.694	<0.001 ***
Wound infection (Deep)	2.568	0.714	3.599	<0.001 ***
Degree of comminution (Moderate)	-0.976	0.307	-3.178	0.001 **
Atypical	1.148	0.456	2.519	0.012 *
Lateral Cortex Gap Size (≥5 mm)	0.947	0.293	3.228	0.001 **
Reduction Angle (Varus 5 – 10 degrees)	0.666	0.344	1.936	0.053 .
Reduction Angle (Varus >10 degrees)	1.130	0.537	2.106	0.035 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 375.10 on 338 degrees of freedom

Residual deviance: 284.59 on 330 degrees of freedom

(2 observations deleted due to missingness)

AIC: 302.59

Number of Fisher Scoring iterations: 5

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.188	0.115	0.307	<0.001
Wound infection (Deep)	13.044	3.221	52.826	<0.001
Degree of comminution (Moderate)	0.377	0.207	0.688	0.001
Atypical	3.153	1.290	7.704	0.012
Lateral Cortex Gap Size (≥5 mm)	2.578	1.451	4.581	0.001
Reduction Angle (Varus 5 – 10 degrees)	1.946	0.992	3.819	0.053
Reduction Angle (Varus >10 degrees)	3.096	1.082	8.863	0.035

3.9.3 Non-union scoring system

Having established the risk factors for non-union of subtrochanteric fractures, the coefficients were used to produce a scoring system by appropriate scaling and rounding, with a maximum of 100 points (**Table 3.18**). To test the possibility of early identification of the patients in risk, a second scoring system was developed, excluding self-dynamisation (**Table 3.19**). To test the validity of the scoring systems, ROC curves were produced and the area under the curve (AUC) was calculated (**Figure 3.17**; **Figure 3.18**). When self-dynamisation was included in the scoring system, AUC was 0.770, compared to 0.766 when self-dynamisation was excluded.

To internally validate each scoring system, the corresponding accuracy was calculated using a repeated 5-fold cross validation model. When self-dynamisation was included in the scoring system, the overall accuracy was 0.798, compared to 0.776 when self-dynamisation was excluded.

Table 3.18 Non-union scoring system, including self-dynamisation

Risk factors	Coefficients	Scoring
Diabetes	0.689	8
Self-dynamisation	2.632	30
Wound infection (Deep)	2.863	33
Degree of comminution (Moderate)	-1.042	-12
Atypical	1.216	14
Lateral Cortex Gap Size (≥5 mm)	1.098	13
Reduction Angle (Varus 5 – 10 degrees)	0.623	7
Reduction Angle (Varus >10 degrees)	1.245	14

Table 3.19 Non-union scoring system, excluding self-dynamisation

Risk factors	Coefficients	Scoring
Wound infection (Deep)	2.568	53
Degree of comminution (Moderate)	-0.976	-20
Atypical	1.148	24
Lateral Cortex Gap Size (≥5 mm)	0.947	20
Reduction Angle (Varus 5 – 10 degrees)	0.666	14
Reduction Angle (Varus >10 degrees)	1.130	23

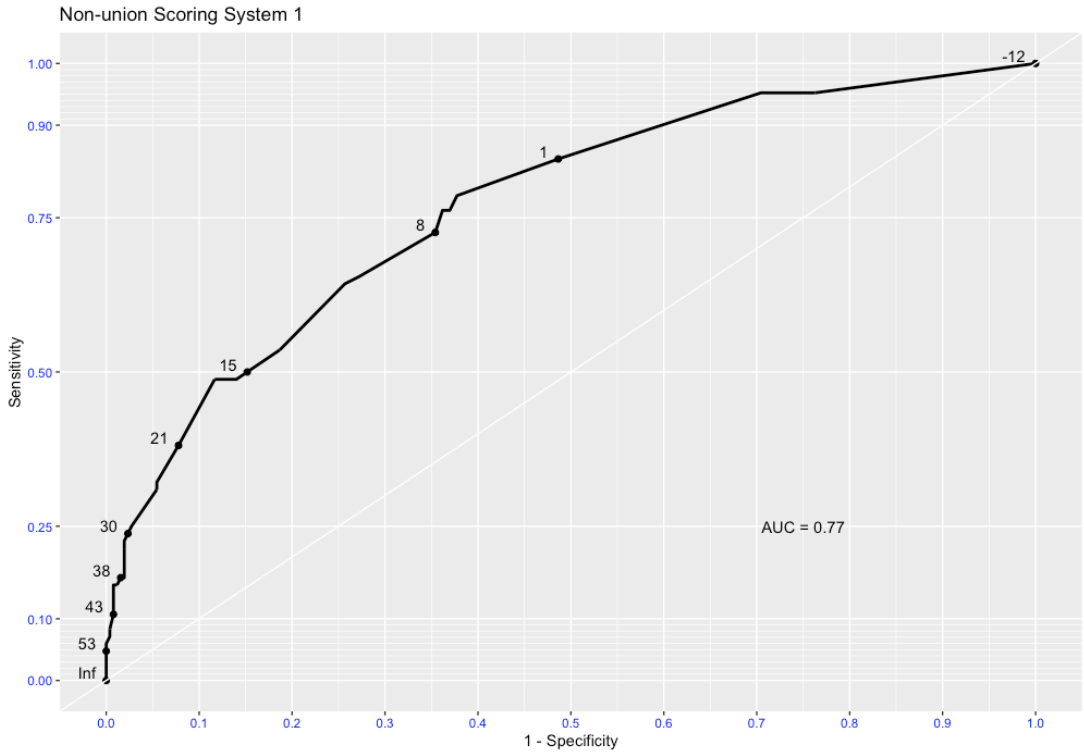


Figure 3.17 ROC curve of non-union scoring system, including self-dynamisation AUC (Area under the curve) = 0.770

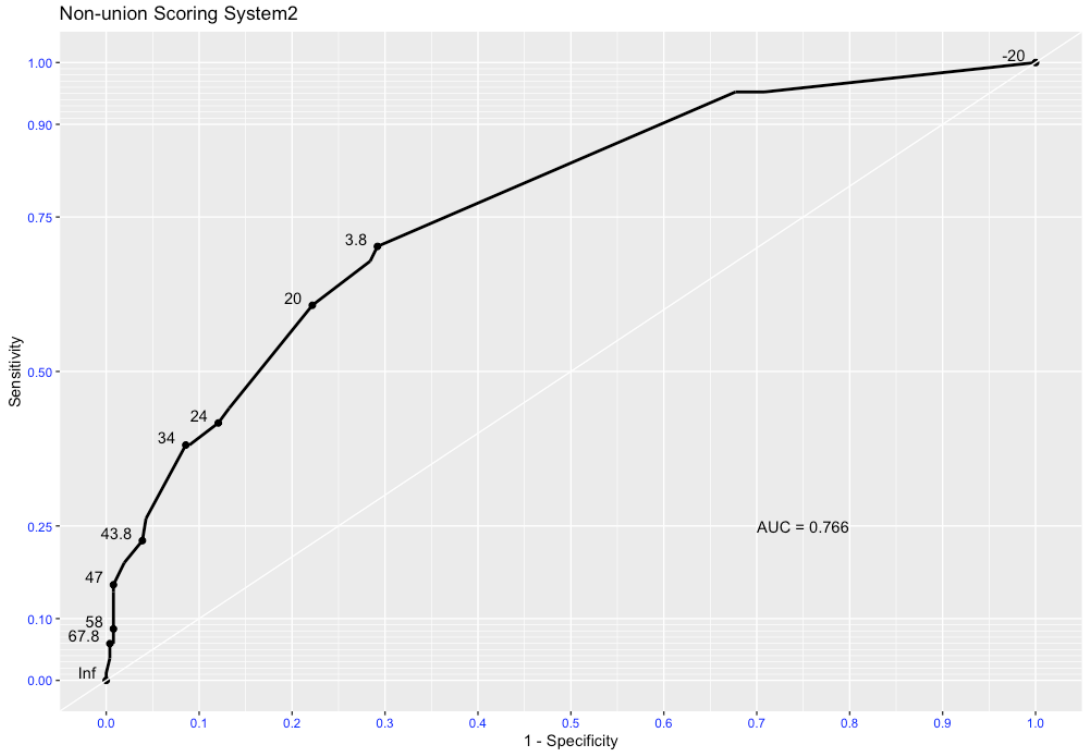


Figure 3.18 ROC curve of non-union scoring system, excluding self-dynamisation AUC (Area under the curve) = 0.766

Subsequently, all patients were scored and the cumulative non-union risk was calculated (**Table 3.20**; **Table 3.21**). Comparing the two groups (union versus non-union), a statistical significance in the mean score per group was evident ($p<0.001$). Overall, in the scoring system including self-dynamisation, 20 out of 34 patients with a score higher than 30 points was diagnosed with a non-union (59%), compared to 64 out of 311 patients having a score of less than 30 points (21%). In the scoring system excluding self-dynamisation, 32 out of 55 patients with a score higher than 30 points was diagnosed with a non-union (58%), compared to 53 out of 286 patients having a score of less than 30 points (19%).

Table 3.20 Number of patients and risk of non-union according to scoring, including self-dynamisation

Score	Union	Non-union	Total	Non-union Risk
-12 – 0	76	4	80	5%
0 – 10	115	26	141	18%
10 – 20	35	14	49	29%
20 – 30	24	21	45	47%
30 – 40	3	7	10	70%
40 – 50	3	8	11	73%
> 50	0	5	5	100%

Table 3.21 Number of patients and risk of non-union according to scoring, excluding self-dynamisation

Score	Union	Non-union	Total	Non-union Risk
-20 – 0	83	4	87	5%
0 – 10	101	23	124	19%
10 – 20	16	6	22	27%
20 – 30	33	20	53	38%
30 – 40	13	13	26	50%
40 – 50	8	7	15	47%
> 50	2	12	14	86%

3.9.4 Infections

The incidence of infection was 6.4%, with 21 patients (3.7%) being diagnosed with a superficial infection, compared to 2.7% being diagnosed with a deep infection (**Table 3.22**). The commonest micro-organism isolated in superficial wound infections (wound swap cultures) was *Staphylococcus aureus*, followed by enteric flora and mixed skin flora. In deep infections, the commonest organisms were *Coliforms*, followed up by *Staphylococcus aureus* and *Escherichia coli*. Interestingly, more than one organisms were present in five patients with superficial infections (5/13: 38.5%) and twelve patients with deep infections (12/15: 80.0%). Finally, in three patients with

deep infections (3/15: 20.0%), no organism was isolated on conventional cultures (the diagnosis of infection was determined by clinical and laboratory findings).

Comparing the patients who had a deep infection with those who did not (regardless of whether a superficial infection was evident), several parameters were identified to contribute to an increased risk healing (**Appendix C; Table C.2**). Deep infections were more prevalent in open fractures (p=0.002), fractures requiring open reduction (p=0.021) and fractures requiring an increased surgical time (p=0.024), as well as fractures extending distally toward the femoral shaft (p=0.012). Regarding the risk of complications, patients having a deep infection had a higher risk of non-union (p<0.001), higher blood loss (p=0.016) and transfusion rate (total transfusion: p=0.016; transfusion within 48-hours post-operatively: p=0.029), increased length of stay (LOS: p<0.001) and need for escalation of care in HDU / ICU (p=0.029). Finally, patients with a history of increased alcohol intake had a tendency of a higher risk for a deep infection (p=0.071).

Progressing to a regressing analysis, the factors identified to be associated to an increased risk of deep infection were open fractures (OR 33.442) and need for massive transfusion (OR 14.191) or post-operative transfusion (OR 12.684) (**Table 3.23**). Moreover, patients presenting with a non-union seemed to have an increased risk of deep infection (OR 8.117), as did patients with a history of increased alcohol intake (OR 3.479) and an increased LOS (OR 1.041).

Table 3.22 Micro-organisms isolated from patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Superficial wound infections		
N	21 patients (13 had microbiology swaps)	
Organisms*	*Staphylococcus aureus*	6 patients
	Enteric flora	5 patients
	Mixed skin flora	5 patients
	Enteroccocus species	1 patient
	Gram -ve bacillus	1 patient
	Beta Haemolytic Streptococcus Group B	1 patient
	Polymicrobial	5 patients

Deep Infections		
N	15 patients (all patients had multiple tissue samples sent to microbiology lab)	
Organisms**	*Coliforms*	5 patients
	Staphylococcus aureus	4 patients
	Escherichia coli	4 patients
	Proteus	3 patients
	Staphylococcus epidermidis	1 patient
	Coagulase negative Staphylococcus	1 patient
	Pseudomonas aeruginosa	1 patient
	Gram -ve bacillus	1 patient
	Beta Haemolytic Dermabacter hominis	1 patient
	No growth	3 patients
	Polymicrobial	12 patients

*Organism isolated as a monomicrobial or a polymicrobial infection
**Organisms were isolated in more than one tissue cultures to be included

Table 3.23 Table A presenting the coefficients and table B presenting the odds ratio estimates of factors associated with development of a deep infection

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.0360	-0.1489	-0.0994	-0.0435	3.2846

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-8.458	1.526	-5.542	<0.001 ***
Alcohol (>10 units / week)	1.247	0.691	1.803	0.071 .
Open fracture	3.510	1.550	2.265	0.024 *
Post-operative Transfusion	2.540	1.277	1.989	0.047 *
Massive Transfusion	2.653	0.885	2.997	0.003 **
Total LOS	0.041	0.010	4.009	<0.001 ***
Non-union	2.094	0.675	3.101	0.002 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for binomial family taken to be 1)
Null deviance: 138.137 on 558 degrees of freedom
Residual deviance: 80.171 on 552 degrees of freedom
(2 observations deleted due to missingness)
AIC: 94.171
Number of Fisher Scoring iterations: 8

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.000	0.000	0.004	<0.001
Alcohol (>10 units / week)	3.479	0.897	13.486	0.071
Open fracture	33.442	1.603	697.500	0.024
Post-operative Transfusion	12.684	1.037	155.105	0.047
Massive Transfusion	14.191	2.504	80.434	0.003
Total LOS	1.041	1.021	1.062	<0.001
Non-union	8.117	2.161	30.494	0.002

3.9.5 Open reduction

Overall, 47.2% of subtrochanteric fractures (265 patients) underwent an open reduction of their fracture to achieve satisfactory reduction and fixation. The mean age of the open reduction group was 70.48 ± 20 years (median 78 y.o.) and the mean ASA was 2.70 (median 3) (**Appendix C; Table C.3**). In contrast, the mean age and mean ASA of the closed reduction group was 75.31 ± 17.14 years (median 81 y.o.) and 2.75 ± 0.79 (median 3) respectively. Of the 36 polytrauma patients, 23 had an open reduction.

In the open reduction group, the time to operation was 1.93 ± 2.01 days, duration of operation was 126.67 ± 44.43 minutes, with a consultant as senior surgeon in 131 cases (23.4%), and a LOS of 21.89 ± 18.76 days. Within the closed reduction group, the time to operation was 2.25 ± 2.89 days, duration of operation was 97.38 ± 37.54 minutes, a consultant as senior surgeon in 123 cases (21.9%) and a LOS of 22.75 ± 19.34 days.

As illustrated in **Appendix C; Table C.3**, when comparing the demographic factors, injury characteristics, comorbidities and operative characteristics of subtrochanteric fractures requiring open reduction against those that had closed reduction, only age ($p=0.002$), gender ($p=0.001$), mechanism of injury ($p=0.002$), ASA ($p=0.030$), number of fracture fragments ($p=0.014$), distal fracture extension to diaphysis ($p<0.001$), duration of operation ($p<0.001$), time from induction to recovery ($p<0.001$) and grade of senior surgeon ($p=0.019$) were statistically different between the two groups. Although there was a variation between the open and closed reduction group in terms of AO / OTA classification, apart from the distal fracture extension to diaphysis, no specific fracture pattern associated with a higher risk of an open reduction was identified.

Of all complications, only infection (superficial or deep), post-operative transfusion and nail related complications were found to be statistically different between the two groups. In the open reduction group, superficial and deep infections occurred in 17 and 12 patients respectively (cf. 4 [superficial infection] and 3 [deep infection] in the closed reduction group, $p<0.001$). Cephalomedullary nail complications (22.6% [open reduction] Vs. 13.5% [closed reduction], $p=0.007$) and the need for post-operative transfusion (22.6% [open reduction] Vs. 13.5% [closed reduction], $p<0.001$) likewise were higher in the open reduction group. Requirement for pre-operative

transfusion, fracture non-union, peri-implant fractures, admission to HDU / ICU, LOS, 30-day and one-year mortality rate were similar between the two groups.

Having adjusted for the different variables associated with open reduction, our linear regression analysis revealed that open reduction was strongly associated with: distal extension of the fracture line to the diaphysis (OR: 2.486), an increased risk of both superficial (OR: 6.756) and deep infections (OR: 3.157), increased risk of post-operative transfusion within the first 48 hours following surgery (OR: 1.761), a surgical time of greater than 120 minutes (OR: 4.111), and a higher likelihood of the senior operating surgeon being a consultant (OR: 1.480) (**Table 3.24**).

Subgroup analysis: 'clamp assisted only' vs. 'cerclage wiring' open reduction
Of the 265 fracture requiring an open reduction, 62 fractures (23.4%) had cerclage wires / cables (**Appendix C; Table C.4**). Even though the proportion of male patients was higher in the cerclage wires group (p=0.029), the demographics were otherwise comparable. With regards to fracture characteristics, comminution (p=0.048), distal extension to the femoral shaft (p<0.001) and lesser trochanter involvement (p=0.018) were more common in the cerclage wiring group. The use of cerclage wiring was also associated with a better reduction as demonstrated by reduced lateral (p=0.008), medial (p=0.035) and anterior (p=0.004) fracture gap sizes post-operatively, as well reduced varus malalignment (p=0.01). Additionally, cerclage wiring was associated with an increased surgical time (p<0.001) and increased Hb drop (p=0.004), whereas mortality seemed to be lower in this group (p=0.039).

A further subgroup regression analysis (**Table 3.25**) of all fractures requiring open reduction revealed that open reduction using cerclage wiring was more commonly performed when there was a distal fracture extension (OR: 8.345) and lesser trochanteric fracture involvement (OR: 2.319). Although open reduction with cerclage wiring was associated with a prolonged surgical time (OR 4.266), it was associated with a smaller risk of developing a non-union when compared to 'clamp assisted only' open reduction (OR: 0.287).

Table 3.24 Table A presenting the coefficients and table B presenting the odds ratio estimates of the associations of open reduction

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.9862	-0.9549	-0.6464	1.0093	1.8268

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.460	0.189	-7.706	<0.001 ***
Wound infection (Superficial)	1.910	0.592	3.226	0.001 **
Wound infection (Deep)	1.150	0.695	1.655	0.098 .
Transfusion within 48 hours post-operatively	0.566	0.190	2.978	0.003 **
Distal Extension	0.911	0.202	4.507	<0.001 ***
Level of senior surgeon present (Consultant)	0.392	0.192	2.039	0.041 *
Surgical Time (> 120 min)	1.414	0.200	7.061	<0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 770.21 on 556 degrees of freedom

Residual deviance: 655.11 on 550 degrees of freedom

(4 observations deleted due to missingness)

AIC: 669.11

Number of Fisher Scoring iterations: 4

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.232	0.160	0.337	<0.001
Wound infection (Superficial)	6.756	2.117	21.561	0.001
Wound infection (Deep)	3.157	0.809	12.318	0.098
Transfusion within 48 hours post-operatively	1.761	1.213	2.556	0.003
Distal Extension	2.486	1.673	3.694	<0.001
Level of senior surgeon present (Consultant)	1.480	1.015	2.158	0.041
Surgical Time (> 120 min)	4.111	2.777	6.087	<0.001

Table 3.25 Table A presenting the coefficients and table B presenting the odds ratio estimates of the associations of cerclage wiring

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4831	-0.6560	-0.3309	-0.1789	3.1543

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.719	0.513	-7.252	<0.001***
Distal Extension	2.122	0.371	5.715	<0.001***
Lesser Trochanter Involvement	0.841	0.390	2.158	0.031*
Surgical Time (> 120 min)	1.451	0.366	3.961	<0.001***
Non-union	-1.249	0.555	-2.251	0.024*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for binomial family taken to be 1)
Null deviance: 284.36 on 261 degrees of freedom
Residual deviance: 215.74 on 257 degrees of freedom
(299 observations deleted due to missingness)
AIC: 225.74

Number of Fisher Scoring iterations: 5

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.024	0.009	0.066	<0.001
Distal Extension	8.345	4.031	17.276	<0.001
Lesser Trochanter Involvement	2.319	1.080	4.978	0.031
Surgical Time (> 120 min)	4.266	2.081	8.745	<0.001
Non-union	0.287	0.097	0.851	0.024

3.9.6 Effect of osteoporosis

In an attempt to examine the impact osteoporosis, the differences between the patients who had a history of previous fragility fracture (N=123; 22%), with those who did not, were investigated (**Appendix C; Table C.5**). Patients having a previous fragility fracture tended to be older than the age of 75 years (p<0.001), with a higher chance of being female (p<0.001). As to be expected, patients with previous history of fragility fractures had a higher incidence of low energy injuries (p<0.001), which were predominantly isolated injuries (p<0.011). On the contrary, an ISS score >16 (polytrauma) was more common in patients with no previous history of fragility fractures (p=0.009).

Additionally, patients with a history of previous fragility fracture were likely to suffer from more co-morbidities, as reflected to a higher ASA grade and a higher CCS (p<0.001). Similarly, patients with previous fragility fractures had a higher incidence of dementia (p=0.003). Regarding social history, patients with previous fragility fractures were less likely to smoke (p=0.002), had a poorer mobility (p<0.001) and a

higher risk of frequent falls (p<0.001). Examining the history of osteoporosis, Singh index was lower in patients with previous fragility fractures indicating higher incidence of osteoporosis, whilst they were more likely to be on bisphosphonates pre-admission (p=0.001), as well as Calcium / Vitamin D both pre-admission and on discharge (p<0.001). Interestingly, the two groups had similar risk of a future fragility fracture (15.3% versus 15.4%; p=1.000).

Comparing the operation characteristics of the two groups, there were no significant differences. No major differences were present in the radiographic measurements either, apart from a lower level of comminution (p=0.038) and smaller chance of greater trochanter involvement (p=0.031) in patients with a previous history of fragility fracture. When it came to post-operative complications however, patients with a previous history of fragility fracture had a higher incidence of urinary tract infection (UTI; p=0.006), pre- and post-operative renal dysfunction (p<0.001 and p=0.004 respectively), and risk of post-operative transfusion (p=0.003). In regards to the biochemical investigations, patients with a previous history of fragility fractures had lower albumin (p=0.017) and higher PTH levels (p=0.030). Finally, LOS and mortality were comparable between the two groups.

Regression to identify the association of previous fragility fractures was then performed, but did not demonstrate any meaningful significant differences between the two groups.

3.9.7 Effect of bisphosphonates

Comparing the group of patients who were on bisphosphonates pre-admission with those who were not, it was demonstrated that that patients on bisphosphonates were more likely to be older (p<0.001), of female gender (p<0.001) and with a higher incidence of bilateral subtrochanteric fractures (different episodes) (p<0.001) (**Appendix C; Table C.6**). Mechanism of injury was more likely to be a low energy injury (p=0.001), isolated (p=0.034) and not part of polytrauma (p=0.011). Patients on bisphosphonate treatment were also noted to have more significant co-morbidities (ASA: p=0.004; CCS: p=0.001). As one would expect, osteoporosis was more prevalent in the bisphosphonates group (lower Singh index: p=0.002), as were osteoporosis related medications (bisphosphonates on discharge: p<0.001; calcium / vitamin D pre-admission: p<0.001; calcium / vitamin D on discharge: p<0.001) and long-term steroids (p<0.001). Pre-operative mobility was poorer in patients on

bisphosphonates, who also presented with a higher incidence of frequent falls ($p<0.001$), but had a lower incidence of smoking ($p<0.001$).

Regarding the operation characteristics, apart from a higher surgical time and lower degree of post-operative weight bearing status in the non-bisphosphonate group ($p=0.026$ and $p=0.021$ respectively), the two groups were comparable. Risk of complications was also similar. Analysing the biochemistry investigations, apart from vitamin D that was less likely to be low in the patients already on bisphosphonates ($p=0.039$), there was no other significant difference between the two groups. Moreover, examining the fracture characteristics of the two groups, patients on bisphosphonates had simpler fracture patterns ($p=0.001$), higher incidence of the fracture not extending outside the subtrochanteric area ($p<0.001$), higher incidence of atypical fractures ($p<0.001$) and lower incidence of distal extension of the fracture (towards the femoral shaft) ($p=0.010$).

When a regression analysis was attempted, several differences between patients using or not using bisphosphonates before admission were demonstrated (**Table 3.26**). Most concerned the bone protection medication, both pre-admission and post-discharge. More specifically, bisphosphonates on discharge and calcium / vitamin D pre-admission were more common in patients who already were on bisphosphonates (OR 66.649, $p<0.001$ and OR 23.509, $p<0.001$ respectively). In contrast, calcium / vitamin D on discharge was less common in patients who already were on bisphosphonates (OR 0.120, $p<0.001$). Regarding the fracture configuration, it was interesting to confirm that patients on bisphosphonates had a higher incidence of isolated subtrochanteric extension and of atypical fractures (OR 4.464, $p=0.001$ and OR 491.281, $p<0.001$ respectively). Finally, smoking was found to be less common in patients on bisphosphonates pre-admission ($p=0.035$).

Table 3.26 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with the use of bisphosphonates pre-admission

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.2467	-0.2157	-0.1289	-0.0447	3.0967

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-4.786	0.526	-9.093	<0.001 ***
Bisphosphonates on discharge	4.199	0.528	7.958	<0.001 ***
Calcium / Vitamin D Pre-admission	3.157	0.486	6.496	<0.001 ***
Calcium / Vitamin D On discharge	-2.120	0.555	-3.821	<0.001 ***
Only Subtrochanteric Extension	1.496	0.433	3.453	0.001 ***
Atypical	6.197	0.998	6.209	<0.001 ***
Smoking	-1.382	0.654	-2.112	0.035 *

Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 470.02 on 520 degrees of freedom

Residual deviance: 182.22 on 514 degrees of freedom

(40 observations deleted due to missingness)

AIC: 196.22

Number of Fisher Scoring iterations: 7

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.008	0.003	0.023	<0.001
Bisphosphonates on discharge	66.649	23.693	187.487	<0.001
Calcium / Vitamin D Pre-admission	23.509	9.068	60.949	<0.001
Calcium / Vitamin D On discharge	0.120	0.040	0.356	<0.001
Only Subtrochanteric Extension	4.464	1.910	10.436	0.001
Atypical	491.281	69.471	3474.226	<0.001
Smoking	0.251	0.070	0.905	0.035

3.9.8 Atypical fractures

In total, 25 fractures (7.4%) were identified as atypical fractures (**Appendix C; Table C.7**). Atypical fractures were commonly bilateral in nature (p<0.001) and were more common in the female population (p=0.002). Furthermore, patients between the age 65 – 75 y.o. were more likely to have an atypical fracture (p=0.023). As per ASBMR criteria, all the atypical fracture patients sustained their injuries following low energy trauma (no trauma or from simple falls). Examining the cohort's comorbidities, use of steroids (p<0.001) and history of malignancy (p=0.003) were more common in the atypical fracture group. Not surprisingly, bone protection medications were more common in the atypical fracture group (bisphosphonates pre-admission: p<0.001; bisphosphonates on discharge: p<0.001; calcium / vitamin D pre-admission:

p<0.001; calcium / vitamin D on discharge: p=0.043). Looking into the fragility fractures before and after the index episode, both were more common in the atypical fracture group (p=0.030 and p=0.026 respectively). Additionally, the Singh index for osteoporosis was higher (i.e. lower degree of osteoporosis) in the atypical fracture group. Finally, a smoking habit was more common in the non-atypical fracture group (p=0.048).

With regards to the operation characteristics, there was no significant difference between the two groups. Investigating the post-operative complications, Hb drop (p=0.034) and transfusion rates (at 48 hours: p=0.022; in total: p=0.006) were lower in the atypical fracture group, whilst the rest of the variables examined were comparable. From the biochemistry results, only albumin was found to be different in the two groups (lower in the non-atypical fracture group, p=0.013). As for the fracture characteristics, not surprisingly, atypical fractures were of simple fracture configuration (p<0.001), were isolated in the subtrochanteric region (p<0.001) and did not extend to the diaphysis (p<0.001). Finally, the nail / canal ratio seemed to be higher in atypical fractures (p=0.003).

Going further into the regression analysis, some strong associations were demonstrated (**Table 3.27**). As per their definition, atypical fractures had a higher incidence of bisphosphonate (OR 812.677; p<0.001) and long-term steroid (OR 42.673; p=0.007) use, and were contained into the subtrochanteric region (OR 213.674; p=0.001). Additionally, patients between the age 65 – 75 y.o. were more likely to have an atypical fracture (OR 25.421; p=0.028). Moreover, the risk of fragility fractures after the index even was higher in the atypical group (OR 58.929; p=0.005). Regarding the risk of complications, the risk of non-union in atypical fractures was significantly increased (OR 64.536; p=0.004), whilst the risk of post-operative transfusion was lower (OR 0.096; p=0.033).

Table 3.27 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with atypical fractures

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.65364	-0.01430	-0.00385	-0.00050	2.14649

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-13.548	3.728	-3.634	<0.001 ***
Age 65 – 75 y.o.	3.236	1.472	2.197	0.028 *
Steroids	3.754	1.402	2.677	0.007 **
Bisphosphonates pre-admission	6.700	1.909	3.509	<0.001 ***
Only Subtrochanteric Extension	5.364	1.669	3.213	0.001 **
Post-operative Transfusion	-2.340	1.098	-2.132	0.033 *
Non-union	4.167	1.431	2.913	0.004 **
Fragility Fractures after index event	4.076	1.465	2.782	0.005 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for binomial family taken to be 1)
Null deviance: 178.465 on 338 degrees of freedom
Residual deviance: 31.192 on 331 degrees of freedom
(2 observations deleted due to missingness)
AIC: 47.192
Number of Fisher Scoring iterations: 10

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.000	0.000	0.002	<0.001
Age 65 – 75 y.o.	25.421	1.419	455.529	0.028
Steroids	42.673	2.734	666.166	0.007
Bisphosphonates pre-admission	812.677	19.259	34293.480	<0.001
Only Subtrochanteric Extension	213.674	8.105	5632.867	0.001
Post-operative Transfusion	0.096	0.011	0.828	0.033
Non-union	64.536	3.908	1065.787	0.004
Fragility fractures post-index episode	58.929	3.336	1040.960	0.005

3.9.9 Comparison of commonest nails

The two commonest cephalomedullary nails used were Gamma nail (Gamma3 long nail; © Stryker, Mahwah, NJ, USA) and Affixus nail (Affixus hip fracture nail; Zimmer Biomet™, Warsaw, IN, USA). Gamma nails were exclusively used until June 2012, after which only Affixus nails were used. For a more accurate representation of the outcomes and complications of these nails, only patients with complete follow-up were included.

Overall, 309 fractures treated with long nails were included into the comparison (**Appendix C; Table C.8**). Of them, 139 fractures were managed with long Gamma nails, as compared to 170 managed with long Affixus nails. The demographics, injury

characteristics, medical comorbidities, social history, length of stay and mortality rates of both groups were comparable. Patients with Affixus nails more often had their operations within 48 hours from presenting to the hospital, whereas bone protection prescription on discharge was better for the Affixus group (Bisphosphonates on discharge: p=0.023; Calcium / Vitamin D on discharge: p=0.043; Vitamin D loading on admission: p<0.001). Fragility fractures before the index episode were commonest in patients having Affixus nails, whilst fragility fractures after the index episode were commonest in the Gamma nail group (p=0.041). The biochemistry results also confirmed that the adjusted calcium was more frequently 'normal' in the Affixus group (p<0.001). The Gamma nail group had a higher incidence of 'high' reading for ALP (alkaline phosphatase) (p<0.001) and a lower incidence of hypoalbuminaemia (p=0.018). Regarding complications, the overall incidence of any complication was higher in the Gamma nail group (p=0.013), but when comparing the individual complications, there was no difference identified. Examining the post-reduction radiographs, the reduction of the lateral cortex was better in the Affixus nail group (p=0.008), but the incidence of the nail touching the anterior cortex was higher in the Gamma nail group (p=0.001).

A regression analysis comparing the baseline characteristics of the patients, as well as the fracture characteristics, demonstrated amongst others that the Affixus nail group had a smaller amount of fractures with distal extension to the femoral shaft (OR 0.497) and a higher incidence of having their operations within 48 hours from presentation (OR 4.590) (**Table 3.28**). A second regression analysis looking into the complications and quality of reduction, showed that patients having an Affixus nail had a better reduction of the lateral cortex as demonstrated by a lower incidence of a gap size ≥5 mm (OR 0.515), a lower incidence of nail complications (OR 0.536) and finally a lower risk of the tip of the nail touching the anterior cortex (OR 0.383) (**Table 3.29**).

Following the regression analysis, all the causes of nail revisions were investigated. A total of 77 nails were revised; 31 were long Affixus nails (18.2%) and 46 were long Gamma nails (33.1%) (**Table 3.30**). The commonest cause of revision was that of a non-union (33.8% of revisions), followed by revision for infection (14.3%). Thereafter, an implant survival curve was produced, demonstrating that Affixus nails performed significantly better (p<0.001) in the first five years following their implantation (**Figure 3.19**).

Investigating the effect of the de-rotation screw in patients receiving an Affixus nail, the two groups were comparable with regards to all variables examined. More specifically, there was no significant difference in fracture reduction, length of operation, complications including non-union, infection and nail failure, as well as revision for any cause.

Table 3.28 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with the type of nail used, only including differences between the patients' characteristics

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.6023	-0.8459	0.2943	0.7723	2.3785

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.338	0.484	-0.697	0.486
Bisphosphonates on Discharge	-1.046	0.362	-2.887	0.004 **
Vitamin-D loading during admission	2.481	0.686	3.619	<0.001 ***
Frequent falls	-0.877	0.361	-2.428	0.015 *
Pre-operative CKD (Moderate / Severe)	-0.729	0.338	-2.154	0.031 *
Adjusted Calcium (Low)	-1.333	0.367	-3.629	<0.001 ***
Albumin (Low)	0.730	0.337	2.165	0.030 *
Fragility fractures pre-index episode	1.256	0.432	2.905	0.004 **
Distal Extension	-0.698	0.334	-2.088	0.037 *
Operation in less than 48 hours	1.524	0.431	3.534	<0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 326.69 on 241 degrees of freedom

Residual deviance: 239.48 on 232 degrees of freedom

(67 observations deleted due to missingness)

AIC: 259.48

Number of Fisher Scoring iterations: 5

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.713	0.276	1.843	0.486
Bisphosphonates on Discharge	0.351	0.173	0.715	0.004
Vitamin-D loading during admission	11.953	3.118	45.823	<0.001
Frequent falls	0.416	0.205	0.844	0.015
Pre-operative CKD (Moderate / Severe)	0.483	0.249	0.937	0.031
Adjusted Calcium (Low)	0.264	0.128	0.542	<0.001
Albumin (Low)	2.076	1.072	4.020	0.030
Fragility fractures pre-index episode	3.511	1.505	8.192	0.004
Distal Extension	0.497	0.258	0.958	0.037
Operation in less than 48 hours	4.590	1.972	10.685	<0.001

Table 3.29 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with the type of nail used, only including differences between the operative characteristics

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.7780	-1.0797	-0.8268	1.1508	1.5745

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.898	0.189	4.760	<0.001***
Lateral Cortex Gap Size (≥5 mm)	-0.664	0.246	-2.693	0.007 **
Nail Complications	-0.624	0.278	-2.241	0.025 *
Touching Anterior Cortex	-0.961	0.270	-3.559	<0.001***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 424.05 on 307 degrees of freedom

Residual deviance: 397.46 on 304 degrees of freedom

(1 observation deleted due to missingness)

AIC: 405.46

Number of Fisher Scoring iterations: 4

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	2.454	1.696	3.551	<0.001
Lateral Cortex Gap Size (≥5 mm)	0.515	0.318	0.835	0.007
Nail Complications	0.536	0.311	0.925	0.025
Touching Anterior Cortex	0.383	0.225	0.650	<0.001

Table 3.30 Causes of revision of original nailing

	Type of Nail	
Cause for Revision	**Long Affixus Nail**	**Long Gamma Nail**
Revision for aseptic non-union		
Removal of Nail + Revision Surgery	9	15
Dynamisation of the nail	2	7
Exchange Nailing	3	3
Biological enhancement	-	2
Revision for infection		
Washout and debridement +/- Cement nail (deep infection)	5	6
Other reasons for revision		
Removal of Nail	3	5
Exchange of lag screw	2	2
Exchange of distal screws	1	2
Removal of Nail + THR	3	1
Peri-implant fracture	2	1
Removal of lag screw (irritation)	1	-
Removal of HO	-	1
Reduction of butterfly fragment	-	1
Total	**31**	**46**

Only first revision considered

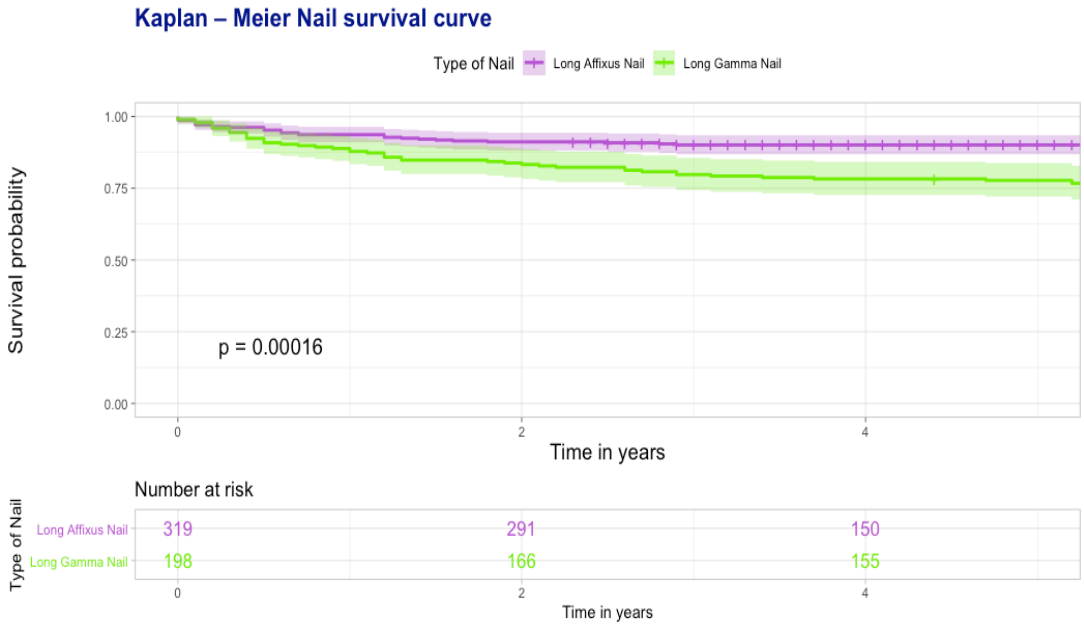


Figure 3.19 Kaplan – Meier survival curve**s of the nail used with 95% confidence intervals, regardless of cause of reoperation**

3.9.10 Transfusion requirements

Blood loss is one of the commonest sequelae of trauma and the subsequent operative management. A total of 48 patients (8.6%) were transfused at least one unit of RBC, before their operations, whilst a further 353 patients (63.3%) were transfused post-operatively (36 patients – 6.5% had both pre- and post- operative transfusions) (**Table 3.31**). The mean Hb drop 28.0 g/L, but was calculated as high as 33.3 g/L when units transfused were taken into account (one unit of RBC was considered to approximately increase Hb by 10 g/L (355, 356)). Massive transfusion was necessary in 13 patients (2.3%), whilst in 20 patients other blood products were transfused. **Figure 3.20** and **Figure 3.21** demonstrate the number of RBC units transfused and at which timeframe.

In an attempt to identify the factors leading to an increased risk of transfusion, patients having an intra-operative transfusion or within the first 48 hours post-operatively (288 patients) were compared to those who did not have any transfusion during the same time (271 patients) (**Appendix C; Table C.9**). Patients having a transfusion tended to be of female gender ($p=0.007$), aged > 75 y.o. ($p<0.001$), with a high ASA and CCS ($p<0.001$), and low albumin levels on admission ($p<0.001$). Interestingly, polytrauma and high energy injuries did not significantly increase the risk of transfusion. From the social history, smoking ($p=0.024$), alcohol ($p=0.006$), reduced mobility ($p<0.001$) and recurrent falls ($p=0.001$), were all correlated with a

reduced risk. Regarding the operation characteristics, open reduction ($p<0.001$) and use of cerclage wires ($p=0.004$) was associated with an increased risk, as was an increased surgical time ($p=0.010$) and a smaller canal / nail ratio ($p=0.034$). Fracture characteristics also seemed to play a role in transfusion requirements, with higher degrees of comminution ($p=0.084$), isolated subtrochanteric ($p=0.011$) and atypical fractures ($p=0.026$) being at higher risk. Post-operatively, self-dynamisation of the nail ($p=0.021$), wound infection ($p<0.022$) and impaired kidney function ($p<0.001$) were also associated with an increased risk. Finally, patients having a transfusion within the first 48 hours post-operatively had a higher incidence of escalation in their care ($p=0.003$), LOS ($p=0.037$) and one-year mortality ($p=0.007$).

A subsequent regression analysis suggested that the most important association of a need for transfusion within the first 48 hours post-operatively was a nail / canal ratio <0.70 (OR 3.681), followed by need for cerclage wiring (OR 3.169) (**Table 3.32**). Regarding the fracture, lesser trochanter involvement was also implicated with an increased risk (OR 1.979). Finally, pre-operative (OR 2.569) and post-operative moderate / severe renal impairment (OR 2.053), as well as presence of low albumin on admission (OR 0.950) were also predictors of an increased risk.

Table 3.31 Blood loss and transfusion requirements of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Transfusion rates	
Pre-operative (within 48 hours)	35 patients (6.2%) RBC units Tx: 2.4 ± 1.0 (2; 1 to 6)
Pre-operative (at any point)	48 patients (8.6%) RBC units Tx: 2.6 ± 1.0 (2; 1 to 6)
Post-operative (within 48 hours)	288 patients (51.3%) RBC units Tx: 2.5 ± 1.4 (2; 1 to 12)
Post-operative (at any point)	353 patients (63.3%) RBC units Tx: 3.1 ± 2.8 (2; 1 to 35)
Both pre- and post-operative	36 patients (6.5%)
Massive transfusion	13 patients (2.3%)
Transfusion of other products	20 patients: - FFP: 15 patients - Platelets: 11 patients - Clotting factors: 4 patients

Hb Values	
Hb value pre-operatively	120.1 ± 18.2 g/L (121 g/L; 61 to 169 g/L)
Hb value post-operatively	92.0 ± 16.4 g/L (92 g/L; 48 to 144 g/L)
Hb change	-28.0 ± 20.9 g/L (-30 g/L; -78 to 38 g/L)
Hb change including Tx	-33.3 ± 19.9 g/L (-33 g/L; -103 to 37 g/L)

One unit of RBC was considered to approximately increase Hb by 10 g/L (355, 356)
Results are presented as: Mean ± SD (Median; Range)

FFP: Fresh frozen plasma
Tx: Transfusion

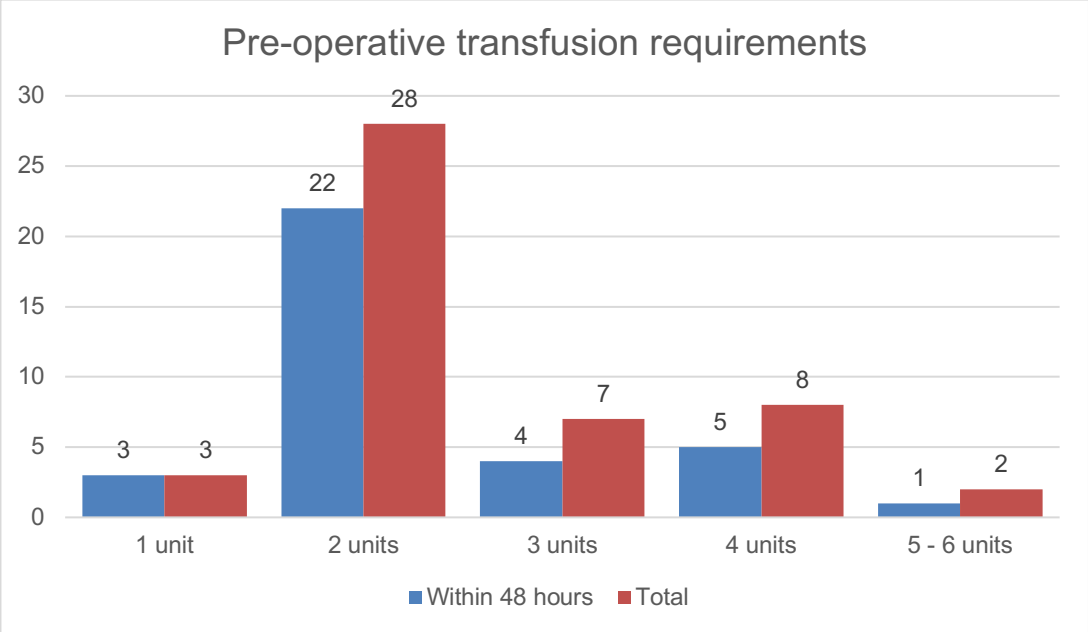


Figure 3.20 Transfusion requirements (within 48 hours from surgery versus total pre-operative transfusion) of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

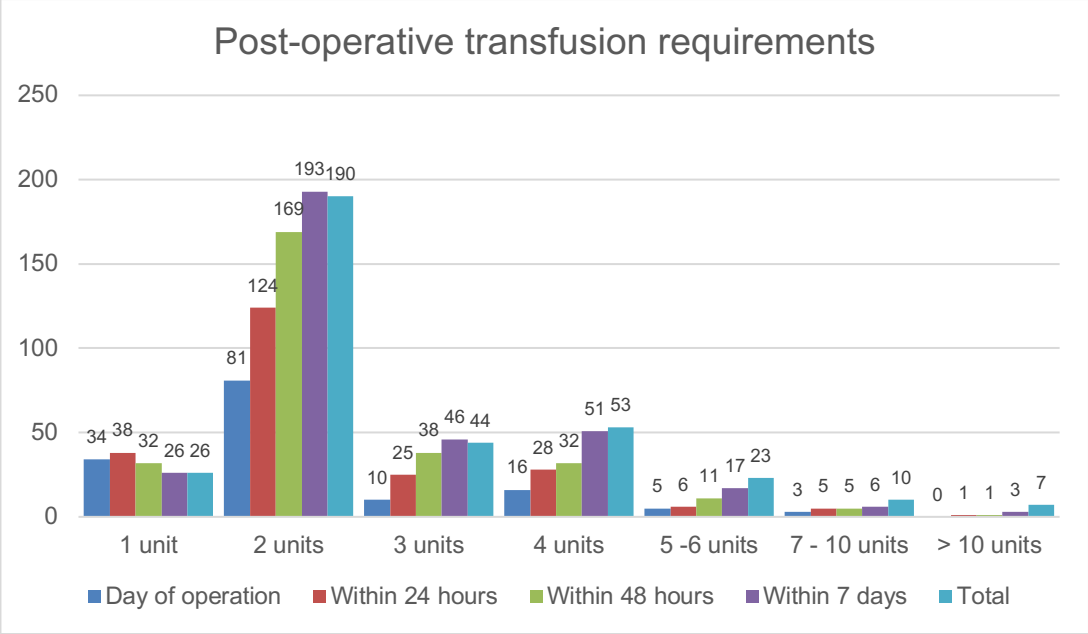


Figure 3.21 Transfusion requirements (within 48 hours from surgery versus total post-operative transfusion) of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Table 3.32 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with the the need for blood transfusion (RBC) within 48 hours post-operatively

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.1590	-0.8840	0.4194	1.0068	1.8094

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.421	0.249	-5.703	<0.001 ***
Pre-operative CKD (Moderate / Severe)	0.944	0.335	2.821	0.005 **
Post-operative CKD (Moderate / Severe)	0.719	0.349	2.063	0.039 *
Albumin (Low)	0.668	0.230	2.904	0.004 **
Lesser Trochanter involvement	0.683	0.225	3.029	0.002 **
Cerclage Wiring	1.153	0.337	3.427	0.001 ***
Nail / Canal Ratio <0.70	1.303	0.493	2.645	0.008 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 587.19 on 424 degrees of freedom

Residual deviance: 502.51 on 418 degrees of freedom

(136 observations deleted due to missingness)

AIC: 516.51

Number of Fisher Scoring iterations: 4

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.242	0.148	0.394	<0.001
Pre-operative CKD (Moderate / Severe)	2.569	1.334	4.950	0.005
Post-operative CKD (Moderate / Severe)	2.053	1.036	4.066	0.039
Albumin (Low)	1.950	1.242	3.061	0.004
Lesser Trochanter involvement	1.979	1.272	3.078	0.002
Cerclage Wiring	3.169	1.638	6.129	0.001
Nail / Canal Ratio <0.70	3.681	1.401	9.670	0.008

3.9.11 Weekend effect

Most patients within our cohort were admitted on a weekday (382 fractures – 69.9%), without any significant difference in terms of patients' demographics being present between patients admitted on either day of the week (**Appendix C; Table C.10**). High-energy injuries were more prevalent over the weekend (19.0% versus 14.7%), as were patients with open fractures (p=0.064). Even though ASA was similar in the two groups, there was a trend for a higher CCS over weekdays (p=0.061). Interestingly, there was no difference to time taken from admission to the operating room. More specifically, weekday admissions had operations performed in less than 48 hours in 78.8% of patients, compared to 79.9% of cases during weekends. In keeping with this there was no difference in the level of the first surgeon performing the surgery, the presence of a consultant in theatres, as well as any differences in the surgical and anaesthetic times. Finally, there was no significant difference in the incidence of complications including HAP, superficial and deep wound infections, VTE or mortality (p>0.100).

Regression was not possible as no significant differences between weekend – weekday admissions were identified. Therefore, no evidence of a so-called 'weekend' effect was demonstrated by these data.

3.9.12 Hospital Acquired Pneumonia

A total of 93 patients suffered from HAP (incidence 16.6%) (**Appendix C; Table C.11**). Age < 65 y.o. (p<0.001) and age > 75 y.o. (p=0.004) were negatively and positively correlated to development of HAP, respectively. Furthermore, the patients' ASA score prior to surgery was also associated with HAP, as was a higher CCS (p<0.001), presence of diabetes (p=0.026) and asthma / COPD (p=0.042). Noteworthy, smoking was not associated with a risk of developing HAP. HAP was also a predictor of HDU / ICU stay (p<0.001), as well as length of hospital stay, and

both 30-day and one-year mortality (p<0.001). Time to surgery, operation characteristics and surgical complications were not associated with an increased incidence of HAP.

Following regression analysis, the OR for developing HAP increased with an increasing CCS (OR 4.190 to 6.309), presence of asthma / COPD (OR 2.355), ICU / HDU stay (OR 2.864) and a length of stay of more than 21 days (OR 2.580) (**Table 3.33**).

Table 3.33 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with the diagnosis of HAP

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4842	-0.6861	-0.4424	-0.2202	2.7319

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.707	0.502	-7.390	<0.001 ***
Charlson Comorbidity Score 3 to 5	1.433	0.524	2.734	0.006 **
Charlson Comorbidity Score 6 to 8	1.548	0.513	3.017	0.003 **
Charlson Comorbidity Score ≥9	1.842	0.540	3.413	0.001 ***
Asthma / COPD	0.857	0.362	2.368	0.018 *
ICU / HDU Stay	1.052	0.313	3.357	0.001 ***
LOS ≥ 21 days	0.948	0.246	3.847	<0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for binomial family taken to be 1)
Null deviance: 503.92 on 560 degrees of freedom
Residual deviance: 448.00 on 554 degrees of freedom
AIC: 462
Number of Fisher Scoring iterations: 5

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.025	0.009	0.066	<0.001
Charlson Comorbidity Score 3 to 5	4.190	1.500	11.700	0.006
Charlson Comorbidity Score 6 to 8	4.701	1.720	12.851	0.003
Charlson Comorbidity Score ≥9	6.309	2.191	18.167	0.001
Asthma / COPD	2.355	1.159	4.786	0.018
ICU / HDU Stay	2.864	1.549	5.294	0.001
LOS ≥ 21 days	2.580	1.592	4.183	<0.001

3.9.13 Myocardial Infarction / Cerebrovascular Accidents

Patients younger than the age of 65 were less likely to have myocardial infarction / cerebrovascular accident (MI / CVA), compared to a higher incidence in patients older than the age of 75 (p=0.040 and p=0.036, respectively) (**Appendix C; Table C.12**). There was no association with smoking or alcohol habits prior to admission.

Surprisingly, a longer surgical time was less predictive of MI / CVA (p=0.047). The ASA grade was however implicated as an associated factor (p=0.002), with higher incidence being seen with higher ASA grades. This was also reflected on CCS (p=0.001). Whilst MI / CVA did not predict ICU / HDU stay, it did predict 30-day and one-year mortality (p<0.001 and p=0.002, respectively). Nevertheless, when a regression analysis model was attempted, no factors that remained significant were identified.

3.9.14 Post-operative delirium

Post-operative delirium was diagnosed in 56 cases within our cohort (incidence 10.0%) (**Appendix C; Table C.13**). Patients aged < 65 y.o. had a lower incidence of post-operative delirium (p=0.002), in contrast to patients aged > 75 y.o. (p<0.001). There was no association with gender, but the incidence was higher in isolated injuries compared to patients sustaining more than one injury (p=0.006). Once more, increasing ASA grade and CCS were implicated with a higher risk (p=0.048 and p<0.001 respectively), as was presence of dementia (p<0.001). Additionally, poor pre-operative mobility (p=0.007) and patients sustaining frequent falls (p=0.009) were also linked to an increased risk. Moreover, delirium was associated with HAP and UTI (p=0.004 and p<0.001 respectively), as well as pre- and post- operative CKD (p=0.008 and p<0.001), and need for post-op transfusion (p=0.017). Whilst there was no association between delirium and mortality, delirium correlated with HDU / ICU stay and increased length of stay in hospital (p=0.012 and p<0.001 respectively).

Following regression analysis, the OR for developing post-operative delirium in patients with dementia was 3.969, compared to 1.983 in patients presenting with a chest infection and 3.587 in patients being diagnosed with a UTI (**Table 3.34**). Patients with a history of asthma / COPD also had an increased risk of post-operative delirium (OR 2.726), but as previously reported, asthma and COPD were also correlated to an increased risk of HAP. Interestingly, patients with a deteriorating renal function post-operatively also had an increased risk of delirium (OR 2.565), as did patients requiring an increased level of care (OR 2.953).

Table 3.34 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with the diagnosis of post-operative delirium

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4018	-0.4186	-0.2992	-0.2136	2.7538

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.769	0.318	-11.852	<0.001 ***
Dementia	1.379	0.324	4.252	<0.001 ***
Asthma / COPD	1.003	0.443	2.265	0.024 *
HAP / CAP	0.685	0.346	1.981	0.048 *
UTI	1.277	0.340	3.753	<0.001 ***
CKD Stage post-operatively (Moderate / Severe)	0.942	0.314	3.002	0.003 **
ICU / HDU Stay	1.083	0.404	2.678	0.007 **

Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 356.75 on 545 degrees of freedom

Residual deviance: 292.49 on 539 degrees of freedom

(15 observations deleted due to missingness)

AIC: 306.49

Number of Fisher Scoring iterations: 6

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.023	0.012	0.043	<0.001
Dementia	3.969	2.103	7.493	<0.001
Asthma / COPD	2.726	1.145	6.493	0.024
HAP / CAP	1.983	1.007	3.905	0.048
UTI	3.587	1.841	6.989	<0.001
CKD Stage post-operatively (Moderate / Severe)	2.565	1.387	4.743	0.003
ICU / HDU Stay	2.953	1.337	6.523	0.007

3.9.15 Venous thromboembolism

A total of 22 VTE events (incidence 3.9%), including PE and DVT, were documented within six months from the index procedure (**Appendix C; Table C.14**). Only open fractures (p=0.016) and superficial wound infections (p=0.035) were positively correlated to development of VTE. Nonetheless, there was no association of VTE with mortality, hospital stay, need for ICU / HDU admission or surgical complications. Regression analysis was not possible as any differences between patients having a VTE and the rest of the cohort were not identified.

3.9.16 Mortality

For calculating the risk of mortality, only the first episode of patients presenting to LTH with bilateral hip fractures was included (12 patient episodes were therefore

excluded). Patients presenting to LTH and having their primary procedures in other institutions were also excluded from the analysis.

Regardless of age, 30-day mortality was 6.0%, compared to 20.8% for one-year mortality (**Table 3.35**, **Figure 3.22**, **Figure 3.23**, **Figure 3.24**, **Figure 3.25**). In patients older than the age of 60 years (434 patients), 30-day mortality increased to 7.6% and one-year mortality to 25.6%. Overall, one-year mortality (regardless of age) was also higher in female patients, even though this was not statistically significant ($p=0.11$), but it was significantly higher with an increasing age ($p<0.001$) (**Figure 3.26**, **Figure 3.27**). Average time to death from injury was 23.7 months (median 18.4 months; SD 22.6 months). Thirty-day mortality was positively correlated with age, with no patient younger than the age of 60 years dying within 30 days and no patient younger than the age of 40 years dying within the first-year post injury. There was no significant difference in mortality based on the day of admission at 30 days or one year.

Comparing the injury characteristics of patients dying within the first year from injury, there was a higher incidence of high energy injuries (**Appendix C; Table C.15**). The incidence of polytrauma (ISS>16) however was lower ($p=0.013$), and the fracture patterns were 'simpler' ($p=0.017$). Regarding the comorbidities, not surprisingly patients who died within the first year post injury, had a higher ASA and CCS scores ($p<0.001$), a higher incidence of malignancy ($p<0.001$) and dementia ($p<0.001$), lower levels of albumin (hypoalbuminaemia; $p<0.001$) and a 'poorer' mobility ($p=0.029$). Following the analysis of the post-operative complications, chest infections ($p<0.001$), deterioration of renal function ($p<0.001$) and transfusion within 48 hours post-operatively ($p=0.010$) were more common in the patients who died. Finally, escalation of treatment was also associated with an increased mortality ($p=0.042$).

Table 3.35 Mortality of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region

Mortality	
Overall mortality	302 / 549 (55.0%)
Inpatient mortality	38 / 549 (6.9%)
30-day mortality	33 / 549 (6.0%)
One-year mortality	114 / 549 (20.8%)
Time injury to death	23.7 ± 22.6 months (18.4 months; 0.1 to 109.6 months)

Results are presented as: Mean ± SD (Median; Range)

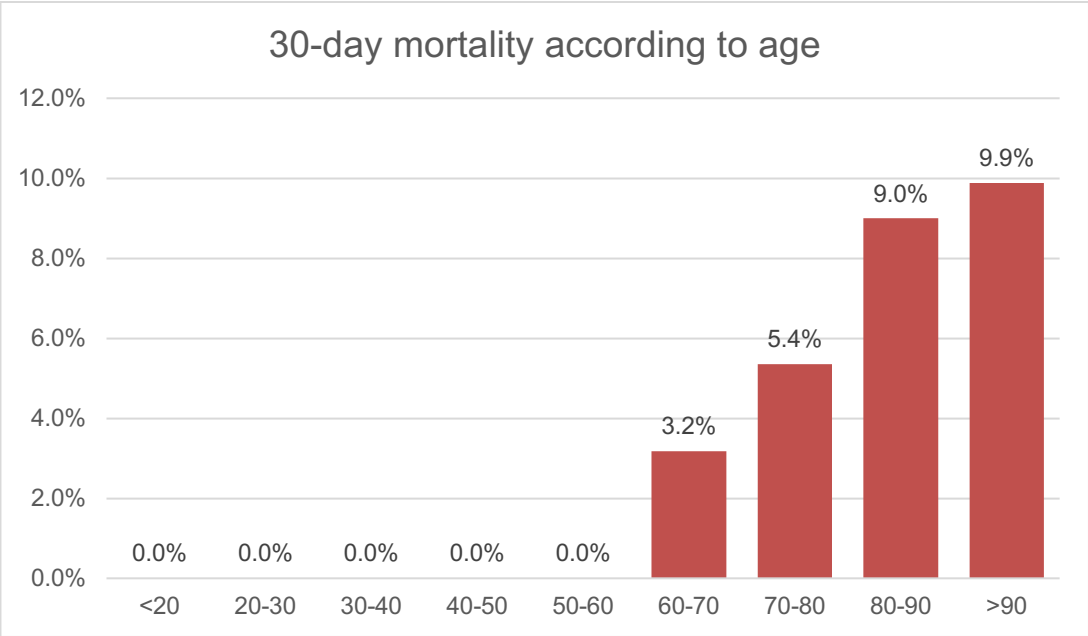


Figure 3.22 30-day mortality of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, according to age

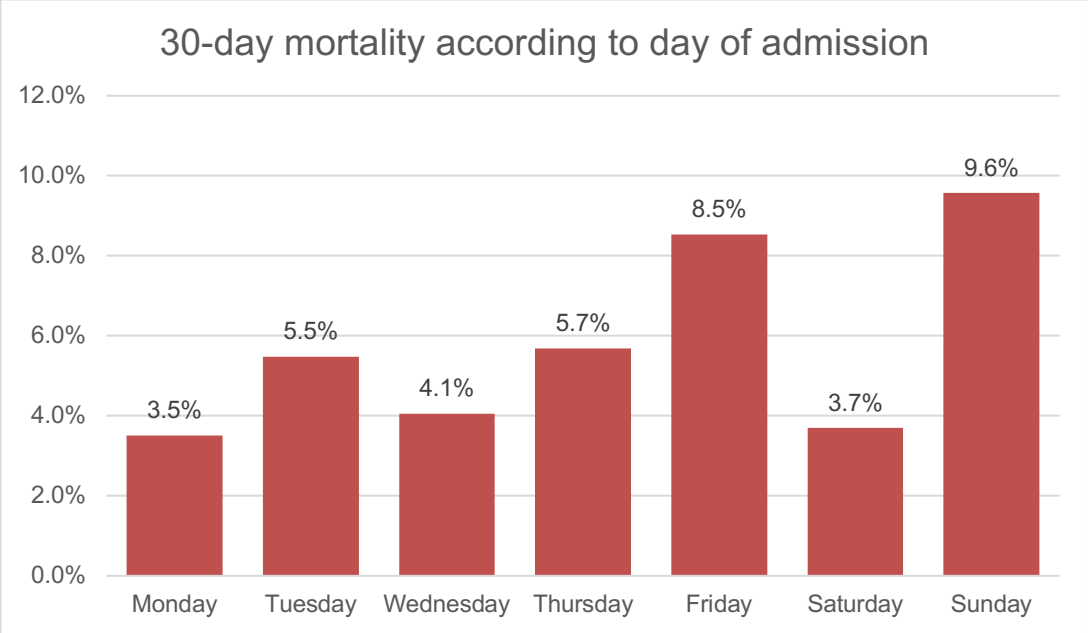


Figure 3.23 30-day mortality of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, according to day of admission

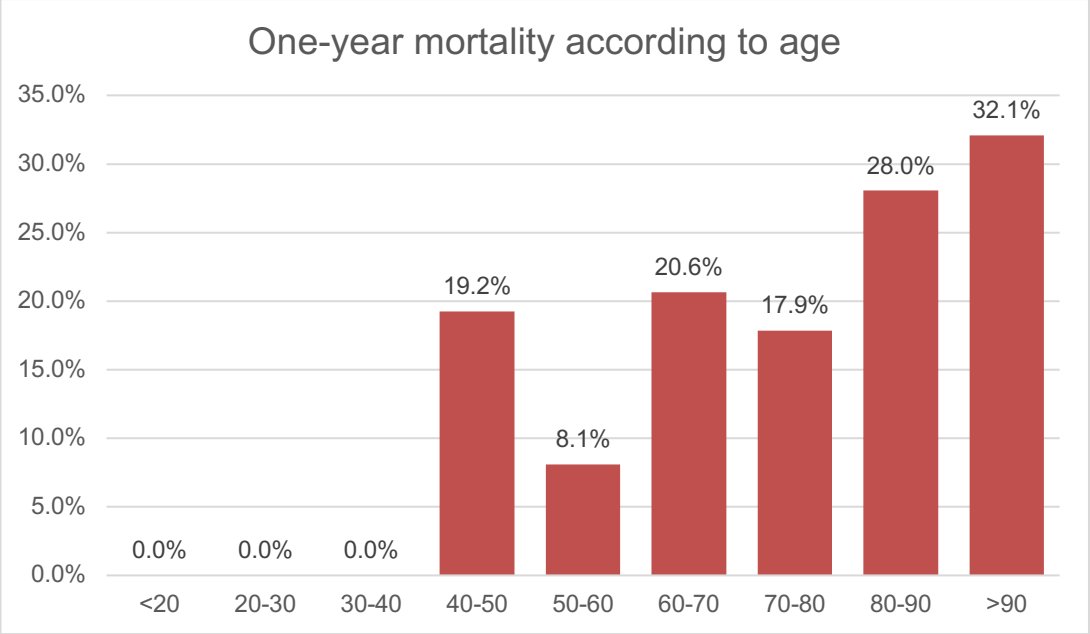


Figure 3.24 One-year mortality of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, according to age

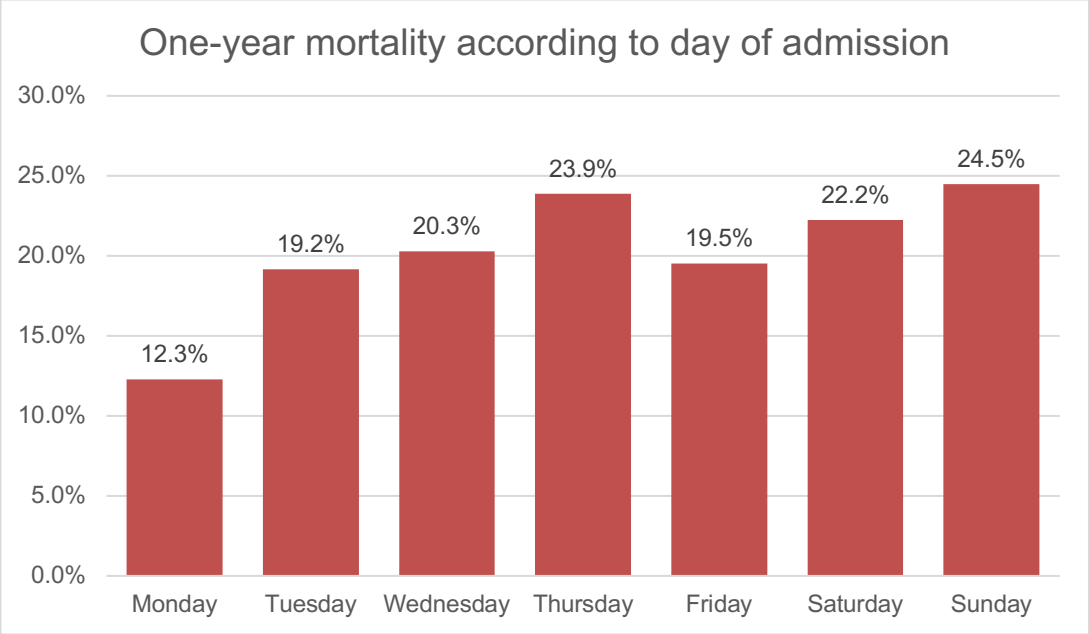


Figure 3.25 One-year mortality of patients presenting to LTH with a proximal femur fracture involving the subtrochanteric region, according to day of admission

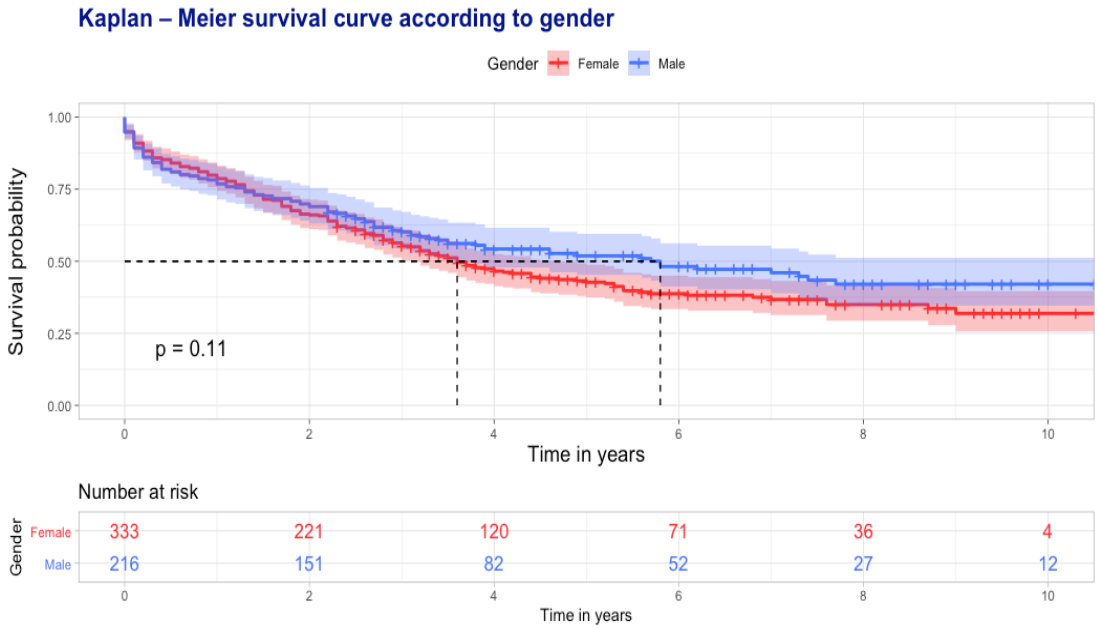


Figure 3.26 Kaplan – Meier survival curves (mortality) according to gender, with 95% confidence intervals

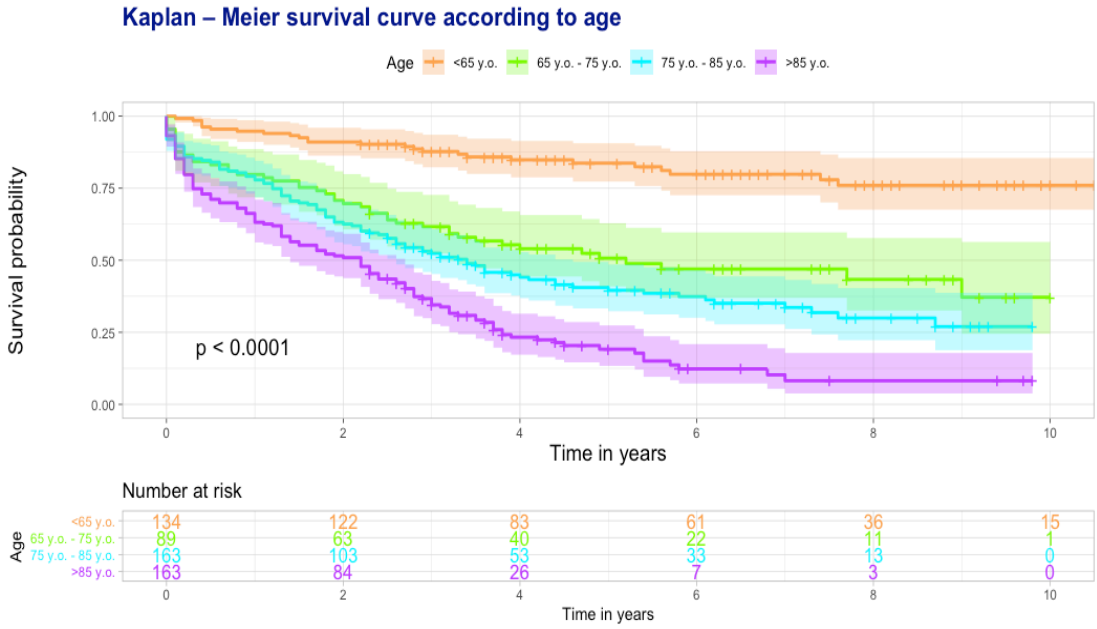


Figure 3.27 Kaplan – Meier survival curves (mortality) according to age, with 95% confidence intervals

Following a regression analysis, several factors were identified to contribute to an increased risk of one-year mortality (**Table 3.36**). Not surprisingly, pathological fractures was the most important risk factor (OR 12.835). A CCS of more than 6 was also implicated with a higher risk (OR 3.607), whilst a low albumin on admission was positively correlated to an increased mortality (OR 3.664). Additionally, dementia had an OR of 3.282 and presence of a chest infection during hospital stay had an OR of 2.645. On the other hand, nail complications seemed to have a 'protective' effect (OR 0.360).

Table 3.36 Table A presenting the coefficients and table B presenting the odds ratio estimates outlining the association of different variables with one-year mortality

A.

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.1875	-0.6874	-0.3921	-0.2044	2.6745

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.810	0.417	-9.130	<0.001 ***
Dementia	1.189	0.278	4.276	<0.001 ***
HAP / CAP	0.973	0.290	3.355	0.001 ***
Charlson Comorbidity Score >6	1.283	0.296	4.338	<0.001 ***
Albumin (Low)	1.299	0.358	3.628	<0.001 ***
Pathological	2.552	0.482	5.294	<0.001 ***
Nail Complications	-1.021	0.467	-2.188	0.029 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 516.38 on 488 degrees of freedom

Residual deviance: 382.04 on 482 degrees of freedom

(60 observations deleted due to missingness)

AIC: 396.04

Number of Fisher Scoring iterations: 5

B.

	OR	Lower	Upper	Pr(>|z|)
(Intercept)	0.022	0.010	0.050	<0.001
Dementia	3.282	1.904	5.660	<0.001
HAP / CAP	2.645	1.499	4.669	0.001
Charlson Comorbidity Score >6	3.607	2.020	6.439	<0.001
Albumin (Low)	3.664	1.817	7.391	<0.001
Pathological	12.835	4.989	33.022	<0.001
Nail Complications	0.360	0.144	0.899	0.029

3.10 Discussion

This study confirms that hip fractures and in particular, subtrochanteric fractures, are associated with high mortality and morbidity. Such injuries are further associated with high costs not only with regards to in-hospital stay but also ongoing care, social services and family input during and after the immediate phase of care (357). Even though a lot of research exists around proximal femoral fractures, there is a paucity of evidence regarding subtrochanteric fractures, especially in reference to their complications. Thereafter, the attempt to investigate the outcomes of operative management of subtrochanteric fractures treated with an intra-medullary nail. This is of particular importance, as by identifying the risk factors leading to these major complications early, not only these could be potentially recognised and treated at their early stages, but in some cases, they could also be prevented.

3.10.1 Basic cohort information

Contrary to the bimodal age and gender distribution (358), and unimodal older male and female distribution reported in the literature (359, 360), a bimodal male, unimodal older female distribution was identified in this cohort. In male patients, the peaks in incidence were noted at the 4th and 9th decade, occurring commonly as a result of high-energy trauma (e.g. following RTCs) and low energy trauma (e.g. fall from standing height, fragility fractures) respectively (222). The unimodal older female distribution reflects the increase in incidence of post-menopausal fragility fractures following low energy injuries (222). In keeping with published literature, the three commonest mechanisms of injury resulting in a subtrochanteric femur fracture in our study population were fall from standing height, unwitnessed falls and RTCs (360).

Regarding the length of hospital stay, the average stay in our cohort was 22.5 days. The literature suggests that risk factors for a prolonged length of stay include increasing age, patients with multiple co-morbidities, deprived socio-economic backgrounds, as well as increased physiotherapy requirements and type of discharge destination (361, 362). Specific comorbidities suggested include diabetes, peptic ulcer disease, fluid and electrolyte disorders, delirium, bleeding disorders, renal failure and paralysis (362). Unsurprisingly, it has been reported that the length of stay is increased in patients who require transfer to medicine once their surgical care is complete (362, 363). Our findings point to similar conclusions. Of interest Lau et al. reported that a multidisciplinary care protocol can lead to a reduced length of stay and better outcomes of patients presenting with hip fractures (364).

Increased length of stay poses a huge burden to every healthcare system (357). The index hospital stay has been previously estimated to cost £8,613, whilst this cost rises to a total of over £10,000 for the first year post fracture (365). These ongoing costs are related to re-admissions mostly secondary to a further hip fracture, other non-hip fragility fractures requiring hospitalisation and hip fracture-related complications (365). This emphasises the importance of prevention or early recognition and management of post-operative complications, adequate bone protection post-operatively, rehabilitation and adequate social input on discharge where this is needed.

3.10.2 Non-unions

Non-union remains one of the most debilitating and difficult to treat complications of subtrochanteric fractures. In our series, 84 out of the 341 fractures (24.6%) with complete follow-up failed to unite, with most being atrophic (78.6%), followed by hypertrophic (14.3%), and septic non-unions (7.1%). In the literature, the reported incidence of non-union of subtrochanteric fractures varies considerably, ranging from 2.3% to 23% (174, 250, 272, 347, 366). This is slightly lower than our findings, but this could possibly be explained by the different definitions of non-union used; the fact that our practice was to intervened 'early' to improve the chances of healing and reduce the symptoms secondary to non-unions; the inclusion of only patients who had a radiographic and clinical confirmation of union, therefore excluding asymptomatic patients who were discharged early; and the inclusion of patients suffering from pathological fractures, a known risk factor for the development of a non-union.

Our regression analysis identified a total of six factors contributing to the development of a non-union: deep infection, self-dynamisation, presence of an atypical fracture, diabetes and malreduction (lateral cortex fracture gap size and varus malalignment). On the other hand, moderate comminution had a protective effect. In the literature however, the evidence identifying risk factors contributing to a non-union remain scarce.

In line with our findings, several authors have suggested deep infection as a causative factor of a non-union (367, 368). This could be explained by the ongoing inflammation which disrupts the fracture callus, increases the gap between the fracture site and inadvertently reduces the BMD around the affected area (368, 369). This results to osteolysis and loosening of the metalwork, leading to mechanical

instability (367). Simpson et al. reported that infection was the cause of non-union in 38% of the cases, whilst occult infection was the case in 5% of the non-unions (367). Similarly, following a multivariate regression analysis, Mehrpour et al. identified infection ($p<0.001$) as a risk factor for non-union of long bones (370).

Another complication directly associated with delayed fracture healing and non-union, is failure of the metalwork. In fact, metalwork failure is considered a consequence rather than a cause of a non-union (26), with non-union featured in all nail failures in previous studies (350, 371). Because of this absolute relationship between nail breakage and non-union, nail breakage was not considered for the regression analysis of our model. Especially in the case of subtrochanteric fractures, their unique biomechanical and anatomical features make them prone to this complication. Johnson et al. investigated the nail failures of proximal femoral fractures treated with IM nails and reported that 21 out of 22 nail breakages were in subtrochanteric fractures (350). The overall incidence of nail breakage in subtrochanteric fractures is estimated between 0.2% and 10% (174, 350, 371, 372), with differences being attributed to the inconsistency in the definition of subtrochanteric fractures, the inclusion of older generation nails where the failure rate was higher and a possible under-reporting because of loss of follow-up / mortality in some of these studies.

The commonest site of the nail breakage is at the nail – lag screw junction, but in older generation nails, the distal barrel taper has also been reported as site of nail failure (350). These are areas of high torque, which is further increased if there is no calcar support because of the fracture configuration. Biomechanical studies have demonstrated that with a decrease in the fracture stability, the stresses in the nail increase, until fatigue fracture (failure following repeated cycles of load, below the ultimate tensile strength of the nail) (350, 373). In more detail, the von Mises stress is the highest around the aperture for the lag screw, and when those become higher than the fatigue strength of the nail, the nail fails at that point (373). Additionally, scratching of the titanium nail whilst drilling for the lag screw can contribute to the risk of nail brakeage (372). Thereafter, meticulous surgical technique and sufficient fracture reduction not only improves the chances of a successful union, but will also reduce the stresses passing through the nail, consequently reducing the risk of nail fatigue failure.

Investigating the risk factors related to nail failure in proximal femoral fractures, Johnson et al. reported that subtrochanteric location of the fracture, low ASA score and pathological fractures were independent risk factors (350). In a subgroup analysis of only the subtrochanteric fractures, the same team concluded that younger age was an additional risk factor (350). Maes et al. on the other hand reported that varus malalignment was the most important risk factor (374).

Self-dynamisation (breakage of the distal locking screws) was another significant risk factor for non-union in our series. Krappinger et al. also reported self-dynamisation of the nail within 12 weeks post-surgery, as a significant risk factor for non-union in subtrochanteric fractures treated by IM nailing (250). More specifically, out of nine patients where self-dynamisation of their nail was observed, eight developed a non-union (88.9%) (250), compared to 15 out of 22 (68.2%) in our series. Self-dynamisation is a general indicator of mechanical instability of the construct, and is often associated with failure of the nail proximally. In a study by Johnson et al., failure of distal screws occurred in 18/22 of patients with subsequent nail failure proximally (350), a mode of failure previously reported by other authors (26, 375).

The presence of an atypical fracture was another factor found to contribute to non-unions in our series. As mentioned, atypical fractures are considered to be caused secondary to inhibition of osteoclastic bone resorption, that in turn can lead to over-mineralisation that makes the bone more brittle, and accumulation of microdamage that increase the risk of pathological fractures (285, 286). Medications related to atypical fractures include bisphosphonates, glucocorticoids, hormone replacement therapy, proton-pump inhibitors (288) and antiresorptive RANKL blocker denosumab (287-291).

Bisphosphonates are the only medications well investigated with regards to atypical fracture causation, whilst the evidence associating other medication to atypical fractures remains poor. Impaired bone healing following atypical fractures is a common finding in most studies, affecting as high as 40% of these fractures (308, 323-326, 331). Healing times have being reported to range between 5 to 10.6 months (34, 325, 326, 329-331), whilst the incidence of revision for any cause as high as 46% (34, 331, 332). Most importantly, the incidence on non-union is increased, with Weil et al. reporting it as high as 46% (332). In another study by Bogdan et al., 12% of atypical femoral fractures failed to unite and required revision surgery (329). Additionally, Donnelly et al. reported a non-union risk of 57.1%, even though

malreduction was identified as an additional risk factor (376). Similarly, Cho et al. reported a non-union risk of 31.3% identifying malreduction with regards to neck-shaft angle and sagittal angulation as additional factors contributing to non-unions (330). Use of Teriparatide has been suggested to improve fracture healing in patients presenting with atypical fractures, whilst the hip function recovery and pain relief is also improved (377).

Interestingly, Krappinger et al. suggested that atypical fractures were not a risk factor for non-union in subtrochanteric fractures treated by IM nailing (250). Similarly, Ego et al. reported delayed healing (time to union: mean of 8.3 months; range, 2 – 18 months), but no increased risk of non-union in patients with atypical fractures of the femur (25 subtrochanteric fractures and 16 femoral shaft fractures), with 63.6% of the patients returning back to baseline status within a year post-injury (378).

Malreduction is another, potentially preventable, cause of non-union. In the current cohort, this was demonstrated by a lateral cortex fracture gap size (more than 5 mm) and a varus malalignment (there was an increase to the risk of non-union with an increase in the varus of fixation). Whilst varus malalignment has been reported as a risk factor for non-unions by a number of authors, lateral cortex gap size has never been identified as such.

In the literature, Shin et al. reported that post-operative varus malalignment was the only risk factor for a non-union (347), whist Krappinger et al. reported that this was one of the risk factors identified (250). Shukla et al. reported that varus malalignment (>10 degrees) was a significant factor for non-union and failure of implant (174), as did Maes et al. (374). Additionally, Liangjun et al. identified that only fracture displacement was a significant risk factor for non-union (all patients with union had a fracture displacement <2.2 cm; all patients with a non-union had fracture displacement >2.5 cm) (348). Moreover, Riehl et al. analysed the effect of the coronal and sagittal displacement, suggesting that malreduction >10 degrees in any plane was a significant risk factor for impaired healing (349). To reduce the risk of malreduction, Robertson et al. suggested provisional plating of the fracture before the insertion of the nail (379). Even though malreduction risk was significantly reduced, these patients had longer operating times and higher blood loss (379).

Other risk factors identified in the literature but not from the analysis of this cohort include: age, which is correlated with time to union (174); and presence of metastatic and bone metabolic disease (374).

Diabetes, a common chronic metabolic disease, has also been identified as a factor related to an increased risk of non-union. On a cellular level, there is an increase in pro-inflammatory mediators in diabetic patients, whilst the downregulation of inflammation is also reduced (380). As a result, following a fracture, the inflammatory process is increased and prolonged, which may in turn lead to an enhanced osteoclastogenesis and decreased osteoblast activity (380, 381). Moreover, insulin has a direct effect on osteoblasts, increasing proliferation, reducing apoptosis, increasing collagen synthesis and enhancing sensitivity to PTH (381, 382), whilst on the contrary, insulin reduces osteoclast activity (383). Hyperglycaemia also alters cellular mechanisms related to bone repair, increasing osteoclast activity while suppressing osteoblastic gene expression (381, 384, 385). Additionally, the macro- and micro- angiopathy secondary to diabetes, increases the risk of impaired healing and wound problems (380, 386).

Even though there is a paucity of evidence regarding the effect of diabetes in subtrochanteric fractures and the risk of non-unions, several authors have reported a clear association of diabetes and delayed union / non-union in fractures of the lower extremity (387, 388). In a systematic review investigating the association of diabetes and impaired healing of lower limb fractures, the risk of non-union was higher in fractures below the level of the knee (i.e. tibial and ankle fractures), but this was not the case for hip fractures (385).

Finally, it was demonstrated that the degree of comminution was significant to the progression to a non-union. More specifically, moderate comminution (three fracture fragments) was associated with a lower risk compared to simple (two fragments) or severe comminution (four fragments or more). In the literature, only lack of medial cortical support (i.e. medial cortical comminution), has been reported as a risk factor for subtrochanteric non-union (250). Nevertheless, high degree of comminution has been associated with a longer time to healing and a higher risk of non-union in tibial fractures (389, 390) and distal femoral fractures (391, 392).

The association of higher degree of comminution and impaired fracture healing may be secondary to the disruption of the blood supply of the fragments, as well as the

subsequent instability at the fracture site, both of which are known factors leading to a non-union. With regards to the 'simple' two-part subtrochanteric fractures, their increased risk of non-union may be secondary to the high incidence of malreduction of these complex fractures, as well as their high association with atypical fractures which was also demonstrated to be a risk factor for a non-union in the herein series.

3.10.3 Non-union scoring system

Following the logistic regression, two non-union scoring systems were produced. The first included 'self-dynamisation' and was used as a control, whereas in an attempt to provide the clinician with an 'early' diagnosis, the second excluded 'self-dynamisation'. Both scoring systems had a good AUC (0.770 and 0.766 respectively). The internal validation (5-fold internal cross validation) confirmed the accuracy of both scoring systems (0.798 and 0.776 respectively).

Using a cut-off of 30 points, **Table 3.37** and **Table 3.38** show the misclassification risk of each scoring system. When self-dynamisation was included, this performed better, even though is slightly lower accuracy could be offset by the ability to use the scoring system at an earlier point. Therefore, both systems can help the treating surgeon to identify the high risk patients and if deemed necessary, to intervene at the early stages. Intervention could be through a minimally invasive procedure (i.e. injection of BM concentrate), or more aggressive procedures such as bone grafting, and even revision of the fixation. Even if that patient would eventually heal without any intervention, time to healing and therefore morbidity associated to it could be successfully reduced.

Table 3.37 Misclassification table for predicting non-union (including self-dynamisation)

	Union	Non-union
<30	79%	21%
≥30	23%	77%

Table 3.38 Misclassification table for predicting non-union (excluding self-dynamisation)

	Union	Non-union
<30	74%	26%
≥30	42%	58%

Note that non-union was observed in 12% of the patients who had no risk factor, as identified by our regression analysis. This could be explained by factors affecting bone healing not documented by our analysis, such as a devascularisation of the fracture fragments during surgery or presence of 'dead' bone not debrided during the operation, both of which can only be documented / reported intra-operatively and therefore if details were not present on the operation note, this was not included into the analysis; low grade infections that were not identified by the conventional microbiology cultures; a possible genetic predisposition; and generally the 'personality' of the fracture.

3.10.4 Infections

To understand the pathogenesis and appropriately treat orthopaedic infections, it is first necessary to understand the interdependence of the human body and microorganisms, as well as the complex but well-orchestrated process of the host defence mechanisms (369). It is also important to define correctly, identify and manage each type of infection, whilst the correct diagnosis is crucial to prevent over- or under- reporting of this complication. According to the Centers for Disease Control and prevention (CDC), surgical site infections (SSIs) are those involving the incision or organ or space, occurring after surgery (393). Depending on the depth of tissues they involve they are classified to superficial and deep SSIs (394). SSIs are largely preventable, so CDC and World Health Organization (WHO) has outlined specific recommendations to limit their incidence, based on evidence-based strategies (393, 395). Nevertheless, patient and operation related factors are still associated with an increased risk of SSIs, whilst their consequences can be devastating for the patients (396-398). They represent the leading or second most common cause of healthcare-associated infections worldwide and are associated with increased morbidity, length of stay in hospital and considerable healthcare costs (399). In fact a recent cost analysis claimed that the median cost in patients with SSI increased to $108,782, compared to $57,418 for uninfected patients (400).

Superficial wound infection (incisional), presents as cellulitis and represents a bacterial infection of the deep dermis and subcutaneous tissue around the incision (394, 401). It is characterised by a poorly demarcated, expanding erythema, and is associated with oedema / swelling, warmth and tenderness around the affected area (401). It can also be associated with increased discharge from the wound and delayed wound healing. This should however be differentiated from the physiological

inflammation associated with wound healing. In contrast, deep infection involves the fascia or muscular layers (394).

In this series, the incidence of infection was 6.4% (superficial infection: 3.7%; deep infection: 2.7%). This is comparable to the findings of Kilinc et al. who reported a 5.77% superficial and 3.85% of deep infection, in their study of 52 patients with subtrochanteric fractures treated with IM nailing and cerclage wiring (276). Other studies report on a lower incidence of infection, but their findings are largely limited by the small sample size and inconsistency in the definitions of infection (0% - 1.7%) (174, 267, 274, 275, 277, 278, 402).

The commonest micro-organism isolated in superficial and deep wound infections was *Staphylococcus aureus* (46.2% and 26.7% respectively). Even though there is no evidence specifically reporting on the microbiology of the subtrochanteric region, this is similar with the findings of post-traumatic osteomyelitis, where the reported incidence ranges between 35% – 50% (403-406). *Coliforms* on the other hand were the commonest group of organisms isolated in deep infections (33.3%). This finding is similar to those reported in the literature, with the incidence of *Enterobacter cloacae* reported as high as 11% – 12% (403, 404), *Klebsiella pneumoniae* 4% – 12%(403, 406), *Citrobacter koseri* 2% (403) and *Klebsiella oxytoca* 1%(403). *Escherichia coli*, a special type of coliform, was isolated in 26.7% of the cases, compared to a 12% – 15% incidence in the literature (403, 404). Of note is that 38.5% of superficial infections and 80.0% of deep infections were polymicrobial (i.e. more than one organism was isolated). This is much higher than the reported 17% – 29% in the literature (403-405), whilst the significance of each organism cannot be fully determined as it may just represent opportunistic colonisation and not active infection. Finally, in 20.0% of deep infections no organism was isolated on conventional cultures, compared to 11% – 32% reported in the literature (405, 406). A possible explanation for negative cultures include the early start of empirical antibiotic therapy following clinical suspicion of infection and before obtaining cultures, or the inability of conventional culture techniques to detect bacteria situated within a biofilm (367, 407). All the above however highlight the complexity of these infections and may explain why antibiotics may not be effective in a lot of these cases.

Investigating the risk factors associated with deep infection, the presence of an open fracture, need for massive transfusion or post-operative transfusion, non-union, increased alcohol intake and increased LOS, were identified as significant factors.

Once more, there is a lack of evidence in the literature with regards to infections in subtrochanteric fractures, but there are many studies reporting on orthopaedic related infections.

Open fractures in general is a well reported risk factor for deep infection, with the incidence of a deep infection following an open fracture being reported as high as 27% (408, 409). Amongst others, this risk is associated with the Gustilo-Anderson classification, the degree of soft tissue injury, the timing of antibiotic treatment and the timing to surgical debridement (408, 409). In our institution, all open fractures were managed according to the BOAST4 (British Orthopaedic Association standards for trauma – 4) guidance, in an attempt to reduce this risk (410). Moreover, transfusion of blood products and especially massive transfusion has been clearly associated with an increased risk of infection (411, 412), which is in line with these findings. The increased risk of infection is thought to be a consequence of the immunosuppression associated with the allogenic blood transfusion (412). Another explanation would be the increased severity of the injury, longer operating time, need for extended tissue dissection etc., all of which can lead to an increased bleeding and a higher risk of infection.

With regards to non-unions, infection is probably the aetiology of the non-union rather than the result. Similarly, increased LOS may be the consequence of a deep infection and therefore the need for additional procedures and prolonged courses of antibiotics. It can however be associated with other conditions that may increase LOS, such as polytrauma, need for HDU / ICU stay, systemic infections (HAP, UTI) etc., that could theoretically be linked to an increased risk of deep infection. Finally, increased alcohol intake was another risk factor demonstrated in this study, a finding previously reported by other authors (394).

3.10.5 Open reduction

The combination of a moderate vascularity of the subtrochanteric region and the high concentration of stresses (233, 413, 414), along with the multiple deforming forces acting on the fracture fragments, increase the risk of complications, especially if anatomic reduction is not achieved. It is therefore of paramount importance to investigate and understand which factors are predictive of the need for an open reduction, and if so, the associated complications that one would perceive to encounter following open reduction.

The majority of subtrochanteric fractures (82.35%) in the herin adult population had comminution, involvement of trochanter(s), distal diaphyseal extension, or pathological fractures – all characteristics of an unstable fracture pattern. This is therefore compatible with the high rate of open reduction demonstrated in this study population (47%), which is in keeping with that reported by other studies (range: 33.3% to 82.2%) (174, 274, 277). When compared against closed reduction, the analysis found open reduction group to be associated with a more complex fracture pattern (open fracture: $p=0.015$; degree of comminution: $p=0.014$; distal fracture extension: $p<0.001$). Analysing all patient and injury factors, distal extension of the fracture (towards the diaphysis) was identified as the most important risk factor predictive of the need for an open reduction in subtrochanteric femur fractures. Furthermore, the regression analysis confirmed that open reduction was associated with a longer surgical time (OR: 4.111), and a higher likelihood of the senior operating surgeon being the orthopaedic consultant (OR: 1.480), both of which further confirm the complexity of the fracture pattern mandating open reduction in the first place.

Nonetheless, there remains a lack of evidence in the literature that compares the outcomes of open versus closed reduction (174, 272, 274, 277, 278). Most of these studies are small in sample size and exclude patients with pathological fractures (174, 272, 274), whilst some assess the elderly age group only (174). None of these studies however have reported on the predictors of open reduction.

Open reduction of long bone fractures, especially in regions of moderate vascularity such as the subtrochanteric region, has been met with some reluctance. This is due to the belief that open reduction may compromise the local fracture biology, and especially the vascular environment (233), therefore raising the threshold amongst orthopaedic surgeons of what should be deemed as an ‘acceptable’ suboptimal reduction. Not surprisingly, subtrochanteric femur fractures treated with IM nailing have a higher non-union rate compared to other long bones treated with an IM device (26, 272, 415).

On the other hand, cerclage wiring can be a useful open reduction tool during IM nailing, allowing the surgeon to gain provisional or definitive fracture reduction. Traditionally, the classic cerclage wiring was believed to cause strangulation of the bone, risking necrosis and ultimately failure of healing (416). Recent studies have however challenged this school of thought. Histological and anatomical studies by Nather et al. (417) and Pazzaglia et al. (418) have both described the periosteal blood

supply as circumferential, entering to nourish the periosteum at multiple levels. Because the periosteal supply is not longitudinal, circumferential cerclage wiring is therefore unlikely to cause strangulation. Lenz et al. in an ex-vivo study using human diaphyseal bone further confirmed this, whereby they found no periosteal compression when using cerclage wire or a cable (419, 420). Furthermore, biomechanical studies have also demonstrated how the use of cerclage wire is advantageous, as it stabilises the medial hinge and therefore raises the threshold of cyclical compressive loading tolerated by the femur before reaching plastic deformation, and was found significantly to decrease the rate of implant failure following IM nailing (273). All these studies therefore support achieving adequate anatomical fracture reduction by open reduction when closed reduction proved difficult, whilst disproving the common misbelief that cerclage wiring inadvertently affects the local blood supply. It is important however to stress the fact that the blood supply is compromised by the extensive stripping of the soft tissues during the open approach of the fracture site, especially if this is circumferential and lengthy. Careful dissection and avoidance of excessive / circumferential stripping especially of the fracture ends, will reduce this risk.

Kilinc et al. reported a 5.77% superficial and 3.85% of deep infection in their study on 52 patients with cerclage wiring of subtrochanteric fractures treated with IM nailing (276). Studies comparing open and closed reduction of subtrochanteric fractures treated with cephalomedullary nailing reported similar infection rates between the two groups, which range between 0% - 1.67% (174, 274, 275, 277, 278). Nevertheless, these studies have their limitation in terms of small sample size, exclusion of pathological fractures and restricted age groups; all of which precludes the affirmative conclusion on whether open reduction indeed poses a higher infection risk. Having overcome the aforementioned limitations, this study has conclusively revealed that there is a statistically significant higher risk of developing superficial wound infection in the open reduction group (OR: 6.756). Contrary to common belief, although there was a trend towards higher risk of deep infection amongst the open reduction group (OR: 3.157), the logistic regression analysis suggested that this was not statistically significant (p=0.098).

There is a paucity of studies in the literature which compared the blood transfusion requirement between subtrochanteric fractures requiring open versus closed reduction. Codesido et al. reported no difference in transfusion requirements between the two groups (277), whereas Shukla et al. reported higher transfusion

requirement in the open reduction group (174). This study supports Shukla et al.'s finding (174). More specifically, it was found that open reduction poses a higher risk of requiring blood transfusion within the initial 48-hour postoperative period (OR: 1.761). The higher transfusion risk could be explained by these findings wherein open reduction was performed for more complicated fracture patterns requiring prolonged surgical time, more extensive surgical dissection, and therefore soft tissue damage – all contributory factors towards a higher bleeding risk.

Collectively, open reduction was found to be associated with a higher risk of developing nail complications than closed reduction (p=0.007). This difference however was no longer significant when each of the nail complications (failure a lag screw junction, cut out, self-dynamisation) was individually assessed. The small sample size for each of these complications, and therefore an underpowered comparison most likely accounts for the lack of statistical difference.

With regards to the non-union rates, these have been reported to be lower in open reduction group compared to the closed reduction groups (274, 277). Karayiannis et al. reported rates of symptomatic non-union to be 5.80% and 6.25% in the open reduction and closed reduction group respectively (274). Codesido reported no non-unions amongst subtrochanteric fractures having an open reduction, whilst 8.33% of the closed reduction group developed non-union (277). Kilinc et al. (cerclage wire reduction) and Mingo-Robinet et al. (clamp assisted reduction only) both reported no non-unions in their cohorts of patients (276, 278). Out of the three main operative factors, Krappinger et al. found that (i) the lack of medial cortical support and (ii) varus malalignment as significant risk factors for non-union in subtrochanteric fractures treated by IM nailing (250). Although this study has demonstrated no difference in the non-union rates between subtrochanteric fractures treated with closed or open reduction, it did however show that open anatomical reduction achieved by cerclage wire / cable to have a lower non-union rate (OR: 0.287), when compared to 'clamp assisted only' open reduction.

3.10.6 Effect of osteoporosis

The association of previous fragility fractures and presence of a subsequent subtrochanteric fracture has been poorly reported in the literature, with most studies focusing on the relationship between bisphosphonate use and the development of subtrochanteric fractures. The risk of further fragility fractures in patients who previously sustained other fragility fractures, has been previously reported as high as

11% at three years, with vertebral and proximal humeral fractures being the most common presentation (421). In this cohort, 22% of patients presenting with subtrochanteric fractures sustained previous fragility fractures. In a similar study by our group investigating the differences between patients presenting with a proximal femoral fracture with or without a history of previous fragility fracture, we reported that patients in both groups had comparable demographics, ASA grades and complication rates (422). This was not however the case in the current cohort, where patients with a history of previous fragility fracture were more likely to be older, of female gender and presenting with a higher ASA grade. Nevertheless, these differences, along with surgical / biochemical differences, were not statistically important when an adjusted model was used, which suggests that the two groups were comparable.

3.10.7 Effect of bisphosphonates

Investigating the effect / associations of bisphosphonates in the current cohort, as one would expect there were differences in bone protection medication, both pre-admission and post-discharge. Not surprisingly, patients on bisphosphonates had a higher incidence of fractures isolated in the subtrochanteric region, and of atypical fractures.

Similar to this study, Das De et al. compared patients who were on bisphosphonate treatment and presented with subtrochanteric fractures, with those who were not on bisphosphonates (12 patients versus 8 patients) (423). They reported demographics comparable to the herein findings, as well as a higher incidence of bilateral fractures in patients on bisphosphonates, that was also identified in this series (423). Additionally, they evaluated bone turnover markers reporting no difference between the two groups, which was comparable to these findings, even though a lower incidence of Vitamin D depletion in patients on bisphosphonates (related to concurrent Vitamin D intake) was demonstrated (423). Prasarn et al. investigated the effect of bisphosphonates in subtrochanteric and femoral shaft fractures, reporting a higher rate of intra- and post- operative complications (424). Looking into their results in more detail, the failures of metalwork they described were only in cases where an extra-medullary device was used (three out of ten extra-medullary fixations failed) (424). In this series on the other hand, the post-operative complications were similar in both groups. Finally, in contrast to these findings, Prasarn et al. reported similar incidence of smoking in both groups (424).

A significant amount of research on bisphosphonates and their association with subtrochanteric fractures assumes complete adherence to treatment. Wang et al., investigated female adherence to bisphosphonate treatment and risk of a subtrochanteric fracture dependent on duration of treatment (425). Results from their study suggest that the highly compliant group (defined as those who picked up 2/3 of prescriptions) had a subtrochanteric hazard risk ratio of 4.06 (95% CI, 1.47-11.9) at 5 years compared to less compliant group (picked up < 1/3 of prescriptions), whilst in their multivariate analysis both increasing age and co-morbidities were associated with increased risk (425).

Black et al. performed a secondary analysis on three large RCTs reporting on bisphosphonate intake (FLEX (426) and FIT (427) investigating the use of oral alendronate; and HORIZON (428) investigating annual zolendronic acid infusions) (429). As compared with placebo, the relative hazard was 1.03 (95% CI, 0.06 to 16.46) for alendronate use in the FIT trial, 1.50 (95% CI, 0.25 to 9.00) for zoledronic acid use in the HORIZON-PFT trial, and 1.33 (95% CI, 0.12 to 14.67) for continued alendronate use in the FLEX trial (429). Although increases in risk were not significant, confidence intervals (CI) were wide. The above findings suggest that there was no significant increase in subtrochanteric fractures associated with bisphosphonate use for up to 10 years (Flex trial duration) (429). Further statistical analysis estimated the risk of fractures in those who did not receive bisphosphonate therapy, would result in an annual rate of 2.3 subtrochanteric or diaphyseal fractures per 10,000 patients. These findings were comparable to a nationwide cohort study performed in Denmark which also assessed the use of alendronate, where a 30% lower risk of subtrochanteric hip fracture was noted (430).

3.10.8 Atypical fractures

Regarding the associations with atypical fractures, not surprisingly, bisphosphonate and steroid intake, as well as isolated subtrochanteric extension were highly significant in our cohort. Therefore, patients presenting with these characteristics should be considered as high risk for having an atypical fracture and managed accordingly. Age between 65 and 75 years old was also significant. A possible explanation could be the relatively high incidence of bisphosphonate intake in this group, along with a more active lifestyle compared to elderly patients, both of which potentially increase the risk of a subtrochanteric fracture. It is also important to mention the increased risk of a fragility fracture in the future, which may be secondary to the abnormal bone metabolism, or commonly the presence of a contralateral

atypical fracture. Most importantly, atypical fractures were also associated with an increased risk of non-union. This was also identified in the regression analysis of risk factors for a non-union and was extensively discussed in section 3.10.2. Finally, atypical fractures were associated with a smaller risk for transfusion. The relatively younger patients affected and lower energy mechanisms of injury may account for this.

With the pervasive use of bisphosphonates in treating osteoporosis, the number of atypical fractures encountered is increasing. Though the benefits of bisphosphonates still outweigh the risk of developing an atypical fracture, this risk should be considered in every patient treated with bisphosphonates. A high suspicion should be present in every patient presenting with typical symptoms and their prompt recognition could lead to better outcomes.

3.10.9 Comparison of commonest nails

Intra-medullary devices remain the 'gold standard' for the treatment of subtrochanteric fractures (230, 250, 251). Even though a lot of implants are available in the market, there is no clear advantage of one over the others, whilst not a lot of studies exist in the literature investigating potential differences between them. By attempting to compare the two commonest nails used in the cohort, an attempt was made to identify the important characteristics in their design that may be associated to, or protect from complications.

The design of both these nails is similar, yet there are some important differences (**Table 3.39**) (431, 432). In the Affixus nail, there is the option of an anti-rotation screw, which can provide some added rotational stability. It however results in an additional 5 mm aperture above the lag screw. This theoretically creates a 'weak' area, but the slightly bigger diameter of the nail (15.6 mm compared to 15.5 mm of the Gamma nail), may reduce this risk. Additionally, the presence of a second screw increases the risk of Z-effect and reverse Z-effect (migration of the two screws in opposite directions). Nevertheless, the option of locking both screws may reduce this risk, whilst to my knowledge no case has been reported in the literature describing this problem in an Affixus nail. Moreover, the thread spacing of the lag screw in the Affixus nail allegedly provides better resistance to cut-out. Finally, Affixus nail claims to be a more 'anatomical' nail having a 1.8 m radius of curvature compared to 1.5 m for the Gamma nail. This would theoretically increase the risk of stress to the anterior cortex in femurs with higher curvature, but a chamfer tip reduces this risk.

Table 3.39 Implant characteristics for Long Gamma and Long Affixus nails

	Long Gamma Nail	**Long Affixus Nail**
Material	Titanium alloy	Titanium alloy
Entry point	Trochanteric entry	Trochanteric entry
Proximal nail diameter	15.5 mm	15.6 mm
Proximal lateral bend	4°	4°
Proximal anteversion	10°	10°
Lag screw angle	120°, 125°, 130°	125°, 130°
Lag screw	10.5 mm	10.5 mm
Anti-rotation screw	n/a	5 mm
Set screw	Not preloaded	Preloaded
Nail length	240 – 480 mm	260 – 460 mm
Nail diameter	10, 11, 13, 15 mm	9, 11, 13, 15 mm
Radius of curvature	1.5 m	1.8 m
Distal bend	3°	3°
Tip of the nail	n/a	Chamfer
Distal screws	5 mm	5 – 6 mm
Locking options	Static - Dynamic	Static - Dynamic
Dynamisation range	5 mm	6 mm

Comparing the basic demographics, medical comorbidities and fracture characteristics of the two groups (Gamma versus Affixus nails), several differences were identified. Most of these differences were related to changes to the protocol of managing the patients presenting with fragility fractures during the study time. This included a better screening for osteoporosis and prescription of bone protection (Vitamin D loading on admission, bisphosphonates and calcium intake), operation within 36 hours in accordance to NHFD guidance, and an improved falls assessment. It is therefore no surprise that patients presenting later to mid 2012, subsequently having an Affixus nail, had a lower risk of presenting with low calcium on admission, higher incidence of Vitamin D loading on admission and lower percentage of bisphosphonates prescription on discharge (bisphosphonates would be prescribed by the GP after Vitamin D loading and confirmation of Vitamin D levels few weeks post discharge), lower incidence of frequent falls and shorter time to operation. Pre-operative impaired renal function incidence was also lower; this could be secondary to an improved community / pre-hospital care, as well as improved fluid resuscitation at the emergency department. Nevertheless, the incidence of hypoalbuminaemia was higher, possibly reflecting to a more infirm population, even though ASA and CCS were not significantly different. Finally, regarding the fracture configuration, distal extension was more common in the Gamma nail group. According to our previous findings, distal extension of the fracture is associated with an increased risk of open reduction and need for cerclage wiring, but it has no obvious effect on the development of complications such as progression to a non-union and development of a wound infection.

The regression analysis also demonstrated that the reduction of the lateral cortex (already shown to be associated with progression to non-union), was better in the Affixus nail. The same applied for the risk of anterior cortex impingement, and therefore potential risk of periprosthetic fracture, around the tip of the nail. Even though the radius of curvature of the Affixus nail is higher, the chamfer end seems to be a beneficial design feature. Additionally, the risk of any type of nail complications in the Affixus group was lower, also demonstrated by the survival curves of the two nails. All the above suggest a superiority of the Affixus nail over the Gamma nail, but this this cannot be claimed with certainty as there was no randomisation of the patients in the two groups, but instead Affixus nails were used at the second half of the study. As our understanding of the complexity of these injuries increases, as well as that of the common types of complications and how to avoid them, orthopaedic surgeons may be becoming better in avoiding such pitfalls and therefore the their devastating sequalae.

In the literature, there are only a few studies reporting on the outcomes of the two nails in question, with most authors reporting on intertrochanteric fractures and a mixture of Gamma nails (long Gamma nail – previous generation; and Gamma3 nail – current nail). Only one study by Persiani et al. investigated the differences between Affixus and Gamma nails for the treatment of trochanteric fractures, reporting no difference in the length of stay, duration of surgery and functional recovery (433).

Even though distal femoral fractures around the tip of the nail were very common in first generations of cephalomedullary nails, changes to their design and improved technique has now reduced this risk (434, 435). In unstable intertrochanteric fractures Gamma nail, the reported failure rate was 13.2%, with gender, TAD > 25 mm and incorrect entry point being associated with failure (436).

Bojan et al. reported on the outcomes of subtrochanteric fractures treated with long Gamma nails (a series of 473 fractures treated with long Gamma and Gamma3 nails) (375). With regards to complications, he reported that the insertion of the nail was difficult in 5.3% of the cases, difficulties with distal screw insertion in 6.6%, intra-operative fractures in 1.3% (three fractures of the lateral cortex and three anterior cortex perforations), post-operative femoral shaft fracture in 0.2%, and need for implant removal 11.8% of the cases (375). He therefore suggested that meticulous adherence to the surgical technique may reduce the risk of failure (375). Buecking et al. in a prospective case series of 90 fractures treated with Gamma nails, reported

an incidence of non-union / cut-out of 1.1%, an incidence of deep infection of 1.1% and an incidence of revision of 4.4% (437).

Kanakaris et al. on the other hand reported on 476 proximal femoral fractures treated with an Affixus nail (included both stable and unstable patterns), a 6.3% non-union rate, a 2.7% cut-out rate, and a 2.5% incidence of revision surgery (438). Finally, Mabrouk et al. investigated Affixus nails for per-trochanteric hip fractures reporting an overall re-operation rate of 3%, cut-out of 1%, backout of 1%, no breakage of nail and no non-unions (439).

3.10.10 Transfusion requirements

Blood loss following trauma and early identification of the possible sites of bleeding is a very important aspect of the initial patient assessment. Most importantly, the prompt management of bleeding, along with appropriate resuscitation will prevent the development of haemorrhagic shock and its sequalae; the lethal triad of coagulopathy, hypothermia and acidosis (280). In the setting of subtrochanteric fractures, there is little evidence with regards to an estimate of blood loss and the risk factors for bleeding. Femoral shaft fractures on the other hand have been associated with substantial blood loss and need for blood transfusion, with a previous report estimating this volume to an average of 1200 ml of blood (440). When accounting for associated injuries however, a recent study suggested that this risk may be overestimated and blood loss following femoral shaft fractures may be less clinically significant than previously thought (441).

In this study, the incidence of blood transfusion was 8.6% pre-operatively (mean: 2.6 units RBC transfused), compared to 63.3% post-operatively (mean: 3.1 units RBC transfused), whilst the estimated Hb drop was 33.3 g/L. A similar study by Shukla et al. reported a mean Hb drop of 30 g/L and a mean of 3.0 units transfused (54% of the patients transfused) for closed reduction; in open reduction the mean Hb drop was 32 g/L and the mean number of units transfused was 3.1 (69% of the patients transfused) (174).

Another study by Persiani et. al investigating the management of intertrochanteric fractures, reported a 95.8% need for transfusion (mean: 1.4 units RBC) in patients managed with a Gamma nail, compared to 100% (mean: 1.7 units RBC) in patients managed with an Affixus nail (433). Wertheimer et al. compared blood loss in femoral shaft fractures to that of ‘extremity’ fractures (70.3% were subtrochanteric fractures),

suggesting that ‘extremity’ fractures had a higher incidence of transfusion (both total transfusion and transfusion within 48 hours), even though these injuries involved a significantly ‘older’ population, sustaining their injuries most commonly from simples falls, and presenting with a lower Hb on admission (280). In their regression analysis examining the risk factors for transfusion within the first 48 hours, only Hb on presentation was found to be important ($p<0.01$), whilst there was also a trend for male gender to require a transfusion ($p=0.08$) (280).

From the regression analysis, a nail / canal ratio <0.70 seemed to be the most important association with transfusion. Even though reaming of the intra-medullary canal increases the diameter of the nail that can be used and therefore the stiffness of the nail (subsequently reducing the risk of failure), it has been associated with adverse effects such as additional bleeding, which correlates to the degree of reaming (442, 443). By using a larger diameter nail, hence increasing the nail / canal ratio, the medullary canal can be tamponaded and the bleeding reduced.

The next most important association with transfusion was the use of cerclage wiring; open reduction alone however did not seem to increase the risk of transfusion. Similarly, Codesido et al. reported no increase in transfusion requirements following open reduction (277), in contrast to Shukla et al. who reported higher transfusion requirements following open reduction (174). Nevertheless, need for cerclage wiring has never been reported as an independent risk factor. Along with lesser trochanteric involvement which was also found to be a risk factor in this cohort, it may suggest that more complex fracture patterns require extensive tissue dissection and are therefore associated with an increase in the risk of bleeding and need for transfusion.

Another association with transfusion was pre- and post-operative moderate / severe renal impairment. It is well established that glomerular filtration rates below 60 mL/minute (i.e. moderate / severe renal impairment), are associated with worsening anaemia (444-446). This is because of the decline of the production of endogenous erythropoietin seen in CKD (445). Additionally, the need for transfusion is higher in older patients and increases with increasing severity of the renal impairment (446). This was in line with the herein findings, along with the observation that elderly patients were more likely to have a transfusion within the first 48 hours post-operatively.

Finally, the presence of low albumin on admission was also linked with an increased risk of transfusion. Aldebeyan et al. reported similar findings in patients undergoing surgery for hip fractures (447), as well as other studies investigating the effect of hypoalbuminaemia in joint replacement surgery (448-450).

3.10.11 Weekend effect

In regards to the so-called 'weekend effect', the literature contains a lot of evidence to suggest that there is in fact no weekend effect in the treatment of patient with hip fractures (451-453). Whilst there has been no specific study to investigate this within the subtrochanteric fracture population, these data correlate with those involving hip fractures in general. Within this cohort there was no difference in the time to surgery or the levels of the leading surgeon between weekend and weekday admission. There was also no difference in the overall outcome of the patient with regards to mortality, HDU / ICU admission, or total length of stay. Overall, any evidence of the so-called 'weekend effect' was not identified.

3.10.12 Hospital Acquired Pneumonia

Approximately one in six of the patients in this cohort (16.6%) developed HAP at one point during their admission. This is significantly higher than the figures quoted in the literature of approximately 4-7% (454-457), but differences in the methodology of the studies may account for this. Furthermore, there has been no study to date looking directly at subtrochanteric fractures and incidence of HAP as opposed to all cause hip fractures. This could therefore potentially represent a higher risk with this specific type of fracture, especially with the highest incidence of weightbearing protection and reduced mobility following the operative management of such complex fractures. Additionally, the incidence of HAP increased with age, a finding previously reported in the literature (455). Risk factors for HAP have been previously identified include male sex, older age (especially ≥90 years), low body mass index, and especially chronic lung disease, all of which are associated with increased risk of complications, consequent escalation of treatment and increased length of stay (454, 458-460). This is supported by the findings of this study, with increasing comorbidities and chronic lung disease being the main risk factors for HAP, whilst this was also associated with an increased risk of HDU / ICU stay and increased length of stay.

3.10.13 Myocardial Infarction / Cerebrovascular Accidents

In the herein study, MI / CVA events related to the index admission was 4.1%. This included, pre-, peri- and post- operative events. This is slightly higher than a large study looking at over 2 million hip fracture operations, which quotes a total number of adverse cardiovascular events to be at 3.3.% (461). This study however only investigated peri-operative events and may have underestimated the risk because of 'under-coding' of these conditions. In line with the herein findings, MI / CVA has been previously reported as a predictor of increased mortality (457, 462, 463). These data also suggests that the ASA grade is implicated with an increasing risk of cardiovascular adverse events. This has previously been reported in hip fracture research, though not within the subtrochanteric fracture population (464).

3.10.14 Post-operative delirium

Delirium has been quoted to be have an accumulated incidence of 24-33% amongst the elderly population with hip fractures (465), compared to 10% in our series. The overall effect of delirium can be very mild to fatal. Not only can delirium increase a patient's length of stay, it can also affect the engagement with physiotherapy which will ultimately affect the post-operative mobility and overall health (466). In their study, McCusker et al. found delirium to be an independent predictor of one-year mortality, with its effect being more serious for patients without pre-existing dementia (8). Furthermore, it has been suggested that the timing of delirium can affect mortality, i.e. immediate post-operative delirium (within 24 hours) is implicated with higher mortality, compared to delayed (>24 hours post operatively) (8). On the contrary, other authors suggest no effect of delirium to the survival of patients with hip fractures (467). Risk factors for developing delirium following a hip fracture include increasing age, pre-existing cognitive decline and polypharmacy, along with infection, MI, CVA, electrolyte abnormalities, pain, constipation, new urinary retention as well as the implementation of new medicines (9). In fact according to Harris et al., dementia was the factor with the greatest risk for developing post-operative delirium (468). The regression analysis suggested that delirium was most likely in patients with underlying dementia (most important predictive factor), infections (UTI and chest infections) and a deteriorating renal function. Therefore, early assessment by geriatrics and monitoring for delirium, prevention / early treatment of infection, correction of biochemical dehydration with adequate fluids and medicines optimisation could potentially reduce this complication.

3.10.15 Venous thromboembolism

Venous thromboembolism is a well-reported complication for any patient following surgical intervention. Within the hip fracture population, work has suggested that the risk of VTE is in keeping with the degree of comorbidity. The more severe the comorbidity, the higher the risk of VTE (469). The literature also suggests that in order to avoid VTE, early surgery (within 48 hours) and pharmacological (or in combination with mechanical) thromboprophylaxis is vital (470, 471). The incidence of VTE in hip fracture patients without thromboprophylaxis is between 42-50%; this has been quoted to fall to 20-30% (up to 60% decrease) after hip arthroplasty (457). Within this cohort, 4% of patients developed VTE. Furthermore, no patient required admission to ICU / HDU, nor did this diagnosis increase their length of hospital. This could suggest a slightly fitter population of patients, which is rather unlikely given the comorbidities of this cohort. The pathway of early mobilisation and prompt review by an orthogeriatric may have contributed to these good outcomes.

3.10.16 Mortality

The 30-day mortality rate in this patient cohort tallied at 6.0%, with one-year mortality rising to 20.8%. When patients younger than the age of 60 years were excluded, mortality increased to 7.6% and 25.6% respectively. This is marginally higher than the national all-cause hip fracture mortality data of 30-day mortality being quoted at 6.9% (cf. 6.7% in 2016) (22). The mortality results are also similar to a Swedish registry study of both subtrochanteric and trochanteric hip fractures, where 30-day mortality was reported 7.7% and one-year mortality at 25.9% (360). With regards to gender, in contrast to the findings of other studies, any increased mortality in male patients was not identified (360, 472). On the contrary, as per the survival curves, mortality was found to be slightly higher in female patients within 10 years, even though this was not statistically significant. A higher proportion of ‘older’ female population with fragility fractures and ‘younger’ male patients with high energy injuries may account for this finding.

Of particular interest is the difference in early mortality between the different age groups. There was no documented death in patients aged 60 or under within the first 30 days, and there was no reported mortality at one year in patients younger than the age of 40. This is likely secondary to the mode of fracture. More specifically, younger patients (i.e. those aged 60 and under) have likely been admitted secondary to high impact, polytrauma injury. Hence, they were either going to be pronounced dead at the scene (and therefore not included in our study) or have such high

physiological reserve that they are able to overcome the first 30 days post injury. In contrast, increasing mortality levels were found to be positively associated with age which likely represents the ever-depleting physiological reserve patients with fragility fractures, a finding previously reported in the literature (472).

With regards to the one-year mortality associations identified, the presence of pathological fractures (secondary to metastatic lesions) unsurprisingly was the most predictive parameter of mortality. Bone is the third commonest site of metastasis (following lung and liver) (473), with the subtrochanteric region in particular being an area prone to metastasis and often associated to high risk of complications (474, 475) and an increasing mortality (463). By the time the metastatic lesions are diagnosed, especially following a pathological fracture, patients have an advanced disease and hence the poor prognosis.

Presence of a low serum albumin level on admission also significantly increased the mortality risk, a finding previously reported by other authors, quoting a 2.5 times increase in mortality in patients with hypoalbuminaemia (476-479). Serum albumin is a marker of 'nutritional state' and low levels are associated with low muscle mass, strength and function. Though, this could also reflect the existing co-morbidities, chronic illness and concurrent infection (478). As albumin is the main transporter of essential molecules like calcium, fatty acids and hormones, nutritional supplementation is warranted in order to reduce both the risk of developing complications, as well as the risk of mortality (480).

The presence of comorbidities (CCS > 6) was another factor significantly increasing the risk of mortality. Comorbidities in general, modify the course of a disease such as in the case of a hip fracture, whilst several authors reported an increasing rate of mortality with an increase in comorbidities (463, 481-483). More specifically, Lunde et al. in an epidemiological study reported a 15% increase in deaths during the first year, in women with a CCS score of ≥ 3 (463), findings comparable to those of Jürisson et al. (483). Dementia in particular, was found to be an independent predictor of mortality in this cohort, confirming previously reported findings (463, 484, 485). The presence of chest infection significantly increased mortality, which was again in line with previous findings (458, 486). On the contrary, nail complications seemed to be a 'protective' factor for one-year mortality, but this can be explained by the fact that immobility and early death of the most infirm patients reduces the risk of nail complications.

3.11 Strengths

To my knowledge, this study is the largest cohort series reported in the literature to date investigating subtrochanteric fractures treated with IM nailing. Not based on a registry (such as NHFD or similar registries used in other countries), it ensures eligibility of patients is comprehensively examined (i.e. no coding concerns). Moreover, with no exclusion criteria posed upon age, comorbidity or fracture aetiology, this study provides a better overview of the overall fracture demographics and epidemiology of subtrochanteric femoral fractures encountered in a Level 1 Trauma Centre serving a metropolitan population. Additionally, through the comprehensive review of the patients' medical records, it was ensured that no documented information was missed, whilst cross reference of different databases ensured information collected was as accurate as possible. The fact that this was a tertiary referral centre also meant that patients with complications presenting to other institutions would be referred back to the same hospital.

Regarding the seniority of the clinician managing these patients, all patients were managed with a close consultant supervision (consultant led care) under set protocols, which ensures good standards in the management of these patients. The comprehensive literature review included in this study also provides a good reference point to compare and evaluate the management and outcomes of this series. Furthermore, this is the first study to report on associations with the most common complications, which are very important to the holistic management of this subgroup of proximal femoral fractures. This study also investigates areas that are poorly recorded, not only limited to surgical complications and mortality, but also including medical complications that are commonly encountered and represent a cause of high morbidity.

Most importantly, this study set a framework for further projects with the ultimate goal of improving patients care and outcomes, through changes in practice and development / improvement of pathways and protocols.

3.12 Limitations

Even though the study design attempted to overcome limitations, avoiding bias and improving the validity of the results, some limitations were identified. Firstly, the retrospective nature of the study meant information collected relied on the accuracy

of documentation entered by medical and nursing staff. A randomised controlled study could have been a superior model, but this is very difficult in terms of study length (eight years), need for dedicated staff to curry this out and its associated costs implications. Blinding to the final outcome would also reduce bias but presence of a second assessor (JV), especially of the radiographic measurements and final outcome, as well as of a blinded individual for the statistical analysis, helped to reduce this risk.

Additionally, during the length of the study there have been changes in the practice and protocols of managing neck of femur fractures. The introduction of best practice tariff and NHFD in 2010 reduced time from admission to surgery, along with potentially improving the overall outcomes. Moreover, the introduction of Major Trauma Centres in 2012 standardised the management of polytrauma patients, also improving the survival and outcomes. Changes in implants used (introduction of Affixus nail in 2012) could also potentially be associated with a learning curve with all the relevant implications. The results are also influenced by the large number of surgeons and their different levels of expertise and / or preferences in management. Note that all of these changes are not only applicable to LTH, but are evident in most institutions around the world and are an important part of the attempt to improve patients' care and outcomes.

The assessment of fracture pattern is also subjected to intra- and inter-observer reliability. Radiographic assessment would also be more accurate with CT scans, but concerns about radiation exposure and additional costs cannot justify their use. An attempt to overcome this limitation by having two independent assessors for the analysis of fracture pattern and radiological measurements was made, whilst templating of the images ensured a more accurate assessment of the calculations.

The number of some complications was limited because of their low frequency, therefore some of the subgroup analysis may be underpowered. On top of this, because of relying on medical records without examining all patients for the purpose of this study, some of the complications may have been over-reported, whilst others may have been under-reported. For example, redness, swelling and mild discharge in the first few post-operative days may represent signs of physiological inflammation of wound healing, but this could easily be clinically over-diagnosed as 'superficial wound infection' by non-orthopaedic physicians, junior doctors, district nurses or advance nursing practitioners who are less experienced in assessing surgical

wounds. On the other hand, VTE, post-operative delirium and others may have been under-reported as special investigations may not have been appropriate or were not performed in some of the patients. Furthermore, a number of patients was lost to follow-up either because they were not given follow-up as in the case of infirm patients, were deceased in the early post-operative period or were followed-up in other institutions (especially polytrauma patients who were repatriated following their operations). Not only this, but follow-up times were not standardised, so time to union was not reported as it would not represent a reliable estimate. Due to the lack of research around some of our outcomes, it is difficult compare the findings with that of the literature given the potential for different risk factors and effects of subtrochanteric fractures to those of all cause hip fractures.

Finally, another limitation of this study is that the unadjusted p-values for the regression analysis were used and no correction for multiple testing was applied. This was because of relatively low numbers of patients included, that did not allow for such testing. The results were however confirmed with additional analysis with patient matching (not reported in this thesis) and no major changes were observed.

All the aforementioned limitations however were considered in the original design of the study and taken into account so that every effort was made to minimise their impact to the analysis and final conclusions.

3.13 Conclusion

The incidence of non-union in subtrochanteric fractures was 24.6%, with atrophic non-unions being the commonest encountered. Associations with the development of a non-union include deep infection, self-dynamisation, presence of an atypical fracture, diabetes, malreduction (lateral cortex fracture gap size and varus malalignment) and fracture comminution (single 2-part fracture or multi-segmented fracture). The development of a risk scoring system was possible, with good predictive value and internal validation.

Additionally, open reduction of subtrochanteric fractures was not associated with an increased risk of deep infection and non-union, even though it was associated with an increased risk of superficial infection, prolonged surgical time and transfusion. The use of cerclage was associated with reduced risk of non-union with little evidence of an increase in complications.

Regarding the incidence of infection, this was 6.4% (superficial infection: 3.7%; deep infection: 2.7%). *Coliforms*, *Staphylococcus aureus* and *Escherichia coli* were the commonest micro-organisms isolated in deep infections. Associations with deep infection included open fractures, need for massive transfusion or post-operative transfusion, whilst presence of a non-union, history of increased alcohol intake and an increased LOS were linked with an increased risk.

Overall 30-day mortality was 6.0%, compared to 20.8% for one-year mortality, increasing to 7.6% and 25.6% respectively in patients older than the age of 60. Presence of dementia, HAP / CAP, a CCS of more than 6, low albumin on admission, presence pathological fractures and nail complications were all related with an increased risk of one-year mortality.

Chapter 4
Biological Characterisation of Non-union Tissue: Literature Review

4.1 Introduction

The exact biological process leading to a non-union remains obscure and it is well accepted that any planned interventions to reverse this process should be well timed and well aimed to restore both biological and mechanical deficiencies (90, 98, 167, 487). It can be postulated that by gaining a better understanding of the underlying mechanisms leading to a non-union, both clinicians and scientists would be allowed to target specific pathways independently, tailoring treatment to each patient's individual requirements (26).

Therefore, a systematic review of the literature was performed, in order to investigate the biological profile of tissue obtained from the non-union site and to analyse any differences or similarities of tissue obtained from different types of non-unions. Moreover, it was attempted to evaluate whether there was adequate evidence to support any interventions on non-union tissue regarding improving its biological characteristics and bone-repair responses.

4.2 Literature review

4.2.1 Eligibility Criteria

Studies selected were original articles fulfilling the following inclusion criteria: (1) the tissue was obtained from a non-union site and examined or processed for defining its characteristics and properties, or tissue was obtained from a distant site and the genetic profile was investigated; (2) only tissue acquired from human subjects was included; and (3) the full text of each article was available. All studies that did not fulfil all eligibility criteria were excluded from further analysis, whereas no publication date restrictions were imposed (last search performed on 15/10/2019).

4.2.2 Information Sources

Studies were identified by searching the following resources / databases: PubMed Medline; Ovid Medline; Embase; Scopus; Google Scholar; and the Cochrane Library,

to retrieve all available relevant articles. The terms used for the search included: non-union(s), nonunion(s), human, tissue, and mesenchymal stem cell(s) (MSCs). The identified articles and their bibliographies including any relevant reviews were manually searched for additional potential eligible studies.

4.2.3 Study Selection

The eligibility assessment was initially performed by MP, then repeated by IP, in an independent, unblinded and standardised manner. Most citations were excluded on the basis of information provided by their respective title or abstract. In any other case, the complete manuscript was obtained, scrutinised by the two reviewers and included if fulfilling the eligibility criteria. Any disagreement between reviewers was resolved by consensus.

4.2.4 Extraction of Data

Relevant information on author's name, publication year, patient demographics, site and duration of non-union, type on the non-union, characteristics and evaluation of tissue samples, culture properties, gene expression, protein expression and effect of additional interventions was carefully extracted.

4.2.5 Data Analysis

All outcomes of interest were inserted in an electronic database and outcomes of different studies were documented. The characteristics of tissue samples were then compared across different studies and the effect of any intervention was evaluated.

4.3 Results

4.3.1 Literature Search

The electronic search of the literature retrieved 2,596 citations, but only 29 of them met the selection criteria (98, 155, 166, 487-512). Another three eligible papers (169, 513, 514) were obtained from the hand search of the references of the eligible studies and relevant review articles, yielding 32 eligible studies for the final analysis (**Figure 4.1**) (98, 155, 166, 169, 487-514).

A further 10 studies investigating the genetic profile of non-unions (non-union related tissue: not analysing tissue directly obtained from non-union, but instead tissue from a distant site in order to investigate its genetic profile), were also identified (96, 170, 217, 515-521). A further study (522), included the same patients with a previous study by the same team (515), and was therefore not included in the final analysis.

All studies were published from 1954 to 2019 and included 1,055 cases (non-union tissue: 712 patients; non-union related tissue: 343 patients) (**Table 4.1**: non-union tissue; **Table 4.2**: non-union related tissue) (96, 98, 155, 166, 169, 170, 217, 487-521, 523). Some of the authors used the same tissue bank for their analysis, but as different investigations were performed in each study, they were included as different studies (98, 487, 491, 499, 500).

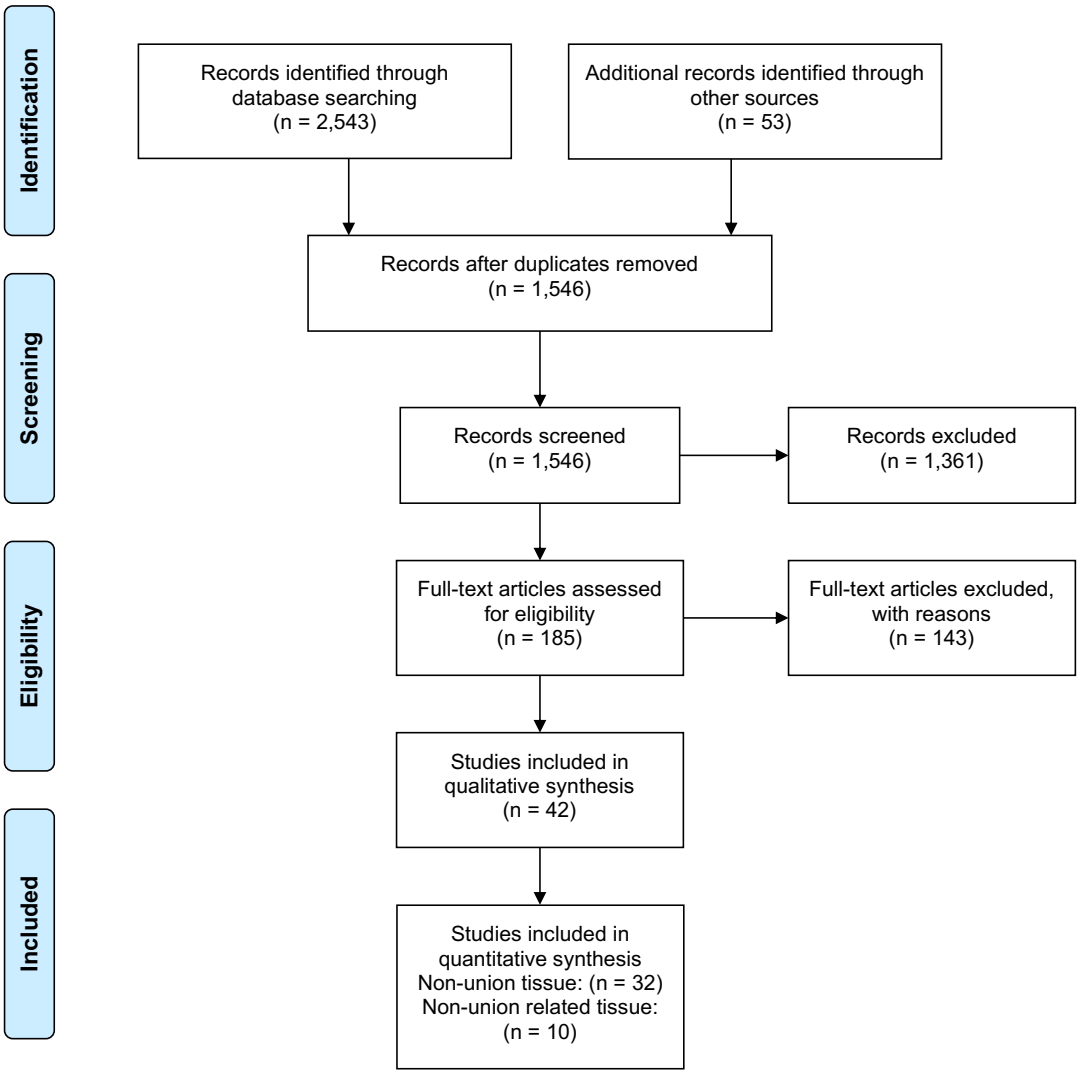


Figure 4.1 Study selection flowchart

Table 4.1 Non-union tissue: Patients' Demographics

Author (Year)	Time Frame	Number of Specimens	Site	Patients' Age (mean +/- SD) (years)	Amount of tissue
Wang (511) (2018)	Not mentioned	8 (compared to 8 with uneventful healing)	Not mentioned	Not mentioned	Not mentioned
Vallim (510) (2018)	Not mentioned	15 (9 male)	Femur: 4; tibia: 3; humerus: 7; ulna: 1	46.4 ± 12.5	Approximately 1cm^3
Takahara (509) (2016)	Not mentioned	4 (2 male)	Femur: 1; humerus: 2; clavicle: 1	65.3 ± 5.4	'Small amount'
Schira (507) (2015)	Not mentioned	80 (77 male)	Scaphoid	24.6 (range,18 to 71)	Not mentioned
Han (505) (2015)	2009 – 2010	11	Not mentioned	40 (range 27 to 81)	Not mentioned
Wang (512) (2014)	Oct 2010 – Mar 2014	Hypertrophic non-union: 20 (15 male); atrophic non-union: 20 (14 male)	Hypertrophic non-unions: femur: 8; femoral neck: 1; tibia: 2; humerus: 9. Atrophic non-unions: femur: 5; tibia: 8; humerus: 7	Hypertrophic non-unions: 39.35 ± 11.67 Atrophic non-unions: 33.75 ± 8.37	Not mentioned
Schwabe (508) (2014)	Not mentioned	Atrophic non-union: 44 (22 male) (histology: 25; GF-quantification: 19); healed fracture: 13 (7 male) (histology: 5; GF-quantification: 8)	Non-union: femur: 16; tibia: 12; clavicle: 9; ulna: 4; humerus: 3. Control group: femur: 2; tibia: 4; ulna: 4; radius: 1; metacarpus: 1	49 (range 20–74)	Not mentioned
Ismail (506) (2013)	Not mentioned	5 (5 male)	Femur: 3; tibia: 1; humerus: 1	27.40 ± 7.64 (range, 18 to 17)	10 mls of BM aspirate
Palmer (488) (2013)	Not mentioned	34 (17 male)	Femur: 12; tibia: 19; humerus: 3	49 (range, 18 to 71)	1 mm^3 biopsies
Koga (489) (2013)	Not mentioned	7	Not mentioned	Not mentioned	'Small amount'
Zimmermann (155) (2012)	Mar 2006 – May 2007	8	Femur: 3; humerus: 3; tibia: 2	48.75 ± 9.63	10 mm x 10 mm x 10 mm
Gille (490) (2012)	Nov 2009 – Mar 2010	23 (15 male)	Tibial shaft	47.4 (range, 20 to 82)	At least 3, each measuring 1 cm^3
Fajardo[a] (98) (2013)	Aug 2007 – Mar 2008	20 (14 male)	Femur: shaft - 2, subtrochanteric - 2, distal - 2; tibia: shaft - 2, proximal - 1, distal - 1; fibula: shaft - 3; clavicle: midshaft - 4; humerus: proximal - 1; ulna: shaft -2	46 (range, 32 to 80)	Approximately 5 mg
Kwong[b] (487) (2009)	Not mentioned	7 (compared to 8 patients with uneventful healing)	Extra-articular fractures	Range, 18 to 87	Not mentioned
Iwakura (166) (2009)	Not mentioned	7 (6 male)	Femoral diaphysis: 3; tibial diaphysis: 2; humeral diaphysis: 1; ulnar diaphysis: 1	53.0 (range, 37 to 74)	'Small amount'
Fajardo[a] (491) (2009)	Aug 2007 – Mar 2008	15 (11 male)	Femur: shaft - 2, subtrochanteric - 2; tibia: shaft - 2, tibial plateau - 1, distal - 1; fibula: shaft - 2; clavicle: midshaft - 3; humerus: proximal - 1; ulna: shaft -1	46 (range, 32 to 80) SD 14	Not mentioned
Bajada (492) (2009)	Not mentioned	8 (3 male)	Femur: 5; tibia: 3	55.6 (range, 26 to 73)	Ranging in wet weight from 120 to 250 mg; mean 162.1 mg
Qu (493) (2008)	Not mentioned	15 (14 male)	Scaphoid bone	29 (range, 17 to 56)	> 1 mm and up to 3 mm of abnormal bone on either side of the non-union

Author (Year)	Time Frame	Number of Specimens	Site	Patients' Age (mean +/- SD) (years)	Amount of tissue
Hofmann (494) (2008)	Not mentioned	10 (4 male) (compared to 10 (5 male) patients with uneventful healing)	Femur: 5; humerus: 3; ulna: 1; pelvis: 1	Non-unions: 59.3 ± 20.3 (range, 25 to 87) Controls: 55.3 ± 15.1 (range, 28 to 75)	Not mentioned
Bajada (495) (2007)	2004	1 (male)	Tibia	34	Not mentioned
Kilian (513) (2004)	Not mentioned	7 (4 male)	Tibia: 4; humerus: 1; radius: 1; ulna: 1	37 (range, 32 to 42)	Not mentioned
Reed[c] (496) (2002)	1993 – 1999	11 (9 male)	Extra-articular fractures. Femur: 2; tibia: 7; fibula: 1; radius: 1	44 (range, 14 to 74)	All biopsies > 5 mm x 5 mm x 5 mm
Reed[c] (496) (2002)	1993 – 1999	11 (8 male)	Extra-articular fractures. Femur: 8; tibia: 3	51 (range, 35 to 81)	All biopsies > 5 mm x 5 mm x 5 mm
Kloen (497) (2002)	Not mentioned	17 non-unions; 4 delayed unions	Femur: 5; tibia: 2; humerus: 12; clavicle: 2	61 (range, 30 to 85)	Not mentioned
Guerkov (498) (2001)	Not mentioned	7 (atrophic group: 1 male; hypertrophic group: 2 male)	Femur: 3; clavicle: 2; tibia: 1; iliac wing: 1	61 (range, 30 to 85)	> 0.5 cm^3
Lawton[b] (499) (1999)	Not mentioned	12 (compared to 15 patients with uneventful healing)	Not mentioned	Normal healing: range, 18 to 87	Not mentioned
Lawton[b] (500) (1997)	Not mentioned	12 (compared to 15 patients with uneventful healing)	Extra-articular long bone fractures	Normal healing: range, 18 to 87	Not mentioned
Santavirta (501) (1992)	Not mentioned	10 (7 male)	Tibia: 8; humerus: 2	48 (range, 27 to 64)	Three parallel representative samples, each about 4 mm x 4 mm
Boyan (502) (1992)	Not mentioned	1 (male)	Tibia	19	Fibrocartilage lying within the fracture gap and periosteal tissue stripped from the edges of the non-union
Quacci (503) (1991)	Not mentioned	2 (male)	Tibia	18 and 23	5 mm biopsy cannula
Milgram (504) (1991)	Not mentioned	Extra-articular: 41; intra-articular: 54	Extra-articular: femur: 10; tibia: 13; other: 18. Intra-articular: femur: 44; patella: 4; other: 6	Not mentioned	Sample tissue included the whole fracture site (intact piece)
Heppenstall (169) (1987)	1970 – 1983	76 (39 males)	Femur: 23; humerus: 29; tibia: 18; clavicle: 3; metatarsal: 1; ulna: 1; radius: 1	39 ± 3	Not mentioned
Urist (514) (1954)	1948 – 1953	85 (19 biopsies between 2 and 7.5 years)	Tibia	Not mentioned	Not mentioned

[a] Both studies used the same samples for their analysis (98, 491)
[b] All three studies used the same samples for their analysis (487, 499, 500)
[c] Same research paper but split into two distinct groups

Table 4.2 Non-union related tissue: Patients' Demographics

Author (Year)	Time Frame	Number of Specimens	Site	Patients' Age (mean +/- SD)	Amount of tissue
McCoy (518) (2019)	Not mentioned (Biobank)	131 (47 male) compared to 1,627 (588 male) with uneventful healing	Upper or lower extremity fractures	Control group: 64.3 ± 15.0; Non-union group: 66.8 ± 12.7	Not applicable
Zhang (520) (2018)	May 2012 – April 2015	24 (11 male) compared to 24 (11 male) with uneventful healing	Fibular head fractures	Control group: 41.5 ± 11.6; Non-union group: 40.4 ± 11.1	Not mentioned
Huang (516) (2018)	2012 – 2016	1,229 (346 non-unions of which 199 males; 883 unions of which 505 males)	Tibial diaphysis: 113/315; femur diaphysis: 98/233; humeral shaft: 82/188; ulnar shaft: 39/117; femoral neck: 14/30 (Non-union/Union)	Non-union: 46.1 ± 8.1; Union: 44.7 ± 8.3	Not applicable
Granchi (515) (2017)	Not mentioned	26 (15 male)	Femur: 11; tibia: 11; humerus: 3; not reported: 1	39.6 ± 14	Not applicable
Sathyendra (519) (2014)	2005 – 2010	Atrophic non-union: 33 (14 male); normal healing: 29 (18 male)	Non-union: femur: 13; tibia; 18; ulna: 2. Normal healing: femur: 10; tibia; 15; humerus: 4.	Atrophic non-union: 48.6; Normal healing: 47.3	Not applicable
Marchelli (517) (2009)	Not mentioned	Atrophic non-union: 16 (16 male); healed - 6 months: 18 (18 males); healing - 1 month: 14 (14 males)	Atrophic non-unions: femur: 3; tibia: 7; radius: 1; radius + ulna: 3; humerus: 2. Healed: femur: 2; tibia: 9; radius: 2; radius + ulna: 4; humerus: 1. Healing: tibia: 8; radius + ulna: 2; humerus: 2;.	Atrophic non-union: 28.1 ± 5.9; healed: 32.2 ± 5.7; healing: 31.4 ± 7.1	Not mentioned
Zeckey (170) (2011)	2000 – 2008	50 compared to 44 patients with uneventful healing	Femur: 21; tibia: 29	37.5 ± 2.0	Not applicable
Dimitriou (217) (2011)	2005 – 2007	62 (45 male) compared to 47 (33 male) with uneventful healing	Femur: 18; tibia: 41; humerus: 2; ulna: 1	43.9 (range, 19 to 65)	Not applicable
Xiong (521) (2009)	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Seebach (523)	2007	20 (14 male)	Not mentioned	Male: 41 ± 15; female: 42 ± 13	Not mentioned
Henle (96) (2005)	Jan 2002 – Jan 2004	15 (12 males) from non-unions and matched group with uncomplicated unions	Femur: 2; tibia: 11; humerus: 1; forearm: 1	47 (range, 20 to 75)	Not applicable

4.3.2 Study Characteristics

The study characteristics of the non-union tissue are outlined in **Table 4.3** (98, 155, 166, 169, 487-514) and **Table 4.4** (96, 98, 155, 166, 169, 170, 217, 487-521, 523). The definition of non-union varied between studies, but it was generally based on the radiographic appearance and clinical examination. Most of the samples were obtained during revision operations for the treatment of the non-unions.

Table 4.3 Study characteristics of non-union tissue

Author	Duration of non-union (months)	Classification	Definition of non-union	Isolation of tissue	Cells / Material Isolation
Wang (511)	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Cell viability; mineralisation assay; gene expression
Vallim (510)	34 (range 9 to 120)	Atrophic	Lack of bone healing 9 months from the fracture	Fibrous tissue interposed between the bone ends was excised, along with adjacent osseous fragments	Histology; population doubling; cell senescence; flow cytometry; osteogenic / adipogenic differentiation
Takahara (509)	14.8 (range 4 to 26)	Pseudarthrosis	(1) Gross motion at the fracture site on physical examination; (2) bridging bone on 0 of 4 cortices on AP and lateral radiographs; (3) CT showing no purposeful cross-sectional area of healing; and (4) evidence showing the existence of pseudocapsule and fluid collection between the fracture gap at the surgery	A small amount of pseudarthrosis tissue (pseudocapsule) was obtained during the surgical treatment	Alizarin Red S staining, ALP activity assay, and RT-PCR after osteogenic induction; chondrogenic differentiation capacity was assessed via Safranin O staining and RT-PCR after chondrogenic induction; histological analysis and cell cultures
Schira (507)	18.3 (range, 3 to 100)	Atrophic	Non-unified fractures >3 months with a resorption zone wider than 1 mm (as determined by a mandatory CT-scan) with no apparent potential to heal without surgical intervention	Non-union tissue (excluding the cortex) and cancellous bone from the ipsilateral radius has been obtained at the time of operative repair	Histology, immunohistochemistry, gene expression
Han (505)	11 (range, 6 to 30)	Not mentioned	Failure of the fracture to heal 6 months or more after surgery or non-surgical treatment	Fracture and scar tissue during surgery, which was divided into bone stump tissue, marrow cavity contents, and sticking bone scars according to the sites	Histology; immunohistochemistry; gene expression
Wang (512)	Hypertrophic non-unions: 19.88 ± 17.88; atrophic non-unions: 14.20 ± 7.42	Atrophic / hypertrophic	Failure of the fracture to heal 9 months or more after the injury	Intra-operative biopsy samples	Immunohistochemistry
Schwabe (508)	Not mentioned	Atrophic	Time span from the initial operation until the revision surgery of a least 6 months	Intra-operative biopsy samples for the treatment of the non-union or removal of metalwork for the control (normal healing)	Histology; immunohistochemistry; ELISA
Ismail (506)	37.2 ± 24.0 (range, 12 to 72)	Not mentioned	Not mentioned	Intra-operative BM from the site adjacent to the non-union, compared to BM from iliac crest	Not mentioned

Author	Duration of non-union (months)	Classification	Definition of non-union	Isolation of tissue	Cells / Material Isolation
Palmer (506)	10	Aseptic / Septic	Radiographic evidence of no progression of healing for at least 3 months, or lack of healing by 9 months since the initial injury	Intra-operative specimens were collected from removed implants, surrounding tissue membrane and local soft tissue	Culture analysis; Ibis' second generation molecular diagnostics; bacterial 16S rRNA-based FISH
Koga (489)	11.0 (range, 9 to 13)	Viable: 2 patients; non-viable: 5 patients	> 9 months had elapsed since the injury, and the fracture had shown no visible progressive signs of healing for 3 months	The non-union site was exposed by careful incision, and care was taken not to contaminate the bone and periosteum	Histological analysis; flow cytometry; cell proliferation; ALP activity assay; ALP mRNA; mRNA analysis; osterix expression; osteocalcin expression; mineralisation assay
Zimmermann (155)	> 9	Not mentioned	> 9 months from injury	Pseudarthrotic tissue was collected out of the fracture gap during regular surgical treatment	mRNA isolation; cDNA arrays
Gille (490)	10.2 (range, 6 to 34)	Aseptic	Absence of healing > 6 months	Intra-operative biopsy samples	Cultures; PCR
Fajardo (98)	16 (range, 6 to 72)	Hypertrophic	Absence of osseous healing > 6 months from injury	Multiple tissue samples from: 1) the non-union site and 2) mineralised fracture callus from the surrounding region	RNA extraction; synthesis of cDNA; real time quantitative PCR; western blot assay (only 8 samples); immunohistochemistry (only 8 samples)
Kwong (487)	Range, 1 to 48	Aseptic	Absence of osseous healing > 9 months after treatment	Fracture biopsies taken at surgery for treatment of malalignment or failure of fixation, as well as acute fractures that were operated upon in a delayed fashion	Immunohistochemical analysis
Iwakura (166)	11 (range, 9 to 14)	Hypertrophic	> 9 months from injury, no visible progressive signs of healing for 3 months	Samples were obtained during revision surgery	Histological analysis; immunophenotyping of non-union cells by flow cytometry; osteogenic induction; chondrogenic induction; adipogenic induction; total RNA extraction and RT-PCR
Fajardo (491)	16 (range, 6 to 72)	Hypertrophic	Absence of osseous healing > 6 months from injury	Multiple tissue samples from: 1) the non-union site and 2) mineralised fracture callus from the surrounding region	RNA extraction; synthesis of cDNA; real time quantitative PCR; western blot assay (only 7 samples); immunohistochemistry (only 7 samples)
Bajada (492)	36 (range, 24 to 60)	Atrophic	Not mentioned	Tissue was excised from the site of non-union between the diaphyseal cortices and below the pseudocapsule	Histological analysis; CD immunoprofiling

Author	Duration of non-union (months)	Classification	Definition of non-union	Isolation of tissue	Cells / Material Isolation
Qu (493)	36 (range, 5 to 156)	Not mentioned	Not mentioned	Bone from either side of the non-union and the fibrocartilaginous central regions were harvested during reconstructive or salvage surgery	Immunocytochemical determination of osteocalcin; ALP enzyme assay
Hofmann (494)	Not mentioned	Hypertrophic	Not mentioned	Endosteal cancellous bone fragments were taken at sites proximal to non-unions during surgery. Control cultures were obtained from healthy individuals from endosteal sites during implant removal after uneventful fracture consolidation	Osteoblast cell viability; formation of ALP-positive and mineralisation-positive CFUs; gene expression
Bajada (495)	108	Hypertrophic	Not mentioned	During operation for grafting	Histology
Kilian (513)	Not mentioned	Atrophic	Not mentioned	Patients surgically treated for resection of atrophic non-union and re-osteosynthesis	Immunohistochemistry; qualitative RT–PCR; LightCycler-based relative mRNA quantification
Reed (496)	27 (range, 11 to 62)	Hypertrophic	A fracture that had not healed within the expected time period, with no progression toward healing on successive radiographs	During surgery, biopsies taken of the material in the non-union gap (interfragmentary tissue) and the cortex immediately adjacent to the gap	Histology; immunohistochemistry; assessment of vascularisation; assessment of vessel density
Reed (496)	34 (range, 12 to 60)	Atrophic	A fracture that had not healed within the expected time period, with no progression toward healing on successive radiographs	During surgery, biopsies taken of the material in the non-union gap (interfragmentary tissue) and the cortex immediately adjacent to the gap	Histology; immunohistochemistry; assessment of vascularisation; assessment of vessel density
Kloen (497)	22 (range, 3.5 to 120)	Not mentioned	Absence of osseous healing > 6 months from treatment	At the time of surgery	Histology; immunohistochemistry
Guerkov (498)	20 (range, 6 to 36)	Atrophic: 4; Hypertrophic: 3	Not mentioned	At the time of revision surgery (central portion of the tissue)	Histology; cell proliferation; [3H]-Thymidine incorporation; ALP specific activity; osteocalcin, collagen and local factor production
Lawton (499)	Range, 4 to 48	Not mentioned (had callus)	Not mentioned	Specimens of fracture callus from normally healing fractures (1-4 weeks after fracture) or non-unions (4-48 months after the fracture)	In situ hybridisation

Author	Duration of non-union (months)	Classification	Definition of non-union	Isolation of tissue	Cells / Material Isolation
Lawton (500)	Range, 4 to 48	Not mentioned (had callus)	Not mentioned	Specimens of fracture callus from normally healing fractures (1-4 weeks after fracture) or non-unions (4-48 months after the fracture)	In situ hybridisation
Santavirta (501)	Range, 4 to 25	Delayed unions; established non-unions	Not mentioned	Tissue from the area between the diaphyseal cortices below the pseudocapsule	Immunopathology (inflammatory-cell analysis, analysis of MMPs); neuroimmunology
Boyan (502)	12	Not mentioned	Not mentioned	During surgical treatment	Histomicrograph; photomicrograph; ALP activity; ELISA; Densitometric analysis of the cytoplasmic dot blots
Quacci (503)	8	Hypertrophic	Not mentioned	Through a 5 mm biopsy cannula	Light and electron microscopy
Milgram (504)	Not mentioned	Not mentioned	Not mentioned	Surgical resections, amputations and a small number of autopsy obtained specimens	Histological analysis
Heppenstall (169)	Tibia: 32.4; humerus: 51.6	Synovial pseudarthrosis	Synovial pseudarthrosis	Biopsies	Light and electron microscopy
Urist (514)	> 18	Not mentioned	> 18 months showing: a bone defect; false motion; sclerosis of the bone ends; rounding, mushrooming, or molding of the fracture surfaces; sealing of the medullary canal with compact bone to form functioning false bone surfaces and an apparent arrest of the process of osteogenesis in the fracture gap	During surgical interventions / autopsy	Histological analysis

Table 4.4 Study characteristics of non-union related tissue

Author	Duration of non-union (months)	Classification	Definition of non-union	Isolation of tissue	Cells / Material Isolation
McCoy (518)	Not mentioned	Not mentioned	Not mentioned	Peripheral blood	DNA was extracted from blood samples
Zhang (520)	Not mentioned	Not mentioned	Not mentioned	Peripheral blood	DNA was extracted from blood samples
Huang (516)	> 9 months	Not mentioned	The cessation of all healing processes and failure to achieve union within 9 months without radiographic signs of progression of the fracture callus	Peripheral blood	DNA was extracted from blood samples
Granchi (515)	> 3 months	Not mentioned	Not mentioned	BM, peripheral blood	Immunoenzymatic assays
Sathyendra (519)	Not mentioned	Not mentioned	Minimal callus formation 6 months after injury requiring additional surgery to achieve union	Buccal mucosal cell harvesting	SNP genotype
Marchelli (517)	Atrophic non-union: 6 to 11 months; healed: 8.5 ± 3.5 months; healing: 0.5 ± 0.5 months	Not mentioned	Not mentioned	Blood samples	ELISAs
Zeckey (170)	> 9 months	Aseptic tibial and femoral shaft non-unions	Clinically and radiologically confirmed unhealed shaft fractures > 9 months following the injury and osteosynthesis treatment	Peripheral venous blood sample	DNA was extracted from blood samples
Dimitriou (217)	Required further intervention to achieve union	Atrophic	Cessation of all healing processes and failure to achieve union after the expected period of time, as seen clinically and radiologically	Peripheral venous blood sample	DNA was extracted from blood samples
Xiong (521)	Not mentioned	Not mentioned	Fracture that does not heal six months after injury	Normal and non-union callous bone samples examined	Gene expression
Seebach (523)	Not mentioned	Atrophic	Not mentioned	BM cells were obtained from the iliac crest aspirate	CFU-F; flow cytometry; osteogenic differentiation
Henle (96)	> 4 months	Atrophic	No bony consolidation of the fracture in conventional X-ray films and the patient continued to report exercise induced pain 4 months after trauma + no bone healing on CT scan	Venous blood	Immunosorbent assays

4.3.3 Macroscopic Structure of non-union Tissue

Urist et al. was the first to hypothesise the mechanism of non-union based on its macroscopic and microscopic characteristics (514). He reported that white soft tissue was interposed between the bone segments, a finding later supported by other authors (504), and explained this as fibrinoid degeneration of the connective tissue in the interior of the callus (514). Han et al. similarly identified tough scars around the non-union site, with only fibrous connections between the fracture fragments, bone sclerosis of the fracture ends and complete obliteration of the medullary canal (505). With regards to synovial pseudarthrosis, a yellow frond-like material was found interposed between the bone fragments, with clear serous fluid filling this space in aseptic cases, whereas in septic cases murky fluid was present (169).

4.3.4 Microscopic Structure of non-union Tissue

4.3.4.1 Histology

The histological findings of non-union tissue are summarised in **Table 4.5** (166, 169, 487, 489, 492, 495-501, 503-505, 507-510, 512, 514). Where relevant information was available, a direct comparison of histological findings between atrophic and hypertrophic non-unions was attempted (**Table 4.6**) (166, 492, 495-498, 503, 507, 508, 510, 512).

Table 4.5 Histological findings of non-union tissue

Author	Classification	Histology
Vallim (510)	Atrophic	Connective tissue with a dense collagenous extracellular matrix, populated by fibroblast-like cells, and areas of vascularisation.
Takahara (509)	Pseudarthrosis	Mainly fibrous tissue with variable amount of fibroblastic cells. Small vessels were sparsely populated. No ossicles or hyaline cartilage were seen.
Schira (507)	Atrophic	Pentachrome staining revealed a heterogeneous mix of different tissues, with a domination of connective tissue and fibroblasts in non-unions, while osteoid was the dominant tissue in cancellous bone. Representative TRAP staining of control cancellous bone and scaphoid non-unions revealed enhanced osteoclasts activity in non-unions.
Han (505)	Not mentioned	Delayed union and non-union areas comprised a mix of different types of tissues: fracture fragments and surrounding tissues were mainly subject to fibrosis, in which the formation of new blood vessels could be seen, and a small amount of woven bone could be seen nearby. In these woven bones, Gergen Bauer's cells grew along the osteoid as cubes, suggesting active bone formations. A large number of cartilage cells existed in the IM tissues, and there was no new bone and neovascularisation. BM occlusion was observed, and in the fibrous tissue of adjacent bone and the gap of bone fractures, there were internal cartilage ossifications and fibrous ossifications. Scattered lamellar bone fragments were observed in some samples; these fractures were surrounded by osteoclasts, and there was a lack of osteoblasts.
Wang (512)	Atrophic / hypertrophic	There were no significant differences in the morphology of atrophic / hypertrophic non-union tissues. They included MSCs, fibrocartilage cells, and hyaline chondrocytes. Some sections showed very few bone islands.
Schwabe (508)	Atrophic	The tissue was a very heterogeneous mixture of fragments of lamellar bone, immature and hypertrophic cartilage, unorganised fibrous tissue and newly formed woven bone. Independent of the group, bone apposition and resorption was seen in the tissue samples. Differences between the groups were not obvious.

Author	Classification	Histology
Koga (489)	Viable: 2 patients; Non-viable: 5 patients	Fibroblast-like morphologic characteristics.
Kwong (487)	Aseptic non-unions	Healing fractures: all consisted of areas of cartilage and significant woven bone formation. Non-healing fractures: in most, cartilaginous areas were accompanied by the presence of small amount of woven bone, but significant fibrous tissue. No notable differences in cellular morphology in the cartilaginous areas of the fractures between healing and non-healing fractures.
Iwakura (166)	Hypertrophic	Mainly fibrous tissue and no ossicles. Non-union tissue contained various amounts of fibroblast-like cells. After a 21-day incubation under chondrogenic conditions, cell pellets had a spherical and glistening transparent appearance.
Bajada (492)	Atrophic	Samples largely consisted of fibrocartilaginous tissue that contained occasional bony islands. In some areas the excised non-union tissue was well populated by fibroblastic cells, but other areas were largely acellular and consisted mostly of a collagenous extracellular matrix. Areas of vascularisation were seen consistently and the presence of osteoclasts within absorption pits was also occasionally notable. After enzymatic treatment to extract cells and their plating out into monolayer culture, the majority of the adherent cells present were stromal in appearance, i.e. bipolar and fibroblastic. Occasional multinucleated osteoclasts were also seen in the early cultures, as were cells with a stellate (possessed multiple cytoplasmic processes) or dendritic appearance.
Bajada (495)	Hypertrophic	Fibrocartilaginous non-union with little evidence of new bone formation and no signs of infection.
Reed (496)	Hypertrophic	Specimens contained fibrous tissue, fibrocartilage, hyaline cartilage and bony islands. Areas of new bone formation by both endochondral and intramembranous ossification. Morphologically samples appeared well vascularised.
Reed (496)	Atrophic	Specimens contained fibrous tissue, fibrocartilage, hyaline cartilage and bony islands. Relatively few areas of new bone formation, predominantly via the endochondral route. Necrotic bone was more prevalent in the atrophic non-union group. Morphologically samples appeared well vascularised.
Kloen (497)	Not mentioned	Delayed unions and non-unions: 11/21 specimens had foci of woven bone (having cuboid-shaped osteoblasts lining the osteoid, suggesting active bone formation) surrounded by large areas of fibrous tissue that was interspersed with areas of numerous blood vessels. 10/21 specimens had similar areas of fibrous tissue but lacked woven bone. Within the samples that contained woven bone, two patterns of bone formation were observed: 1) bone appeared to be forming directly from fibrous tissues; 2) bone seemed to be forming from cartilage. Other observations included scattered lamellar bone fragments surrounded by osteoclasts and a paucity of lining osteoblasts. Some specimens also showed villous projections resembling synovial pseudarthroses with lining cells resembling synoviocytes.
Guerkov (498)	Atrophic: 4; Hypertrophic: 3	Mainly fibrous tissue with organised collagen bundles. No ossicles were seen in any of the sections examined. All sections from atrophic non-unions were oligocellular and contained few vessels, whereas those from hypertrophic non-unions were more cellular, with little evidence of cartilaginous tissue.
Lawton (499)	Not mentioned (had callus)	Human fracture callus: heterogeneous appearance with several of the elements of normal fracture healing (haematoma, fibrous tissue, woven and compact lamellar bone, and cartilage) being present in close proximity in any one section. Non-union gap: tissues consisted largely of vascularised fibrous tissue or avascular cartilage.
Lawton (500)	Not mentioned (had callus)	Areas of old bone, new bone formation, non-union gap (either fibrous, cartilaginous, or both), and an interface between the gap and bony material.
Santavirta (501)	Delayed union; established non-unions	The morphology of the samples was not dependent on the duration of delayed union / non-union. All samples contained connective tissue of varying density, in which tissue fibroblast-like mononuclear cells seemed to predominate. The cellularity varied inside each sample from poorly cellular, tight connective tissue areas to highly cellular strands with occasional cartilage or bony islets.
Quacci (503)	Hypertrophic	Light microscopy: non-union tissue was composed of connective tissue, cartilage (had a hypertrophic aspect and frequently presented degenerative aspects) and fragmented osteoid-like trabeculae.
Milgram (504)	Not mentioned	Extra-articular locations: presence of non-mineralised fibrous or fibrocartilaginous tissue between the ends of the bone at the old fracture site. Also demonstrated a spectrum of clefts at the site of non-union ranging from tiny microscopic spaces within the soft tissue of the non-union to dominant clefts that completely separated the ends of the fracture (i.e. frank pseudarthrosis). Intra-articular locations: demonstrated the same sequence of changes occurring in 24 of the cases. 30 of them however demonstrated no tissues of a fibrous non-union.
Heppenstall (169)	Synovial pseudarthrosis	Light microscopy (62 patients): hyaline cartilage, synovial-like lining cells, or synovium and fibrous tissue was present.
Urist (514)	Not mentioned	When healing does not occur <18 months, the interior of the callus is more likely to show: inflammatory and fibrous connective tissue; failure of fibrous tissue to regress; fibrinoid and hyaline degeneration.

Table 4.6 Comparison of histological findings between atrophic – hypertrophic non-unions

	Atrophic	Hypertrophic
Type of tissue		
Fibrocartilaginous tissue	(492, 496)	(495, 496)
Fibrous tissue	(496, 498, 508)	(166, 496)
Cartilaginous tissue	(508)	(496, 498, 503)
Collagenous extracellular matrix / connective tissue	(492, 498, 507, 510)	(492, 498, 503)
Bone tissue	No ossicles (498); occasional bony islands (492, 496, 512); mixture of lamellar and woven bone (508)	No ossicles (166, 498); bony islands (495, 496, 503, 512)
Necrotic bone	More prevalent (496)	-
Bone production	Predominantly via the endochondral route (496)	Bone formation by both endochondral and intramembranous ossification (496)
Cells	- Generally oligocellular (498); some areas acellular (492) - Fibroblasts: majority of cells (492, 507, 510) - osteoclasts: occasionally (492) or enhanced activity (507) - bipolar cells: majority of cells (492) - cells with a stellate (possessed multiple cytoplasmic processes) or dendritic appearance (492) - Include MSCs, fibrocartilage cells and hyaline chondrocytes (512)	- More cellular (498) - Fibroblast-like (166) - Include MSCs, fibrocartilage cells and hyaline chondrocytes (512)
Vascularisation	Well vascularised (492, 496, 497); few vessels (498, 510)	Well vascularised (496)

4.3.4.2 Immunohistochemistry

The immunohistochemical findings of non-union tissue are summarised in **Table 4.7** (98, 487, 491, 496, 497, 499-501, 505, 507, 508, 512, 513). Interestingly, BMP's were present in the non-union tissue, even though their expression was reduced (487, 491, 497), and was locally generated (505), whilst their BMP antagonists were present in 'normal' and non-union tissue (508). Moreover, matrix metalloproteinases (MMPs) were also reported to be present in the non-union tissue, not localised in a particular cell type or cellular component (98, 501).

Table 4.7 Immunohistochemistry Findings

Author	Classification	Immunohistochemistry
Schira (507)	Atrophic	ALP: higher levels in non-unions as opposed to cancellous bone. Likewise, immunofluorescence for phosphorylated SMAD2/3 revealed increased activity in non-unions.
Han (505)	Not mentioned	- BMP-2 was locally generated: BMP-2 staining in the cytoplasm increased with increasing proximity to the new bone formation region, with some staining of the Golgi apparatus. - Osteogenesis: a wide variety of cells, including epithelial cells, smooth muscle cells around the small blood vessels, fusiform fibroblast-like cells, and chondrocyte cells, showed positive staining in the fibrous tissues. - There was no difference in the immunostaining of fibrous tissue between the samples with and without new bone. - There was no positive BMP staining in the extracellular matrix or the fibrous tissue space. - Sub-parts of view, fracture fragments were mainly fibrotic tissues and BMP-2 staining was negative. In the surrounding tissues, especially in the sticking scars and posted plate scars, neovascular and woven bone filled in a lot of the fibrous tissues, and in the vicinity there were stained cells, indicating BMP-2 expression. - There was a small amount of cartilage with positive staining in the cytoplasm, without expression in fibrous tissues of the closed medullary cavity. - DCN expression was extensive in the interstitial fracture fragments. There was no positive staining of cartilage cells in the medullary cavity. DCN expression in the sticking scars was close to perivascular. - The rate of expression of BMP-2 was highest in the posted bone scar group, and was low in the bone ends and canal content group ($p < 0.05$). - The fracture fragment group had the highest DCN expression, with significant differences from the other two groups; the least significant difference analysis showed that between the fracture fragment group and the other groups ($p < 0.05$).
Wang (512)	Atrophic / hypertrophic	- There was no significant difference in the mean optical density of BMP-2 between atrophic and hypertrophic non-union tissue. - There was no significant difference in the mean optical density of BMP-2 between 20 to 35 years old group, 35 to 50 years old group and > 50 years old group. - There was no significant difference in the mean optical density of BMP-2 between the 9–12 months group, the 13–24 months group and the > 24 months group.
Schwabe (508)	Atrophic	Bone morphogenic antagonists were demonstrated in non-union and control tissue.
Fajardo (98)	Hypertrophic	MMP-7 and MMP-12 were found to be stained within the substance of the non-union tissue and not localised within a particular cell type or cellular component. Both enzymes were likewise not visualised in the bone callus specimens.
Kwong (487)	Aseptic	There was a significant reduction in BMP-2 and BMP-14 expression in cartilaginous areas of non-healing fractures compared to healing fractures, but no statistical differences in the endogenous expression of noggin and chordin (BMP inhibitors).
Fajardo (491)	Hypertrophic	BMP-7: absent in the non-union specimens but present in the fracture callus specimens. BMP-2: positive immunostaining was restricted consistently to the fibrous tissue of the non-union tissue.
Kilian (513)	Atrophic	Immunostaining appeared in close vicinity to immature osteoid trabeculae. EDB+ fibronectin immunostaining was negative for scFvL19 antibody.
Reed (496)	Atrophic / Hypertrophic	No statistically significant difference in median vessel counts between atrophic, hypertrophic and normal unions.
Kloen (497)	Not mentioned	- The most consistent expression was that of BMP-2, BMP-4, and BMP-7 in the osteoblasts lining the newly formed osteoid. The staining was cytoplasmic and, in certain specimens, was specifically located in the Golgi apparatus, illustrating local production of BMP. No correlation between the location of the delayed union or non-union and staining. In the areas of dense fibrous tissue the presence of staining for all BMP isoforms tested was the same as or less than that in the areas close to bone at all time-points after the fracture. - Expression of Type-IA, Type-IB, and Type-II BMP Receptors: positive staining was observed in the osteoblasts lining the ossified tissue, in the areas near the ossification sites, and in the fibrous tissue. As observed for the BMP antibodies, there was a trend toward decreased staining in areas remote from bone formation. There was no clear trend between a decreased percentage of positive staining and an increased duration of the non-union. - Expression of pSmad1: in the osteoblasts lining the areas of reactive bone formation as well as in osteoclasts, fibroblast-like cells, and chondroblast-type cells.
Lawton (499)	Not mentioned (had callus)	In normally healing fractures, mature osteoblasts on woven bone were negative for MGP mRNA, but positive for osteonectin, osteopontin, and osteocalcin mRNA molecules. In non-unions, osteoblasts displayed a novel phenotype: they were positive for MGP mRNA, in addition to osteonectin, osteopontin, and osteocalcin mRNA molecules.

Author	Classification	Immunohistochemistry
Lawton (500)	Not mentioned (had callus)	In areas of new bone covered by plump osteoblasts, the matrix was either stained uniformly or in a superficial zone, indicating the presence of collagen type III. Fibrous tissue in the fracture gap was also immunostained positively.
Santavirta (501)	Delayed union; established non-unions	- Most inflammatory cells were CD4 T-lymphocytes and their number was always twice that of the CD8 positive cells. - Staining for CD11b positive monocyte/macrophages showed in all samples positive cells scattered in the connective tissue stroma with perivascular enrichments. - Mast cells were absent or very rare. - Almost all resident cells seem to be involved in tissue remodelling as suggested by their content of fibroblast-type MMP-1 and its proteolytic activator MMP-3 or stromelysin, whereas MMP-8 was rare or absent.

4.3.4.3 Neuroimmunohistochemistry

Only one study performed neuroimmunohistochemical analysis revealing paucity or total lack of peripheral innervation in the non-union tissue (501).

4.3.4.4 Analysis of vessel density

Blood vessels were present in cases of hypertrophic non-unions, with a varying density (**Table 4.8**) (496, 501, 503, 507, 508). When comparing however atrophic and hypertrophic non-union tissue, an interesting finding was that the number of fields containing no blood vessels, some blood vessels and hot-spots was very similar. More specifically, atrophic non-unions were demonstrated to be well vascularised (492, 496, 497) or having 'few vessels' (498, 510). Hypertrophic non-unions were similarly well vascularised (496). This was also confirmed with immunohistochemistry studies, where no significant difference was evident in the median vessel count between atrophic / hypertrophic non-unions and normal unions (496). Moreover, histological findings confirmed the presence of vascular tissue in both types of non-unions ((**Table 4.5** (492, 496, 498, 499, 505, 508-510); (**Table 4.6** (492, 496-498, 510)).

Table 4.8 Analysis of vessel density

Author	Analysis of vessel density
Schira (507)	Angiogenesis in scaphoid non-unions is similar to cancellous bone. Blood vessels and endothelial cells were detected by immunohistochemical staining of PECAM-1 in non-unions and controls revealing similar levels of angiogenesis in both tissues.
Schwabe (508)	Histology: Vessels were present in all investigated samples without a difference between the tissue from non-union and control patients. Immunohistochemistry: well vascularised but also unvascularised areas with no difference between the non-union and the control tissue.
Reed (496)	The number of fields containing no blood vessels, some blood vessels and hot-spots was very similar in the atrophic and hypertrophic non-union groups.
Santavirta (501)	Samples mostly consisted of vascularised connective tissue of varying density.
Quacci (503)	A lot of blood vessels were present in the tissue, often appearing free of blood and occluded by thrombi at different organisation stages.

4.3.4.5 Electron microscopy

Only two studies performed ultrastructural examination of the non-union tissue by the means of electron microscopy (**Table 4.9**) (169, 503). In a study by Quacci et al., it was reported that the non-union tissue contained normal fibroblasts and chondrocytes (503). Additionally, Heppenstall et al. who examined synovial pseudarthrosis reported large amounts of surface fibrin and densely packed collagen (169).

Table 4.9 Ultrastructural Examination of non-union tissue

Author	Electron microscopy (Ultrastructural Examination)
Quacci (503)	Fibroblasts and chondrocytes found in the non-union tissue seemed normal, with a good secretion apparatus. The cell membranes were able to produce matrix vesicles. Hydroxyapatite crystals could be observed in the cell matrix or inside matrix vesicles.
Heppenstall (169)	Large amounts of surface fibrin. Some cells had profuse rough endoplasmic reticulum and resembled fibrocytes or Type B synovial lining cells. Some of these cells contained prominent lipid droplets and intermediate filaments. There were also phagocytic cells with vacuoles containing granular and cellular debris, resembling to Type A lining cells or moncyte-macrophages. Surrounding the cells were some necrotic cells, clusters of apatite crystals and occasional clumps of collagen fibres infiltrated with more fibrin-like material. Deeper was more densely packed collagen.

4.3.5 Bacteriology of the non-union

Palmer et al. analysed 34 samples obtained from patients with non-unions (488). Even though eight samples had a positive conventional culture, only four out of 34 cases were negative following analysis of bacterial DNA using a combination of Ibis molecular diagnostics and FISH techniques. Similarly, Gille et al. examined culture negative samples of 23 patients and reported the presence of bacterial RNA following analysis with PCR in 2 patients (8.7%) (490).

4.3.6 Evaluation of Tissue Sample

4.3.6.1 Cell surface protein expression

Six studies performed flow Cytometry to determine the presence of specific proteins on the cell surface (**Table 4.10**) (166, 489, 492, 506, 509, 510). The non-union tissue was found to be positive for MSC's related markers CD13 (166), CD29 (166, 489), CD44 (166, 489), CD73 (506, 510), CD90 (166, 506, 510), CD105 (166, 489, 492, 506, 509, 510) and CD166 (166, 489), but negative for haematopoietic markers CD14 (166, 489, 506), CD34 (489, 506), CD45 (166, 489, 492, 506, 509), CD143 (166, 489) and HLA-DR (506).

Table 4.10 Cell surface protein expression

Author	Cell surface protein expression (flow Cytometry)
Vallim (510)	Compared to BM MSCs and osteoblasts, non-union MSCS: 1. Homogeneously expressed CD90 and CD73. 2. The percentage of cells expressing CD105 was significantly lower in comparison to BM MSCs, and similar to that of osteoblasts. 3. CD146+ positive cells was lower compared to BM MSCs. 4. Evaluating the percentage of cells simultaneously expressing both markers, confirmed that NUSC contained cells of the osteoblastic lineage, whose surface markers profile resembles that of cells in late-stage differentiation.
Takahara (509)	Consistently positive for MSC-related markers such as CD29, CD44, CD105, and CD166. The cells were negative for haematopoietic-lineage markers such as CD31, CD34, CD45, and CD133.
Ismail (506)	There was positive expression of CD105, CD73, CD90 for at least 95%, negative expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR.
Koga (489)	Strongly positive for the MSC's–related markers CD29, CD44, CD105, and CD166 but negative for the haematopoietic markers CD14, CD34, CD45, and CD133.
Iwakura (166)	Positive for MSC's-related markers CD13, CD29, CD44, CD90, CD105, and CD166, but negative for haematopoietic markers CD14, CD34, CD45, and CD133.
Bajada (492)	Less than 1% of NUSC and BMSC were positive for CD34 and CD45, whilst 78% ± 14% of NUSC and 92% ± 7% of BMSC were positive for CD105.

4.3.6.2 Cell Senescence

Bajada et al. was the first author to report on the cell senescence of NUSC (non-union stromal cells) (492). According to his findings, from passage I (P1) onwards, many of the cells developed an appearance that was less bipolar and more spread along with the development of prominent stress fibres. Further passages lead to prolonged culture doubling times (phenotypic changes are consistent with the onset of cell senescence). When examining the proportion of SA-β gal positive cells, that was significantly greater in the NUSC when compared to the BMSC, but that did not correlate with the patient's age, number of previous operative procedures or time between original fracture and operative management. Additionally, Vallim et al. compared the non-union MSCs senescence rates to those of BM MSCs and osteoblasts, reporting no differences between each group (510).

4.3.7 Cultures Characteristics

4.3.7.1 Properties

Cell morphology, viability and proliferation are outlined in **Table 4.11** (166, 489, 492-494, 498, 502, 506, 509-511).

4.3.7.2 Alkaline Phosphatase activity (ALP) assay – mRNA evaluation

ALP activity and mRNA evaluation is outlined in **Table 4.12** (166, 489, 492-494, 498, 502, 503, 507, 509, 515, 517).

Table 4.11 Cell culture characteristics

Author	Classification	Intervention	Cell Morphology	Cell Viability (MTT-Test)	Cell Proliferation
Wang (511)	Not mentioned	Chordin, Noggin and Gremlin expression knockdown	Not applicable	The cell viability of MSCs remained unchanged with PSI. By contrast, the cell viability of PEI25kDa-treated MSCs dramatically dropped to 20% of the original value when the polymer concentration reached 15 μg/mL.	Not applicable
Vallim (510)	Atrophic	Non-union MSCs, BM MSCs and osteoblasts were transplanted into the subcutaneous of immunodeficient mice	Not applicable	Not applicable	Non-union MSCs had proliferative and senescence rates comparable to BM MSCs and osteoblasts. The percentage of cells staining positive for b-galactosidase activity in non-union MSCs cultures was comparable to those observed in BM MSCs and osteoblasts.
Takahara (509)	Pseudarthrosis	Not applicable	Fibroblast-like spindle shape	Not applicable	Could be cultured through at least 10 passages, with minimal decline in their proliferative capacity.
Ismail (506)	Not mentioned	Not applicable	Not applicable	Non-union: viability of 87.1% (81.7% to 90.8%); iliac crest: 89.8% (84.7% to 94.5%). No differences was found between the two sources of MSCs.	Not applicable
Koga (489)	Viable: 2 patients; Non-viable: 5 patients	Group A: BMP-7 alone; Group B: BMP-7 + LIPUS	Not applicable	Not applicable	No significant difference in the DNA concentration between the two groups on days 3, 5, and 7.
Iwakura (166)	Hypertrophic	Not applicable	Not applicable	Not applicable	Proliferation capacity of non-union cells was significantly inferior to that of fracture haematoma cells.

Author	Classification	Intervention	Cell Morphology	Cell Viability (MTT-Test)	Cell Proliferation
Bajada (492)	Atrophic	Not applicable	Not applicable	Not applicable	Both non-union and BMSC differentiated along each mesenchymal lineage, forming ALP positive cells (i.e. osteoblastic differentiation), oil red O positive cells (adipocytic differentiation) and depositing an extra-cellular matrix in pellet culture that stained metachromatically with toluidine blue and was immunopositive for type II collagen (chondrogenic differentiation).
Qu (493)	Not mentioned	rhBMP-2	Not applicable	Not applicable	Osteoblastic cell populations isolated from bone harvested from the ilium and the three regions of the scaphoid non-unions had similar proliferative capacity.
Hofmann (494)	Hypertrophic	Not applicable	Although the morphology of confluent cells did not differ between controls and non-unions, there were significantly more bone nodules in the controls group.	At day four the mitochondrial succinyldehydrogenase enzyme activity was significantly higher in human osteoblast cultures (compared to human non-union osteoblasts), indicating that the number of metabolically active (viable) cells was higher in this group.	At four weeks, all cultures in both groups were confluent monolayers, and there was no significant difference in cell numbers between the groups.
Guerkov (498)	Atrophic: 4; Hypertrophic: 3	PEFS	Atrophic non-unions: cells formed a uniform monolayer of elongated cells that had few cellular extensions. Hypertrophic non-unions: also consisted of elongated cells, but the cells were more cuboidal, having cellular extensions in a multilayer. After the cells were treated with PEFS for 4 days, cells from the atrophic non-unions were small, elongated, or cuboidal, whereas cells from the hypertrophic non-unions were multi-layered, mostly cuboidal, and had cellular extensions connecting with adjacent cells. Cells that were not stimulated remained elongated and fibroblastic.	Not applicable	PEFS had no significant effect on the proliferation of hypertrophic and atrophic non-union cultures, at any of the times examined.
Boyan (502)	Not mentioned	BMP (bovine or dog)	Following BMP treatment cells became elongated and more fibroblast like, with no distinct foci of aggregated cells.	Not applicable	Incubation with BMP resulted in an inhibition in cell proliferation in periosteal (significant at 2mg/ml BMP); 3.7-fold inhibition and fibrocartilage cells (significant at 1mg/ml BMP; four-fold inhibition).

Table 4.12 ALP activity and ALP related mRNA expression

Author	Classification	Intervention	ALP Activity Assay	ALP mRNA
Granchi* (515)	Not mentioned	Regenerative approach consisted in a minimally invasive administration of autologous BM cells expanded in GMP facilities	After regenerative treatment: 1. At the time of BM harvesting, levels generally tended to be higher than reference values of healthy individuals. 2. After 6 and 12 weeks from surgery, a significant increase was observed. 3. At 24 weeks, concentrations were similar to those observed before treatment. Bone-specific ALP correlated to the imaging results collected at 12 and 24 weeks. Its variation along the healing course differed in patients who had an early consolidation (at 12 weeks). A remarkable decrease in ALP was observed at all time points in a single patient who experienced a treatment failure.	Not applicable
Takahara (509)	Pseudarthrosis	Not applicable	ALP activity increased with time and declined on day 28. By contrast, under control conditions, ALP activity in culture remained low between days 7 and 28. ALP activity under osteogenic conditions was significantly higher than control conditions on days 14 and 21.	Its expression under osteogenic conditions was upregulated compared with those under control conditions, and had a similar pattern to that shown by BMSCs.
Schira (507)	Not mentioned	Not applicable	Not applicable	ALP was significantly up-regulated across all non-unions
Marchelli* (517)	Not mentioned	Not applicable	Serum ALP levels in non-unions were similar to healed and healing fractures.	Not applicable
Koga (489)	Viable: 2 patients; Non-viable: 5 patients	Group A: BMP-7 alone; Group B: BMP-7 + LIPUS	The ALP activity of the non-union tissue-derived cells in Group B was significantly higher by 57% and 32% than that in Group A group on days 7 and 14, respectively.	In Group B, the expression level of ALP mRNA was significantly up-regulated by 55%, 24%, 50%, and 49% compared with the BMP-7–alone group on days 3, 7, 10, and 14, respectively.
Iwakura (166)	Hypertrophic	Not applicable	The level of ALP activity under osteogenic conditions was significantly higher than under control conditions on day 21. ALP activity of non-union cells was significantly higher than that of fracture haematoma cells under differentiated conditions.	The expression of ALP under osteogenic conditions was higher than under undifferentiated conditions in the control group.
Bajada (492)	Atrophic	Not applicable	The ALP activity of the NUSC cultures appeared markedly lower than that for BMSC cultures.	Not applicable
Qu (493)	Not mentioned	rhBMP-2	Baseline ALP activity was similar amongst cell populations isolated from all regions of the scaphoid non-unions and the ilium after 14 days of culture. rhBMP-2 treatment resulted in a significant increase in ALP activity in all groups (proximal: 1.7-fold; central: 2.1-fold; distal: 1.9-fold; iliac: 1.5-fold).	Not applicable
Hofmann (494)	Hypertrophic	Not applicable	The comparison of CFU-ALP as an early marker for osteoblast differentiation at day 7 did not show significant differences compared to controls.	Not applicable
Guerkov (498)	Atrophic: 4; hypertrophic: 3	PEFS	There was a time-dependent increase in ALP specific activity in all cultures that was significant in the cell layers and in isolated cells at 4 days after confluence. Exposure of the cultures to PEFS had no effect on the enzyme activity in either the cell layers or isolated cells. At Day 4, enzyme specific activity in the cell layer had increased in PEFS treated and control cultures by 99% and 90%, respectively. The time-dependent increases in the isolated cells were comparable. In addition, no differences between cultures from atrophic or hypertrophic non-unions were observed.	Not applicable
Boyan (502)	Not mentioned	BMP (bovine or dog)	There was significant reduction in ALP specific activity in matrix vesicles and plasma membranes from human fibrocartilage and	Incubation with BMP resulted in dose-dependent increase in transcription of ALP.

Author	Classification	Intervention	ALP Activity Assay	ALP mRNA
			periosteal cells incubated with 2mg/ml BMP (not at 1mg/ml BMP). As with connective tissue cells, ALP activity in the plasma membrane did not differ from that of the matrix vesicle membranes, before or after the exposure to BMP. Baseline ALP activity in cultures of human periosteal cells was comparable to fibrocartilage cells delivered from human non-union tissue.	
Quacci (503)	Hypertrophic	Not applicable	Some matrix vesicles presented ALPase activity inside them, but the main enzymatic activity was present outside and strictly connected to the vesicle membrane.	Not applicable

* Non-union related tissue

4.3.7.3 Osterix

Koga et al. has studied the effect of LIPUS on non-union cells cultured with the presence of BMP-7 and reported no significant difference in the expression of osterix (489). Takahara et al. on the other hand reports that the expression of osterix under osteogenic conditions was upregulated compared to controls, a finding similar to that demonstrated by the BMSCs (509).

4.3.7.4 Osteocalcin

Osteocalcin expression is outlined in **Table 4.13** (166, 489, 492-494, 498, 499, 507, 509, 515, 517).

4.3.7.5 Osteonectin

Osteonectin expression was investigated by Lawton et al. (499). Osteonectin was found to be strongly positive in non-cuboidal and induced osteoblasts of early woven bone, as well as cuboidal osteoblasts of later woven bone. Included osteoblasts and flattened lining cells on lamellar bone were only weakly positive, whereas endothelial cells were consistently negative.

4.3.7.6 Osteopontin

Lawton et al. investigated osteopontin expression during the different stages of repair (499). Osteopontin was found to be weakly positive in non-cuboidal osteoblasts on early woven bone, and moderately positive in cuboidal osteoblasts on the surface of woven bone later in repair. Multinucleate resorptive cells were associated with a strong signal, in comparison with most flattened cells on the surface of lamellar bone and endothelial cells that were negative.

4.3.7.7 Bone sialoprotein

Iwakura et al. studied the expression of Bone Sialoprotein under osteogenic conditions and found it to be higher in the non-union cells than under undifferentiated conditions in the human dermal fibroblasts (controls) (166). Takahara et al. also reported that its expression under osteogenic conditions was upregulated compared with those under control conditions, and had a similar pattern to that shown by BMSCs (509).

4.3.7.8 Mineralisation assay

Mineralisation assay outcomes are outlined in **Table 4.13** (166, 489, 492-494, 498, 499, 507, 509-511, 515, 517).

4.3.7.9 Dkk -1 expression

According to Bajada et al., both non-union and BMSC secreted Dkk-1 (Dickkopf 1) into conditioned medium at comparable levels under control (i.e. non-stimulated) conditions (492). This was in similar to the findings of Schira et al. who reported similar expression patterns in non-union tissue and controls (507). On the other hand, Dkk-1 levels detected in stimulated NUSC conditioned medium were markedly and significantly greater than those found in stimulated BMSC cultures (492).

4.3.7.10 RANKL expression

Schira et al. identified a significant up-regulation of RANKL in non-unions (20-fold), as also for the expression of the soluble decoy receptor of RANKL OPG (osteoprotegerin) and NFATc1 (Nuclear Factor of Activated T-cells, cytoplasmic 1), regardless of the duration of the non-union (507). The RANKL receptor RANK (receptor activator of nuclear factor κB) and macrophage colony-stimulating factor (M-CSF) was slightly but not significantly up-regulated (507). On the other hand, the expression of ATF4 (Activating Transcription Factor 4) was unchanged (507).

Table 4.13 Osteocalcin expression and mineralisation assay

Author	Classification	Intervention	Osteocalcin	Mineralisation Assay
Wang (511)	Not mentioned	Chordin, Noggin and Gremlin expression knockdown	Not applicable	The osteogenic diferenciation of MSCs isolated from non-unions was lower than those isolated for patients with uncomplicated healing.
Vallim (510)	Not mentioned	Non-union MSCs, BM MSCs and osteoblasts were transplanted into the subcutaneous of immunodeficient mice	Not applicable	Non-union MSCs deposited mineralised matrix positive for Von Kossa, similarly as BM MSCs and osteoblasts.
Granchi (515)	Not mentioned	Regenerative approach consisted in a minimally invasive administration of autologous bone marrow cells expanded in GMP facilities	After regenerative treatment: 1. At the time of BM harvesting, intact osteocalcin and N-terminal/midregion osteocalcin levels were comparable to the reference values of healthy individuals. 2. N-terminal/midregion osteocalcin decreased after 6 weeks. 3. At 24 weeks, concentrations were similar to those observed before treatment. Intact osteocalcin and N-terminal/midregion osteocalcin levels were significantly decreased at 6 weeks in patients healed after 24 weeks, to increase afterward, with changes not significantly different from baseline values.	Not applicable
Takahara (509)	Pseudarthrosis	Not applicable	Its expression under osteogenic conditions was upregulated compared with those under control conditions, and had a similar pattern to that shown by BMSCs.	Formed a mineralised matrix as observed on Alizarin Red S staining, contrasting with the absence of a mineralised matrix under control conditions after the same duration.
Schira (507)	Not mentioned	Not applicable	Similar expression pattern in non-union tissue and controls	Not applicable
Marchelli (517)	Not mentioned	Not applicable	Serum osteocalcin levels in non-unions were similar to healed and healing fractures	Not applicable
Koga (489)	Viable: 2 patients; non-viable: 5 patients	Group A: BMP-7 alone; Group B: BMP-7 + LIPUS	No significant differences	The intensity of Alizarin Red S staining in the Group B was significantly higher by 30% than in Group A at day 2.

Author	Classification	Intervention	Osteocalcin	Mineralization Assay
Iwakura (166)	Hypertrophic	Not applicable	The expression of osteocalcin under osteogenic conditions was higher than under undifferentiated conditions in the control group.	After a 21-day incubation under osteogenic conditions, induced non-union cells formed a mineralised matrix (mineralisation significantly higher than that of fracture haematoma cells), contrasting with an absence of mineralised matrix under undifferentiated conditions after the same duration.
Bajada (492)	Atrophic	Not applicable	Not applicable	Although non-union stromal cells elevated their expression of these markers in response to osteogenic stimuli, there was a marked and significant reduction in their capacity to differentiate along an osteoblastic lineage compared to BMSC.
Qu (493)	Not mentioned	rhBMP-2	All populations had low numbers of osteocalcin-positive cells (7–9%) when grown in the presence of standard medium. There was no statistical difference in the number of osteoblasts between any of the three regions of the scaphoid and the ilium amongst cells grown under standard conditions, nor was there any correlation between the number of osteoblasts and the duration of the non-union. Cell populations originating from the central fibrocartilagenous part of the non-union had the greatest variability in osteocalcin staining. Significant increases in osteocalcin expression were observed in all groups in response to treatment with rhBMP-2 (ilium: 2.9-fold increase; proximal and distal: 2.3-fold increase; central: 2.0-fold increase).	Cell populations derived from scaphoid non-unions formed an extracellular matrix that showed very little bone nodule formation when maintained in culture for 28 days. Treatment with rhBMP also resulted in a significant increase in the number of bone nodules for all groups (proximal: 3.5-fold; central: 10.5-fold; distal: 4.9-fold; iliac: 3.4-fold).
Hofmann (494)	Hypertrophic	Not applicable	Not applicable	The mineralisation of extracellular matrix (CFU-M) was very low in human non-union osteoblast cultures that were cultured under the same culture conditions and was significantly less than that in human osteoblast cultures.
Guerkov (498)	Atrophic: 4; Hypertrophic: 3	PEFS	Osteocalcin was expressed at very low levels by the cultures, indicating the fourth passage cultures contained few, if any, committed osteoblasts. PEFS did not affect production of osteocalcin by non-union cells.	Not applicable
Lawton (499)	Not mentioned (had callus)	Not applicable	Weakly positive in flattened lining cells on lamellar bone. Positive in multinucleate resorptive cells. Consistently negative in endothelial cells.	Not applicable

4.3.7.11 Gene expression and genetic predisposition to fracture non-union

Several authors have examined the expression of different genes in the non-union tissue. A summary of their results is outlined in **Table 4.14** (98, 155, 166, 489, 491, 494, 499, 502, 505, 507, 509, 511, 513), and **Table 4.15** (500, 502). Additionally, a number of studies investigated the theory of genetic predisposition to fracture non-union and identified numerous polymorphisms associated with an increased risk of developing non-union (**Table 4.16**) (170, 217, 516, 518-521).

Table 4.14 Gene expression of non-union tissue

Author	Gene Expression
Wang (511)	- The expression of Chordin, Noggin and Gremlin was higher in bone non-union isolated MSCs, whilst the expression of BMP-7 was lower. - The expression of ID1 and ID3 was down-regulated in non-union MSCs. - Chordin knockdown enhances the osteogenic differentiation of MSCs in patients with bone non-union.
Takahara (509)	- The expression of RUNX2 under osteogenic conditions was upregulated compared with those under control conditions, and had a similar pattern to that shown by BMSCs. - The mRNA of aggrecan, Col II, Col X, SOX5, and SOX9 after a 21-day chondrogenic induction was not expressed. - Glycosaminoglycan was extensively present in sections from BMSC pellets, and a high expression of those chondrocyte-related genes was observed in BMSC pellets after a 21-day chondrogenic induction.
Schira (507)	- Noggin was significantly down-regulated in non-union tissue. - BMP-7 and pro-osteogenic FGFs, FGF-9 and FGF-18, were neither detectable in non-unions nor in control cancellous bone. FGF-2 was not differentially expressed. - Cyclin D1 was significantly up-regulated in non-unions. - WNT3A expression was not detectable in both tissues, whilst WNT5A was up-regulated in non-unions. - MMP-9 as well as MMP-13 expression were found to be significantly up-regulated in non-unions. - Quantification of gene expression levels of PECAM-1 by qRT- PCR revealed similar expression levels in non-unions and controls. - The expression of RUNX2 was hardly detectable in non-unions and controls.
Han (505)	- BMP-2 was expressed in non-union tissue, and was highest in the posted bone scar and lowest in the bone ends. The expression in the posted bone scar was significantly different to the canal content and bone ends groups (bone ends < marrow cavity < posted bone scar). - Decorin was expressed in three different parts of the non-union area, and was highest in the bone ends. The expression level in the bone ends group was significantly different to the canal content and posted bone scar groups ($p < 0.05$).
Koga (489)	The expression level of RUNX2 mRNA in BMP-7 + LIPUS group was significantly higher by 49% and 134% compared with the BMP-7–alone group on days 10 and 14, respectively.
Zimmermann (155)	Genes expressed more than two times than in normal tissue: CDO1; PDE4DIP; COMP; FMOD; CLU; FN1; ACTA2; TSC22D1.
Fajardo (98)	MMP-7 and MMP-12 were significantly elevated in non-union tissue when compared with local mineralised callus from the same site.
Iwakura (166)	It showed the expression of mRNA of Col II, Col X, SOX9, and aggrecan chondrogenic conditions after a 21-day induction. Under adipogenic conditions after a 21-day culture period, it showed the expression of LPL and PPAR-g2 (higher than under undifferentiated conditions in the control group).
Fajardo (491)	- BMP antagonist genes (Drm/Gremlin, follistatin, noggin): upregulated in non-union tissue when compared to fracture callus tissue. - BMP receptors (R1A, R1B, R2): expressed but did not demonstrate any significant differences. - BMP-4: upregulated in non-union tissue. - BMP-7: increased in the fracture callus tissue.
Hofmann (494)	- Gene terms significantly upregulated in human non-union osteoblast cultures: Skeletal development; response to wounding; organ morphogenesis; vasculature development; proteinaceous extracellular matrix; extracellular space; cytokine activity; glycosaminoglycan binding; GF activity; IGF binding. - Genes significantly downregulated in human non-union osteoblast cultures: IGF-2, FGF-1, FGF-R2, BMP-4, TGF-β2, PDGF, Wnt-induced proteins (WISP2 and 3), β-catenin, and prostaglandin E2 receptor EP4.
Kilian (513)	- EDA+ fibronectin mRNA was detectable at low levels. - EDB+ fibronectin mRNA transcription was not detected.
Lawton (499)	- Osteoblasts in non-unions: positive for MGP mRNA signal (in the zone of new bone formation and in the interface zone; old bone zone: almost always negative; gap zone: rarely contained osteoblasts). Small and large chondrocytes in non-unions: negative. Small and large chondrocytes in normal fractures: positive for MGP mRNA. - Osteoblasts in normal fractures: never detected.
Boyan (502)	- Addition of BMP resulted in an increase in mRNA levels for ALP and collagen Type I (two-fold) and Type II (not significant) in periosteal cells but not in fibrocartilage cells. - Actin mRNA levels did not change.

Table 4.15 Collagen gene expression

Author	Intervention	Type I	Type II	Type III
Lawton (500)	Not applicable	Signal for procollagen type I mRNA over fibroblasts and over osteoblasts on woven bone was uniformly strong in most non-unions and normal fractures.	Not applicable	Non-unions: in the zone of new bone formation and the interface zone, a population of surface and included osteoblasts was strongly positive for the procollagen type III mRNA signal; osteoblasts in the old zone were usually negative, while the gap zone contained osteoblasts only rarely; fibroblasts were frequently positive in the gap zone and interface. Normal fractures: procollagen type III mRNA was seen in the very early granulation tissue, where most of the positive cells were mesenchymal spindle cells (a cell population that includes osteoblast precursors); osteoblasts were in the vast majority negative; small areas of fibrous tissue in which fibroblasts were either negative or weakly positive.
Boyan (502)	BMP (bovine or dog)	There was no stimulation of Type I collagen message in the non-union fibrocartilage cells. Non-union periosteal cells were found to be more strongly activated by BMP.	The increase in mRNA levels of Type II collagen was not significant compared to controls.	Not applicable

Table 4.16 Gene predisposition to non-unions

Author	Genetic predisposition
McCoy (518)	The most strongly-associated SNP is located in Calcyon. Among the loci associated with non-union, one notable region spans the TACR1 gene.
Zhang (520)	- CtBP2, but not CtBP1 (only slightly increased), is significantly upregulated in atrophic non-union tissue compared to healthy controls, which is also translated to a protein level. Osteoblast isolated from non-union tissue also had the same upregulation compared to healthy controls. - Expression of SPHK1, Dkk-1 and CDH2 were significantly upregulated whilst p300, RUNX2 and BMP2 were dramatically downregulated in all atrophic non-union tissues compared to the healthy controls. - CtBP2 forms a transcriptional complex with p300 and RUNX2. More specifically, CtBP2 plays an inhibitory role in regulating p300-RUNX2 complex formation. - The CtBP2-p300-RUNX2 transcriptional complex inhibits the expression of genes involved in bone formation and differentiation. - An elevated NADH level upregulates RUNX2 target gene levels in osteoblasts. - HDACs are not required for the transcription of RUNX2 target genes.
Huang (516)	- SNP rs2297514 showed significant association with the fracture healing process after adjusting for age and gender (OR = 1.38, p = 0.0005). - The T allele of rs2297514 significantly increased the risk of a non-union during the fracture healing process by 38% compared to the C allele. - Significance could only be observed in the tibial diaphysis subgroup (not for femur / humerus / ulna).
Sathyendra (519)	Five SNPs on four genes were significant, with three having an OR >1, indicating that the presence of the allele increased the risk of non-union. The rs2853550 SNP, which had the largest effect (OR = 5.9, p = 0.034), was on the IL1B gene, which codes for IL1 beta. The rs2297514 SNP (OR = 3.98, p = 0.015) and the rs2248814 SNP (OR = 2.27, p = 0.038) were on the NOS2 gene coding for nitric oxide synthase. The remaining two SNPs had an OR of <1, indicating that the presence of the allele may be protective against non-union. The rs3819089 SNP (OR = 0.26, p = 0.026) was on the MMP13 gene for MMP13, and the rs270393 SNP (OR = 0.30, p = 0.015) was on the BMP6 gene for BMP6.
Zeckey (170)	An influence of a PDGF haplotype is significantly associated with long bone non-unions of the lower limb following fracture. No major influence of single polymorphisms only within the genes encoding for the other observed mediators involved in fracture healing. There was a trend however towards an association of uneventful healing and a polymorphism of MMP-13.
Dimitriou (217)	Two specific genotypes (G/G genotype of the rs1372857 SNP, located on NOGGIN and T/T genotype of the rs2053423 SNP, located on SMAD6) are associated with a greater risk of fracture non-union.
Xiong (521)	The ADAMTS18 level is significantly lower in subjects with non-union fractures as compared to subjects with normal-healing fractures. Decreased in vivo ADAMTS18 expression might thus potentially contribute to the non-healing of skeletal fractures. The TGFBR3 level is significantly lower in normal skeletal fracture subjects as compared to non-union skeletal fracture subjects. This is opposite of the expression pattern for ADAMTS18, suggesting different physiological roles for these two genes in the healing of bone fractures.

4.3.7.12 Western Blot assay

Western Blot assay was used to detect the presence of specific proteins in the tissue under examination. Fajardo et al. investigated the presence of MMPs and reported that MMP-7 and MMP-12 were present in both non-union and mineralised callus tissue; however, the signal intensity of both enzymes was stronger in the non-union tissue (98). In another study, the same team reported that BMP-2 was present in both non-union and mineralised callus tissue; BMP-4 was detected in non-union samples but decreased in healing bone samples; and BMP-7 was detected in the healing bone but was absent in the non-union samples (491). Wang et al. on the other hand, demonstrated that the expression of p-SMAD1/5/8 was decreased in MSCs isolated from patients with bone non-union, whilst chordin knockdown rescued the osteogenic capacity of MSCs isolated from patients with bone non-union (511).

4.3.7.13 Comparison between atrophic and hypertrophic non-union tissue

Table 4.6 (166, 492, 495-498, 503, 507, 508, 510, 512) and **Table 4.17** (166, 491, 492, 494, 496, 498, 507, 508, 512, 513) compare the characteristics of tissue obtained from atrophic and hypertrophic non-unions.

Table 4.17 Comparison between atrophic / hypertrophic non-union tissue

Type of Analysis	Atrophic	Hypertrophic
Histology	**Table 4.6**	
Immunohistochemistry	SMAD2/3 revealed increased activity in non-unions (507) Close vicinity to immature osteoid trabeculae (513)	-
Vessel Density	No difference in the median vessel count between atrophic / hypertrophic non-unions (496)	
Cell surface antigen profile	Less than 1% of NUSC and BMSC were positive for CD34 and CD45, whilst 78% ± 14% of NUSC and 92% ± 7% of BMSC were positive for CD105 (492)	Positive for MSC's-related markers CD13, CD29, CD44, CD90, CD105, and CD166, but negative for hematopoietic markers CD14, CD34, CD45, and CD133 (166)
Cell morphology	Cells formed a uniform monolayer of elongated cells that had few cellular extensions (498)	Also consisted of elongated cells, but the cells were more cuboidal, having cellular extensions in a multilayer (498)
Cell Proliferation	Cells differentiate along each mesenchymal lineage (492)	Significantly inferior to that of fracture haematoma cells (166)
ALP Activity	No differences between atrophic / hypertrophic non-unions (498) Higher levels in scaphoid non-unions as opposed to cancellous bone (507) Markedly lower than that for BMSC cultures (492)	No differences between atrophic / hypertrophic non-unions (498) No difference with controls (494)
Osteocalcin	Very low levels (498)	Very low levels (498); higher than in human dermal fibroblasts (166) The expression of osteocalcin under osteogenic conditions was higher than under undifferentiated conditions in the control group (166)
BMPs	No significant difference in BMP-2 levels between atrophic / hypertrophic non-unions (512) BMPs antagonists present in non-union tissue and controls (508)	No significant difference in BMP-2 levels between atrophic / hypertrophic non-unions (512) BMP-2: present in the fibrous tissue of the non-union (491) BMP-7: absent (491)
MMPs	-	MMP-7 and MMP-12 were present (98)
Mineralisation Assay	Significant reduction in the MSCs capacity to differentiate along an osteoblastic lineage compared to BMSC (492)	Higher than haematoma cells (166) Very low mineralisation potential and significantly lower than 'normal' human osteoblasts (494) Under osteogenic conditions, mineralisation was significantly higher than that of fracture haematoma cells, in contrast to undifferentiated conditions (166)

4.3.7.14 Effect of Interventions to the non-union tissue

Table 4.18 (489, 493, 498, 502, 516) outlines the effects of interventions on the non-union tissue.

Table 4.18 Effect of interventions

Author	Wang	Koga	Qu	Guerkov	Boyan
Type of Intervention	Chordin, Noggin and Gremlin knockdown	Group A: BMP-7 alone; Group B: BMP-7 + LIPUS	rhBMP-2	PEFS	BMP (bovine or dog)
Proliferation	Not applicable	No effect	No effect	No effect	Inhibition in periosteal and fibrocartilage cells
ALP	Not applicable	ALP activity higher in Group B. ALP mRNA: Upregulated by 55%, 24%, 50%, and 49% compared with the Group A on days 3, 7, 10, and 14, respectively	Significant increase in all regions	No effect: cell layers or isolated cells. At Day 4, enzyme specific activity in the cell layer had increased in PEFS treated and control cultures by 99% and 90% respectively (comparable increase).	Reduction: matrix vesicles and plasma membranes from human fibrocartilage and periosteal cells incubated with 2mg/ml BMP (not at 1mg/ml BMP). No effect: connective tissue cells, plasma membrane, matrix vesicle membranes.
mRNA	Not applicable	The expression level of RUNX2 mRNA in Group B was significantly higher	Not applicable	Not applicable	The relative amounts of each type of mRNA differed (ALP, Collagen Type I and II).
Osterix	Promoted by Chordin knockdown, more strongly than Gremlin. Decreased by Noggin knockdown	No effect	Not applicable	Not applicable	Not applicable
Osteocalcin	Promoted by Chordin knockdown, more strongly than Gremlin. Decreased by Noggin knockdown	No effect	Significant increases in osteocalcin expression in all groups	No effect	Not applicable
Mineralization Assay	Chordin knockdown rescued the osteogenic ability of BMSCs isolated from patients with non-union	Significantly higher by 30% than in Group A at day 2	Significant increase in the number of bone nodules for all groups.	Not applicable	Not applicable
Collagen Expression	Col1a1: promoted by Chordin knockdown, more strongly than Gremlin. Decreased by Noggin knockdown	Not applicable	Not applicable	No effect on collagen synthesis	Type I: No effect in non-union fibrocartilage cells but increase in periosteal cells Type II: No effect
Other	Not applicable	Not applicable	Not applicable	TGF-β1: time-dependent effect Prostaglandin E2: no effect	Glycosaminoglycan: increased

4.3.8 Discussion

Non-unions represent a significant public health problem and have been associated with devastating consequences for the patients, their family and the society as a whole (524). The mechanism behind the progression of a fracture to a non-union state is multifactorial and as a consequence the treatment can be very challenging. The treatment of non-unions has evolved over the years from prolonged immobilisation (514) to the use of biological stimulation and polytherapy. Such a strategy attempts to address all the elements of a compromised fracture healing response (90, 167).

With regards to the macroscopic appearance of non-unions, a common finding is the interposition of soft tissue between the bone fragments (504, 505, 514). Additionally, Han et al. reported bone sclerosis at the fracture ends and complete obliteration of the medullary canal (505). Moreover, in aseptic non-unions, this tissue is whiter in colour, occasionally surrounded by clear fluid, compared to infected non-unions where this tissue becomes more yellowish and frequently surrounded by murky fluid (169). The above characteristics are confirmed in the author's experience from obtaining non-union tissue, and in fact the macroscopic appearance of the non-union tissue is used as an additional marker for suspecting / confirming an underlying septic process.

As for the culture characteristics of the non-union tissue, there was an inconsistency in the reported findings. This may be because of the different types of non-union tissue examined (i.e. atrophic and hypertrophic), as well as because of the different topography of the non-unions from where samples were obtained.

Several similarities were reported in the histological analysis of atrophic and hypertrophic non-unions. The main types of tissues involved include fibrous, cartilaginous and connective tissue in varying degree (166, 492, 495, 496, 498, 503, 507, 508, 510). In atrophic non-unions, bony islands were not always (498) or occasionally present (492, 496, 512) and with a mixture of lamellar and woven bone (508), whereas necrotic bone was more prevalent (496) and bone production was predominantly via the endochondral route (496). Similarly, in hypertrophic non-unions no ossicles (166, 498) or few bony islands were present (495, 496, 503, 512), with bone formation through both endochondral and intramembranous ossification (496). Generally, the cellular density of atrophic non-unions was lower compared to hypertrophic non-unions, whilst some areas were completely acellular (492, 498).

Moreover, the majority of the cells in both atrophic and hypertrophic non-unions were fibroblast like (166, 492, 507, 510), but an important finding was that MSCs were present in both tissues (512). All the above suggest a different cellular background and local environment, which may correspond to the higher failure rate following revision surgery of atrophic non-unions (167).

More importantly, Bajada et al. were the first to report that non-union tissue contained cells positive for MSCs-related markers and negative for haemopoietic markers (492). This finding was later confirmed by other authors, thereby highlighting the fact that non-union tissue contains biologically active cells with the potential to differentiate to osteoblastic, adipogenic and chondrocytic lineages (166, 489, 506, 509, 510).

In contrast to the common preconception that atrophic non-unions are relatively avascular and inert (496, 525), several authors have confirmed the presence of vascular tissue in histological examination of atrophic (492, 496-498, 510) and hypertrophic (496) non-union tissue, with no major differences between the two types on non-union (496). Analysing the vessel density of non-union tissue (496, 501, 503, 507, 508) also revealed similar levels of angiogenesis in non-unions and cancellous (507) or healing bone (496, 508). This biological finding may be of importance, as it suggests that treatments targeting to the enrichment and restoration of local angiogenesis could be applied as an effective treatment modality in the clinical setting.

Low-grade infection represents a challenge for the treating surgeon, as laboratory markers (such as CRP (C-Reactive Protein), ESR, WBC) and conventional cultures of intra-operative samples can be negative (488, 490). A possible explanation for this phenomenon could be the presence of biofilms (bacteria adhere on implants and tissues around the fracture site, forming matrix-enclosed communities), which are resistant to ‘normal’ concentrations of systemic antibiotics (488). Palmer et al. and Gille et al. have reported the benefit of utilising molecular based techniques to identify these infections (488, 490). This can be very important, as distinguishing between septic and aseptic non-union is essential for determining the course of treatment. Limitations of their use in clinical practice however include: the fact that single-primer PCR can only detect one target organism (488); concerns for oversensitivity with regards to clinical relevance (488, 526); and associated cost implications. Other situations where conventional cultures may be negative include infections by

mycobacteria, fungal infections or infections caused by a small amount of low virulence organisms (369).

Cell senescence is known to play an important role in healing and tissue regeneration (527). In essence, the senescence of adult stem cells or more differentiated cells present in the non-union tissue may represent one of the main mechanisms of the loss of the regenerative potential, leading to healing impairment (527). As already mentioned, Bajada et al. reported that an increased proportion of NUSC were senescent when compared to BMSC, which did not correlate with the patient's age (492). On the other hand, Vallim et al. identified no differences between non-union MSCs senescence rates to those of BM MSCs and osteoblasts (510). Nonetheless, the pathways leading to this genomic damage and the contribution of several factors (such as repeated cellular replication and the consequent cell stress (492)) are yet to be determined.

BMP's are some of the major signalling molecules, promoting the differentiation of MSC's into chondrocytes or osteoblasts and have been extensively studied because of their important role in bone healing (103, 104). Kloen et al. reported evidence of on-going BMP signalling in the non-union tissue, where endogenous BMP's, their receptors and molecules involved in their signal transduction were present in the tissue (497). Additionally, Wang et al. reported no difference in BMP expression between atrophic and hypertrophic non-unions, and no difference with increasing duration of the non-union (512). Han et al. also reported that BMP-2 was locally generated in non-unions MSCs, with no BMP presence in the extracellular matrix and low expression in the bone ends and canal contents (505). With regards to the gene expression of Chordin, Noggin and Gremlin, these were found to be elevated in non-union tissue (491, 511), whilst the gene expression of BMP-7 was reduced (491, 511). Therefore, some authors suggest that imbalance in the expression of BMP's and their inhibitors Chordin, Noggin, Gremlin and Follistatin, might account for the impaired bone forming ability (487, 491, 508).

Investigating further the effect of BMPs on non-union tissue, Boyan et al. added BMP in non-union cells cultures, reporting a dose dependant decrease in cell number, a decrease in matrix vesicle and plasma membrane ALP and an increase in mRNA levels for ALP and collagen genes (502). Similarly, Qu et al. added rhBMP-2 in cell cultures identifying that MSC's differentiated into functional osteoblasts with an increase in ALP, osteocalcin expression and generally an improved mineralisation

potential (493). On the other hand Wand et al. investigated the effect of BMP inhibitors Chordin, Noggin and Gremlin knockdown (511). More specifically he and his team reported an increased expression of osterix, osteocalcin and collagen with Chordin and Gremlin knockdown, in contrast to Noggin knockdown where a decrease was evident (511). Moreover, Chordin knockdown rescued the osteogenic ability of non-union cells (511). Even though treatments regulating concentrations of BMP's have already been used in clinical practice with encouraging results (such as BMP-2 and BMP-7 (167)), further research is needed to investigate their effects, as well as those of similar agonist molecules and their inhibitors.

MMP's are proteases that play an important role in bone remodelling and bone repair. When the MMP's or their inhibitors are disrupted, disorders of fracture healing may occur (98). In a study by Fajardo et al., MMP-7 and MMP-12 genes were reported to be significantly upregulated within the tissue of hypertrophic non-unions (98). When the hypertrophic non-union tissue was examined *in vitro*, it was found that the same proteins directly bounded to and degraded BMP-2, a highly osteoinductive agent (98). This action of the MMP's may be responsible for the impaired fracture healing in the case of hypertrophic non-unions, even though the same finding may not correlate to atrophic fracture non-unions. Additionally, in a study by Schira et al., MMP-9 and MMP-13 expression was significantly up-regulated in non-unions, further suggesting their role in the non-unions' pathogenesis (507).

Several reports suggest that LIPUS stimulates bone healing, even though the mechanism behind this remains obscure (528, 529). When applying LIPUS in non-union cells cultures, it was suggested that there was a significant effect on the osteogenic differentiation rather than proliferation of non-union tissue cells (489). In addition, GF synthesis and release was stimulated (498). The use of LIPUS can potentially improve union rates and accelerate the healing process. Interestingly, in a double-blinded RCT Simpson et al. suggested that LIPUS does not influence the rate of bone healing by distraction osteogenesis (530).

Dkk-1, a secreted protein acting as an antagonist of the Wnt signaling pathway, has been reported to suppress fracture repair by inhibiting osteogenic differentiation (492, 531). Non-union tissue has been reported to have similar expression of Dkk-1 with BMSC (492) and 'normal' bone (507). Bajada et al. then compared the levels of Dkk-1 in atrophic NUSC and BMSC cultured under osteogenic conditions, reporting an increased secretion by the non-union cells, associated with reduced osteoblastic

differentiation (492). When recombinant human Dkk-1 was then added in the culture medium of the BMSC, the effect on osteogenic differentiation remained inhibitory (492). This finding suggests that Dkk-1 may play an important role in the development of non-unions, however further research is needed to shed more light on the underlying mechanism of an increased Dkk-1 production by non-union cells, as well as its exact mechanism on action and role, if any, in the development of a non-union.

In the last few years, there is emerging evidence that genetic predisposition may play an important role in the development of non-unions. A number of studies have investigated the differences between patients developing a non-unions with those with uneventful healing (170, 217, 516, 518-521). Numerous polymorphisms have therefore been associated with non-unions, with some involving the BMP (217, 519) and MMP pathways (170, 519). Nevertheless, most of these studies have significant limitations as they are underpowered, include a small number of patients and investigate a small number of SNPs. A more comprehensive analysis of the genome in the years to come may reveal genes / SNPs which may play a crucial role in fracture healing acceleration or inhibition, and these could be targeted as part of novel ‘gene therapies’.

The herein literature review has some limitations. Firstly, it excludes studies involving experimental animal models. Nevertheless, the outcomes of such studies should be treated with caution, as they cannot be translated directly to the clinical scenarios. Secondly, there is an inconsistency in defining non-union, and as such the timing of tissue harvesting would be slightly different, which might be responsible for some of the differences reported amongst similar studies. Moreover, as the term MSC’s is fairly recent, studies performed in earlier years used a different terminology for the same cells, such as osteoprogenitors, skeletal stem cells etc. As a result, their findings could not be compared to those of more recent studies.

Strengths of the review include the systematic approach of analysing the results and the detailed careful analysis of the data obtained. Collectively, this chapter presents our current understanding of the molecular and cellular pathways that can be involved in the development of non-union. Direct recommendations to be applied in the clinical setting cannot be safely made with the available evidence. It is essential that a widely accepted definition of the timeframe for non-unions should be set allowing an earlier intervention in such cases. The conceptual frame of the ‘diamond concept’ for a successful fracture healing response should be considered in cases where bone

repair is desirable (92). Cellular therapies and inductive molecules with scaffolds have a role to play in future treatment strategies, as would do tissue engineering approaches (532). Although still under intense investigation genetic therapy could be another treatment option in the foreseeable future.

4.3.9 Conclusion

In conclusion, failure of fracture healing and progression to non-union represents a not uncommon clinical complication which carries devastating consequences. The histopathological appearance of non-union tissue between atrophic and hypertrophic non-union indicates that both types of non-unions are not avascular and contain a potentially active population of MSC's. Pathways believed to be involved in their pathogenesis include an imbalance in the expression of BMP's and their inhibitors, and an upregulated expression of several substances such as that of the MMP's and Dkk-1which can block the BMP and Wnt pathways respectively. Emmerging evidence also support a genetic predisposition in this patient group.

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Chapter 5
Biological Characterisation of Non-union Tissue: Laboratory work

5.1 Introduction

The exact biological process leading to a non-union remains obscure (90, 98, 167, 487). A better understanding of the underlying mechanisms leading to a non-union, including the role of MSCs, would help clinicians to target specific pathways with an aim to improve clinical outcomes (26). Therefore, the ultimate main objective of this project was to investigate the factors contributing to the development of long bone non-unions and the biological mechanisms behind this clinical entity.

5.2 Aims, hypothesis and objectives

Hypothesis:

In a subset of patients who have no other risk factors for non-union, phenotypic and functional differences of the MSCs in healthy and non-union tissues exist.

In order to address the hypothesis, the following aim was set:

To investigate the biological and molecular profile of fracture non-union tissue.

5.3 Materials and methods

5.3.1 Patients

Following ethics committee approval (06/Q1206/127 – NRES (National Research Ethics Service) Committee Yorkshire & The Humber – Leeds East; 12/04/2012; **Appendix D**) and with informed consent, ten patients undergoing revision surgery as part of their treatment for atrophic non-union were recruited. This research project was carried out in compliance to the principles of the Declaration of Helsinki.

5.3.1.1 Inclusion and exclusion criteria

The inclusion criteria were the following:

i. Patients having revision surgery for an atrophic non-union of the femur or tibia. Atrophic non-union was defined as incomplete fracture healing at least six months following injury, along with typical radiographic features of an atrophic non-union (little or no callus) on serial radiographs over the course of three consecutive months, with the fracture site being not freely mobile.
ii. Age 18 to 65 years old.

The exclusion criteria were the following:

i. Septic non-union, defined as clinical or laboratory evidence of on-going infection (abnormal WCC and CRP within six months from index procedure).
ii. Patients where number of MSCs isolated from initial samples was poor.
iii. Patients with head injury or patients unable to give an informed consent at the time of surgery.
iv. Pathological fractures or any history of previous or current neoplasia.
v. Open fractures, segmental bone loss requiring complex management including bone transport or large structural allograft, and fractures complicated with vascular or neural injury.
vi. Smokers and those with a history of alcohol abuse.
vii. Patients with a history of infectious diseases, history of HIV / hepatitis B / hepatitis C infection, presence of diabetes or autoimmune inflammatory disease, bone turnover modifying medication taken six months prior or at any point after the original injury (bisphosphonates, steroids and immunosuppressive medication).

5.3.2 Collection of tissue samples

All patients were managed by a multidisciplinary team specialised in lower limb reconstruction. Tissue samples were obtained intra-operatively by the lead surgeon, and were immediately processed according to a standardised protocol.

More specifically, the following types of tissue were collected from each patient (**Figure 5.1**):

1. Non-union tissue:

Non-union tissue was harvested from the site of the non-union, between the opposed cortices (minimum volume of 1 cm^3). Half of the harvested tissue was embedded in OCT (optimal cutting temperature compound) and stored in -80^oC for future

histological analysis. The other half was processed for isolation of the cells, after removing any periosteum and bone attached to the non-union tissue.

A part of the non-union tissue was also sent for microbiological analysis, including microscopy, conventional cultures and sensitivities. If the result was positive, the patient samples were excluded from this project. Peri-operative antibiotics were only administered after all samples were obtained.

2. Bone:

Cortical and underlying cancellous bone was harvested from a site distant to the non-union site (minimum volume of 0.5 cm^3) and was then processed for isolation of the cells. This was preferred over RIA reamings, to avoid collection of damaged cells during the RIA process, as well as to reduce the number of cells coming from the bone marrow and collected by RIA.

3. Bone marrow:

Bone marrow aspirates were obtained after perforation of the anterior superior iliac crest using a standardised technique (533). The MarrowStim kit was used (Biomet biologics, INC), volume of aspirate (12 ml) and draw method (single 12 ml draw), was the same for all patients (**Appendix E**). Bone marrow aspirates were immediately transferred to 5 ml vacutainer tubes (BD Vacutainer®, #367928) containing EDTA to prevent clotting and processed within two hours of collection. In the laboratory, any existing clots were removed by filtering aspirates through a Cell Strainer and transferring then into 50 ml centrifuge tubes where they were diluted (1:2) with sterile PBS. The diluted BM aspirates were layered on the top of equal amount of Lymphoprep (Axis-Shield, #1114545) and centrifuged for 25 minutes at 1800 rpm. The interface zone containing the mononuclear cells (MNCs) was then collected with a sterile Pasteur pipette and the cells were frozen using a standardised technique.

4. Serum:

For the isolation of serum, whole peripheral blood was collected (20 ml) and immediately transferred to vacutainer tubes (BD Vacutainer®, #367955). Subsequently the blood was allowed to clot for 30 minutes, by leaving it undisturbed at room temperature. The clot was then removed by centrifuging at 800 g for 15 minutes in a refrigerated centrifuge. Following centrifugation, the resulting supernatant (serum) was immediately transferred into a clean polypropylene tube using a Pasteur pipette, labelled and stored in –80°C.

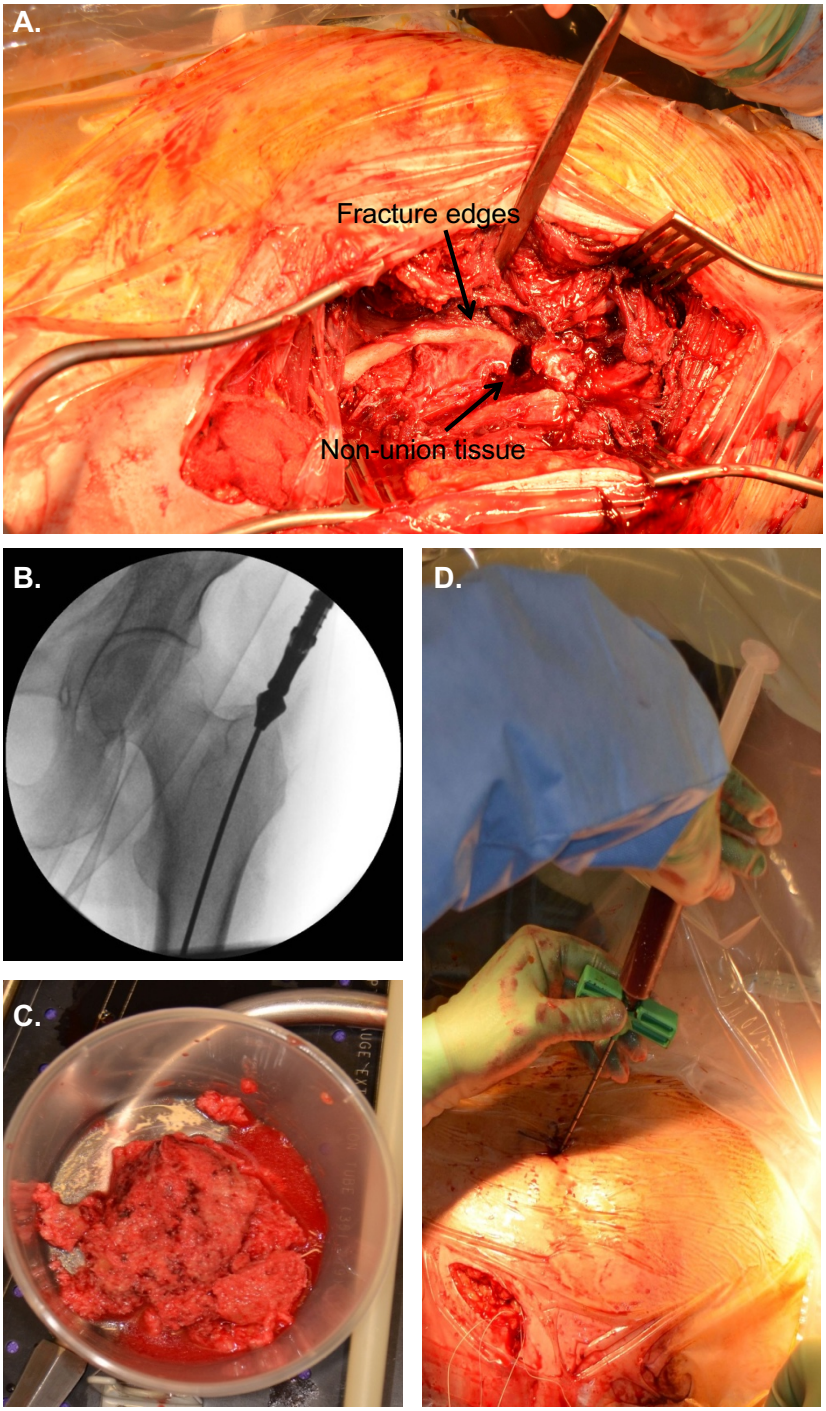


Figure 5.1 Collection of tissue samples

A. Non-union tissue: following surgical approach to the non-union site, tissue was collected between the fracture ends with care not to collect any 'normal' tissue.

B - C. Bone: bone was collected either from the iliac crest or from the medullary canal using the RIA technique.

D. Bone marrow: collected from the iliac crest.

5.3.3 General reagents and tissue culture plastics

Unless otherwise stated, all tissue culture reagents, including Dulbecco's Modified Eagle's Medium (DMEM) (#61965-026), Dulbecco's Phosphate Buffered Saline (PBS) (#14190-094), Trypsin / Ethylenediaminetetraacetic acid (EDTA) solution (0.05%/0.02% EDTA, #15400-054), and Penicillin / Streptomycin solution (#15140-122) were from Invitrogen. Tissue culture plastic was from Corning, including 15 ml centrifuge tubes (#430790), 50ml centrifuge tubes (#430828), 96 well plates (# 3599), 48 well plates (# 3548), 24 well plates (# 3524), 6 well plates (# 3516), and pipettes (#4251 – 25 ml, #4101 – 10 ml, #4051 – 5 ml). Polypropylene microtubes were from Trefflab (#564225.9.01 – 0.5 ml and 96.8160.9.01 – 1.5 ml), pipette tips were from Gilson (#F167104, #F167103, #F167101 – blue, yellow and white, respectively) and 12 well plates were from Nunclon (# 150628).

5.3.4 Culture media

1. Standard MSC culture media

89% DMEM supplemented, with 10% FBS (foetal bovine serum) optimised for MSC cultures, and antibiotics (1%; penicillin and streptomycin) (**Appendix F**).

2. Patient own serum MSC media

89% DMEM supplemented, with 10% serum derived from same patient, and antibiotics (1%; penicillin and streptomycin).

5.3.5 Enzymatic digestion of tissues

Following the collection of the non-union tissue and bone, samples were processed within two hours. The resident cells were retrieved following 4-hour enzymatic digestion at 37°C with 0.25% collagenase (Life Technologies). The contents were then filtered through a cell strainer and transferred into 50 ml centrifuge tubes, thereafter they were washed twice with PBS for collection of any remaining cells. The collected suspension was then centrifuged for 5 minutes at 1800 rpm and the cells were frozen using a standardised technique.

5.3.6 Freezing procedure

Cells were required to be kept frozen prior to experiments. For freezing the MSCs, a freezing medium was used, composed of DMSO (dimethylsulfoxide) (10%), DMEM

(45%) and FCS (45%). Before initiating the freeze procedure the freezing medium was mixed and stored at 2°C to 8°C until use. Following the collection and processing of the cells (either through enzymatic release from tissue or after being trypsinised for cultured cells), the collected suspension was centrifuged for 5 minutes at 1800 rpm. The cell pellet was then resuspended in cold freezing medium and dispensed into previously labelled cryogenic storage vials. The cells were then frozen in a controlled rate freezing apparatus (CoolCell LX®, BioCision), decreasing the temperature approximately 1°C per minute and stored at –80°C. Finally, frozen cells were transferred to -150°C, where they were stored for future use.

5.3.7 Thaw procedure – Cell resuscitation

For thawing the samples, the vials containing MSCs were first placed in 37°C water bath until the sides were thawed but the centre remained frozen. The partially frozen cells were then transferred to a 15 ml centrifuge tube and 10 ml of warm PBS (37°C) was added. The suspension was then centrifuged for 5 minutes at 1800 rpm to wash the remaining DMSO and the resulting cell pellet was then resuspended in warm culture media (37°C).

5.3.8 Establishment of the MSCs cultures

Cells were placed into 25 cm^2 flasks in 5 ml standard tissue culture medium. Culture flasks were placed in the incubator set at 37°C, 95% humidified atmosphere and 5% CO_2. The cells were left overnight to allow adherent cell attachment. Non-adherent cells were removed by decanting the medium, washing with PBS and cultures were maintained in standard culture medium that was subsequently changed every three days. When the adherent cell population had reached approximately 80% confluency, the cells were trypsinised and passaged as 1:2 splits in 25 cm^2 flasks until passage 3 (P3). At P3 they were frozen in -150ºC for further use.

5.3.9 Trypsinisation technique

For removing the adherent cells from the culture surface, the culture medium was first removed from the culture flasks which were then washed with 5 ml of PBS, twice, to remove all traces of serum. Subsequently, 5 ml of Trypsin / EDTA solution (0.05% / 0.02%) were added in the culture flasks and placed in the incubator set at 37°C, 95% humidified atmosphere and 5% CO_2, for 2 minutes. Then, 5 ml of standard culture medium was added to the cell suspension as soon as possible to inhibit

further tryptic activity which may damage cells. The culture flasks were checked under the microscope to ensure no cells were still adherent on the culture surface. For cell isolation, the collected suspension was centrifuged for 5 minutes at 1800 rpm.

5.3.10 Cell counting

The determination of cell concentration in a medium was performed by manually enumeration in a haemocytometer. More specifically, following trypsinisation, the collected suspension was centrifuged for 5 minutes at 1800 rpm. The centrifugation solution and pellet were obtained, and the supernatant was tipped off. An additional 230 μl of standard culture medium was added to the remaining liquid and pellet (average residual volume of 270 μl), resulting to a total volume of 0.5 ml. Care was then taken to ensure the pellet was fully dissolved in the medium and the tube was kept in ice to ensure best possible survival of the remaining cells. Subsequently, 10 μl of the cell suspension was mixed with 10 μl of viability die (trypan blue; 1:1 dilution) and a haemocytometer (Neubauer®) was used to count the cells (**Figure 5.2**).

Cell count formula (cells/ml):

$$(\boldsymbol{N\ in\ 4\ squares} \times \mathbf{10^4} \times \mathbf{2}\ (dilution\ factor\ for\ Trypan\ Blue) \times \mathbf{0.5}\ (volume\ of\ cell\ suspention) \\ /\ \mathbf{4}$$

Where N=Number of alive cells counted

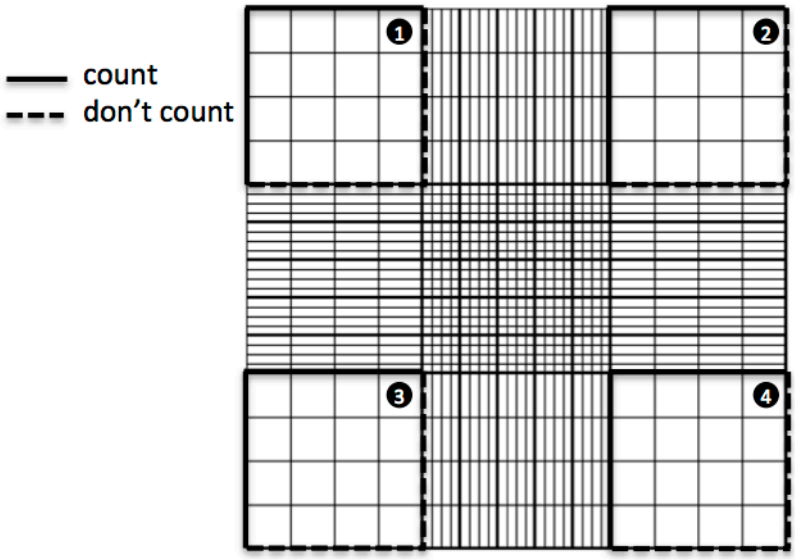


Figure 5.2 Haemocytometer

Cells in the four big squares were counted (1 to 4)
Figure adapted from https://www.hemocytometer.org/hemocytometer-protocol/

5.3.11 Cell proliferation XTT assay

For the determination of cellular proliferation, viability and activation, a non-radioactive colorimetric assay (XTT based) was used (Roche Diagnostics, #11465015001). XTT is only absorbed by viable cells, therefore correlating directly to the number of metabolically active cells in the culture. Cells were grown in a 96 well tissue culture plate (1,000 cells / well – cultured for 7 days; 4,000 / well cultured for 72 h), in triplicates. Feeding of the cells was every 72 h, where half the volume of medium was removed and replaced with fresh medium.

After completion of the cultures, the cells were then incubated with the XTT solution for 4 h at 37°C. Following the incubation period, the resulting formazan solution was spectrophotometrically quantified using an ELISA reader at 450–500 nm with a reference wavelength at 650 nm.

5.3.12 Cell proliferation CFU-F assay

The CFU-F (Colony Forming Units – Fibroblast) assay was performed to enumerate MSCs volumetrically. Using methods described in previous sections, 1,000 cells were seeded into 6-well plates and were incubated at 37ºC, 5% CO_2. After 24 h original culture medium and non-adherent cells were removed and fresh media was added. Subsequently, half media changes were performed every 72 h. At the end of the culture period (7 days), adherent cells were washed with PBS and fixed with 1 ml of 1% paraformaldehyde (Aldrich, #533998) for 15 minutes. Colonies were stained with 0.5 ml Crystal Violet (1% in water, BD Lab Supplies, #C142555) for two minutes; a colony was defined as a tight macroscopically-visible cluster of five or more cells with a defined focal origin (clusters of less than five cells and diffuse patches of single cells were excluded). Two people (MP and IP) counted the colonies and an average of the values was taken. In cases of variation of >20% a third count was performed.

5.3.13 *In vitro* osteogenic differentiation

Osteogenic differentiation was assessed with calcium and ALP assays.

5.3.13.1 Calcium colorimetric assays

For calcium assays, 10,000 cells were seeded in 96-well plates, in triplicates. After 24 h original culture medium and non-adherent cells were removed and fresh osteogenic media was added (Gibco, StemPro® Osteocyte / Chondrocyte Differentiation Basal Medium, #A10069-01; Gibco, StemPro® Osteogenesis Supplement, #A10066-01). Subsequently, half media changes were performed every 72 h. At 21 days, the osteogenic medium was removed and cells were washed with PBS twice. Then 50 μl of 0.5N HCl was added and cells were left for 5 minutes at room temperature. Using a pastette, the cells were gently scraped off the surface of the plates. The resulting solution was then transferred to 1.5 ml Eppendorf tubes and was mixed for 4 h at 4°C using a rotator. Using the calcium colorimetric assay kit (Sigma-Aldrich, #MAK022-1KT), the calcium ion concentration was determined.

5.3.13.2 ALP activity assays

For the determination of ALP activity, osteogenic cultures in 24-well plates using the same methods described in previous sections, were terminated on day 10. All samples were analysed in duplicates. The media was then aspirated from the wells and plates washed with PBS. A lysing solution composed of 150 μl of 0.5% Triton X100 was added for 30 minutes and subsequently adherent cell layers were scraped with pipette tip. During this time plates were kept in ice and mixed in a horizontal shaker. The resulting mixture was collected into 1.5 ml Eppendorf tubes. Using the ALP colorimetric assay kit (Temecula, #CA 92590), the ALP concentration was determined.

5.3.14 Gene expression

To investigate potential differences in osteogenesis of the different MSCs groups, a pathway-focused gene expression analysis was used (Qiagen, RT² Profiler™ PCR Array Human Osteogenesis – #330231). More specifically, samples were sent to Qiagen where mature RNA was isolated using an RNA extraction kit, RNA quality was determined using a spectrophotometer (**Table 5.1**) and was reverse transcribed using a cDNA conversion kit and cDNA was used on the real-time RT^2 Profiler PCR Array (QIAGEN, Cat. no. PAHS-026Z) in combination with RT^2 SYBR® Green qPCR Mastermix (Cat. no. 330529). A table of CT values was produced and used for further analysis. A list with the gene table used is found in **Appendix G**. Additionally, the

subnetwork around the identified genes was also examined using the protein-protein interaction (PPI) database STRING (https://string-db.org/) (534).

Table 5.1 Quality, concentration and integrity of samples

RIN Value	RNA Quality	Results	Recommendation
> 7	Good	High quality profiling results	Use
5 - 7	Medium	Can give high quality profiling results	Consider if sample is similar to other samples in experiment. Possibly replace samples.
< 7	Poor	RNA quality may interfere with the profiling results	Consider to replace sample

5.3.15 Statistics

Statistical analysis was undertaken using the computing environment R (R version 3.6.0; R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.). Data from assays and mRNA analysis were analysed for differences using a two-tailed paired t-test. The Benjamini Hochberg procedure was used to account for multiple testing. A mixed linear model (random intercepts at patient and plate levels) was also used to check the effect of origin of MSCs and time point of osteogenesis, also calculating the patient variance. For analysing gene expression data from RT^2 Profiler PCR Arrays, the relevant online software was used (535). A p-value < 0.05 was considered significant.

5.4 Results

5.4.1 Patients

A total of ten patients (8 males; age: mean 46.2 y.o., SD 12.1 years, median 48.9 years) were recruited for this study. The average duration of non-union was 18.7 months (SD 12.3 months, median 14.9 months) (**Table 5.2**). None of the patients had any obvious risk factors that would increase the risk of developing non-union, such as smoking, diabetes, osteoporosis, presence of metabolic syndromes or long term use of steroids.

Table 5.2 Demographics and fracture characteristics of eligible patients.

Patient	Gender	DOB	Age at time of samples (years)	Duration of non-union (months)	Mechanism of Injury	Site	Side	AO Classification
MP001	Male	08/07/1983	31.2	22.6	RTC - Pedestrian Versus Car	Tibia	Right	41-A3
MP003	Female	02/08/1957	57.5	25.1	Fell from standing height*	Tibia	Left	41-A2
MP004	Male	16/08/1975	39.5	32.1	RTC - Motorcycle Versus Car	Femur	Right	32-C1.1
MP005	Male	12/04/1973	41.8	6.7	Fell from standing height*	Femur	Left	32-C3.1
MP006	Male	27/04/1959	55.8	8.7	Fell from standing height*	Femur	Left	32-C3.1
MP007	Male	08/04/1961	53.9	44.1	RTC - Bike Versus Car	Femur	Left	32-B2
MP010	Male	02/11/1954	60.5	9.3	Polytrauma - Motorcycle	Femur	Right	33-C3
MP011	Male	16/11/1962	52.5	8.5	RTC - Motorcycle Versus Car	Tibia	Right	41-C3
MP012	Female	01/03/1970	45.2	17.1	Fell from standing height*	Tibia	Left	43-A3
MP014	Male	05/08/1992	23.8	12.8	RTC - Motorcycle Versus Car	Femur	Left	32-B3

DOB: Date Of Birth

* Fell from standing height: this mechanism of injury involved falls with direct injury to the limb or twisting injury resulting to a fracture. This was however not related to fragility or pathological fractures.

5.4.2 Establishment of the MSCs cultures

After all samples were cultured, MSCs reached P3 in comparable times. The microscopic appearance of the MSCs was also similar and at no point it was indicated that these cells were close to apoptosis or arrest of proliferation.

5.4.3 Cell proliferation

All of the samples demonstrated satisfactory cell proliferation, both at 3 days and 7 days of culture (4,000 cells and 1,000 cells respectively) (**Figure 5.3**). The variance between the triplicates was low, confirming the validity / reliability of the results.

When comparing the MSCs isolated from bone to those isolated from non-union tissue, there was no difference between the two groups when medium containing patient's own serum was used (**Table 5.3**). Conversely, when medium containing commercial serum was used, MSCs isolated from bone had superior proliferation rate at 3 days following culture ($p=0.016$); this was not the case at the 7 days culture point ($p=0.407$). With regards to the comparison of the proliferation of MSCs cultured in medium containing patient's own serum, this was superior than the medium containing commercial serum, both in MSCs isolated from bone and MSCs isolated from non-union tissue ($p<0.001$ and $p=0.006$ respectively).

Using a mixed-effects linear model, the formula predicting the amount of XTT from the MSCs was calculated as:

XTT =	1.318		
	- 0.115	if non-union	(p=0.440; 95% CI: -0.408 to 0.178)
	+ 0.864	if own serum	(p=0.000; 95% CI: 0.571 to 1.157)
	+ 0.086	if 3 days	(p=0.565; 95% CI: -0.207 to 0.379)
	+ error due to patient*		(Variance 0.000; Std. Error: 0.000)
	+ error due to plate		(Variance 0.331; Std. Error: 0.031)

* Patient did not appear to contribute to overall variation

Table 5.3 Cell proliferation assays

	3 days of culture (4,000 cells)	7 days of culture (1,000 cells)
Comparison	**p-value**	
Non-union Vs Bone (own serum)	0.242	0.448
Non-union Vs Bone (commercial serum)	**0.016**	0.407
Own Serum Vs Commercial Serum (non-union)	**0.001**	**0.016**
Own Serum Vs Commercial Serum (bone)	**<0.001**	**0.006**

The statistically significant results ($p<0.05$) are presented in bold font

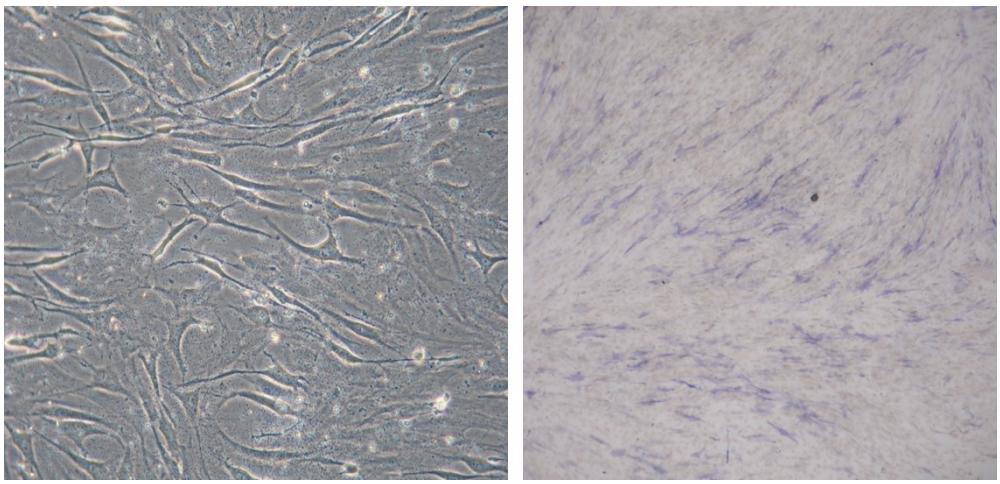


Figure 5.3 Morphology and osteogenic differentiation of MSCs

A. Morphology of MSCs at P3; B. ALP staining following osteogenic differentiation

5.4.4 CFU-F assay

When counting the colonies for CFU-F assay, there was no statistical difference between the different groups (**Table 5.4**).

Using a mixed-effects linear model, the formula predicting the amount of CFU-F values was calculated as:

CFU-F = 28.07 - 0.115 if non-union (p=0.440; 95% CI: -0.408 to 0.178)
+ 1.38 if own serum (p=0.891; 95% CI: -18.77 to 21.53)
+ error due to patient * (Variance 0.000; Std. Error: 0.000)
+ error due to plate (Variance 562.37; Std. Error: 119.90)

* Patient did not appear to contribute to overall variation

Table 5.4 CFU-F assays

Comparison	p-value
Non-union Vs Bone (own serum)	0.899
Non-union Vs Bone (commercial serum)	0.846
Own Serum Vs Commercial Serum (non-union)	0.179
Own Serum Vs Commercial Serum (bone)	0.418

5.4.5 *In vitro* osteogenic differentiation

The osteogenic differentiation of the MSCs was comparable in the MSCs isolated from bone and those isolated from non-union tissue.

5.4.5.1 Calcium colorimetric assays

There was no statistical difference in the amount of calcium produced at 21 days of culture, in MSCs isolated from bone and those isolated from non-union tissue (p=0.446).

Using a mixed linear model, the formula predicting the amount of Ca produced by the MSCs was calculated as:

Ca = 1.198 - 0.079 if non-union (p=0.153; 95% CI: -0.188 to 0.030)
+ error due to patient (Variance 0.061; Std. Error: 0.000)
+ error due to plate (Variance 0.091; Std. Error: 0.012)

5.4.5.2 ALP assays

The ALP activity in the MSCs cultures at 10 days of culture was comparable in those isolated from bone and those isolated from non-union tissue (p=0.963).

Using a mixed-effects linear model, the formula predicting the amount of ALP released by MSCs was calculated as:

ALP = 1.651	+ 0.023	if non-union	(p=0.952; 95% CI: -0.743 to 0.789)
	+ error due to patient*		(Variance 0.000; Std. Error: 0.000)
	+ error due to plate		(Variance 2.961; Std. Error: 0.474)

* Patient did not appear to contribute to overall variation

5.4.6 Gene expression

5.4.6.1 Quality, concentration and integrity of the samples

The total RNA yields for all samples were calculated as per **Table 5.5**. The majority of the samples had a RIN value of > 7 (i.e. good RNA quality, high quality profiling results). Some of the samples had a RIN value of 5 – 7 (i.e. medium RNA quality, can give high quality profiling results). None of the samples had a RIN value of < 5 (i.e. poor RNA quality).

Table 5.5 Samples quality, RNA concentration and integrity

Sample	Sample ID	Conc. (ng/µl)	A260	A280	260 /280	260 /230	Yield (µg)	RIN	Sample	Sample ID	Conc. (ng/µl)	A260	A280	260 /280	260 /230	Yield (µg)	RIN
1	MP001 NU	795.6	19.89	9.85	2.02	2.04	11.14	9.9	21	MP001 NU Osteo	161.4	4.04	1.99	2.02	1.1	2.26	6.3
2	MP001 B	353.2	8.83	4.46	1.98	1.78	4.94	9.6	22	MP001 B Osteo	180.5	4.51	2.22	2.03	0.76	2.53	6.5
3	MP003 NU	234.4	5.86	2.9	2.02	1.98	3.28	9.7	23	MP003 NU Osteo	84.72	2.12	0.98	2.16	0.25	1.19	7.8
4	MP003 B	85.5	2.14	1.09	1.97	1.69	1.2	9.8	24	MP003 B Osteo	19.63	0.49	0.21	2.34	0.23	0.27	5.5
5	MP004 NU	350.6	8.77	4.4	1.99	1.34	4.91	9.5	25	MP004 NU Osteo	299.8	7.49	3.73	2.01	1.75	4.2	7.2
6	MP004 B	215.6	5.39	2.66	2.03	1.91	3.02	9.7	26	MP004 B Osteo	246.7	6.17	3.05	2.02	1.86	3.45	7.5
7	MP005 NU	713.8	17.85	8.66	2.06	1.52	9.99	10	27	MP005 NU Osteo	133	3.33	1.62	2.05	0.74	1.86	6.2
8	MP005 B	194.1	4.85	2.4	2.02	1.48	2.72	9.7	28	MP005 B Osteo	86.06	2.15	1.05	2.04	0.23	1.2	7
9	MP006 NU	418.5	10.46	5.31	1.97	1.75	5.86	10	29	MP006 NU Osteo	735	18.38	9.08	2.02	2.12	10.29	5.6
10	MP006 B	287.7	7.19	3.55	2.03	1.56	4.03	9.6	30	MP006 B Osteo	584.4	14.61	8.56	1.71	1.8	8.18	6.4
11	MP007 NU	296.5	7.41	3.67	2.02	1.85	4.15	9.6	31	MP007 NU Osteo	353.8	8.85	4.43	2	1.45	4.95	6
12	MP007 B	450	11.25	5.84	1.93	1.41	6.3	9.4	32	MP007 B Osteo	577.4	14.44	7.53	1.92	2.15	8.08	5.8
13	MP010 NU	380	9.5	4.86	1.95	1.06	5.32	9.6	33	MP010 NU Osteo	657.4	16.44	8.33	1.97	2.19	9.2	6.8
14	MP010 B	501.1	12.53	6.72	1.86	1.29	7.02	9.6	34	MP010 B Osteo	593.7	14.84	7.3	2.03	2.09	8.31	6.7
15	MP011 NU	436.9	10.92	5.65	1.93	1.15	6.12	9.7	35	MP011 NU Osteo	620.3	15.51	7.65	2.03	1.53	8.68	7.4
16	MP011 B	61.63	1.54	0.72	2.14	0.27	0.86	9.9	36	MP011 B Osteo	58.64	1.47	0.71	2.08	0.71	0.82	5.5
17	MP012 NU	582.4	14.56	7.42	1.96	1.26	8.15	10	37	MP012 NU Osteo	638.7	15.97	8.06	1.98	2.14	8.94	7.8
18	MP012 B	103.9	2.6	1.23	2.11	0.77	1.45	9.4	38	MP012 B Osteo	70.13	1.75	0.84	2.08	1.04	0.98	8
19	MP014 NU	341.4	8.53	4.25	2.01	1.62	4.78	9.5	39	MP014 NU Osteo	378.6	9.47	4.75	1.99	1.67	5.3	6.8
20	MP014 B	644.6	16.12	8.24	1.96	1.93	9.02	9.9	40	MP014 B Osteo	850.3	21.26	10.59	2.01	2.14	11.9	7.6

Samples with 'medium' RNA quality are presented in red font. No sample had a 'poor' quality of RNA

5.4.6.2 Gene expression data

5.4.6.2.1 Non-union versus Bone (control) at baseline

The expression of the housekeeping (internal control; **Appendix G**) genes used for the normalisation of the samples is demonstrated in **Figure 5.4**, confirming that there was no significant variance between the sample groups.

A clustergram demonstrating a 'heat map' of the associated genes is found in **Figure 5.5**, whilst **Figure 5.6** and **Figure 5.7** show a scatter plot and a volcano plot of the significant genes ($p<0.05$).

The expression of ICAM1, MMP10 and GLI1 was significantly higher in non-union derived MSCs compared to bone derived MSCs at baseline, even though another four genes were over-expressed more than two-fold, but did not reach statistical significance (COL15A1, FLT1, GDF10, TNF) (**Table 5.5**). On the contrary, the expression of EGF, IGF2, MMP8 and COL14A1 was significantly lower, whilst another seven genes were under-expressed more than two-fold, but did not reach statistical significance (BMP4, CD36, DLX5, FGFR2, TGFB2, TNFSF11, VCAM1). When the Benjamini Hochberg (BH) correction was applied however, these failed to reach significance. Finally, a power calculation of the samples required to show a significant difference, is demonstrated in **Table 5.6**. Finally, a power calculation of the samples required to show a significant difference, is demonstrated in **Table 5.7**.

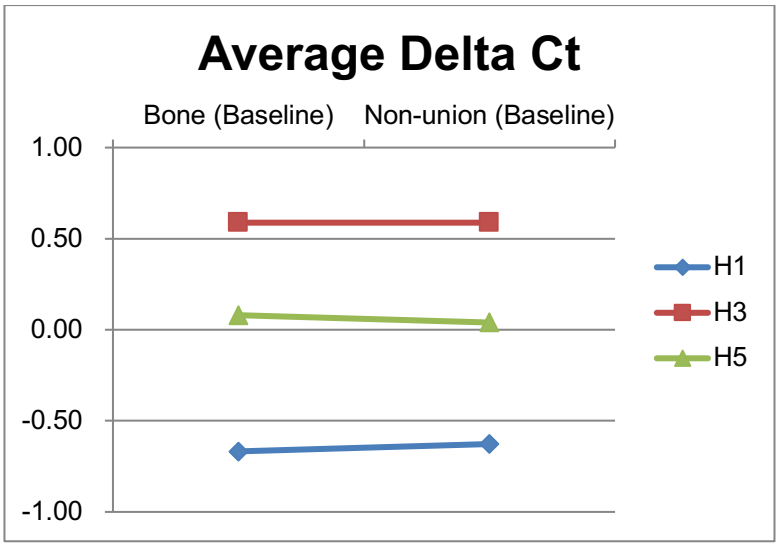


Figure 5.4 Non-union versus Bone (control) at baseline: Expression of housekeeping (internal control) genes used for the normalisation of the samples.

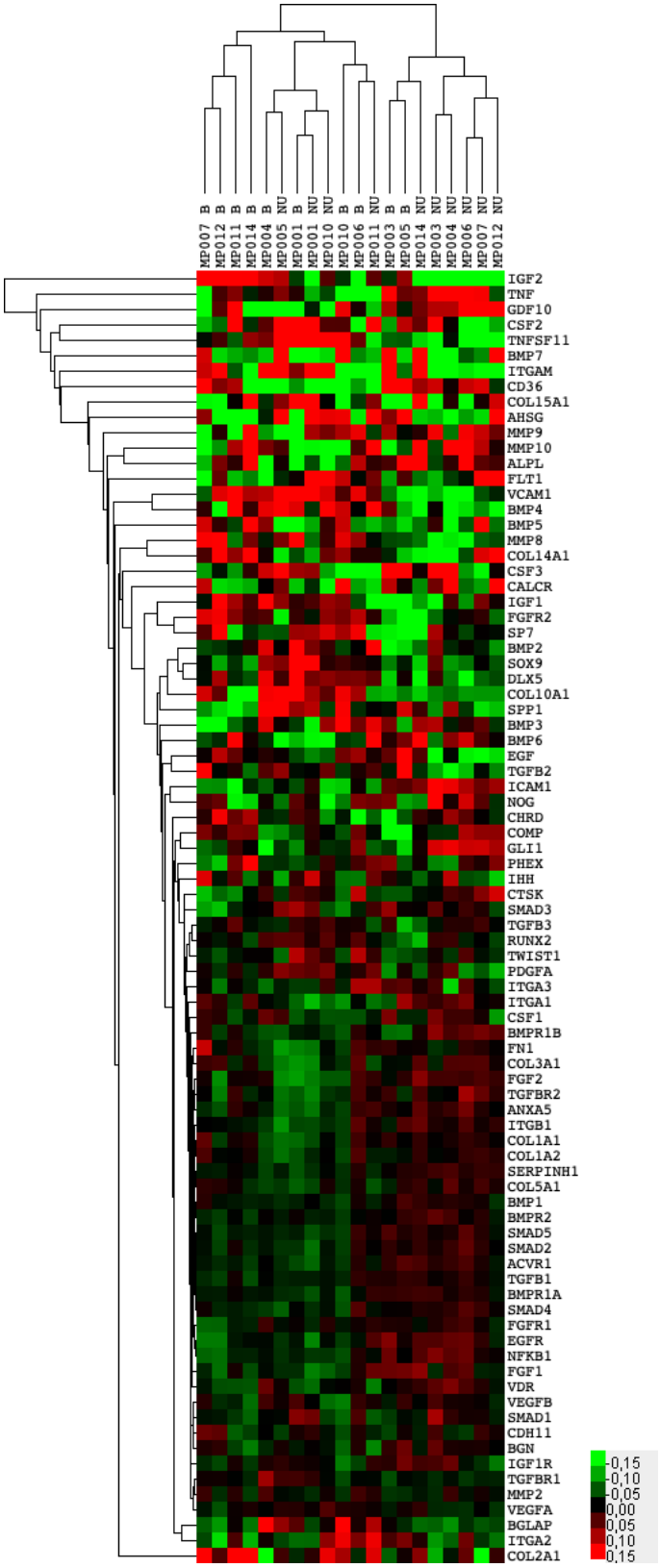
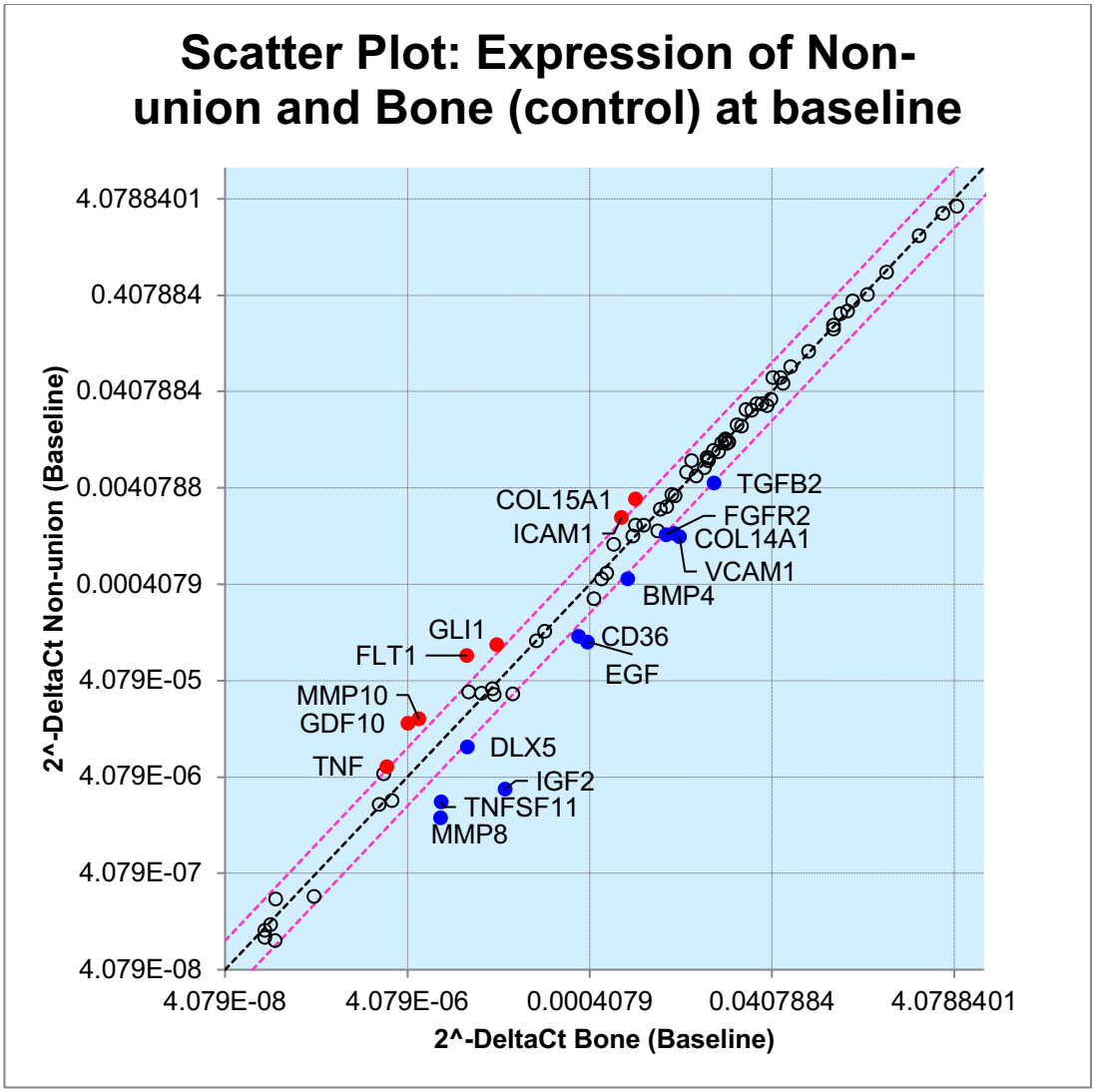


Figure 5.5 Clustergram: Non-union and Bone (control) at baseline

A non-supervised hierarchical clustering of all samples to display a heat map with dendrograms indicating co-regulated genes across groups or individual samples was performed.



- Up-regulated genes (fold difference >2)
- Down-regulated genes (fold difference <-2)
- Unregulated genes (fold difference -2 to 2)

Figure 5.6 Scatter plot comparing the normalised expression of every gene on the array between Non-union and Bone (control) at baseline.

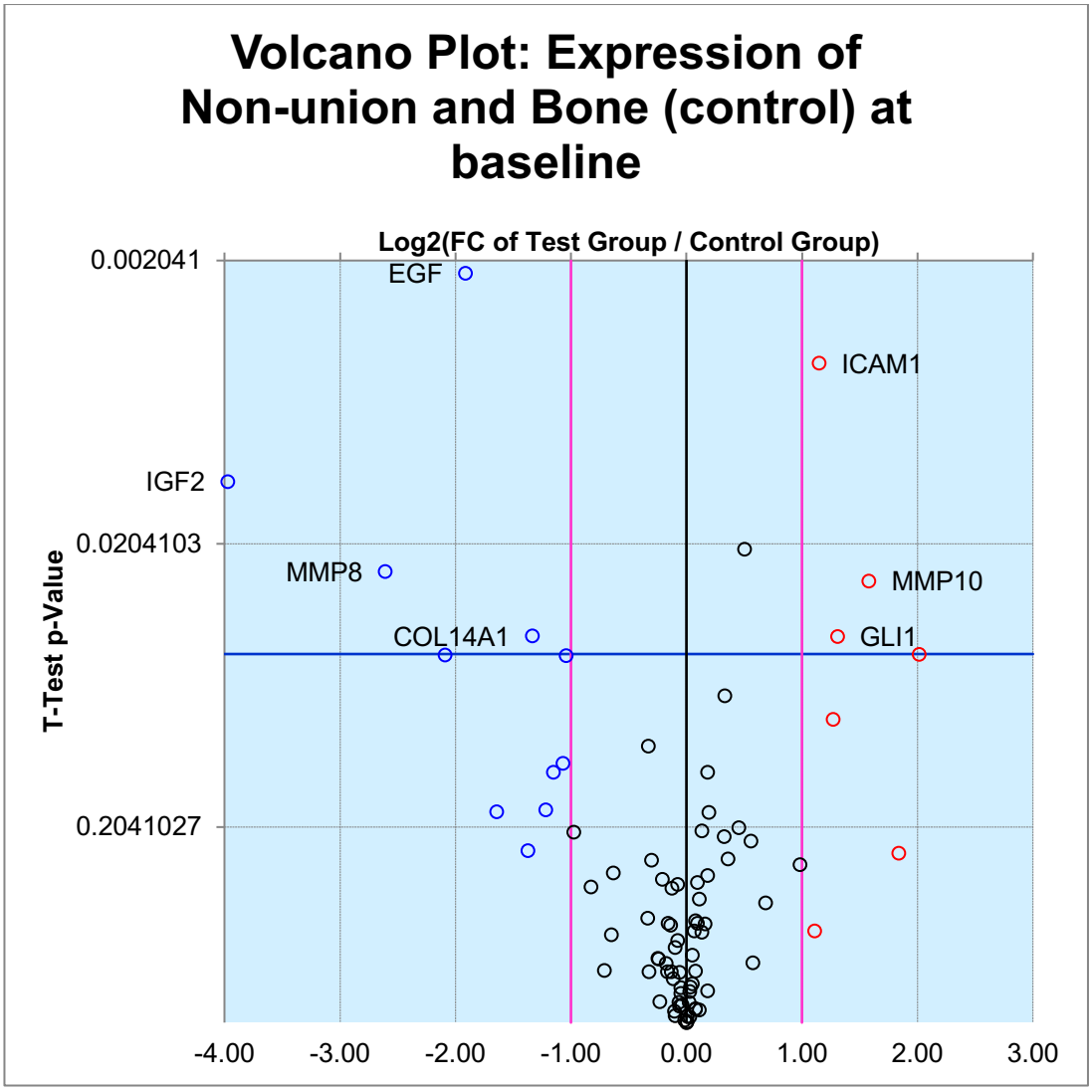


Figure 5.7 Volcano plot demonstrating significant gene expression changes between Non-union and Bone (control) at baseline.

Table 5.6 Fold difference of genes differentially expressed between Non-union and Bone (control) at baseline

Genes Over-Expressed in Non-union Vs Bone (control) at baseline				
Position	**Gene Symbol**	**Fold Difference**	**p-value**	**BH p-value**
D5	FLT1	4.046	0.05	0.388
D7	GDF10	3.574	0.253	0.764
E7	MMP10	2.992	**0.028**	0.386
D8	GLI1	2.474	**0.043**	0.388
B11	COL15A1	2.409	0.085	0.551
D9	ICAM1	2.216	**0.005**	0.198
G6	TNF	2.163	0.475	0.84
Genes Under-Expressed in Non-union Vs Bone (control) at baseline				
Position	**Gene Symbol**	**Fold Difference**	**p-value**	**BH p-value**
D12	IGF2	-15.704	**0.012**	0.345
E9	MMP8	-6.092	**0.026**	0.386
G7	TNFSF11	-4.249	0.05	0.388
C11	EGF	-3.764	**0.002**	0.19
G9	VCAM1	-3.12	0.18	0.761
B6	CD36	-2.593	0.247	0.764
B10	COL14A1	-2.52	**0.043**	0.388
A10	BMP4	-2.326	0.178	0.761
C10	DLX5	-2.226	0.131	0.646
D4	FGFR2	-2.102	0.122	0.646
G2	TGFB2	-2.056	0.051	0.388

* The statistically significant results ($p<0.05$) are presented in bold font

Table 5.7 Power calculation of number of samples per group at p=5%; 80% confidence and p=1%; 95% confidence

Gene ID	PowerSize (N; p=5%; 80% confidence)	PowerSize (N; p=1%; 95% confidence)	Gene ID	PowerSize (N; p=5%; 80% confidence)	PowerSize (N; p=1%; 95% confidence)
IGF2	12	26	BMP7	12694	28809
CSF2	22939	52061	ITGA1	229	518
COMP	99	222	BGLAP	64	142
FLT1	23	50	DLX5	35	78
GDF10	44	97	SERPINH1	39	87
TNF	101	227	PHEX	17289	39239
ICAM1	12	25	BMP3	88	197
IGF1	184	416	SMAD1	911	2067
BMP2	925	2097	ITGB1	1026	2328
CSF3	19710	44733	BMP1	163	368
NOG	1174735	2666229	TWIST1	5521	12529
ITGAM	255	576	COL1A1	565	1281
MMP8	15	31	TGFBR2	132	297
EGF	8	16	ALPL	5847	13270
IHH	167	376	SP7	504	1142
CTSK	43	96	COL1A2	57717	130996
FGFR2	40	88	SMAD5	1238	2809
BMP6	4241	9624	COL2A1	1709	3877
GLI1	12	25	ITGA3	2814	6385
MMP9	70	158	RUNX2	366	829
SMAD3	82	184	CDH11	160	362
TNFSF11	35	79	IGF1R	883	2002
SOX9	512	1160	FGF1	144075408	326999913
CD36	56	126	FGFR1	190	430
MMP10	26	57	EGFR	36	80
BMP5	102	230	NFKB1	21	45
VCAM1	35	78	SMAD2	94	210
TGFB2	23	50	COL3A1	2067	4690
COL14A1	31	69	PDGFA	567	1284
TGFB3	2182	4951	ACVR1	1158	2626
CSF1	355	803	COL5A1	1130	2564
CHRD	95625	217032	TGFBR1	84	189
COL15A1	60	135	MMP2	209	472
COL10A1	57	128	VEGFA	453	1027
SPP1	717	1624	ANXA5	38	85
BMPR1B	17	37	SMAD4	129	291
FN1	51	114	BMPR2	79	178
AHSG	278	629	BMPR1A	125	282
CALCR	5197	11793	VDR	302	683
VEGFB	183	413	ITGA2	1159	2628
BMP4	48	108	BGN	693	1571
FGF2	88	197	TGFB1	302	684

N: Number of samples per group

5.4.6.2.2 Non-union versus Bone (control), following osteogenic stimulation

The expression of the housekeeping (internal control) genes used for the normalisation of the samples is demonstrated in **Figure 5.8**, confirming that there was no significant variance between the sample groups.

A clustergram demonstrating a 'heat map' of the associated genes is found in **Figure 5.9**, whilst **Figure 5.10** and **Figure 5.11** show a scatter plot and a volcano plot of the significant genes ($p<0.05$).

The expression of IGF1 and CALCR was higher in non-union derived MSCs compared to bone derived MSCs following osteogenic stimulation (more than two-fold), but did not reach statistical significance (**Table 5.8**). On the contrary, seven genes were under-expressed (IGF2, EGF, FGFR2, AHSG, CSF2, MMP10, MMP9), but only IGF2 and EGF genes reached statistical significance. When the BH correction was applied however, all genes failed to reach significance. Finally, a power calculation of the samples required to show a significant difference, is demonstrated in **Table 5.9**.

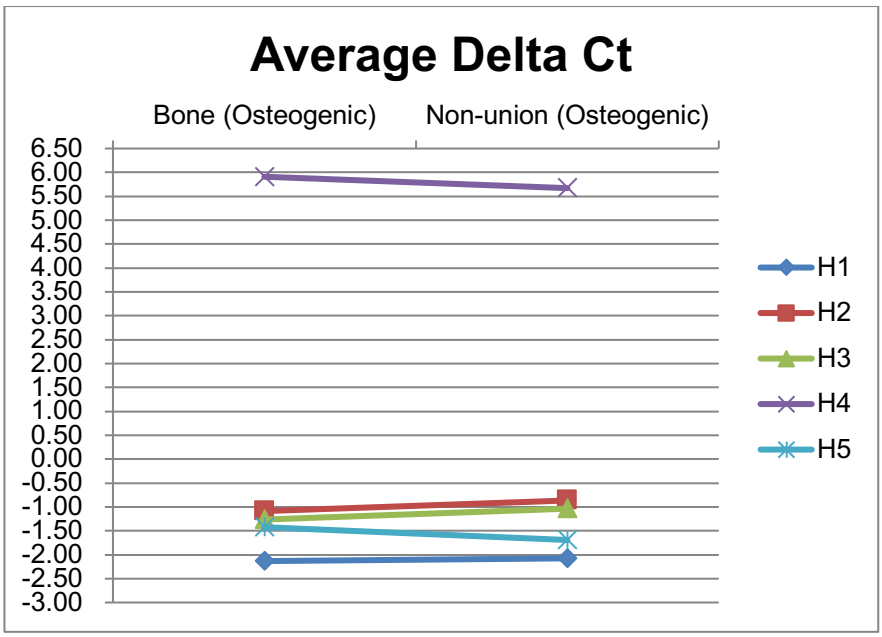


Figure 5.8 Non-union versus Bone (control) following osteogenic stimulation: Expression of housekeeping (internal control) genes used for the normalisation of the samples.

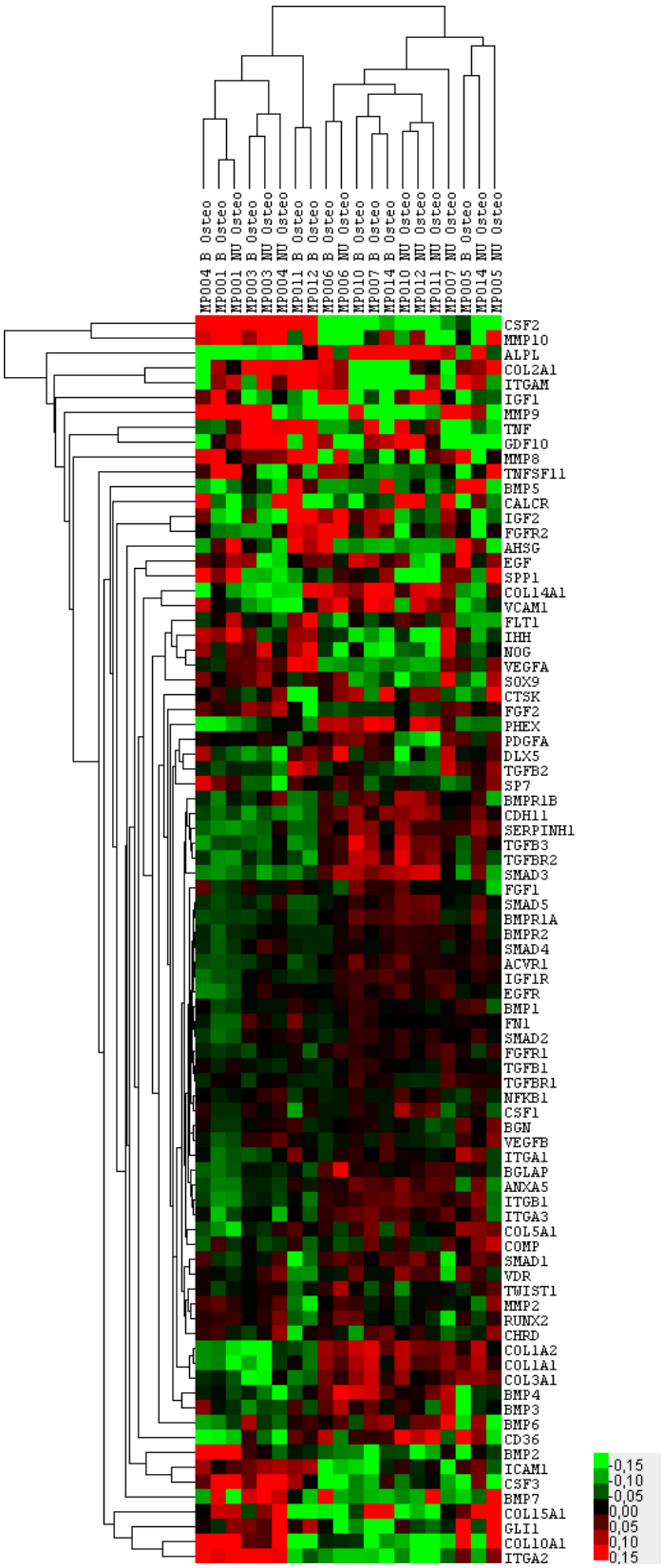
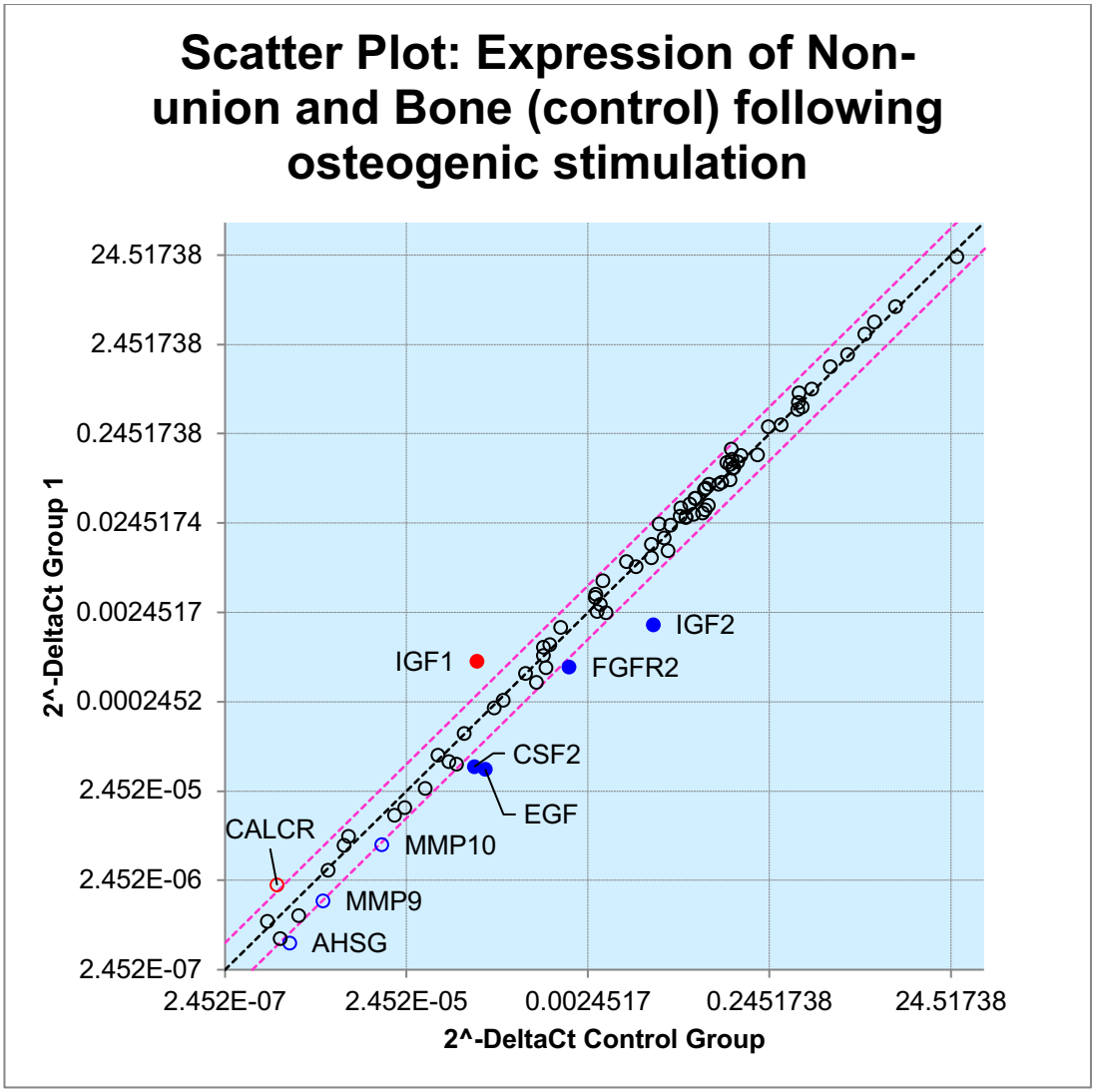


Figure 5.9 Clustergram: Non-union and Bone (control) following osteogenic stimulation

A non-supervised hierarchical clustering of all samples to display a heat map with dendrograms indicating co-regulated genes across groups or individual samples was performed.



- Up-regulated genes (fold difference >2)
- Down-regulated genes (fold difference <-2)
- Unregulated genes (fold difference -2 to 2)

Figure 5.10 Scatter plot comparing the normalised expression of every gene on the array between Non-union and Bone (control) following osteogenic stimulation.

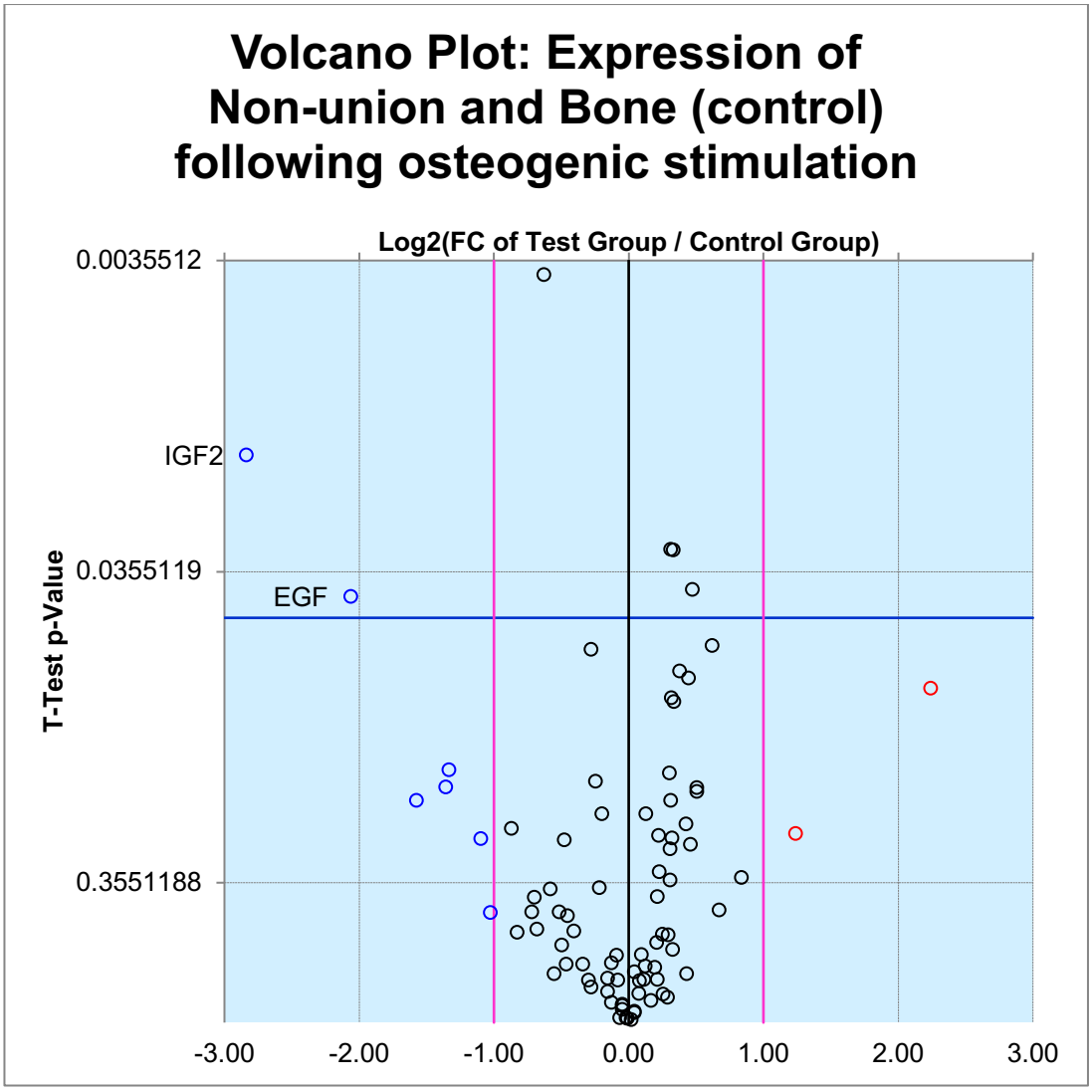


Figure 5.11 Volcano plot demonstrating significant gene expression changes between Non-union and Bone (control) following osteogenic stimulation.

Table 5.8 Fold difference of genes differentially expressed between Non-union and Bone (control) following osteogenic stimulation

Genes Over-Expressed in Non-union and Bone (control) at baseline				
Position	**Gene Symbol**	**Fold Difference**	**p-value**	**BH p-value**
D10	IGF1	4.724	0.084	0.599
B5	CALCR	2.356	0.246	0.723
Genes Under-Expressed in Non-union and Bone (control) at baseline				
Position	**Gene Symbol**	**Fold Difference**	**p-value**	**BH p-value**
D12	IGF2	-7.159	**0.015**	0.598
C11	EGF	-4.181	**0.043**	0.598
C7	CSF2	-2.976	0.193	0.723
A2	AHSG	-2.561	0.175	0.723
D4	FGFR2	-2.52	0.154	0.723
E7	MMP10	-2.143	0.257	0.723
E10	MMP9	-2.037	0.442	0.863

* The statistically significant results ($p<0.05$) are presented in bold font

Table 5.9 Power calculation of Number of samples per group at p=5%; 80% confidence and p=1%; 95% confidence

Gene ID	PowerSize (N; p=5%; 80% confidence)	PowerSize (N; p=1%; 95% confidence)	Gene ID	PowerSize (N; p=5%; 80% confidence)	PowerSize (N; p=1%; 95% confidence)
IGF2	12	24	BMP7	1196	2713
CSF2	34	75	ITGA1	324	732
COMP	18	38	BGLAP	36	80
FLT1	123	277	DLX5	120	271
GDF10	40	89	SERPINH1	78	176
TNF	59	132	PHEX	114	256
ICAM1	50	112	BMP3	635	1439
IGF1	176	398	SMAD1	2080	4718
BMP2	148	334	ITGB1	264	598
CSF3	95	213	BMP1	148	333
NOG	60	135	TWIST1	545	1234
ITGAM	175	395	COL1A1	3092	7015
MMP8	209	472	TGFBR2	7121	16161
EGF	129	290	ALPL	1401	3179
IHH	206	465	SP7	493	1117
CTSK	310	701	COL1A2	3267	7413
FGFR2	11	23	SMAD5	260	589
BMP6	33	72	COL2A1	1676	3802
GLI1	107	241	ITGA3	620	1405
MMP9	521	1181	RUNX2	448	1014
SMAD3	162	366	CDH11	527	1194
TNFSF11	32	72	IGF1R	955	2166
SOX9	53	119	FGF1	1134	2573
CD36	212	479	FGFR1	1859	4218
MMP10	98	221	EGFR	34663	78670
BMP5	15	33	NFKB1	15589	35380
VCAM1	630	1427	SMAD2	41532	94261
TGFB2	98	220	COL3A1	7321	16614
COL14A1	124	279	PDGFA	12363	28058
TGFB3	52	116	ACVR1	456	1033
CSF1	944	2141	COL5A1	46567	105689
CHRD	170	384	TGFBR1	68931	156446
COL15A1	131	296	MMP2	157394	357226
COL10A1	64	143	VEGFA	64263	145853
SPP1	334	756	ANXA5	1196	2713
BMPR1B	29	64	SMAD4	324	732
FN1	16	35	BMPR2	36	80
AHSG	282	638	BMPR1A	120	271
CALCR	310	701	VDR	78	176
VEGFB	37	81	ITGA2	114	256
BMP4	17	38	BGN	635	1439
FGF2	35	77	TGFB1	2080	4718

N: Number of samples per group

5.5 Discussion

Bone healing is a process regulated by several distinct biological phenomena, involving a number of cells and mediators. MSCs in particular possess vast proliferative potential, whilst retaining the capacity of self-renewal and differentiation to different cell populations. By further investigating the pathways involved in bone healing, as well as those involved in non-unions, this clinical complication may be reversed or the treating team could even predict the high risk patients and individualise their treatment. Therefore, by analysing the characteristics and properties of the non-union tissue and compare them with that of 'normal' tissue, it was attempted to identify areas that could be targeted in future treatments, adding more information to the current pool of evidence.

5.5.1 Patients

In order to have a homogenous sample with similar characteristics, ten patients with atrophic non-unions of the tibia or the femur were recruited. The choice of the anatomical location of the non-union ensured that similar biomechanical parameters were present (both are long weight bearing bones), whereas by only including atrophic non-unions it was suggested that the aetiology of the non-union was not lack of mechanical stability, but instead a deficiency of the local environment and / or the host. Smoking, diabetes, osteoporosis, presence of metabolic syndromes and long term steroid use were also excluded, as all are known causes of impaired fracture healing (155, 158, 173-176).

Zhou et al. investigated the effects of age in human MSCs in patients aged between 17y.o. to 90 y.o. (536). They suggested that MSCs isolated from older patients had a longer doubling time (1.7-fold; $p=0.002$), contained more apoptotic cells ($p=0.016$) and had a lower osteoblast differentiation potential ($p<0.001$) (536). Other authors reported similar findings and further suggested a poor response of these cells to therapeutic interventions (537-540). For these reasons, the age of our cohort was limited and ranged between 23.8 y.o to 60.5 y.o. (mean 46.2 y.o.) at the time of obtaining the samples (<60.0 y.o. at the time of injury). This reduced the risk of variation because of increasing age of the patients and therefore reduced proliferative and differentiation capacity of the isolated MSCs. Moreover, by obtaining samples of non-union derived MSCs and comparing them with 'normal' MSCs isolated from bone away from the non-union site, from the same patient, the effect of age, co-morbidities, genetic background and other patient's factors was counterweighed.

5.5.2 Establishment of MSCs cultures

Even though one of the exclusion criteria was patients where inadequate number of MSCs was isolated or the cells failed to expand, there was no patient / sample where this was the case. This confirms the correct collection of samples and expansion techniques, and removed potential bias of excluding these patients. It also confirms that no other factors preventing MSCs' proliferation and differentiation were present, such as infection or inappropriate isolation / culturing techniques resulting in cell senescence.

It is well accepted that expansion of MSCs *in vitro* accelerates cell aging (541, 542). Following extended passaging, the proliferation potential decreases (537, 539, 543, 544), the number of senescent cells increases (537, 538), and the osteogenic differentiation potential decreases (545), whilst their morphology also changes (543). Takahara et al. cultured non-union MSCs (pseudarthrosis tissue) and reported minimal decline in their proliferative capacity through at least 10 passages (509), whilst Hofmann et al. used third passage cells for their experiments (494). In view of the above, cells were only expanded to passage 3 (P3) and closely monitored to ensure their shape and properties were optimal (i.e. there was no evidence of change in shape, increased apoptosis or arrest of proliferation).

5.5.3 Cell proliferation assay

Proliferation of the cells was satisfactory in all of the samples. When using autologous serum as the culture medium, there was no difference between non-union and bone derived MSCs. This highlights the fact that MSCs isolated from non-unions have the same proliferative capacity with bone MSCs, when cultured in autologous serum. When however the cells were cultured with commercial serum, the proliferation of the bone derived MSCs was superior at 3 days of culture (4,000 cells) but not at 7 days (1,000 cells). Investigating the literature, the findings involving human non-union cells are inconsistent. Vallim et al. compared the population doubling time of atrophic NU MSCs to that of BM MSCs and osteoblasts cultured with commercial serum (493). Even though the population doubling time was slightly lower in BM MSCs compared to NU MSCs, this did not reach statistical significance, therefore, the authors concluded that there was no difference between the samples (510). In another study, Qu et al. reported no difference in proliferation capacity of non-union and bone derived cells (type of non-union not specified) (493). Similarly Hofmann et al. investigated the proliferation of hypertrophic non-unions and did not identify any significant difference at four weeks of culture; yet, at day 4 the viable cells number

was higher in the bone derived cells (494). Finally, Iwakura et al. reported that the proliferation of hypertrophic non-union MSCs was inferior to that of fracture haematoma's (166).

Furthermore, the results indicate that autologous serum was associated with superior proliferation properties in both non-union and bone MSCs. The concept of using autologous serum for MSCs expansion is not new and has been previously reported by our group (546). Further studies reported that autologous serum is at least as good as FBS of the same concentration (i.e. 10%) (547), whilst others showed a clear superiority of autologous serum without loss of chondrogenic and osteogenic potential (548). Most importantly, in clinical applications on expanded MSCs, autologous serum could be used to ensure no risk of disease transmission or immunologic reactions (548).

5.5.4 CFU-F assay

One of the basic characteristics of MSCs is their ability to produce colonies after being seeded at low densities, relying on the presence of the early progenitor cells in the cell population (539). The CFU-F assays revealed good proliferation of all samples, with no difference between non-union and bone MSCs, regardless of the serum used (commercial versus autologous). This reinforces the fact that both groups have cells of comparable proliferative potential. In the literature, only Seebach et al. performed CFU-F assays, comparing non-union MSCs to those of polytrauma patients and 'healthy' individuals, reporting lower values in the non-union patients (523). The non-union and bone samples obtained however were not from the same patient.

5.5.5 *In vitro* osteogenic differentiation

The results of the calcium colorimetric assays revealed no significant difference between non-union and bone MSCs. Similarly, Vallim et al. performed mineralisation assays reporting that NU MSCs deposited mineralised matrix in a similar manner to BM MSCs and osteoblasts (510). On the other hand, Wang et al. (511), Bajada et al. (492) and Hofmann et al. (494) reported inferior osteogenic differentiation of the non-union MSCs compared to controls. Of note, Iawkura et al. suggested that in MSCs isolated from hypertrophic non-unions, mineralisation was superior than that of fracture haematoma (166). Of all of these studies, only that of Vallim et al. analysed samples taken from the same patient, whilst the rest included comparisons with 'healthy' individuals (510). Therefore, one could assume that cells isolated from the

same patient (non-union versus bone or bone marrow) have similar mineralisation potential.

As per the calcium colorimetric assays, ALP assays did not show any difference between non-union and bone MSCs. These findings are in line with previous reports by Qu et al. (493) and Hofmann et al. (494), but in contrast to the findings of Bajada et al. who suggested that the ALP activity of the NU MSCs was lower than that of BM MSCs (492). Iwakura et al. on the other hand reported superior ALP activity of the NM MSCs compared to that of fracture haematoma (166).

Contrary to these findings, in an animal study published by Tawonsawatruk et al., it was observed that non-union derived MSCs were not able to form colonies or differentiate under adipogenic conditions, even though they could differentiate under osteogenic conditions (549). They therefore suggested that these cells had characteristics of osteochondral progenitor cells, with the microenvironment in the atrophic non-union site possibly being responsible for their altered behaviour (549).

5.5.6 Gene expression

Recently, there has been an increasing interest in the patient's genome and how this is related to several pathological processes, including that of impaired bone healing. Several polymorphisms have therefore been associated with non-unions, but a paucity of strong evidence to support this still exists (170, 217, 516, 518-521). Even though some authors attempted to identify differences between the expression of the non-union cells compared to BM and bone MSCs (166, 491, 494, 499, 507, 511), this has not generated much attention.

From the results of the proliferation and osteogenic differentiation assays, there was no evidence to support any superiority of MSCs isolated from bone compared to those isolated from the non-union tissue. Therefore, it was hypothesised that local factors may alter the expression of key genes and proteins in the non-union tissue. To investigate this hypothesis, the gene expression of a number of proteins involved in the osteogenic pathway was analysed, trying to identify potential differences between non-union and bone MSCs.

5.5.6.1 Non-union versus Bone (control) at baseline

Comparing the samples at baseline, several genes were found to be over- or under-expressed in the non-union MSCs. More specifically, ICAM1, MMP10, GLI1,

COL15A1, FLT1, GDF10 and TNF were found to be over-expressed in non-union MSCs (more than 2-fold), but only the expression of ICAM1, MMP10 and GLI1 was statistically significant. The under-expressed genes in non-union MSCs included EGF, IGF2, MMP8, COL14A1, BMP4, CD36, DLX5, FGFR2, TGFB2, TNFSF11 and VCAM1 (more than 2-fold), with only EGF, IGF2, MMP8 and COL14A1 being statistically significant.

5.5.6.1.1 Role / action of involved genes

ICAM-1 is a protein that mediates cellular interactions by binding to leukocyte adhesion protein LFA-1 (lymphocyte function-associated antigen - 1) and Mac-1 (550), being upregulated in inflammatory microenvironments (551). Additionally, ICAM-1 has been reported to regulate bone remodelling by promoting osteoclastogenesis (552) and inhibiting the osteogenic differentiation of MSCs (550). In a study by Xu et al., ICAM-1 expression was upregulated in an inflammatory culture environment (presence of inflammatory cytokines) with reduced capacity of MSCs to differentiate into osteoblasts, whilst enhancing proliferation of MSCs (550). When ICAM-1 was knocked down, the osteogenic differentiation recovered (550).

MMP10 (Stromelysin-2) is a protein induced by inflammation, shown to have a profibrinolytic function on several types of gelatines, collagens and to fibronectin (553). It is also involved in bone growth (554, 555), tissue repair (556, 557) and wound healing (558). Combination of MMP-10 with BMP-2, an important cytokine that regulates osteoblast differentiation (553), has also been reported to enhance the repair process and significantly increase mineralisation compared to BMP-2 alone (553), as well as augment the differentiation of myoblastic cells into osteoblastic cells (559). The mechanism of MMP-10 action is not yet known, but Reyes et al. suggested it could be secondary to increased recruitment of osteoprogenitor cells through CXCR4/SDF1 signalling (553).

GLI1 is not only a transcription activator but also a target gene of GLI proteins, thus amplifying the transcriptional response to Hedgehog signalling (560). In animal studies, it has been demonstrated that haploinsufficiency of GLI1 negatively affects bone mass and leads to an uncoupling of bone metabolism, resulting to an impaired bone formation, accelerated bone resorption and reduced fracture healing potential (561). The same team suggested that GLI1 acts as a downstream of Hedgehog signalling promoting osteoblast differentiation, repressing the osteoblast maturation toward osteocytes, therefore maintaining normal bone homeostasis (561). In another

animal study by Shi et al., they discovered that GLI1 marks mesenchymal progenitor cells responsible for both normal bone formation and fracture repair (560). More specifically, during bone fracture healing chondrocyte and osteoblast populations derived from GLI1 positive cells rapidly expand in response to the fracture (560).

EGF is known to stimulate bone repair, increasing proliferation of several cell lines including osteoblast growth and bone formation (562). With regards to MSCs, it stimulates their proliferation, without altering their differentiation process and potential and at the same time promotes their paracrine activity (539, 563). In an animal study, Lee et al. reported that EGF interacts synergistically with BMP-2 accelerating bone formation at the early stages of healing (121), whilst in another animal study, EGF carried by liposomes was associated with faster healing of tooth sockets (562). Moreover, Liu et al. reported that EGF enhanced BMP9-induced early and late osteogenic action MSCs *in vitro*, and significantly augmented BMP9-induced bone formation *in vivo* (564). Interestingly, some authors report an inhibitory action of EGF on proliferation and osteogenic differentiation (565).

IGF2 is a major foetal GH and plays a key role in regulating embryonic development, as well as in post-natal bone development (chondrocyte maturation and perichondral cell differentiation and survival) (566). It also stimulates bone formation by increasing the proliferation of osteoblastic lineage cells and enhancing their differentiation to osteoblasts (567). Gangji et al. also suggested that any inhibition of IGF2 synthesis may result in a decreased collagen synthesis which may in turn result in a decrease in bone matrix (567). In an animal study by Chen et al., it was reported that the relative endogenous expression of IGF2 in MSCs is relatively low, but had a positive effect on BMP-9 induced ectopic bone formation, as well as the endochondral ossification (568). A potential mechanism of IGF2 action could be through co-ordination with TGF-β and local amplification of IGF1 bioavailability through controlled IGFBP-4 proteolysis, which translates to an increased bone formation (569).

MMP-8 is mainly secreted from neutrophils and in lower amounts by fibroblasts, chondrocytes, endothelial cells, smooth muscle cells and mononuclear phagocytes (570-572). MMP-8 is more active against type I and III collagens and less in type II collagen (571). Its expression is upregulated by low doses of IL-1β (interleukin - 1β) and downregulated by TGF-β1 (573, 574). Dejonckheere et al. reported that it plays a role in the development of an inflammatory response, but also has an anti-inflammatory action during recovery which may be crucial for normal healing (575).

Previous studies also suggest that it is the predominant collagenase in healing wounds, whilst its overexpression has been associated with impaired wound healing (576, 577). Additionally, in an MMP-8 deficient mice wound healing was also delayed, probably because of MMP-8 acting by contributing to the resolution of inflammation (578). In other animal study by Itagaki et al., MMP-8 was found in osteoblasts and osteocytes expressing collagen type I in early stages of bone healing, suggesting that MMP8 plays a role in the remodelling of the extra-cellular matrix during the healing of defective bone (579). In a study on human non-union tissue, MMP-8 serum levels were significantly elevated in the non-unions compared to patients with uneventful healing (96).

Not a lot of research exists around COL14A1. It has been previously reported to be expressed by the end of embryonic development in every collagen I-containing tissue, including skeletal muscle and periosteum (580), and generally in areas of high mechanical stresses, therefore affecting the mechanical properties of these tissues (581). In a COL14A1 knockout mouse, Ansorge et al. implicate COL14A1 with the regulation of fibrillogenesis and further suggest that it functions in the integration of fibrils into fibres (582). Moreover, Minarikova et al. previously reported an increase in its expression during alveolar bone formation (583).

5.5.6.1.2 Potential pathway

The interactions between the identified genes by our study are demonstrated in **Figure 5.12**. Apart from COL14A1, there seems to be a link between all the rest of the proteins. The role of COL14A1 in bone healing and regeneration remains obscure, therefore interactions with the other proteins may still be present but not well understood. It is important to note that apart from GLI1 which is an intracellular protein, all the rest involve the extracellular component (**Table 5.10**). Additionally, four genes (MMP10, ICAM1, MMP8, COL14A1) are involved in extracellular matrix organisation. Another interesting finding is that three genes are associated with inflammation; the overregulated ICAM1 and MMP10 being induced in inflammatory environments, whilst underregulated MMP8 has an inflammatory action. These findings not only confirm the presence of low grade inflammation at the non-union site, but also raises the question of role of inflammation toward failure of healing.

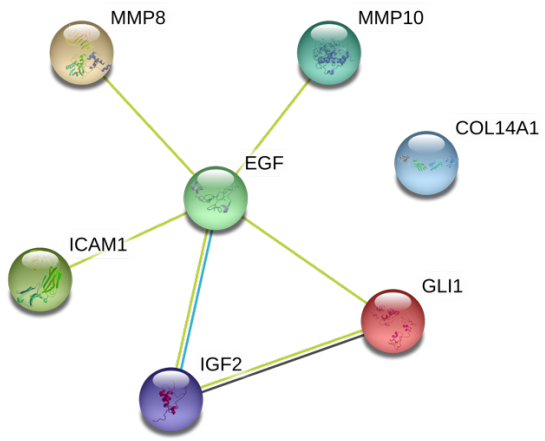


Figure 5.12 Diagram of the network between the significantly different expressed genes (non-union versus bone at baseline)
Diagram produced by https://string-db.org

Table 5.10 Pathways / functions of the isolated genes (non-union versus bone at baseline)

	Functional enrichments of the network	Genes over-expressed	Genes under-expressed
Biological process	Extracellular matrix organisation	MMP10, ICAM1	MMP8, COL14A1
	Regulation of cell migration	MMP10, ICAM1, GLI1	EGF
	Osteoblast differentiation	GLI1	IGF2
	Canonical Wnt signalling pathway	GLI1	EGF
	Regulated exostosis	-	MMP8, EGF, IGF2
	Positive regulation of cell proliferation	GLI1	EGF, IGF2
	Cell surface receptor signalling pathway	ICAM1, GLI1	EGF, IGF2
Cellular component	Cellular component part: extracellular	ICAM1, MMP10	EGF, IGF2, MMP8, COL14A1
	Cellular component part: intracellular	GLI1	-
Reactome pathways	Extracellular matrix organisation	MMP10, ICAM1	MMP8, COL14A1
	Collagen degradation	MMP10	MMP8, COL14A1

Over-expressed genes: ICAM1, MMP10, GLI1
Under-expressed genes: EGF, IGF2, MMP8, COL14A1

5.5.6.2 Non-union versus Bone (control) following osteogenic stimulation

Comparing non-union versus bone MSCs following osteogenic stimulation (i.e. osteoblastic differentiation), only IGF2 and EGF were significantly downregulated in non-union MSCs. These two genes were also downregulated in the baseline comparison of the two groups. As mentioned in previous sections, both are key GF which lead to an increased proliferation of MSCs and at the same time enhance their differentiation to osteoblasts. Their downregulation can therefore be linked to a reduced proliferation and differentiation of the MSCs and therefore inability of the fracture to consolidate. Nonetheless, this only happens at a local level. As described in the previous section, the findings of the comparison of non-union and bone MSCs at baseline suggest that an inflammatory environment may be the cause of their downregulation, a finding which may support that the cause of the non-union may be local and not systemic. In other words, the patients have otherwise 'normal' MSCs which can proliferate and differentiate, but at the site of the non-union their functions are impaired. Continuous attempts for healing and 'aging' of the cells may also contribute to the altered phenotype.

5.6 Strengths

This study has several strengths that increase the validity and weight of the results. Firstly, it involves human cells, in contrast to most of the literature investigating animal derived MSCs, with their results not necessarily being extrapolated to the human genome. Additionally, the inclusion and exclusion criteria were very strict, in an attempt to reduce variation between the samples because of age, comorbidities, history of malignancy, smoking and medication intake, all of which are well known to alter the process of 'physiological' bone healing. To increase the homogeneity of the samples further and account for differences in the local environment and biomechanics of each region, only long weight bearing bone non-unions were selected (tibia and femur), excluding intra-articular fractures, where the process of healing and operative characteristics differ. For the same reason, only atrophic non-unions were selected, as in the case of hypertrophic non-unions lack of mechanical stability is commonly the underlying cause of the non-union. Septic non-unions were also excluded as the inflammatory process caused by the infective process alters the topical environment, is cytotoxic to local cell populations and exerts a negative effect to the proliferation and differentiation of the MSCs.

In addition, the number of recruited patients was higher than most studies in the field. To further reduce the risk of technical errors and variation in processing of the samples and completing the experiments, all were performed by the same person (MP) in accordance to comprehensive and well established SOPs (standard operating procedures). Furthermore, the samples collected were from the same patient (i.e. non-union, bone, bone marrow and serum), to ensure differences reflect in local changes in gene expression instead of variation between the genome of the patients. Further to this, cultures were performed up to P3 to ensure no aging of the population or osteogenic differentiation of the MSCs and therefore reduction of their proliferation capacity. To ensure isolated cells not only had the ability to self-renew and proliferate but could also differentiate, osteogenic differentiation studies were also performed, whilst in an attempt to investigate the effect of origin of serum on proliferation, autologous serum was also used. Regarding the gene expression studies, all of the samples scored well in terms of quality of isolated RNA, while the comparison performed was both at baseline and following the osteogenic stimulation (i.e. osteoblastic differentiation). Adjustment for multiple testing was also performed, to reduce the risk of false positive results in each comparison. Finally, the only funding obtained for this project was to cover the consumables and expenses for gene expression and was received from an independent source, therefore removing any risk of bias often associated with industry funding.

5.7 Limitations

It is recognised that this study had some limitations. Firstly, *in vitro* investigations do not include the interactions of the local environment of the cells, consequently, the conclusions of the herein study cannot be extrapolated to *in vivo* conditions and therefore need further validation. In addition, even though compared to other studies in the literature the number of patients was good, the power analysis revealed that more samples were needed to reach statistical significance. The limitation of the number of samples however was not only because of the high costs of each experiment, but also because of the difficulty in recruiting eligible patients secondary to the strict eligibility criteria selected. Furthermore, it is not known if the peri-operative medication (anaesthetic, analgesics) do have an effect on the harvested tissues / MSCs. Finally, harvested cells were cryopreserved until thawed for the experiments. Even though this meant all the environmental variables were the same for all samples, inadvertently a number of cells was lost because of the freezing / thawing procedure. Nevertheless, the number of MSCs harvested from bone, bone

marrow and non-union tissue was limited and culture was the only way to provide the necessary numbers required for all the experiments.

Chapter 6
Future Work Plan

6.1 Subtrochanteric fractures

The work contained in the thesis sets an important framework for further research around subtrochanteric fractures, as well proximal femoral fractures in general. Firstly, the non-union scoring system developed should be validated in other hospitals, preferably both in the UK and abroad. This would confirm its validity in different patient populations and healthcare systems. A smartphone application could also be developed providing healthcare practitioners not only with a quick calculation of the risk, but also informing clinicians with the relevant up-to-date literature.

Additionally, another 19 patients (19 fractures) were referred to our tertiary referral centre with a diagnosis of a non-union. All of these were very challenging to treat, and most required more than one procedure to achieve union. The characteristics of these patients will be analysed and compared to the existing series, proposing an algorithm on how to manage recalcitrant atrophic non-unions, as well as septic non-unions. This could also guide orthopaedic surgeons managing complex subtrochanteric fractures along with their sequelae, and advice early referral to a tertiary centre in case of presence of significant non-union risk factors.

Many fracture classification systems have been developed to describe subtrochanteric fractures, but these are not always reliable or reproducible, while most have significant interobserver variations. Additionally, none of the existing classification systems can successfully determine treatment or successfully predict outcomes. Using the existing cohort, we will try and develop a new classification system that will not only be based on the fracture configuration, but also correlate to the outcome and more specifically to risk of non-union. This could be achieved by the comparison of the characteristics of the primary and secondary fracture lines in patients developing a non-union with those with uncomplicated healing, and identification of the fracture patterns associated to this risk.

The incidence of post-operative transfusion within the first 48 h was reported as high as 51.5% in our series. Therefore, any attempt to minimise this risk, would not only reduce the risks associated to transfusion, but also enhance recovery. Tranexamic acid has been previously demonstrated to significantly reduce peri-operative

bleeding. Especially in the elderly, in the setting of trauma and the associated coagulopathy related to it, the increased risk of thromboembolic events is an issue of concern. These risks can be minimised by the use of topical tranexamic acid administered in the IM canal following reaming. Designing an RCT could provide us with sufficient evidence regarding its safety and efficacy. If successful, it could be then applied in nailing of other long bones, such as the tibia and the humerus.

It would also be interesting to investigate the validity of the results (degree of comminution, lateral cortex gap size, reduction size) on a biomechanical level. Commercially available synthetic bone could be utilised for this reason and bending / torsional / axial stiffness could be calculated. Going even further, a finite element analysis could be performed to explore if any alterations to the nail design (i.e. an increased proximal nail diameter) carry a positive effect.

Furthermore, comparing subtrochanteric with intertrochanteric fractures also treated with IM nails could help demonstrate the challenges of subtrochanteric fractures, and also investigate whether the two types of fractures have comparable outcomes and complications. This would further guide clinicians and associated health practitioners on how to manage these common injuries.

Finally, using these findings as a guide, a study scrutinising several already established national databases can be performed, attempting to identify risk factors of these complications using large datasets. This will increase the power of the study, whilst at the same time correction for cofounders and multiple testing can be added to the statistical model, thus increase the validity and accuracy of the results.

The findings of this thesis, as well as those of all future projects associated to this, could be used to modify the neck of femur fracture protocol, aiming to the earlier recognition and prevention of these devastating complications. If successful, it could potentially reduce the morbidity and mortality, as well as reduce the costs of managing these complications which would be of great benefit of our already stretched healthcare system.

6.2 Biological Characterisation of Non-union Tissue

Following a comprehensive literature review it becomes apparent that the pathways leading to a non-union are complex and not well understood. Even though this study provides some further insight into the characteristics of the MSCs at the non-union site, further investigations are still required to confirm these findings and establish the possible pathways.

The first step following this study would be to confirm the findings of the gene expression. Using the commercially available colorimetric ELISA assays, the concentrations of MMP-8, MMP-9, MMP-10, ICAM-1, EGF, COL14A1, Dkk-1 and IGF-2 will be determined in in culture supernatants at the eight time points of culture (at baseline and every three days of culture under osteogenic stimulation, up to 21 days of culture). The concentration of the same molecules will also be calculated in the stored patients' own serum. Positive findings could direct our research to developing a scaffold loaded with a blocking antibody or an enhancing molecule depending to the pathway's negative or positive action towards bone healing, or be injected into the non-union site through a percutaneous / minimally invasive approach. If successful, this could revolutionise the treatment of these patients.

Additionally, all the expanded samples were stored and a 'bank of MSCs' is now available (bone, bone marrow, non-union MSCs; patient serum; non-union tissue embedded in OCT). This bank will be used to provide samples for further projects around bone healing and non-unions. Non-union tissue samples will also be histologically processed in order to create 3D models of their structure, including details about their vascular network, presence of different proteins and characteristics of the matrix and cellular composition.

As this project investigated a subset of patients who have no other risk factors for non-union, the same protocol could be repeated investigating patients with a history of diabetes or heavy smokers. This could help to identify any differences in the *in-vitro* activity of the MSCs, both in 'normal' bone and non-union tissue. Potential differences could identify altered pathways of bone healing secondary to the effect of smoking and diabetes.

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Appendix A

Study Documents (Clinical work)

Clinical audit approval

Audit Approved

Clinical Audit Database <caad@leedsth.nhs.uk>

Mon 14/09/2015 09:45

To:Panteli Michalis (LEEDS TEACHING HOSPITALS NHS TRUST) <michalis.panteli@nhs.net>;

Dear Michalis Panteli,

The Audit "Management and Outcomes of Subtrochanteric Fractures: Our Institutional Experience" has been reviewed and approved by Theodoros Tosounidis. You may now carry out the Audit.

Please do not reply to this e-mail. It is from an automated and unmonitored account; your e-mail will not be read or actioned

http://auditaction.leedsth.nhs.uk

Regards,

Clinical Audit Database

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Appendix B
Patients' management

The initial patient management depended on the mechanism of injury (high energy versus low energy injuries), presence of associated injuries (i.e. polytrauma) and medical co-morbidities. More specifically, patients presenting with high energy injuries (e.g. RTCs, fall from high etc.), were initially managed at the emergency department according to the ATLS® principles, by the multidisciplinary trauma team. After the initial resuscitation and correcting any haemodynamic instability, all patients underwent a trauma CT (computed tomography) scan. In those cases where haemodynamic instability could not be reversed, the trauma CT was delayed and patients underwent immediate surgical intervention where deemed necessary. Otherwise, surgery was performed in a planned list, as soon as possible. In case of additional injuries requiring surgical fixation, these were either managed simultaneously or on a planned list before or after the femoral fracture fixation (save life first, then limb), by a surgeon with the appropriate expertise.

In patients with significant blood loss, metabolic and coagulation functions were closely monitored to prevent the lethal triad of hypothermia, acidosis, and coagulopathy, whilst activating the protocol for massive transfusion (584). Massive transfusion was defined as: transfusion of ≥ 10 units of red blood cells (RBC) (equivalent of the total blood volume of an average adult patient) within 24 hours; transfusion of > 4 units of RBC within 1 hour with anticipation of continued need for transfusion; or replacement of > 50% of the total blood volume by blood products within 3 hours (584-586).

Patients presenting with low energy injuries such as fragility fractures, were managed by a multidisciplinary team including orthogeriatricians, orthopaedic surgeons, anaesthetists, nursing and allied health professionals, and according to a standardised hip fracture protocol. Correctable co-morbidities were identified and treated immediately so that surgery was not delayed. Surgery was performed on the day of, or the day after, admission on a planned trauma list, with a consultant or senior orthopaedic surgeon and anaesthetist present in theatres .

Patients presenting with pathological or suspected pathological fractures, were referred to the local oncology team and underwent further investigations including additional imaging investigations as appropriately. Surgery was then performed when

the patient was deemed fit by the anaesthetic team and a post-operative tumour management plan was set in place by the oncology team. Intra-operatively, samples (bone and / or reamings) were send to histology for confirmation of the diagnosis.

B.1. Surgical management

A standardised surgical technique was implemented. The choice of anaesthesia was determined according to the patient's comorbidities and wishes, as well as the anaesthetist's preference. Following administration of pre-operative prophylactic antibiotics according to the departmental protocol, patients were positioned in the supine position on a fracture table, with a perineal post and with the contralateral leg in lithotomy position and secured to the leg holder (**Figure B.1**). Fluoroscopic check was then performed to ensure adequacy of intra-operative imaging (AP and lateral views). Following removal of any hair where appropriate, the leg was prepped with an alcoholic solution (iodine based or chlorhexidine) and a large Ioban™ Drape (3M™) was applied, followed by a shower curtain-type drape.

Closed anatomic reduction was then attempted using simple reduction manoeuvres, including traction, rotation and indirect pressure at the fracture site. If closed reduction was unsuccessful, indirect reduction using external fixator pins or the reduction spoon was attempted. Nonetheless, there was a low threshold for opening the fracture site and attempting direct reduction in order to achieve anatomical reduction.

Open reduction was then performed by first exposing the fracture site through an incision, most commonly through the direct lateral approach. This would then allow traction and rotational adjustment both under direct vision and fluoroscopic guidance. Most importantly, open reduction was attempted with great care in order to minimise the soft-tissue trauma and further injury to the periosteum and bone. Following exposure of the fracture, Hey Groves bone holding forceps, pointy reduction clamps and other reduction instruments were utilised to hold reduction, supplemented by cerclage wire(s) / cable(s) as deemed appropriate.

Regarding the choice of IM device utilised, this depended on the age of the patient, the fracture configuration, associated injuries and the surgeon's preference. Generally, in case of fragility fractures or were the fracture line extended into the femoral neck, a cephalomedullary nail was used. In more distal fracture

configurations and in younger patients with good bone quality, antegrade or retrograde nails were used, with locking mechanisms avoiding the placement of a lag screw into the femoral neck.

In case of cephalomedullary nailing, the surgical approach was through a 3-4 cm longitudinal incision proximally and slightly posterior to the greater trochanter. Access to the femur was gained through blunt dissection, ensuring there was no extensive damage to the surrounding tissues, therefore reducing bleeding and risk of development of post-operative haematoma. The entry point for the nail was then identified under fluoroscopic guidance; this was at the junction of the anterior and middle thirds of the greater trochanter on the lateral view, and the tip of the greater trochanter on the AP view (**Figure B.2**). Care was taken to ensure the entry point was not too lateral, or lateralised during reaming. An entry point placed too laterally risks the fracture falling into varus; whereas an entry point placed too posteriorly could lead to the tip of the nail touching or breaching the anterior cortex distally.

The IM nail was then inserted and locked, according to the specifications of each type of device. Reaming was avoided in cases were the medullary canal was spacious, especially in the elderly population or in patients presenting with associated chest trauma where the risk of pulmonary complications was high. In cases were reaming was performed, a nail diameter 1.5 – 2 mm smaller than the diameter of reaming was used to ensure good fit in the IM canal. Cerclage wires were used in cases where the stability around the fracture site was deemed inadequate, either before or after the insertion of the IM device. For the distal locking of the nail and therefore setting of rotation of the leg, great care was taken to avoid malrotation, by referencing with the contralateral leg (this was checked pre-operatively). Intra-operative radiographs were taken to guide the implant placement and were subsequently saved on PACS (Picture Archiving and Communication System).

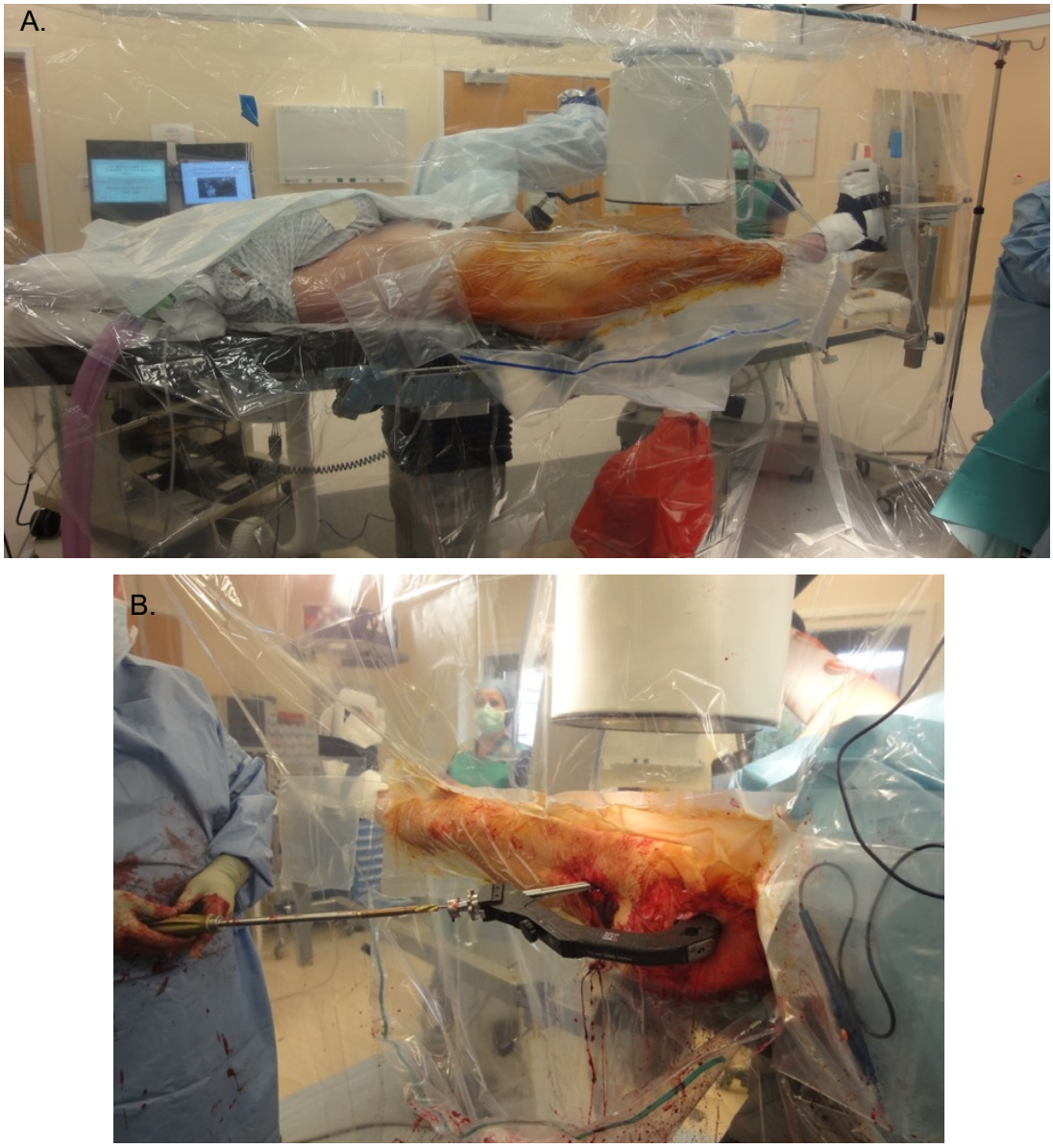
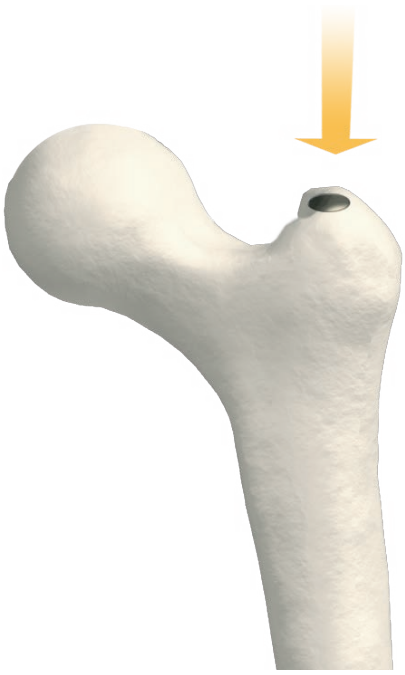


Figure B.1 Patient positioning

A. Patients were positioned in the supine position on a fracture table, with a perineal post and with the contralateral leg in lithotomy position and secured to the leg holder. The image intensifier was situated on the contralateral site, enabling good access whilst ensuring sterility was not compromised.

B. Intra-operative image with the application of the nailing apparatus.

A.



B.

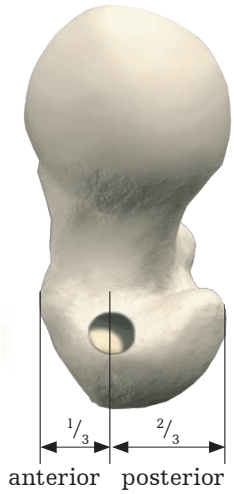


Figure B.2 Nail entry point

The entry point of the nail is located:

A. Anteroposterior (AP) view: on the tip of the greater trochanter

B. Lateral (LAT) view: on the junction between anterior 1/3 and posterior 2/3

Image adapted from Gamma3 long nail R1.5 and R2.0: Operative technique (432).

B.2. Post-operative management

Post-operatively, all patients were closely monitored for femoral compartment syndrome (especially in high energy mechanism of injury) and neurovascular injuries. The degree of weight bearing was decided by the operating surgeon according to the stability of the fixation, the bone quality and the potential compliance of the patient, and clear instructions were given to the nursing and physiotherapy teams. Unless other injuries were present, mobilisation was commenced on day of operation or the day after, depending of the time of operation and type of anaesthesia. Routine thromboprophylaxis in the form of low weight molecular heparin (LWMH) and mechanical compression was offered to the patients according the Departmental guidelines, unless any contraindications were present.

Standard AP radiographs of the pelvis and LAT radiographs of the hip and femur were obtained within 48 hours post-operatively where necessary to assess the implant positioning, as well as during the follow-up appointments to assess progression of healing and presence of any metalwork / fracture related complications. The patients were followed-up until fracture healing and resolution of symptoms. Osteoporosis and falls assessment, as well as medical treatment was offered to all patients. Implant removal was only offered in cases of failed metalwork, deep infection or implant related symptoms such as impingement or local tissue irritation.

Appendix C

Additional Tables

Table C.1 Table presenting the demographics / characteristics of patients having their operation in LTH, with complete follow-up, stratified according to the progression to a non-union

Demographics		**Union**	**Non-union**	**p-value**
Total number		257 (75.4%)	84 (24.6%)	-
Bilateral		18 (7.0%)	4 (4.8%)	0.638
Age < 65 y.o.		86 (33.5%)	27 (32.1%)	0.929
Age 65 – 75 y.o		41 (16.0%)	18 (21.4%)	0.324
Age > 75 y.o.		130 (50.6%)	39 (46.4%)	0.592
Gender	Male	102 (39.7%)	34 (40.5%)	1.000
	Female	155 (60.3%)	50 (59.5%)	
Injury Characteristics		**Union**	**Non-union**	**p-value**
Mechanism of Injury	Low energy	193 (75.1%)	59 (70.2%)	0.334
	High energy	53 (20.6%)	18 (21.4%)	
	Pathological	11 (4.3%)	7 (8.3%)	
Isolated		211 (82.1%)	73 (86.9%)	0.392
ISS > 16		20 (7.8%)	8 (9.5%)	0.783
Side	Left	128 (49.8%)	45 (53.6%)	0.636
	Right	129 (50.2%)	39 (46.4%)	
Open fracture		4 (1.6%)	3 (3.6%)	0.492
Medical Comorbidities		**Union**	**Non-union**	**p-value**
ASA	1	39 (15.2%)	5 (6.0%)	0.143
	2	71 (27.6%)	28 (33.3%)	
	3	115 (44.7%)	42 (50.0%)	
	4	32 (12.5%)	9 (10.7%)	
Charlson Comorbidity Score		4.55 (3.10)	4.76 (3.06)	0.583
Diabetes		30 (11.7%)	17 (20.2%)	0.073
Steroids		14 (5.4%)	4 (4.8%)	1.000
Malignancy		54 (21.0%)	21 (25.0%)	0.539
Dementia		40 (15.6%)	5 (6.0%)	**0.038**
Osteoporosis		**Union**	**Non-union**	**p-value**
Bisphosphonates pre-admission		47 (18.3%)	20 (23.8%)	0.343
Bisphosphonates on discharge		71 (27.6%)	23 (28.0%)	1.000
Calcium / Vitamin D pre-admission		69 (26.8%)	25 (29.8%)	0.705
Calcium / Vitamin D on discharge		118 (45.9%)	39 (47.6%)	0.894
Vitamin D loading on admission		34 (13.2%)	8 (9.8%)	0.523
Fragility Fractures Before		45 (17.5%)	16 (19.3%)	0.841
Fragility Fractures After		52 (20.2%)	18 (21.7%)	0.898
DEXA Result	Normal	3 (9.1%)	2 (18.2%)	0.111
	Osteopenia	9 (27.3%)	6 (54.5%)	
	Osteoporosis	21 (63.6%)	3 (27.3%)	
Singh Index	1	21 (8.9%)	6 (8.2%)	0.891
	2	51 (21.7%)	13 (17.8%)	
	3	46 (19.6%)	15 (20.5%)	
	4	54 (23.0%)	18 (24.7%)	
	5	25 (10.6%)	11 (15.1%)	
	6	38 (16.2%)	10 (13.7%)	
Social History		**Union**	**Non-union**	**p-value**
Smoking		55 (21.4%)	19 (22.6%)	0.934
Alcohol >10 units / week		50 (19.5%)	23 (27.4%)	0.166

Social History		Union	Non-union	p-value
Pre-operative Mobility				
	Independent	147 (57.2%)	45 (53.6%)	
	Stick(s) / Crutch(es)	66 (25.7%)	32 (38.1%)	0.185
	Frame	33 (12.8%)	5 (6.0%)	
	Wheelchair / Hoisted	11 (4.3%)	2 (2.4%)	
Frequent falls		69 (26.8%)	19 (22.6%)	0.532
Operation Characteristics		**Union**	**Non-union**	**p-value**
Operation in less than 48 hours		203 (79.0%)	65 (77.4%)	0.874
Simultaneous procedures		26 (10.1%)	5 (6.0%)	0.350
Type of Nail	Long Affixus Nail	134 (52.1%)	36 (42.9%)	
	Long Gamma Nail	101 (39.3%)	38 (45.2%)	0.302
	Others	22 (8.6%)	10 (11.9%)	
Nail Diameter	9	11 (4.3%)	9 (10.8%)	
(mm)	10	6 (2.4%)	3 (3.6%)	0.145
	11	169 (66.3%)	49 (59.0%)	
	13	69 (27.1%)	22 (26.5%)	
Open reduction		117 (45.5%)	47 (56.0%)	0.125
Use of cerclage wires		38 (14.8%)	6 (7.1%)	0.104
Post-op Mobilisation	FWB	122 (47.5%)	35 (41.7%)	
(first 6 weeks)	PWB	64 (24.9%)	33 (26.2%)	0.056
	TTWB	46 (17.9%)	10 (11.9%)	
	NWB	25 (9.7%)	17 (20.2%)	
Surgical time (min)		112.2 (44.4)	118.2 (45.6)	0.289
Anaesthetic Time (min)		47.6 (23.0)	48.9 (23.1)	0.652
Time from induction to recovery (min)		179.2 (49.6)	186.6 (52.3)	0.247
Level of First Surgeon				
	Registrar	157 (61.1%)	51 (62.2%)	0.961
	Consultant	100 (38.9%)	31 (37.8%)	
Level of Senior Surgeon Present				
	Registrar	145 (56.4%)	47 (57.3%)	0.988
	Consultant	112 (43.6%)	35 (42.7%)	
Complications		**Union**	**Non-union**	**p-value**
Nail complications*		41 (16.0%)	44 (52.4%)	**<0.001**
Failure at lag screw junction**		1 (0.4%)	23 (27.4%)	**<0.001**
Self-dynamisation		7 (2.7%)	15 (17.9%)	**<0.001**
Cut-out		3 (1.2%)	5 (6.0%)	**0.036**
Nail infection		3 (1.2%)	2 (2.4%)	0.779
Peri-implant fracture		8 (3.1%)	1 (1.2%)	0.574
HAP / CAP		36 (14.0%)	11 (13.1%)	0.977
UTI		35 (13.6%)	10 (11.9%)	0.828
Wound infection	Superficial	6 (2.3%)	6 (7.1%)	**<0.001**
	Deep	3 (1.2%)	9 (10.7%)	
Washout / Revision for Infection		4 (36.4%)	7 (43.8%)	1.000
CKD Stage pre-operatively				
	Mild	189 (75.3%)	51 (62.2%)	**0.031**
	Moderate / Severe	62 (24.7%)	31 (37.8%)	
CKD Stage post-operatively				
	Mild	192 (77.7%)	57 (69.5%)	0.175
	Moderate / Severe	55 (22.3%)	25 (30.5%)	
Pre-operative Transfusion		21 (8.2)	4 (4.8)	0.438
Post-operative Transfusion (48 hours)		122 (47.5)	42 (50.6)	0.711

Complications		Union	Non-union	p-value
Post-operative Transfusion (total)		150 (58.4)	54 (65.1)	0.340
Hb Drop (g/L)		44.95 (18.34)	45.69 (18.25)	0.753
Biochemistry		**Union**	**Non-union**	**p-value**
Adjusted Calcium	Normal	153 (78.5%)	40 (61.5%)	**0.011**
	Low	42 (21.5%)	25 (38.5%)	
Albumin	Normal	87 (39.0%)	45 (62.5%)	0.928
	Low	136 (61.0%)	27 (37.5%)	
Alkaline Phosphatase	High	44 (20.0%)	15 (20.8%)	0.970
	Normal	162 (73.6%)	52 (72.2%)	
	Low	14 (6.4%)	5 (6.9%)	
Phosphate	Normal / High	163 (83.2%)	53 (80.3%)	0.733
	Low	33 (16.8%)	13 (19.7%)	
TSH	High	10 (8.7%)	4 (11.4 %)	0.662
	Normal	103 (89.6%)	31 (88.6%)	
	Low	2 (1.7%)	0 (0.0%)	
Free T4	High	18 (15.8%)	3 (8.8 %)	0.354
	Normal	93 (81.6%)	31 (91.2%)	
	Low	3 (2.6%)	0 (0.0%)	
PTH	High	50 (50.5%)	15 (38.5%)	0.277
	Normal	49 (49.5%)	24 (61.5%)	
Total 25OH Vitamin D	Normal	17 (14.9%)	4 (10.3%)	0.646
	Low	97 (85.1%)	35 (89.7%)	
Radiographic measurements		**Union**	**Non-union**	**p-value**
Femoral Neck Shaft Angle				0.513
	Normal	168 (66.4%)	59 (72.0)%)	
	Coxa Valga	75 (29.6%)	19 (23.2%)	
	Coxa Vara	10 (4.0%)	4 (4.9%)	
Number of fragments (Comminution)	Simple	72 (28.0%)	30 (36.6%)	**<0.001**
	Moderate	133 (51.8%)	22 (26.8%)	
	Severe	52 (20.2%)	30 (36.6%)	
Only Subtrochanteric Extension		44 (17.1%)	16 (19.5%)	0.743
Atypical		12 (4.7%)	13 (15.9%)	**0.002**
Pathological		7 (2.7%)	4 (4.9%)	0.548
Distal Extension		97 (37.7%)	32 (39.0%)	0.938
Greater Trochanter Fracture		24 (9.3%)	9 (11.0%)	0.825
Lesser Trochanter Fracture		164 (63.8%)	49 (59.8%)	0.596
Medial Calcar Comminution		17 (6.6%)	5 (6.1%)	1.000
AO/ OTA Classification		-	-	0.756
Lateral Cortex Gap Size (mm)	≤4	167 (65.0%)	32 (38.1%)	**<0.001**
	5-9	57 (22.2%)	37 (44.0%)	
	≥10	33 (12.8%)	15 (17.9%)	
Medial Cortex Gap Size (mm)	≤4	184 (71.6%)	44 (52.4%)	**0.004**
	5-9	49 (19.1%)	29 (34.5%)	
	≥10	24 (9.3%)	11 (13.1%)	
Anterior Cortex Gap Size (mm)	≤4	169 (65.8%)	45 (53.6%)	0.070
	5-9	54 (21.0%)	20 (23.8%)	
	≥10	34 (13.2%)	19 (22.6%)	
Posterior Cortex Gap Size (mm)	≤4	202 (78.6%)	46 (54.8%)	**<0.001**
	5-9	37 (14.4%)	30 (35.7%)	
	≥10	18 (7.0%)	8 (9.5%)	

Radiographic measurements		Union	Non-union	p-value
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	197 (76.7%)	45 (53.6%)	**0.001**
	Valgus >5	13 (5.1%)	7 (8.3%)	
	Varus 5-10	37 (14.4%)	23 (27.4%)	
	Varus >10	10 (3.9%)	9 (10.7%)	
Antirotation Screw		90 (35.7%)	26 (31.7%)	0.597
TAD (mm)	<25	217 (87.5%)	66 (80.5%)	0.164
	≥25	31 (12.5%)	16 (19.5%)	
Distal locking (Number of Screws)	1	11 (4.3%)	1 (1.2%)	0.321
	2	246 (95.7%)	83 (98.8%)	
Method of locking				0.320
Static Locking		170 (66.7%)	51 (60.7%)	
Secondary Dynamisation		82 (32.2%)	33 (39.3%)	
Dynamic		3 (1.2%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	171 (67.1%)	47 (56.0%)	0.104
	Lateral ≥5	44 (17.3%)	23 (27.4%)	
	Medial ≥5	40 (15.7%)	14 (16.7%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	202 (79.2%)	70 (83.3%)	0.543
	Anterior ≥5	49 (19.2%)	12 (14.3%)	
	Posterior ≥5	4 (1.6%)	2 (2.4%)	
Distance of tip of the nail from knee (mm)	<10	3 (1.2 %)	0 (0%)	0.100
	10 to 19	17 (6.7%)	11 (13.1%)	
	20-29	85 (33.3%)	21 (25.0%)	
	≥30	153 (60.0%)	52 (61.9%)	
Nail / Canal Ratio		0.82 (0.07%)	0.83 (0.07%)	0.736
Hospital stay / Mortality		**Union**	**Non-union**	**p-value**
HDU / ICU stay		23 (8.9%)	15 (17.9%)	**0.040**
Total length of hospital stay (days)		20.57 (17.99)	22.69 (22.22)	0.378
Weekend admission		84 (32.7%)	29 (34.5%)	0.859
Died within a year		13 (5.1%)	5 (6.0%)	0.970

Continuous variables are presented as mean (SD)

* 'Nail complications' variable was not included in the logistic regression as the individual factors were examined.

** Failure at lag screw junction was not included in the logistic regression as by definition, a non-union should be present for the nail to fail at the nail – lag screw junction.

Significant parameters are presented in bold ($p < 0.05$)

Table C.2 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the deep infection

Demographics		**No Deep Infection**	**Deep Infection**	**p-value**
Total number		546	15	-
Bilateral		26 (4.8%)	0 (0.0 %)	0.808
Age < 65 y.o.		132 (24.2%)	6 (40.0 %)	0.271
Age 65 – 75 y.o		87 (15.9%)	3 (20.0 %)	0.947
Age > 75 y.o.		327 (59.9%)	6 (40.0 %)	0.200
Gender	Male Female	215 (39.4%) 331 (60.6%)	5 (33.3 %) 10 (66.7 %)	0.838
Injury Characteristics		**No Deep Infection**	**Deep Infection**	**p-value**
Mechanism of Injury	Low energy High energy Pathological	424 (77.7%) 86 (15.8%) 36 (6.6%)	9 (60.0 %) 4 (26.7 %) 2 (13.3 %)	0.265
Isolated		465 (85.2%)	11 (73.3 %)	0.370
ISS > 16		33 (6%)	3 (20.0 %)	0.101
Side	Left Right	285 (52.2%) 261 (47.8%)	10 (66.7 %) 5 (33.3 %)	0.398
Open fracture		5 (0.9%)	2 (13.3 %)	**0.002**
Medical Comorbidities		**No Deep Infection**	**Deep Infection**	**p-value**
ASA	1 2 3 4	48 (8.8%) 139 (25.5%) 272 (49.8%) 87 (15.9%)	1 (6.7 %) 6 (40.0 %) 6 (40.0 %) 2 (13.3 %)	0.656
Charlson Comorbidity Score		5.29 (3.11%)	4.73 (3.59 %)	0.499
Diabetes		74 (13.6%)	3 (20.0 %)	0.737
Steroids		28 (5.1%)	1 (6.7 %)	1.000
Malignancy		133 (24.4%)	4 (26.7 %)	1.000
Dementia		123 (22.5%)	2 (13.3 %)	0.596
Osteoporosis		**No Deep Infection**	**Deep Infection**	**p-value**
Bisphosphonates pre-admission		92 (16.8%)	2 (13.3 %)	0.993
Bisphosphonates on discharge		135 (26.5%)	1 (7.1 %)	0.186
Calcium / Vitamin D pre-admission		155 (28.4%)	3 (20.0 %)	0.673
Calcium / Vitamin D on discharge		245 (48.1%)	6 (42.9 %)	0.905
Vitamin D loading on admission		84 (16.5%)	3 (21.4 %)	0.901
Fragility Fractures Before		121 (22.2%)	2 (13.3 %)	0.615
Fragility Fractures After		84 (15.4%)	2 (13.3 %)	1.000
DEXA Result	Normal Osteopenia Osteoporosis	6 (12.5%) 15 (31.2%) 27 (56.2%)	0 (0 %) 0 (0 %) 0 (0 %)	-
Singh Index	1 2 3 4 5 6	42 (8.6%) 113 (23.2%) 105 (21.5%) 113 (23.2%) 61 (12.5%) 54 (11.1%)	0 (0.0 %) 1 (7.7 %) 2 (15.4 %) 5 (38.5 %) 1 (7.7 %) 4 (30.8 %)	0.135
Social History		**No Deep Infection**	**Deep Infection**	**p-value**
Smoking		107 (19.6%)	6 (40.0 %)	0.106
Alcohol >10 units / week		99 (18.1%)	6 (40.0 %)	0.071

Social History		No Deep Infection	Deep Infection	p-value
Pre-operative Mobility				
	Independent	283 (51.8%)	10 (66.7 %)	0.616
	Stick(s) / Crutch(es)	144 (26.4%)	2 (13.3 %)	
	Frame	93 (17%)	2 (13.3 %)	
	Wheelchair / Hoisted	26 (4.8%)	1 (6.7 %)	
Frequent falls		147 (26.9%)	6 (40.0 %)	0.408
Operation Characteristics		**No Deep Infection**	**Deep Infection**	**p-value**
Operation in less than 48 hours		113 (20.7%)	4 (26.7 %)	0.811
Simultaneous procedures		34 (6.2%)	3 (20.0 %)	0.111
Type of Nail	Long Affixus Nail	311 (57%)	8 (53.3 %)	0.924
	Long Gamma Nail	192 (35.2%)	6 (40.0 %)	
	Others	43 (7.9%)	1 (6.7 %)	
Nail Diameter (mm)	9	29 (5.3%)	2 (13.3 %)	0.616
	10	13 (2.4%)	0 (0.0 %)	
	11	335 (61.7%)	10 (66.7 %)	
	12	2 (0.4%)	0 (0.0 %)	
	13	164 (30.2%)	3 (20.0 %)	
Open reduction		253 (46.3%)	12 (80.0 %)	**0.021**
Use of cerclage wires		62 (11.4%)	3 (20.0 %)	0.533
Post-op Mobilisation (first 6 weeks)	FWB	300 (54.9%)	7 (46.7 %)	0.216
	PWB	117 (21.4%)	5 (33.3 %)	
	TTWB	73 (13.4%)	0 (0.0 %)	
	NWB	56 (10.3%)	3 (20.0 %)	
Surgical time (min)		110.92 (44.17)	137.53 (64.43)	**0.024**
Anaesthetic Time (min)		48.19 (21.43)	58.27 (26.89)	0.075
Time from induction to recovery (min)		177.41 (48.26)	228.73 (77.18)	**<0.001**
Level of First Surgeon				
	Registrar	324 (59.7%)	8 (53.3 %)	0.821
	Consultant	219 (40.3%)	7 (46.7 %)	
Level of Senior Surgeon Present				
	Registrar	298 (54.9%)	8 (53.3 %)	1.000
	Consultant	245 (45.1%)	7 (46.7 %)	
Complications		**No Deep Infection**	**Deep Infection**	**p-value**
Nail complications		94 (17.2%)	6 (40.0 %)	0.053
Failure at lag screw junction		23 (4.2%)	1 (6.7 %)	1.000
Self-dynamisation		25 (4.6%)	0 (0.0 %)	0.831
Cut-out		13 (2.4%)	0 (0.0 %)	1.000
Non-union		75 (13.7%)	9 (60.0 %)	**<0.001**
Peri-implant fracture		13 (2.4%)	1 (6.7 %)	0.833
HAP / CAP		103 (18.9%)	3 (20.0 %)	1.000
UTI		76 (13.9%)	2 (13.3 %)	1.000
CKD Stage pre-operatively				
	Mild	372 (69.5%)	12 (80.0 %)	0.558
	Moderate / Severe	163 (30.5%)	3 (20.0 %)	
CKD Stage post-operatively				
	Mild	385 (72.5%)	12 (80.0 %)	0.727
	Moderate / Severe	146 (27.5%)	3 (20.0 %)	
Pre-operative Transfusion		46 (8.5%)	2 (13.3 %)	0.843

Complications		No Deep Infection	Deep Infection	p-value
Post-operative Transfusion (48 hours)		275 (50.6%)	13 (86.7 %)	**0.012**
Post-operative Transfusion (total)		339 (62.3%)	14 (93.3 %)	**0.029**
Hb Drop (g/L)		46.87 (18.57)	35.13 (17.15)	**0.016**
VTE	No DVT PE	102 (82.3%) 12 (9.7%) 10 (8.1%)	5 (100.0 %) 0 (0.0 %) 0 (0.0 %)	0.586
Biochemistry		**No Deep Infection**	**Deep Infection**	**p-value**
Adjusted Calcium	Normal Low	343 (77.1%) 102 (22.9%)	5 (38.5 %) 8 (61.5 %)	**0.004**
Albumin	Normal Low	156 (32%) 331 (68%)	2 (13.3 %) 13 (86.7 %)	0.210
Alkaline Phosphatase	High Normal Low	94 (19.4%) 348 (71.9%) 42 (8.7%)	2 (13.3 %) 10 (66.7 %) 3 (20.0 %)	0.301
Phosphate	Normal / High Low	362 (80.8%) 86 (19.2%)	11 (91.7 %) 1 (8.3 %)	0.565
TSH	High Normal Low	30 (10.9%) 239 (87.2%) 5 (1.8%)	1 (14.3 %) 6 (85.7 %) 0 (0.0 %)	0.906
Free T4	High Normal Low	38 (14.2%) 223 (83.5%) 6 (2.2%)	1 (14.3 %) 6 (85.7 %) 0 (0.0 %)	0.923
PTH	High Normal	127 (51.6%) 119 (48.4%)	3 (42.9 %) 4 (57.1 %)	0.941
Total 25OH Vitamin D	Normal Low	33 (12.3%) 236 (87.7%)	2 (25.0 %) 6 (75.0 %)	0.597
Radiographic measurements		**No Deep Infection**	**Deep Infection**	**p-value**
Femoral Neck Shaft Angle	Normal Coxa Valga Coxa Vara	356 (66.5%) 142 (26.5%) 37 (6.9%)	10 (66.7 %) 4 (26.7 %) 1 (6.7 %)	0.999
Number of fragments (Comminution)	Simple Moderate Severe	160 (29.4%) 265 (48.7%) 119 (21.9%)	3 (20.0 %) 8 (53.3 %) 4 (26.7 %)	0.719
Only Subtrochanteric Extension		96 (17.6%)	3 (20.0 %)	1.000
Atypical		30 (5.5%)	0 (0.0 %)	0.723
Pathological		30 (5.5%)	0 (0.0 %)	0.723
Distal Extension		176 (32.4%)	10 (66.7 %)	**0.012**
Greater Trochanter Fracture		55 (10.1%)	4 (26.7 %)	0.103
Lesser Trochanter Fracture		349 (64.2%)	11 (73.3 %)	0.646
Medial Calcar Comminution		29 (5.3%)	2 (13.3 %)	0.445
AO/ OTA Classification		-	-	0.991
Lateral Cortex Gap Size (mm)	≤4 5-9 ≥10	336 (61.8%) 138 (25.4%) 70 (12.9%)	7 (50.0 %) 4 (28.6 %) 3 (21.4 %)	0.569
Medial Cortex Gap Size (mm)	≤4 5-9 ≥10	362 (66.5%) 131 (24.1%) 51 (9.4%)	9 (64.3 %) 3 (21.4 %) 2 (14.3 %)	0.820

Radiographic measurements		No Deep Infection	Deep Infection	p-value
Anterior Cortex Gap Size (mm)	≤4	348 (63.9%)	7 (50.0 %)	0.072
	5-9	121 (22.2%)	2 (14.3 %)	
	≥10	76 (13.9%)	5 (35.7 %)	
Posterior Cortex Gap Size (mm)	≤4	419 (76.9%)	8 (57.1 %)	0.178
	5-9	96 (17.6%)	4 (28.6 %)	
	≥10	30 (5.5%)	2 (14.3 %)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	395 (72.6%)	9 (64.3 %)	0.508
	Valgus >5	36 (6.6%)	2 (14.3 %)	
	Varus 5-10	28 (5.1%)	0 (0.0 %)	
	Varus >10	85 (15.6%)	3 (21.4 %)	
TAD (mm)	<25	462 (86.8%)	12 (85.7 %)	1.000
	≥25	70 (13.2%)	2 (14.3 %)	
Distal locking (Number of Screws)	1	18 (3.3%)	0 (0.0 %)	1.000
	2	527 (96.7%)	14 (100.0 %)	
Method of locking				
Static Locking		346 (64%)	10 (71.4 %)	0.826
Secondary Dynamisation		192 (35.5%)	4 (28.6 %)	
Dynamic		3 (0.6%)	0 (0.0 %)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	345 (64%)	10 (71.4 %)	0.507
	Lateral ≥5	90 (16.7%)	3 (21.4 %)	
	Medial ≥5	104 (19.3%)	1 (7.1 %)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	422 (78%)	12 (85.7 %)	0.299
	Anterior ≥5	106 (19.6%)	1 (7.1 %)	
	Posterior ≥5	13 (2.4%)	1 (7.1 %)	
Touching Anterior Cortex		136 (25.2%)	1 (7.1 %)	0.218
Distance of tip of the nail from knee (mm)	<10	7 (1.3%)	0 (0.0 %)	0.383
	10 to 19	45 (8.4%)	3 (21.4 %)	
	20-29	176 (32.8%)	4 (28.6 %)	
	≥30	309 (57.5%)	7 (50.0 %)	
Nail / Canal Ratio		0.83 (0.08%)	0.82 (0.06 %)	0.647
Hospital stay / Mortality		**No Deep Infection**	**Deep Infection**	**p-value**
HDU / ICU stay		62 (11.4%)	5 (33.3 %)	**0.029**
Total length of hospital stay (days)		21.76 (17.41)	49.87 (35.38)	**<0.001**
Weekend admission		174 (31.9%)	5 (33.3%)	1.000
Died within a year		114 (20.9%)	1 (6.7 %)	0.307

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.3 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to open reduction

Demographics		**Closed Reduction**	**Open Reduction**	**p-value**
Total number		296	265	-
Bilateral		18 (6.1%)	8 (3%)	0.128
Age < 65 y.o.		65 (22.0%)	73 (27.5%)	0.151
Age 65 – 75 y.o		40 (13.5%)	50 (18.9%)	0.107
Age > 75 y.o.		191 (64.5%)	142 (53.6%)	**0.011**
Gender	Male	96 (32.4%)	124 (46.8%)	**0.001**
	Female	200 (67.6%)	141 (53.2%)	
Injury Characteristics		**Closed Reduction**	**Open Reduction**	**p-value**
Mechanism of Injury	Low energy	242 (81.8%)	191 (72.1%)	**0.002**
	High energy	22 (7.4%)	16 (6%)	
	Pathological	32 (10.8%)	58 (21.9%)	
Isolated		254 (85.8%)	222 (83.8%)	0.580
ISS > 16		13 (4.4%)	23 (8.7%)	0.058
Side	Left	160 (54.1%)	135 (50.9%)	0.514
	Right	136 (45.9%)	130 (49.1%)	
Open fracture		0 (0.0%)	7 (2.6%)	**0.015**
Medical Comorbidities		**Closed Reduction**	**Open Reduction**	**p-value**
ASA	1	17 (5.7%)	32 (12.1%)	**0.030**
	2	87 (29.4%)	58 (21.9%)	
	3	147 (49.7%)	131 (49.4%)	
	4	45 (15.2%)	43 (16.2%)	
	5	0 (0.0%)	1 (0.4%)	
Charlson Comorbidity Score		5.48 (2.98)	5.04 (3.26)	0.099
Diabetes		34 (11.5%)	43 (16.2%)	0.132
Steroids		13 (4.4%)	16 (6%)	0.491
Malignancy		75 (25.3%)	62 (23.4%)	0.663
Dementia		65 (22.0%)	60 (22.6%)	0.927
Osteoporosis		**Closed Reduction**	**Open Reduction**	**p-value**
Bisphosphonates pre-admission		55 (18.6%)	39 (14.7%)	0.267
Bisphosphonates on discharge		83 (30.0%)	53 (21.5%)	**0.037**
Calcium / Vitamin D pre-admission		81 (27.4%)	77 (29.1%)	0.726
Calcium / Vitamin D on discharge		135 (48.7%)	116 (47.2%)	0.784
Vitamin D loading on admission		57 (20.6%)	30 (12.2%)	**0.014**
Fragility Fractures Before		63 (21.3%)	60 (22.7%)	0.757
Fragility Fractures After		58 (19.6%)	28 (10.6%)	**0.005**
DEXA Result	Normal	2 (6.7%)	4 (22.2%)	0.119
	Osteopenia	8 (26.7%)	7 (38.9%)	
	Osteoporosis	20 (66.7%)	7 (38.9%)	
Singh Index	1	26 (9.7%)	16 (6.9%)	**<0.001**
	2	83 (30.9%)	31 (13.4%)	
	3	53 (19.7%)	54 (23.3%)	
	4	58 (21.6%)	60 (25.9%)	
	5	34 (12.6%)	28 (12.1%)	
	6	15 (5.6%)	43 (18.5%)	

Social History		Closed Reduction	Open Reduction	p-value
Smoking		59 (19.9%)	54 (20.4%)	0.979
Alcohol >10 units / week		56 (18.9%)	49 (18.5%)	0.983
Pre-operative Mobility				
	Independent	146 (49.3%)	147 (55.5%)	
	Stick(s) / Crutch(es)	80 (27.0%)	66 (24.9%)	0.321
	Frame	52 (17.6%)	43 (16.2%)	
	Wheelchair / Hoisted	18 (6.1%)	9 (3.4%)	
Frequent falls		83 (28.0%)	70 (26.4%)	0.736
Operation Characteristics		**Closed Reduction**	**Open Reduction**	**p-value**
Operation in less than 48 hours		237 (80.1%)	207 (78.1%)	0.642
Simultaneous procedures		13 (4.4%)	24 (9.1%)	**0.040**
Type of Nail	Long Affixus Nail	185 (62.5%)	134 (50.6%)	
	Long Gamma Nail	87 (29.4%)	111 (41.9%)	**0.008**
	Others	24 (8.1%)	20 (7.5%)	
Nail Diameter	9	8 (2.7%)	23 (8.7%)	
(mm)	10	9 (3.1%)	4 (1.5%)	
	11	178 (60.3%)	167 (63.5%)	**0.005**
	12	2 (0.7%)	0 0%)	
	13	98 (33.2%)	69 (26.2%)	
Use of cerclage wires		0 (0.0%)	62 (23.4%)	**<0.001**
Post-op Mobilisation	FWB	200 (67.6%)	107 (40.4%)	
(first 6 weeks)	PWB	57 (19.3%)	65 (24.5%)	**<0.001**
	TTWB	21 (7.1%)	52 (19.6%)	
	NWB	18 (6.1%)	41 (15.5%)	
Surgical time (min)		95.49 (33.68)	129.73 (49.02)	**<0.001**
Anaesthetic Time (min)		47.65 (20.55)	49.37 (22.78)	0.349
Time from induction to recovery (min)		160.37 (40.12)	199.45 (51.64)	**<0.001**
Level of First Surgeon				
	Registrar	187 (63.4%)	145 (55.1%)	0.058
	Consultant	108 (36.6%)	118 (44.9%)	
Level of Senior Surgeon Present				
	Registrar	176 (59.7%)	130 (49.4%)	**0.019**
	Consultant	119 (40.3%)	133 (50.6%)	
Complications		**Closed Reduction**	**Open Reduction**	**p-value**
Nail complications		40 (13.5%)	60 (22.6%)	**0.007**
Failure at lag screw junction		9 (3.0%)	15 (5.7%)	0.186
Self-dynamisation		12 (4.1%)	13 (4.9%)	0.777
Cut-out		6 (2.0%)	7 (2.6%)	0.840
Non-union		37 (12.5%)	47 (17.7%)	0.106
Peri-implant fracture		5 (1.7%)	9 (3.4%)	0.306
HAP / CAP		59 (19.9%)	47 (17.7%)	0.579
UTI		37 (12.5%)	41 (15.5%)	0.372
Wound infection	Superficial	4 (1.4%)	17 (6.4%)	**<0.001**
	Deep	3 (1.0%)	12 (4.5%)	
CKD Stage pre-operatively				
	Mild	200 (69.0%)	184 (70.8%)	0.714
	Moderate / Severe	90 (31.0%)	76 (29.2%)	
CKD Stage post-operatively				
	Mild	219 (75.5%)	178 (69.5%)	0.141
	Moderate / Severe	71 (24.5%)	78 (30.5%)	

Complications		Closed Reduction	Open Reduction	p-value
Pre-operative Transfusion		19 (6.4%)	29 (11%)	0.078
Post-operative Transfusion (48 hours)		129 (43.7%)	159 (60.2%)	**<0.001**
Post-operative Transfusion (total)		164 (55.6%)	189 (71.6%)	**<0.001**
Hb Drop (g/L)		50.47 (17.07)	42.15 (19.32)	**<0.001**
VTE	No DVT PE	56 (84.8%) 6 (9.1%) 4 (6.1%)	51 (81%) 6 (9.5%) 6 (9.5%)	0.754
Biochemistry		**Closed Reduction**	**Open Reduction**	**p-value**
Adjusted Calcium	Normal Low	201 (79.8%) 51 (20.2%)	147 (71.4%) 59 (28.6%)	**0.047**
Albumin	Normal Low	181 (67.3%) 88 (32.7%)	163 (70%) 70 (30%)	0.585
Alkaline Phosphatase	High Normal Low	54 (80.2%) 189 (70.8%) 24 (9.0%)	42 (18.1%) 169 (72.8%) 21 (9.1%)	0.834
Phosphate	Normal / High Low	202 (20.2%) 50 (19.8%)	171 (82.2%) 37 (17.8)	0.660
TSH	High Normal Low	19 (12.3%) 132 (85.2%) 4 (2.6%)	12 (9.5%) 113 (89.7%) 1 (0.8%)	0.390
Free T4	High Normal Low	22 (14.8%) 123 (82.6%) 4 (2.7%)	17 (13.6%) 106 (84.8%) 2 (1.6%)	0.790
PTH	High Normal	77 (53.8%) 66 (46.2%)	53 (48.2%) 57 (51.8%)	0.443
Total 25OH Vitamin D	Normal Low	21 (13.4%) 136 (86.6%)	14 (11.7%) 106 (88.3%)	0.809
Radiographic measurements		**Closed Reduction**	**Open Reduction**	**p-value**
Femoral Neck Shaft Angle	Normal Coxa Valga Coxa Vara	182 (62.5%) 87 (29.9%) 22 (7.6%)	184 (71%) 59 (22.8%) 16 (6.2%)	0.106
Number of fragments (Comminution)	Simple Moderate Severe	83 (28.0%) 160 (54.1%) 53 (17.9%)	80 (30.4%) 113 (43%) 70 (26.6%)	**0.014**
Only Subtrochanteric Extension		51 (17.2%)	48 (18.3%)	0.838
Atypical		17 (5.7%)	13 (4.9%)	0.817
Pathological		20 (6.8%)	10 (3.8%)	0.174
Distal Extension		69 (23.3%)	117 (44.5%)	**<0.001**
Greater Trochanter Fracture		32 (10.8%)	27 (10.3%)	0.943
Lesser Trochanter Fracture		187 (63.2%)	173 (65.8%)	0.580
Medial Calcar Comminution		19 (6.4%)	12 (4.6%)	0.440
AO/ OTA Classification		-	-	**0.002**
Lateral Cortex Gap Size (mm)	≤4 5-9 ≥10	190 (64.6%) 65 (22.1%) 39 (13.3%)	153 (58%) 77 (29.2%) 34 (12.9%)	0.154
Medial Cortex Gap Size (mm)	≤4 5-9 ≥10	202 (68.7%) 67 (22.8%) 25 (8.5%)	169 (64%) 67 (25.4%) 28 (10.6%)	0.473

Radiographic measurements		Closed Reduction	Open Reduction	p-value
Anterior Cortex Gap Size (mm)	≤4	185 (62.7%)	170 (64.4%)	0.915
	5-9	66 (22.4%)	57 (21.6%)	
	≥10	44 (14.9%)	37 (14%)	
Posterior Cortex Gap Size (mm)	≤4	234 (79.3%)	193 (73.1%)	0.224
	5-9	46 (15.6%)	54 (20.5%)	
	≥10	15 (5.1%)	17 (6.4%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	214 (72.8%)	190 (72.0%)	0.671
	Valgus >5	23 (7.8%)	15 (5.7%)	
	Varus 5-10	43 (14.6%)	45 (17%)	
	Varus >10	14 (4.8%)	14 (5.3%)	
TAD (mm)	<25	258 (89.0%)	216 (84.4%)	0.146
	≥25	32 (11.0%)	40 (15.6%)	
Distal locking (Number of Screws)	1	14 (4.7%)	4 (1.5%)	0.057
	2	282 (95.3%)	259 (98.5%)	
Method of locking				0.820
Static Locking		106 (36.1%)	90 (34.5%)	
Secondary Dynamisation		186 (63.3%)	170 (65.1%)	
Dynamic		2 (0.7%)	1 (0.4%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	199 (68.2%)	156 (59.8%)	**0.011**
	Lateral ≥5	36 (12.3%)	57 (21.8%)	
	Medial ≥5	57 (19.5%)	48 (18.4%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	220 (74.8%)	214 (82%)	**0.016**
	Anterior ≥5	69 (23.5%)	38 (14.6%)	
	Posterior ≥5	5 (1.7%)	9 (3.4%)	
Touching Anterior Cortex		83 (28.3%)	54 (20.7%)	**0.048**
Distance of tip of the nail from knee (mm)	<10	3 (1.0%)	4 (1.5%)	0.529
	10 to 19	30 (10.3%)	18 (6.9%)	
	20-29	94 (32.3%)	86 (33.1%)	
	≥30	164 (56.4%)	152 (58.5%)	
Nail / Canal Ratio		0.82 (0.08)	0.83 (0.08)	0.512
Hospital stay / Mortality		**Closed Reduction**	**Open Reduction**	**p-value**
HDU / ICU stay		30 (10.1%)	37 (14%)	0.206
Total length of hospital stay (days)		22.04 (18.31)	23.05 (18.98)	0.521
Weekend admission		92 (31.1%)	87 (32.8%)	0.724
Died within a year		58 (35.6%)	57 (39.3%)	0.577

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.4 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to presence of cerclage wiring

Demographics		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Total number		203	62	-
Bilateral		6 (3%)	2 (3.2%)	1.000
Age < 65 y.o.		54 (26.6%)	19 (30.6%)	0.644
Age 65 – 75 y.o		42 (20.7%)	8 (12.9%)	0.236
Age > 75 y.o.		107 (52.7%)	35 (56.5%)	0.710
Gender	Male Female	87 (42.9%) 116 (57.1%)	37 (59.7%) 25 (40.3%)	**0.029**
Injury Characteristics		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Mechanism of Injury	Low energy High energy Pathological	144 (70.9%) 43 (21.2%) 16 (7.9%)	47 (75.8%) 15 (24.2%) 0 (0.0%)	0.073
Isolated		170 (83.7%)	52 (83.9%)	1.000
ISS > 16		18 (8.9%)	5 (8.1%)	1.000
Side	Left Right	104 (51.2%) 99 (48.8%)	31 (50.0%) 31 (50.0%)	0.980
Open fracture		7 (3.4%)	0 (0.0%)	0.303
Medical Comorbidities		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
ASA	1 2 3 4	21 (10.3%) 42 (20.7%) 105 (51.7%) 35 (17.2%)	11 (17.7%) 16 (25.8%) 26 (41.9%) 9 (14.5%)	0.278
Charlson Comorbidity Score		5.26 (3.27%)	4.34 (3.17%)	0.052
Diabetes		34 (16.7%)	9 (14.5%)	0.825
Steroids		14 (6.9%)	2 (3.2%)	0.449
Malignancy		53 (26.1%)	9 (14.5%)	0.086
Dementia		49 (24.1%)	11 (17.7%)	0.379
Osteoporosis		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Bisphosphonates pre-admission		32 (15.8%)	7 (11.3%)	0.506
Bisphosphonates on discharge		40 (21.4%)	13 (22.0%)	1.000
Calcium / Vitamin D pre-admission		61 (30%)	16 (25.8%)	0.628
Calcium / Vitamin D on discharge		89 (47.6%)	27 (45.8%)	0.923
Vitamin D loading on admission		19 (10.2%)	11 (18.6%)	0.132
Fragility Fractures Before		48 (23.8%)	12 (19.4%)	0.582
Fragility Fractures After		20 (9.9%)	8 (12.9%)	0.663
DEXA Result	Normal Osteopenia Osteoporosis	3 (21.4%) 5 (35.7%) 6 (42.9%)	1 (25.0%) 2 (50.0%) 1 (25.0%)	0.804
Singh Index	1 2 3 4 5 6	12 (6.9%) 24 (13.7%) 42 (24%) 46 (26.3%) 22 (12.6%) 29 (16.6%)	4 (7.0%) 7 (12.3%) 12 (21.1%) 14 (24.6%) 6 (10.5%) 14 (24.6%)	0.862
Social History		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Smoking		42 (20.7%)	12 (19.4%)	0.962
Alcohol >10 units / week		36 (17.7%)	13 (21.0%)	0.699

Social History	No Cerclage Wiring	Cerclage Wiring	p-value
Pre-operative Mobility			
Independent	114 (56.2%)	33 (53.2%)	0.765
Stick(s) / Crutch(es)	49 (24.1%)	17 (27.4%)	
Frame	32 (15.8%)	11 (17.7%)	
Wheelchair / Hoisted	8 (3.9%)	1 (1.6%)	
Frequent falls	54 (26.6%)	16 (25.8%)	1.000
Operation Characteristics	**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Operation in less than 48 hours	154 (75.9%)	53 (85.5%)	0.153
Simultaneous procedures	19 (9.4%)	5 (8.1%)	0.954
Type of Nail Long Affixus Nail	100 (49.3%)	34 (54.8%)	0.127
Long Gamma Nail	84 (41.4%)	27 (43.5%)	
Others	19 (9.4%)	1 (1.6%)	
Nail Diameter (mm) 9	19 (9.5%)	4 (6.5%)	0.416
10	4 (2%)	0 (0.0%)	
11	123 (61.2%)	44 (71.0%)	
13	55 (27.4%)	14 (22.6%)	
Post-op Mobilisation (first 6 weeks) FWB	84 (41.4%)	23 (37.1%)	0.400
PWB	53 (26.1%)	12 (19.4%)	
TTWB	36 (17.7%)	16 (25.8%)	
NWB	30 (14.8%)	11 (17.7%)	
Surgical time (min)	121.85 (46.45)	155.82 (48.64)	**<0.001**
Anaesthetic Time (min)	49.54 (20.01)	48.82 (30.39)	0.829
Time from induction to recovery (min)	192.02 (50.36)	224.05 (48.45)	**<0.001**
Level of First Surgeon			
Registrar	108 (53.5%)	37 (60.7%)	0.399
Consultant	94 (46.5%)	24 (39.3%)	
Level of Senior Surgeon Present			
Registrar	96 (47.5%)	34 (55.7%)	0.328
Consultant	106 (52.5%)	27 (44.3%)	
Complications	**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Nail complications	50 (24.6%)	10 (16.1%)	0.220
Failure at lag screw junction	13 (6.4%)	2 (3.2%)	0.526
Self-dynamisation	10 (4.9%)	3 (4.8%)	1.000
Cut-out	7 (3.4%)	0 (0.0%)	0.303
Non-union	41 (20.2%)	6 (9.7%)	0.088
Peri-implant fracture	8 (3.9%)	1 (1.6%)	0.628
HAP / CAP	34 (16.7%)	13 (21.0%)	0.568
UTI	33 (16.3%)	8 (12.9%)	0.661
Wound infection Superficial	15 (7.4%)	2 (3.2%)	0.503
Deep	9 (4.4%)	3 (4.8%)	
CKD Stage pre-operatively			
Mild	136 (68.3%)	48 (78.7%)	0.163
Moderate / Severe	63 (31.7%)	13 (21.3%)	
CKD Stage post-operatively			
Mild	128 (65.6%)	50 (82.0%)	**0.024**
Moderate / Severe	67 (34.4%)	11 (18.0%)	
Pre-operative Transfusion	22 (10.9%)	7 (11.3%)	1.000
Post-operative Transfusion (48 hours)	117 (57.9%)	42 (67.7%)	0.217

Complications		No Cerclage Wiring	Cerclage Wiring	p-value
Post-operative Transfusion (total)		141 (69.8%)	48 (77.4%)	0.316
Hb Drop (g/L)		43.62 (17.89)	35.89 (19.83)	**0.004**
VTE	No	35 (81.4%)	16 (80.0%)	0.991
	DVT	4 (9.3%)	2 (10.0%)	
	PE	4 (9.3%)	2 (10.0%)	
Biochemistry		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Adjusted Calcium	Normal	112 (70.9%)	35 (72.9%)	0.928
	Low	46 (29.1%)	13 (27.1%)	
Albumin	Normal	49 (28%)	21 (36.2%)	0.309
	Low	126 (72%)	37 (63.8%)	
Alkaline Phosphatase	High	32 (18.4%)	10 (17.2%)	0.968
	Normal	126 (72.4%)	43 (74.1%)	
	Low	16 (9.2%)	5 (8.6%)	
Phosphate	Normal / High	129 (81.1%)	42 (85.7%)	0.603
	Low	30 (18.9%)	7 (14.3%)	
TSH	High	11 (11.1%)	1 (3.7%)	0.085
	Normal	88 (88.9%)	25 (92.6%)	
	Low	0 0%)	1 (3.7%)	
Free T4	High	14 (14.3%)	3 (11.1%)	0.678
	Normal	82 (83.7%)	24 (88.9%)	
	Low	2 (2%)	0 (0.0%)	
PTH	High	41 (49.4%)	12 (44.4%)	0.821
	Normal	42 (50.6%)	15 (55.6%)	
Total 25OH Vitamin D	Normal	11 (12.1%)	3 (10.3%)	1.000
	Low	80 (87.9%)	26 (89.7%)	
Radiographic measurements		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
Femoral Neck Shaft Angle				0.154
	Normal	134 (68%)	50 (80.6%)	
	Coxa Valga	50 (25.4%)	9 (14.5%)	
	Coxa Vara	13 (6.6%)	3 (4.8%)	
Number of fragments (Comminution)	Simple	69 (34.3%)	11 (17.7%)	**0.045**
	Moderate	81 (40.3%)	32 (51.6%)	
	Severe	51 (25.4%)	19 (30.6%)	
Only Subtrochanteric Extension		42 (20.9%)	6 (9.7%)	0.070
Atypical		13 (6.5%)	0 (0.0%)	0.086
Pathological		10 (5%)	0 (0.0%)	0.158
Distal Extension		68 (33.8%)	49 (79.0%)	**<0.001**
Greater Trochanter Fracture		22 (10.9%)	5 (8.1%)	0.679
Lesser Trochanter Fracture		124 (61.7%)	49 (79.0%)	**0.018**
Medial Calcar Comminution		7 (3.5%)	5 (8.1%)	0.245
AO/ OTA Classification		-	-	**0.017**
Lateral Cortex Gap Size (mm)	≤4	107 (53%)	46 (74.2%)	**0.008**
	5-9	64 (31.7%)	13 (21.0%)	
	≥10	31 (15.3%)	3 (4.8%)	
Medial Cortex Gap Size (mm)	≤4	121 (59.9%)	48 (77.4%)	**0.035**
	5-9	56 (27.7%)	11 (17.7%)	
	≥10	25 (12.4%)	3 (4.8%)	
Anterior Cortex Gap Size (mm)	≤4	119 (58.9%)	51 (82.3%)	**0.004**
	5-9	50 (24.8%)	7 (11.3%)	
	≥10	33 (16.3%)	4 (6.5%)	

Radiographic measurements		No Cerclage Wiring	Cerclage Wiring	p-value
Posterior Cortex Gap Size (mm)	≤4	146 (72.3%)	47 (75.8%)	0.830
	5-9	43 (21.3%)	11 (17.7%)	
	≥10	13 (6.4%)	4 (6.5%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	133 (65.8%)	57 (91.9%)	**0.001**
	Valgus >5	14 (6.9%)	1 (1.6%)	
	Varus 5-10	42 (20.8%)	3 (4.8%)	
	Varus >10	13 (6.4%)	1 (1.6%)	
TAD (mm)	<25	159 (82%)	57 (91.9%)	0.092
	≥25	35 (18%)	5 (8.1%)	
Distal locking (Number of Screws)	1	4 (2%)	0 (0.0%)	0.599
	2	197 (98%)	62 (100.0%)	
Method of locking				0.211
Static Locking		124 (62.3%)	46 (74.2%)	
Secondary Dynamisation		74 (37.2%)	16 (25.8%)	
Dynamic		1 (0.5%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	119 (59.8%)	37 (59.7%)	0.535
	Lateral ≥5	41 (20.6%)	16 (25.8%)	
	Medial ≥5	39 (19.6%)	9 (14.5%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	164 (82.4%)	50 (80.6%)	0.789
	Anterior ≥5	29 (14.6%)	9 (14.5%)	
	Posterior ≥5	6 (3%)	3 (4.8%)	
Touching Anterior Cortex		45 (22.6%)	9 (14.5%)	0.232
Distance of tip of the nail from knee (mm)	<10	2 (1%)	2 (3.3%)	0.630
	10 to 19	14 (7%)	4 (6.6%)	
	20-29	65 (32.7%)	21 (34.4%)	
	≥30	118 (59.3%)	34 (55.7%)	
Nail / Canal Ratio		0.83 (0.07)	0.82 (0.09)	0.234
Hospital stay / Mortality		**No Cerclage Wiring**	**Cerclage Wiring**	**p-value**
HDU / ICU stay		29 (14.3%)	8 (12.9%)	0.948
Total length of hospital stay (days)		23.45 (19.44)	21.73 (17.50)	0.532
Weekend admission		63 (31%)	24 (38.7%)	0.331
Died within a year		50 (24.6%)	7 (11.3%)	**0.039**

All patients had an open reduction of their fracture
Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.5 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the presence of a fragility fracture in the past

Demographics		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Total number		437	123	-
Bilateral		14 (3.2%)	12 (9.8%)	**0.005**
Age < 65 y.o.		126 (28.8%)	11 (8.9%)	**<0.001**
Age 65 – 75 y.o		74 (16.9%)	16 (13.0%)	0.364
Age > 75 y.o.		237 (54.2%)	96 (78.0%)	**<0.001**
Gender	Male	194 (44.4%)	25 (20.3%)	**<0.001**
	Female	243 (55.6%)	98 (79.7%)	
Injury Characteristics		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Mechanism of Injury	Low energy	321 (73.5%)	112 (91.1%)	**<0.001**
	High energy	85 (19.5%)	4 (3.3%)	
	Pathological	31 (7.1%)	7 (5.7%)	
Isolated		362 (82.8%)	114 (92.7%)	**0.011**
ISS > 16		34 (7.8%)	1 (0.8%)	**0.009**
Side	Left	234 (53.5%)	61 (49.6%)	0.501
	Right	203 (46.5%)	62 (50.4%)	
Open fracture		7 (1.6%)	0 (0.0%)	0.341
Medical Comorbidities		**No previous fractures**	**Hx of fragility fractures**	**p-value**
ASA	1	49 (11.2%)	0 (0.0%)	**<0.001**
	2	117 (26.8%)	28 (22.8%)	
	3	203 (46.5%)	74 (60.2%)	
	4	68 (15.6%)	21 (17.1%)	
Charlson Comorbidity Score		4.98 (3.28%)	6.33 (2.16%)	**<0.001**
Diabetes		55 (12.6%)	22 (17.9%)	0.174
Steroids		20 (4.6%)	9 (7.3%)	0.326
Malignancy		100 (22.9%)	37 (30.1%)	0.128
Dementia		85 (19.5%)	40 (32.5%)	**0.003**
Osteoporosis		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Bisphosphonates pre-admission		61 (14%)	33 (26.8%)	**0.001**
Bisphosphonates on discharge		98 (24.1%)	38 (32.8%)	0.081
Calcium / Vitamin D pre-admission		99 (22.7%)	59 (48.0%)	**<0.001**
Calcium / Vitamin D on discharge		172 (42.4%)	79 (68.1%)	**<0.001**
Vitamin D loading on admission		63 (15.5%)	24 (20.7%)	0.239
Fragility Fractures After		67 (15.3%)	19 (15.4%)	1.000
DEXA Result	Normal	5 (15.2%)	1 (6.7%)	0.712
	Osteopenia	10 (30.3%)	5 (33.3%)	
	Osteoporosis	18 (54.5%)	9 (60.0%)	
Singh Index	1	29 (7.4%)	13 (12.1%)	**0.001**
	2	84 (21.3%)	30 (28.0%)	
	3	82 (20.8%)	25 (23.4%)	
	4	89 (22.6%)	29 (27.1%)	
	5	53 (13.5%)	9 (8.4%)	
	6	57 (14.5%)	1 (0.9%)	
Social History		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Smoking		101 (23.1%)	12 (9.8%)	**0.002**
Alcohol >10 units / week		87 (19.9%)	18 (14.6%)	0.233

Social History		No previous fractures	Hx of fragility fractures	p-value
Pre-operative Mobility				
Independent		250 (57.2%)	42 (34.1%)	**<0.001**
Stick(s) / Crutch(es)		105 (24%)	41 (33.3%)	
Frame		61 (14%)	34 (27.6%)	
Wheelchair / Hoisted		21 (4.8%)	6 (4.9%)	
Frequent falls		97 (22.2%)	56 (45.5%)	**<0.001**
Operation Characteristics		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Operation in less than 48 hours		341 (78%)	102 (82.9%)	0.292
Simultaneous procedures		32 (7.3%)	5 (4.1%)	0.280
Type of Nail	Long Affixus Nail	232 (53.1%)	87 (70.7%)	**<0.001**
	Long Gamma Nail	164 (37.5%)	34 (27.6%)	
	Others	41 (9.4%)	2 (1.6%)	
Nail Diameter (mm)	9	24 (5.5%)	7 (5.7%)	0.111
	10	12 (2.8%)	0 (0.0%)	
	11	276 (63.4%)	69 (56.6%)	
	12	1 (0.2%)	1 (0.8%)	
	13	122 (28%)	45 (36.9%)	
Open reduction		204 (46.7%)	60 (48.8%)	0.757
Use of cerclage wires		53 (12.1%)	12 (9.8%)	0.571
Post-op Mobilisation (first 6 weeks)	FWB	228 (52.2%)	79 (64.2%)	0.086
	PWB	98 (22.4%)	24 (19.5%)	
	TTWB	63 (14.4%)	10 (8.1%)	
	NWB	48 (11%)	10 (8.1%)	
Surgical time (min)		111.97 (46.03)	110.45 (41.13)	0.741
Anaesthetic Time (min)		48.63 (22.33)	47.87 (19.01)	0.730
Time from induction to recovery (min)		179.45 (50.59)	176.46 (47.27)	0.558
Level of First Surgeon				
	Registrar	261 (60%)	71 (57.7%)	0.726
	Consultant	174 (40%)	52 (42.3%)	
Level of Senior Surgeon Present				
	Registrar	238 (54.7%)	68 (55.3%)	0.992
	Consultant	197 (45.3%)	55 (44.7%)	
Complications		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Nail complications		83 (19%)	17 (13.8%)	0.234
Failure at lag screw junction		19 (4.3%)	5 (4.1%)	1.000
Self-dynamisation		21 (4.8%)	4 (3.3%)	0.624
Cut-out		11 (2.5%)	2 (1.6%)	0.810
Non-union		67 (15.3%)	16 (13.0%)	0.619
Nail infection		5 (1.1%)	0 (0.0%)	0.516
Peri-implant fracture		11 (2.5%)	3 (2.4%)	1.000
HAP / CAP		81 (18.5%)	25 (20.3%)	0.751
UTI		51 (11.7%)	27 (22.0%)	**0.006**
Wound infection	Superficial	18 (4.1%)	3 (2.4%)	0.480
	Deep	13 (3%)	2 (1.6%)	
Washout / Revision for Infection		12 (33.3%)	2 (28.6%)	1.000

Complications		No previous fractures	Hx of fragility fractures	p-value
CKD Stage pre-operatively				
	Mild	316 (73.7%)	68 (56.2%)	**<0.001**
	Moderate / Severe	113 (26.3%)	53 (43.8%)	
CKD Stage post-operatively				
	Mild	322 (75.8%)	75 (62.0%)	**0.004**
	Moderate / Severe	103 (24.2%)	46 (38.0%)	
Pre-operative Transfusion		38 (8.7%)	10 (8.1%)	0.982
Post-operative Transfusion (48 hours)		210 (48.2%)	78 (63.4%)	**0.004**
Post-operative Transfusion (total)		261 (59.9%)	92 (74.8%)	**0.003**
Hb Drop (g/L)		46.09 (19.08)	48.14 (16.86)	0.287
VTE	No	82 (80.4%)	25 (92.6%)	0.206
	DVT	10 (9.8%)	2 (7.4%)	
	PE	10 (9.8%)	0 (0.0%)	
Biochemistry		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Adjusted Calcium	Normal	263 (76.9%)	85 (73.3%)	0.507
	Low	79 (23.1%)	31 (26.7%)	
Albumin	Normal	131 (34.4%)	27 (22.3%)	**0.017**
	Low	250 (65.6%)	94 (77.7%)	
Alkaline Phosphatase	High	71 (18.8%)	25 (20.7%)	0.806
	Normal	274 (72.5%)	84 (69.4%)	
	Low	33 (8.7%)	12 (9.9%)	
Phosphate	Normal / High	278 (81%)	95 (81.2%)	1.000
	Low	65 (19%)	22 (18.8%)	
TSH	High	26 (13%)	5 (6.2%)	0.222
	Normal	170 (85%)	75 (92.6%)	
	Low	4 (2%)	1 (1.2%)	
Free T4	High	29 (14.8%)	10 (12.8%)	0.728
	Normal	162 (82.7%)	67 (85.9%)	
	Low	5 (2.6%)	1 (1.3%)	
PTH	High	82 (46.6%)	48 (62.3%)	**0.030**
	Normal	94 (53.4%)	29 (37.7%)	
Total 25OH Vitamin D	Normal	20 (10.5%)	15 (17.2%)	0.172
	Low	170 (89.5%)	72 (82.8%)	
Radiographic measurements		**No previous fractures**	**Hx of fragility fractures**	**p-value**
Femoral Neck Shaft Angle				
	Normal	281 (65.5%)	85 (70.2%)	0.490
	Coxa Valga	119 (27.7%)	27 (22.3%)	
	Coxa Vara	29 (6.8%)	9 (7.4%)	
Number of fragments (Comminution)	Simple	126 (28.9%)	37 (30.1%)	**0.038**
	Moderate	204 (46.8%)	69 (56.1%)	
	Severe	106 (24.3%)	17 (13.8%)	
Only Subtrochanteric Extension		75 (17.2%)	24 (19.5%)	0.646
Atypical		20 (4.6%)	10 (8.1%)	0.189
Pathological		27 (6.2%)	3 (2.4%)	0.160
Distal Extension		148 (33.9%)	38 (30.9%)	0.599
Greater Trochanter Fracture		53 (12.2%)	6 (4.9%)	**0.031**
Lesser Trochanter Fracture		281 (64.4%)	79 (64.2%)	1.000
Medial Calcar Comminution		24 (5.5%)	7 (5.7%)	1.000
AO/ OTA Classification		-	-	0.881

Radiographic measurements		No previous fractures	Hx of fragility fractures	p-value
Lateral Cortex Gap Size (mm)	≤4	260 (59.9%)	83 (67.5%)	0.085
	5-9	110 (25.3%)	31 (25.2%)	
	≥10	64 (14.7%)	9 (7.3%)	
Medial Cortex Gap Size (mm)	≤4	286 (65.9%)	85 (69.1%)	0.692
	5-9	108 (24.9%)	26 (21.1%)	
	≥10	40 (9.2%)	12 (9.8%)	
Anterior Cortex Gap Size (mm)	≤4	281 (64.6%)	74 (60.2%)	0.122
	5-9	88 (20.2%)	35 (28.5%)	
	≥10	66 (15.2%)	14 (11.4%)	
Posterior Cortex Gap Size (mm)	≤4	332 (76.3%)	95 (77.2%)	0.371
	5-9	75 (17.2%)	24 (19.5%)	
	≥10	28 (6.4%)	4 (3.3%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	322 (74.2%)	82 (66.7%)	0.424
	Valgus >5	27 (6.2%)	10 (8.1%)	
	Varus 5-10	20 (4.6%)	8 (6.5%)	
	Varus >10	65 (15%)	23 (18.7%)	
Antirotation Screw		159 (37.3%)	53 (43.1%)	0.293
TAD (mm)	<25	362 (85.8%)	111 (90.2%)	0.257
	≥25	60 (14.2%)	12 (9.8%)	
Distal locking (Number of Screws)	1	9 (2.1%)	9 (7.3%)	**0.009**
	2	426 (97.9%)	114 (92.7%)	
Method of locking				0.199
Static Locking		270 (62.5%)	86 (70.5%)	
Secondary Dynamisation		159 (36.8%)	36 (29.5%)	
Dynamic		3 (0.7%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	271 (63%)	83 (68.0%)	0.592
	Lateral ≥5	75 (17.4%)	18 (14.8%)	
	Medial ≥5	84 (19.5%)	21 (17.2%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	337 (78%)	96 (78.7%)	0.987
	Anterior ≥5	84 (19.4%)	23 (18.9%)	
	Posterior ≥5	11 (2.5%)	3 (2.5%)	
Touching Anterior Cortex		109 (25.3%)	27 (22.1%)	0.551
Distance of tip of the nail from knee (mm)	<10	6 (1.4%)	1 (0.8%)	0.394
	10 to 19	33 (7.7%)	15 (12.4%)	
	20-29	140 (32.6%)	40 (33.1%)	
	≥30	250 (58.3%)	65 (53.7%)	
Nail / Canal Ratio		0.83 (0.07%)	0.82 (0.09%)	0.210
Hospital stay / Mortality		**No previous fractures**	**Hx of fragility fractures**	**p-value**
HDU / ICU stay		55 (12.6%)	11 (8.9%)	0.343
Total length of hospital stay (days)		22.11 (19.18)	23.81 (16.46)	**0.004**
Weekend admission		142 (32.5%)	37 (30.1%)	0.691
Died within a year		85 (19.5%)	30 (24.4%)	0.284

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.6 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the use of bisphosphonates pre-admission

Demographics		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Total number		467	94	-
Bilateral		14 (3%)	12 (12.8%)	**<0.001**
Age < 65 y.o.		133 (28.5%)	5 (5.3%)	**<0.001**
Age 65 – 75 y.o		74 (15.8%)	16 (17.0%)	0.897
Age > 75 y.o.		260 (55.7%)	73 (77.7%)	**<0.001**
Gender	Male	203 (43.5%)	17 (18.1%)	**<0.001**
	Female	264 (56.5%)	77 (81.9%)	
Injury Characteristics		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Mechanism of Injury	Low energy	350 (74.9%)	83 (88.3%)	**0.001**
	High energy	87 (18.6%)	3 (3.2%)	
	Pathological	30 (6.4%)	8 (8.5%)	
Isolated		389 (83.3%)	87 (92.6%)	**0.034**
ISS > 16		36 (7.7%)	0 (0.0%)	**0.011**
Side	Left	248 (53.1%)	47 (50.0%)	0.662
	Right	219 (46.9%)	47 (50.0%)	
Open fracture		6 (1.3%)	1 (1.1%)	1.000
Medical Comorbidities		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
ASA	1	49 (10.5%)	0 (0.0%)	**0.004**
	2	118 (25.3%)	27 (28.7%)	
	3	232 (49.7%)	46 (48.9%)	
	4	68 (14.6%)	21 (22.3%)	
Charlson Comorbidity Score		5.08 (3.22)	6.22 (2.37)	**0.001**
Diabetes		65 (13.9%)	12 (12.8%)	0.895
Steroids		15 (3.2%)	14 (14.9%)	**<0.001**
Malignancy		108 (23.1%)	29 (30.9%)	0.145
Dementia		106 (22.7%)	19 (20.2%)	0.695
Osteoporosis		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Bisphosphonates on discharge		66 (15.1%)	70 (80.5%)	**<0.001**
Calcium / Vitamin D pre-admission		87 (18.6%)	71 (75.5%)	**<0.001**
Calcium / Vitamin D on discharge		186 (42.7%)	65 (74.7%)	**<0.001**
Vitamin D loading on admission		78 (17.9%)	9 (10.3%)	0.117
Fragility Fractures After		69 (14.8%)	17 (18.1%)	0.517
DEXA Result	Normal	5 (16.1%)	1 (5.9%)	0.181
	Osteopenia	7 (22.6%)	8 (47.1%)	
	Osteoporosis	19 (61.3%)	8 (47.1%)	
Singh Index	1	31 (7.5%)	11 (12.5%)	**0.002**
	2	95 (23%)	19 (21.6%)	
	3	92 (22.3%)	15 (17.0%)	
	4	87 (21.1%)	31 (35.2%)	
	5	51 (12.3%)	11 (12.5%)	
	6	57 (13.8%)	1 (1.1%)	
Social History		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Smoking		107 (22.9%)	6 (6.4%)	**<0.001**
Alcohol >10 units / week		92 (19.7%)	13 (13.8%)	0.235
Pre-operative Mobility				**0.027**
Independent		255 (54.6%)	38 (40.4%)	
Stick(s) / Crutch(es)		112 (24%)	34 (36.2%)	
Frame		80 (17.1%)	15 (16.0%)	
Wheelchair / Hoisted		20 (4.3%)	7 (7.4%)	
Frequent falls		110 (23.6%)	43 (45.7%)	**<0.001**

Operation Characteristics		No $PO(OH)_2$	$PO(OH)_2$	p-value
Operation in less than 48 hours		370 (79.2%)	74 (78.7%)	1.000
Simultaneous procedures		34 (7.3%)	3 (3.2%)	0.219
Type of Nail	Long Affixus Nail	265 (56.7%)	54 (57.4%)	0.342
	Long Gamma Nail	162 (34.7%)	36 (38.3%)	
	Others	40 (8.6%)	4 (4.3%)	
Nail Diameter (mm)	9	26 (5.6%)	5 (5.4%)	0.071
	10	13 (2.8%)	0 (0.0%)	
	11	295 (63.4%)	50 (53.8%)	
	12	2 (0.4%)	0 (0.0%)	
	13	129 (27.7%)	38 (40.9%)	
Open reduction		226 (48.4%)	39 (41.5%)	0.267
Use of cerclage wires		57 (12.2%)	8 (8.5%)	0.398
Post-op Mobilisation (first 6 weeks)	FWB	245 (52.5%)	62 (66.0%)	**0.021**
	PWB	102 (21.8%)	20 (21.3%)	
	TTWB	69 (14.8%)	4 (4.3%)	
	NWB	51 (10.9%)	8 (8.5%)	
Surgical time (min)		113.54 (46.52)	102.21 (35.04)	**0.026**
Anaesthetic Time (min)		47.99 (21.96)	50.81 (19.85)	0.249
Time from induction to recovery (min)		180.49 (51.02)	170.37 (42.85)	0.073
Level of First Surgeon				0.411
	Registrar	272 (58.6%)	60 (63.8%)	
	Consultant	192 (41.4%)	34 (36.2%)	
Level of Senior Surgeon Present				0.176
	Registrar	248 (53.4%)	58 (61.7%)	
	Consultant	216 (46.6%)	36 (38.3%)	
Complications		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Nail complications		86 (18.4%)	14 (14.9%)	0.505
Failure at lag screw junction		19 (4.1%)	5 (5.3%)	0.789
Self-dynamisation		20 (4.3%)	5 (5.3%)	0.865
Cut-out		10 (2.1%)	3 (3.2%)	0.809
Non-union		5 (1.1%)	0 (0.0%)	0.685
Nail infection		86 (18.4%)	14 (14.9%)	0.505
Peri-implant fracture		11 (2.4%)	3 (3.2%)	0.911
HAP / CAP		85 (18.2%)	21 (22.3%)	0.429
UTI		62 (13.3%)	16 (17.0%)	0.427
Wound infection	Superficial	17 (3.6%)	4 (4.3%)	0.903
	Deep	13 (2.8%)	2 (2.1%)	
Washout / Revision for Infection		12 (33.3%)	2 (28.6%)	1.000
CKD Stage pre-operatively				**0.024**
	Mild	328 (71.9%)	56 (59.6%)	
	Moderate / Severe	128 (28.1%)	38 (40.4%)	
CKD Stage post-operatively				0.137
	Mild	335 (74.1%)	62 (66.0%)	
	Moderate / Severe	117 (25.9%)	32 (34.0%)	
Pre-operative Transfusion		43 (9.2%)	5 (5.3%)	0.299
Post-operative Transfusion (48 hours)		237 (51%)	51 (54.3%)	0.639
Post-operative Transfusion (total)		294 (63.2%)	59 (62.8%)	1.000
Hb Drop (g/L)		45.7 (19.22)	50.6 (14.88)	0.020
VTE	No	80 (80%)	27 (93.1%)	0.254
	DVT	11 (11%)	1 (3.4%)	
	PE	9 (9%)	1 (3.4%)	

Biochemistry		No $PO(OH)_2$	$PO(OH)_2$	p-value
Adjusted Calcium	Normal	290 (77.3%)	58 (69.9%)	0.195
	Low	85 (22.7%)	25 (30.1%)	
Albumin	Normal	123 (30%)	35 (38.0%)	0.168
	Low	287 (70%)	57 (62.0%)	
Alkaline Phosphatase	High	81 (19.9%)	15 (16.5%)	0.629
	Normal	292 (71.6%)	66 (72.5%)	
	Low	35 (8.6%)	10 (11.0%)	
Phosphate	Normal / High	310 (82.2%)	63 (75.9%)	0.239
	Low	67 (17.8%)	20 (24.1%)	
TSH	High	24 (10.8%)	7 (12.1%)	0.503
	Normal	194 (87%)	51 (87.9%)	
	Low	5 (2.2%)	0 (0.0%)	
Free T4	High	30 (13.7%)	9 (16.4%)	0.422
	Normal	183 (83.6%)	46 (83.6%)	
	Low	6 (2.7%)	0 (0.0%)	
PTH	High	101 (49.3%)	29 (60.4%)	0.218
	Normal	104 (50.7%)	19 (39.6%)	
Total 25OH Vitamin D	Normal	23 (10.4%)	12 (21.8%)	**0.039**
	Low	199 (89.6%)	43 (78.2%)	
Radiographic measurements		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Femoral Neck Shaft Angle				
	Normal	300 (65.8%)	66 (70.2%)	0.661
	Coxa Valga	123 (27%)	23 (24.5%)	
	Coxa Vara	33 (7.2%)	5 (5.3%)	
Number of fragments (Comminution)	Simple	122 (26.2%)	41 (43.6%)	**0.001**
	Moderate	231 (49.7%)	42 (44.7%)	
	Severe	112 (24.1%)	11 (11.7%)	
Only Subtrochanteric Extension		67 (14.4%)	32 (34.0%)	**<0.001**
Atypical		5 (1.1%)	25 (26.6%)	**<0.001**
Pathological		28 (6%)	2 (2.1%)	0.202
Distal Extension		166 (35.7%)	20 (21.3%)	**0.010**
Greater Trochanter Fracture		54 (11.6%)	5 (5.3%)	0.104
Complications		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
Lesser Trochanter Fracture		308 (66.2%)	52 (55.3%)	0.058
Medial Calcar Comminution		27 (5.8%)	4 (4.3%)	0.725
AO/ OTA Classification		-	-	**0.001**
Lateral Cortex Gap Size (mm)	≤4	292 (62.8%)	51 (54.8%)	0.315
	5-9	113 (24.3%)	29 (31.2%)	
	≥10	60 (12.9%)	13 (14.0%)	
Medial Cortex Gap Size (mm)	≤4	311 (66.9%)	60 (64.5%)	0.897
	5-9	110 (23.7%)	24 (25.8%)	
	≥10	44 (9.5%)	9 (9.7%)	
Anterior Cortex Gap Size (mm)	≤4	299 (64.3%)	56 (59.6%)	0.629
	5-9	99 (21.3%)	24 (25.5%)	
	≥10	67 (14.4%)	14 (14.9%)	
Posterior Cortex Gap Size (mm)	≤4	358 (77%)	69 (73.4%)	0.190
	5-9	78 (16.8%)	22 (23.4%)	
	≥10	29 (6.2%)	3 (3.2%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	342 (73.5%)	62 (66.7%)	0.058
	Valgus >5	35 (7.5%)	3 (3.2%)	
	Varus 5-10	22 (4.7%)	6 (6.5%)	
	Varus >10	66 (14.2%)	22 (23.7%)	

Complications		No $PO(OH)_2$	$PO(OH)_2$	p-value
Antirotation Screw		187 (40.7%)	26 (28.6%)	**0.039**
TAD (mm)	<25	391 (86.1%)	83 (90.2%)	0.374
	≥25	63 (13.9%)	9 (9.8%)	
Distal locking (Number of Screws)	1	13 (2.8%)	5 (5.3%)	0.345
	2	452 (97.2%)	89 (94.7%)	
Method of locking				0.507
Static Locking		293 (63.4%)	63 (67.7%)	
Secondary Dynamisation		167 (36.1%)	29 (31.2%)	
Dynamic		2 (0.4%)	1 (1.1%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	296 (64.2%)	59 (64.1%)	0.982
	Lateral ≥5	78 (16.9%)	15 (16.3%)	
	Medial ≥5	87 (18.9%)	18 (19.6%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	366 (79%)	68 (73.9%)	0.463
	Anterior ≥5	85 (18.4%)	22 (23.9%)	
	Posterior ≥5	12 (2.6%)	2 (2.2%)	
Touching Anterior Cortex		111 (24%)	26 (28.3%)	0.467
Distance of tip of the nail from knee (mm)	<10	5 (1.1%)	2 (2.2%)	0.665
	10 to 19	40 (8.7%)	8 (8.8%)	
	20-29	147 (32%)	33 (36.3%)	
	≥30	268 (58.3%)	48 (52.7%)	
Nail / Canal Ratio		0.82 (0.08%)	0.84 (0.08%)	0.112
Hospital stay / Mortality		**No $PO(OH)_2$**	**$PO(OH)_2$**	**p-value**
HDU / ICU stay		60 (12.8%)	7 (7.4%)	0.194
Total length of hospital stay (days)		22.73 (19.23)	21.47 (15.28)	0.551
Weekend admission		154 (33%)	25 (26.6%)	0.276
Died within a year		101 (21.6%)	14 (14.9%)	0.182

$PO(OH)_2$: bisphosphonates
Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.7 Table presenting the demographics / characteristics of patients having their operation in LTH, with complete follow-up, stratified according presence of an atypical fracture

Demographics		**Non-atypical**	**Atypical**	**p-value**
Total number		314	25	-
Bilateral		13 (4.1%)	9 (36.0%)	**<0.001**
Age < 65 y.o.		107 (34.1%)	4 (16.0%)	0.103
Age 65 – 75 y.o		50 (15.9%)	9 (36.0%)	**0.023**
Age > 75 y.o.		157 (50.0%)	12 (48.0%)	1.000
Gender	Male	132 (42.0%)	2 (8.0%)	**0.002**
	Female	182 (58.0%)	23 (92.0%)	
Injury Characteristics		**Non-atypical**	**Atypical**	**p-value**
Mechanism of Injury	Low energy	234 (74.5%)	25 (100.0%)	-
	High energy	69 (22.0%)	0 (0.0%)	
	Pathological	11 (3.5%)	0 (0.0%)	
Isolated		260 (82.8%)	24 (96.0%)	0.150
ISS > 16		26 (8.3%)	0 (0.0%)	0.268
Side	Left	163 (51.9%)	10 (40.0%)	0.348
	Right	151 (48.1%)	15 (60.0%)	
Open fracture		7 (2.2%)	0 (0.0%)	0.981
Medical Comorbidities		**Non-atypical**	**Atypical**	**p-value**
ASA	1	44 (14.0%)	0 (0.0%)	0.218
	2	91 (29.0%)	7 (28.0%)	
	3	142 (45.2%)	14 (56.0%)	
	4	37 (11.8%)	4 (16.0%)	
Charlson Comorbidity Score		4.55 (3.11)	5.56 (2.58)	0.115
Diabetes		43 (13.7%)	4 (16.0%)	0.984
Steroids		8 (2.5%)	10 (40.0%)	**<0.001**
Malignancy		63 (20.1%)	12 (48.0%)	**0.003**
Dementia		43 (13.7%)	2 (8.0%)	0.616
Osteoporosis		**Non-atypical**	**Atypical**	**p-value**
Bisphosphonates pre-admission		69 (13.0%)	25 (83.3%)	**<0.001**
Bisphosphonates on discharge		78 (25.0%)	16 (64.0%)	**<0.001**
Calcium / Vitamin D pre-admission		77 (24.5%)	17 (68.0%)	**<0.001**
Calcium / Vitamin D on discharge		140 (44.9%)	17 (68.0%)	**0.043**
Vitamin D loading on admission		41 (13.1%)	1 (4.0%)	0.309
Fragility Fractures Before		52 (16.6%)	9 (36.0%)	**0.030**
Fragility Fractures After		60 (19.1%)	10 (40.0%)	**0.026**
DEXA Result	Normal	4 (11.1%)	1 (12.5%)	0.143
	Osteopenia	10 (27.8%)	5 (62.5%)	
	Osteoporosis	22 (61.1%)	2 (25.0%)	
Singh Index	1	27 (9.5%)	0 (0.0%)	**0.002**
	2	59 (20.7%)	5 (21.7%)	
	3	58 (20.4%)	3 (13.0%)	
	4	59 (20.7%)	13 (56.5%)	
	5	34 (11.9%)	2 (8.7%)	
	6	48 (16.8%)	0 (0.0%)	
Social History		**Non-atypical**	**Atypical**	**p-value**
Smoking		73 (23.2%)	1 (4.0%)	**0.047**
Alcohol >10 units / week		68 (21.7%)	4 (16.0%)	0.681

Pre-operative Mobility			
Independent	173 (55.1%)	17 (68.0%)	0.394
Stick(s) / Crutch(es)	91 (29.0%)	7 (28.0%)	
Frame	37 (11.8%)	1 (4.0%)	
Wheelchair / Hoisted	13 (4.1%)	0 (0.0%)	
Frequent falls	83 (26.4%)	5 (20.0%)	0.639
Operation Characteristics	**Non-atypical**	**Atypical**	**p-value**
Operation in less than 48 hours	247 (78.7%)	19 (76.0%)	0.953
Simultaneous procedures	31 (9.9%)	0 (0.0%)	0.198
Type of Nail Long Affixus Nail	157 (50.0%)	13 (52.0%)	0.672
Long Gamma Nail	128 (40.8%)	11 (44.0%)	
Others	29 (9.2%)	1 (4.0%)	
Nail Diameter (mm) 9	17 (5.4%)	3 (12.5%)	0.426
10	7 (2.2%)	0 (0.0%)	
11	202 (64.7%)	16 (66.7%)	
13	86 (27.6%)	5 (20.8%)	
Open reduction	150 (47.8%)	12 (48.0%)	1.000
Use of cerclage wires	44 (14.0%)	0 (0.0%)	0.090
Post-op Mobilisation (first 6 weeks) FWB	141 (44.9%)	16 (64.0%)	0.268
PWB	82 (26.1%)	4 (16.0%)	
TTWB	54 (17.2%)	2 (8.0%)	
NWB	37 (11.8%)	3 (12.0%)	
Surgical time (min)	113.62 (45.20)	113.68 (39.49)	0.995
Anaesthetic Time (min)	47.42 (22.91)	53.8 (23.66)	0.182
Time from induction to recovery (min)	180.74 (50.79)	184.4 (45.76)	0.727
Level of First Surgeon			
Registrar	192 (61.3%)	15 (60.0%)	1.000
Consultant	121 (38.7%)	10 (40.0%)	
Level of Senior Surgeon Present			
Registrar	176 (56.2%)	15 (60.0%)	0.876
Consultant	137 (43.8%)	10 (40.0%)	
Complications	**Non-atypical**	**Atypical**	**p-value**
Nail complications	82 (26.1%)	3 (12.0%)	0.184
Failure at lag screw junction	22 (7.0%)	2 (8.0%)	1.000
Self-dynamisation	20 (6.4%)	2 (8.0%)	1.000
Cut-out	7 (2.2%)	1 (4.0%)	1.000
Nail infection	5 (1.6%)	0 (0.0%)	1.000
Peri-implant fracture	9 (2.9%)	0 (0.0%)	0.832
HAP / CAP	45 (14.3%)	2 (8.0%)	0.561
UTI	40 (12.7%)	5 (20.0%)	0.469
Wound infection Superficial	10 (3.2%)	2 (8.0%)	0.290
Deep	12 (3.8%)	0 (0.0%)	
Washout / Revision for Infection	6 (8.8%)	0 (0.0%)	0.855
CKD Stage pre-operatively			
Mild	225 (73.3%)	14 (56.0%)	0.105
Moderate / Severe	82 (26.7%)	11 (44.0%)	
CKD Stage post-operatively			
Mild	230 (75.9%)	18 (72.0%)	0.845
Moderate / Severe	73 (24.1%)	7 (28.0%)	
Pre-operative Transfusion	25 (8.0%)	0 (0.0%)	0.285
Post-operative Transfusion (48 hours)	157 (50.0%)	6 (24.0%)	**0.022**
Post-operative Transfusion (total)	195 (62.1%)	8 (32.0%)	**0.006**

Complications		Non-atypical	Atypical	p-value
Hb Drop (g/L)		44.43 (18.49)	52.48 (13.21)	**0.034**
VTE	No	61 (84.7%)	5 (100.0%)	
	DVT	6 (8.3%)	0 (0.0%)	0.640
	PE	5 (6.9%)	0 (0.0%)	
Biochemistry		**Non-atypical**	**Atypical**	**p-value**
Adjusted Calcium	Normal	176 (74.6%)	17 (73.9%)	1.000
	Low	60 (25.4%)	6 (26.1%)	
Albumin	Normal	98 (36.4%)	16 (64.0%)	**0.013**
	Low	171 (63.6%)	9 (36.0%)	
Alkaline Phosphatase	High	57 (21.4%)	2 (8.0%)	
	Normal	193 (72.6%)	20 (80.0%)	0.177
	Low	16 (6.0%)	3 (12.0%)	
Phosphate	Normal / High	196 (82.4%)	20 (87.0%)	0.788
	Low	42 (17.6%)	3 (13.0%)	
TSH	High	13 (9.5%)	1 (7.7%)	
	Normal	122 (89.1%)	12 (92.3%)	0.885
	Low	2 (1.5%)	0 (0.0%)	
Free T4	High	21 (15.4%)	0 (0.0%)	
	Normal	112 (82.4%)	12 (100.0%)	0.283
	Low	3 (2.2%)	0 (0.0%)	
PTH	High	56 (46.7%)	9 (50.0%)	0.991
	Normal	64 (53.3%)	9 (50.0%)	
Total 25OH Vitamin D	Normal	14 (10.4%)	7 (38.9%)	**0.003**
	Low	121 (89.6%)	11 (61.1%)	
Radiographic measurements		**Non-atypical**	**Atypical**	**p-value**
Femoral Neck Shaft Angle				
	Normal	210 (67.7%)	17 (68.0%)	0.576
	Coxa Valga	88 (28.4%)	6 (24.0%)	
	Coxa Vara	12 (3.9%)	2 (8.0%)	
Number of fragments (Comminution)	Simple	81 (25.8%)	21 (84.0%)	
	Moderate	153 (48.7%)	2 (8.0%)	**<0.001**
	Severe	80 (25.5%)	2 (8.0%)	
Only Subtrochanteric Extension		40 (12.7%)	20 (80.0%)	**<0.001**
Pathological		11 (3.5%)	0 (0.0%)	0.715
Distal Extension		128 (40.8%)	1 (4.0%)	**0.001**
Greater Trochanter Fracture		33 (10.5%)	0 (0.0%)	0.175
Lesser Trochanter Fracture		211 (67.2%)	0 (0%)	<0.001
Medial Calcar Comminution		21 (6.7%)	0 (0%)	0.918
AO/ OTA Classification		-	-	**<0.001**
Lateral Cortex Gap Size (mm)	≤4	185 (58.9%)	13 (52.0%)	
	5-9	83 (26.4%)	10 (40.0%)	0.291
	≥10	46 (14.6%)	2 (8.0%)	
Medial Cortex Gap Size (mm)	≤4	212 (67.5%)	15 (60.0%)	
	5-9	69 (22.0%)	9 (36.0%)	0.207
	≥10	33 (10.5%)	1 (4.0%)	
Anterior Cortex Gap Size (mm)	≤4	199 (63.4%)	14 (56.0%)	
	5-9	69 (22.0%)	5 (20.0%)	0.458
	≥10	46 (14.6%)	6 (24.0%)	
Posterior Cortex Gap Size (mm)	≤4	230 (73.2%)	17 (68.0%)	
	5-9	59 (18.8%)	7 (28.0%)	0.456
	≥10	25 (8.0%)	1 (4.0%)	

Radiographic measurements		Non-atypical	Atypical	p-value
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	224 (71.3%)	17 (68.0%)	0.488
	Valgus >5	19 (6.1%)	0 (0.0%)	
	Varus 5-10	54 (17.2%)	6 (24.0%)	
	Varus >10	17 (5.4%)	2 (8.0%)	
Antirotation Screw		113 (36.7%)	2 (8.3%)	**0.010**
TAD (mm)	<25	259 (85.5%)	23 (92.0%)	0.547
	≥25	44 (14.5%)	2 (8.0%)	
Distal locking (Number of Screws)	1	9 (2.9%)	3 (12.0%)	0.069
	2	305 (97.1%)	22 (88.0%)	
Method of locking				0.861
Static Locking		205 (65.7%)	16 (64.0%)	
Secondary Dynamisation		104 (33.3%)	9 (36.0%)	
Dynamic		3 (1.0%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	197 (63.1%)	19 (76.0%)	0.284
	Lateral ≥5	65 (20.8%)	2 (8.0%)	
	Medial ≥5	50 (16.0%)	4 (16.0%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	251 (80.4%)	19 (76.0%)	0.589
	Anterior ≥5	55 (17.6%)	6 (24.0%)	
	Posterior ≥5	6 (1.9%)	0 (0.0%)	
Distance of tip of the nail from knee (mm)	<10	3 (1.0%)	0 (0.0%)	0.801
	10 to 19	24 (7.7%)	1 (4.0%)	
	20-29	98 (31.4%)	7 (28.0%)	
	≥30	187 (59.9%)	17 (68.0%)	
Nail / Canal Ratio		0.82 (0.08)	0.87 (0.06)	**0.003**
Hospital stay / Mortality		**Non-atypical**	**Atypical**	**p-value**
HDU / ICU stay		36 (11.5%)	1 (4.0%)	0.413
Total length of hospital stay (days)		21.31 (18.96)	17.4 (21.19)	0.326
Weekend admission		106 (33.8%)	7 (28.0%)	0.713
Died within a year		18 (5.7%)	0 (0.0%)	0.443

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.8 Table presenting the demographics / characteristics of patients having their operation in LTH, with complete follow-up, stratified according to the type of nail used

Demographics		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Total number		139	170	-
Bilateral		8 (5.8%)	12 (7.1%)	0.817
Age < 65 y.o.		43 (30.9%)	42 (24.7%)	0.275
Age 65 – 75 y.o		26 (18.7%)	32 (18.8%)	1.000
Age > 75 y.o.		70 (50.4%)	96 (56.5%)	0.339
Gender	Male	49 (35.3%)	62 (36.5%)	0.918
	Female	90 (64.7%)	108 (63.5%)	
Injury Characteristics		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Mechanism of Injury	Low energy	117 (84.2%)	127 (74.7%)	0.064
	High energy	18 (12.9%)	29 (17.1%)	
	Pathological	4 (2.9%)	14 (8.2%)	
Isolated		122 (87.8%)	144 (84.7%)	0.543
ISS > 16		7 (5.0%)	11 (6.5%)	0.771
Side	Left	69 (49.6%)	92 (54.1%)	0.503
	Right	70 (50.4%)	78 (45.9%)	
Open fracture		1 (0.7%)	2 (1.2%)	1.000
Medical Comorbidities		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
ASA	1	13 (9.4%)	15 (8.8%)	0.466
	2	43 (30.9%)	50 (29.4%)	
	3	61 (43.9%)	87 (51.2%)	
	4	22 (15.8%)	18 (10.6%)	
Charlson Comorbidity Score		5.09 (2.98)	4.87 (2.92)	0.523
Diabetes		19 (13.7%)	27 (15.9%)	0.702
Steroids		6 (4.3%)	11 (6.5%)	0.565
Malignancy		35 (25.2%)	37 (21.8%)	0.568
Dementia		23 (16.5%)	21 (12.4%)	0.376
Osteoporosis		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Bisphosphonates pre-admission		29 (20.9%)	34 (20.0%)	0.964
Bisphosphonates on discharge		50 (36.2%)	40 (23.7%)	**0.023**
Calcium / Vitamin D pre-admission		40 (28.8%)	53 (31.2%)	0.739
Calcium / Vitamin D on discharge		79 (57.2%)	76 (45.0%)	**0.043**
Vitamin D loading on admission		3 (2.2%)	39 (23.1%)	**<0.001**
Fragility Fractures Before		19 (13.7%)	42 (24.7%)	**0.023**
Fragility Fractures After		38 (27.3%)	29 (17.1%)	**0.041**
DEXA Result	Normal	1 (5.6%)	2 (8.7%)	0.641
	Osteopenia	8 (44.4%)	7 (30.4%)	
	Osteoporosis	9 (50.0%)	14 (60.9%)	
Singh Index	1	14 (11.1%)	13 (8.3%)	0.475
	2	27 (21.4%)	36 (23.1%)	
	3	32 (25.4%)	28 (17.9%)	
	4	28 (22.2%)	43 (27.6%)	
	5	10 (7.9%)	19 (12.2%)	
	6	15 (11.9%)	17 (10.9%)	

Social History		Long Gamma Nail	Long Affixus Nail	p-value
Smoking		31 (22.3%)	31 (18.2%)	0.456
Alcohol >10 units / week		26 (18.7%)	37 (21.8%)	0.602
Pre-operative Mobility				
	Independent	76 (54.7%)	85 (50.0%)	
	Stick(s) / Crutch(es)	46 (33.1%)	52 (30.6%)	0.394
	Frame	13 (9.4%)	24 (14.1%)	
	Wheelchair / Hoisted	4 (2.9%)	9 (5.3%)	
Frequent falls		46 (33.1%)	39 (22.9%)	0.063
Operation Characteristics		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Operation in less than 48 hours		93 (66.9%)	152 (89.4%)	**<0.001**
Simultaneous procedures		10 (7.2%)	13 (7.6%)	1.000
Nail Diameter (mm)	9	0 (0.0%)	14 (8.3%)	**0.001**
	11	99 (72.3%)	103 (60.9%)	
	13	38 (27.7%)	52 (30.8%)	
Open reduction		67 (48.2%)	81 (47.6%)	1.000
Use of cerclage wires		20 (14.4%)	23 (13.5%)	0.959
Post-op Mobilisation (first 6 weeks)	FWB	58 (41.7%)	93 (54.7%)	0.129
	PWB	41 (29.5%)	35 (20.6%)	
	TTWB	25 (18.0%)	25 (14.7%)	
	NWB	15 (10.8%)	17 (10.0%)	
Surgical time (min)		107.96 (47.20)	113.86 (40.37)	0.237
Anaesthetic Time (min)		45.58 (26.72)	51.65 (18.81)	**0.020**
Time from induction to recovery (min)		175.42 (54.09)	183.32 (45.69)	0.166
Level of First Surgeon				
	Registrar	86 (61.9%)	106 (62.7%)	0.972
	Consultant	53 (38.1%)	63 (37.3%)	
Level of Senior Surgeon Present				
	Registrar	79 (56.8%)	99 (58.6%)	0.847
	Consultant	60 (43.2%)	70 (41.4%)	
Complications		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Nail complications		45 (32.4%)	33 (19.4%)	**0.013**
Failure at lag screw junction		12 (8.6%)	12 (7.1%)	0.764
Self-dynamisation		13 (9.4%)	7 (4.1%)	0.103
Cut-out		5 (3.6%)	2 (1.2%)	0.299
Non-union		38 (27.3%)	36 (21.2%)	0.259
Nail infection		4 (2.9%)	1 (0.6%)	0.257
Peri-implant fracture		5 (3.6%)	3 (1.8%)	0.516
HAP / CAP		17 (12.2%)	28 (16.5%)	0.374
UTI		13 (9.4%)	30 (17.6%)	0.054
Wound infection	Superficial	5 (3.6%)	5 (2.9%)	0.948
	Deep	5 (3.6%)	6 (3.5%)	
Washout / Revision for Infection		6 (46.2%)	4 (36.4%)	0.945
CKD Stage pre-operatively				
	Mild	85 (63.0%)	126 (75.0%)	**0.032**
	Moderate / Severe	50 (37.0%)	42 (25.0%)	
CKD Stage post-operatively				
	Mild	99 (74.4%)	121 (72.5%)	0.799
	Moderate / Severe	34 (25.6%)	46 (27.5%)	
Pre-operative Transfusion		12 (8.6%)	10 (5.9%)	0.476

Complications		Long Gamma Nail	Long Affixus Nail	p-value
Post-operative Transfusion (48 hours)		74 (53.2%)	78 (45.9%)	0.241
Post-operative Transfusion (total)		91 (65.5%)	99 (58.2%)	0.237
Hb Drop (g/L)		44.29 (18.90)	42.40 (17.34)	0.366
VTE	No DVT PE	30 (85.7%) 3 (8.6%) 2 (5.7%)	31 (83.8%) 3 (8.1%) 3 (8.1%)	0.923
Biochemistry		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Adjusted Calcium	Normal Low	63 (63.0%) 37 (37.0%)	122 (83.6%) 24 (16.4%)	**<0.001**
Albumin	Normal Low	54 (47.0%) 61 (53.0%)	51 (32.1%) 108 (67.9%)	**0.018**
Alkaline Phosphatase	High Normal Low	31 (27.0%) 84 (73.0%) 0 (0.0%)	27 (17.2%) 113 (72.0%) 17 (10.8%)	**<0.001**
Phosphate	Normal / High Low	83 (82.2%) 18 (17.8%)	124 (83.8%) 24 (16.2%)	0.873
TSH	High Normal Low	8 (16.7%) 40 (83.3%) 0 (0.0%)	5 (5.3%) 88 (92.6%) 2 (2.1%)	0.053
Free T4	High Normal Low	10 (20.8%) 37 (77.1%) 1 (2.1%)	11 (11.8%) 81 (87.1%) 1 (1.1%)	0.312
PTH	High Normal	8 (32.0%) 17 (68.0%)	57 (51.4%) 54 (48.6%)	0.126
Total 25OH Vitamin D	Normal Low	4 (16.7%) 20 (83.3%)	17 (13.4%) 110 (86.6%)	0.917
Radiographic measurements		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
Femoral Neck Shaft Angle	Normal Coxa Valga Coxa Vara	91 (65.5%) 39 (28.1%) 9 (6.5%)	118 (70.7%) 44 (26.3%) 5 (3.0%)	0.303
Number of fragments (Comminution)	Simple Moderate Severe	37 (26.6%) 64 (46.0%) 38 (27.3%)	53 (31.2%) 83 (48.8%) 34 (20.0%)	0.296
Only Subtrochanteric Extension		19 (13.7%)	31 (18.2%)	0.353
Atypical		11 (7.9%)	13 (17.6%)	1.000
Pathological		3 (2.2%)	8 (4.7%)	0.371
Distal Extension		60 (43.2%)	56 (32.9%)	0.084
Greater Trochanter Fracture		13 (9.4%)	19 (11.2%)	0.737
Lesser Trochanter Fracture		93 (66.9%)	109 (64.1%)	0.695
Medial Calcar Comminution		10 (7.2%)	12 (7.1%)	1.000
AO/ OTA Classification		-	-	**0.040**
Lateral Cortex Gap Size (mm)	≤4 5-9 ≥10	69 (49.6%) 48 (34.5%) 22 (15.8%)	114 (67.1%) 38 (22.4%) 18 (10.6%)	**0.008**
Medial Cortex Gap Size (mm)	≤4 5-9 ≥10	98 (70.5%) 29 (20.9%) 12 (8.6%)	110 (64.7%) 40 (23.5%) 20 (11.8%)	0.509

Radiographic measurements		Long Gamma Nail	Long Affixus Nail	p-value
Anterior Cortex Gap Size (mm)	≤4	89 (64.0%)	110 (64.7%)	0.713
	5-9	27 (19.4%)	37 (21.8%)	
	≥10	23 (16.5%)	23 (13.5%)	
Posterior Cortex Gap Size (mm)	≤4	98 (70.5%)	136 (80.0%)	0.077
	5-9	29 (20.9%)	28 (16.5%)	
	≥10	12 (8.6%)	6 (3.5%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	96 (69.1%)	122 (71.8%)	0.396
	Valgus >5	5 (3.6%)	12 (7.1%)	
	Varus 5-10	9 (6.5%)	9 (5.3%)	
	Varus >10	29 (20.9%)	27 (15.9%)	
TAD (mm)	<25	124 (89.2%)	147 (87.0%)	0.673
	≥25	15 (10.8%)	22 (13.0%)	
Distal locking (Number of Screws)	1	4 (2.9%)	7 (4.1%)	0.782
	2	135 (97.1%)	163 (95.9%)	
Method of locking				
Static Locking		86 (61.9%)	115 (68.0%)	0.526
Secondary Dynamisation		52 (37.4%)	53 (31.4%)	
Dynamic		1 (0.7%)	1 (0.6%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	85 (61.2%)	111 (65.7%)	0.706
	Lateral ≥5	30 (21.6%)	33 (19.5%)	
	Medial ≥5	24 (17.3%)	25 (14.8%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	106 (76.3%)	138 (81.7%)	0.327
	Anterior ≥5	31 (22.3%)	27 (16.0%)	
	Posterior ≥5	2 (1.4%)	4 (2.4%)	
Touching Anterior Cortex		51 (36.7%)	33 (19.5%)	**0.001**
Distance of tip of the nail from knee (mm)	<10	1 (0.7%)	1 (0.6%)	0.255
	10 to 19	7 (5.0%)	16 (9.5%)	
	20-29	39 (28.1%)	57 (33.7%)	
	≥30	92 (66.2%)	95 (56.2%)	
Nail / Canal Ratio		0.83 (0.08%)	0.82 (0.07%)	0.177
Hospital stay / Mortality		**Long Gamma Nail**	**Long Affixus Nail**	**p-value**
HDU / ICU stay		10 (7.2%)	22 (12.9%)	0.144
Total length of hospital stay (days)		22.55 (19.14)	21.11 (19.97)	0.519
Weekend admission		46 (33.1%)	57 (33.5%)	0.936
Died within a year		10 (7.2%)	7 (4.1%)	0.353

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.9 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the need for blood transfusion (RBC) within 48 hours post-operatively

Demographics		**No Tx**	**Tx within 48h**	**p-value**
Total number		271	288	-
Bilateral		16 (5.9%)	10 (3.5%)	0.245
Age < 65 y.o.		84 (31.0%)	53 (18.4%)	**0.001**
Age 65 – 75 y.o		52 (19.2%)	38 (13.2%)	0.070
Age > 75 y.o.		135 (49.8%)	197 (68.4%)	**<0.001**
Gender	Male	120 (44.3%)	98 (34%)	**0.017**
	Female	151 (55.7%)	190 (66%)	
Injury Characteristics		**No Tx**	**Tx within 48h**	**p-value**
Mechanism of Injury	Low energy	201 (74.2%)	231 (80.2%)	0.171
	High energy	47 (17.3%)	42 (14.6%)	
	Pathological	23 (8.5%)	15 (5.2%)	
Isolated		229 (84.5%)	246 (85.4%)	0.854
ISS > 16		16 (5.9%)	19 (6.6%)	0.870
Side	Left	147 (54.2%)	148 (51.4%)	0.555
	Right	124 (45.8%)	140 (48.6%)	
Open fracture		4 (1.5%)	3 (1%)	0.935
Medical Comorbidities		**No Tx**	**Tx within 48h**	**p-value**
ASA	1	30 (11.1%)	19 (6.6%)	**<0.001**
	2	87 (32.1%)	58 (20.1%)	
	3	123 (45.4%)	154 (53.5%)	
	4	31 (11.4%)	57 (19.8%)	
Charlson Comorbidity Score		4.66 (3.07)	5.83 (3.04)	**<0.001**
Diabetes		29 (10.7%)	48 (16.7%)	0.055
Steroids		17 (6.3%)	12 (4.2%)	0.352
Malignancy		76 (28.0%)	60 (20.8%)	0.059
Dementia		55 (20.3%)	69 (24%)	0.347
Osteoporosis		**No Tx**	**Tx within 48h**	**p-value**
Bisphosphonates pre-admission		43 (15.9%)	51 (17.7%)	0.639
Bisphosphonates on discharge		68 (26.3%)	68 (26%)	1.000
Calcium / Vitamin D pre-admission		65 (24.0%)	93 (32.3%)	**0.037**
Calcium / Vitamin D on discharge		115 (44.4%)	136 (51.9%)	0.104
Vitamin D loading on admission		38 (14.7%)	49 (18.7%)	0.264
Fragility Fractures Before		45 (16.6%)	78 (27.1%)	**0.004**
Fragility Fractures After		39 (14.4%)	47 (16.3%)	0.607
DEXA Result	Normal	4 (14.8%)	2 (9.5%)	0.851
	Osteopenia	8 (29.6%)	7 (33.3%)	
	Osteoporosis	15 (55.6%)	12 (57.1%)	
Singh Index	1	21 (8.6%)	21 (8.2%)	0.435
	2	55 (22.6%)	59 (23.0%)	
	3	47 (19.3%)	59 (23.0%)	
	4	62 (25.5%)	56 (21.8%)	
	5	25 (10.3%)	37 (14.4%)	
	6	33 (13.6%)	25 (9.7%)	
Social History		**No Tx**	**Tx within 48h**	**p-value**
Smoking		66 (24.4%)	47 (16.3%)	**0.024**
Alcohol >10 units / week		64 (23.6%)	41 (14.2%)	**0.006**

Social History		No Tx	Tx within 48h	p-value
Pre-operative Mobility				
	Independent	168 (62.0%)	123 (42.7%)	
	Stick(s) / Crutch(es)	64 (23.6%)	82 (28.5%)	**<0.001**
	Frame	29 (10.7%)	66 (22.9%)	
	Wheelchair / Hoisted	10 (3.7%)	17 (5.9%)	
Frequent falls		215 (79.3%)	191 (66.3%)	**0.001**
Operation Characteristics		**No Tx**	**Tx within 48h**	**p-value**
Operation in less than 48 hours		214 (79.0%)	228 (79.2%)	1.000
Simultaneous procedures		15 (5.5%)	22 (7.6%)	0.407
Type of Nail	Long Affixus Nail	158 (58.3%)	161 (55.9%)	
	Long Gamma Nail	89 (32.8%)	108 (37.5%)	0.382
	Others	24 (8.9%)	19 (6.6%)	
Nail Diameter	9	17 (6.3%)	14 (4.9%)	
(mm)	10	5 (1.9%)	7 (2.4%)	
	11	167 (61.9%)	177 (61.9%)	0.945
	12	1 (0.4%)	1 (0.3%)	
	13	80 (29.6%)	87 (30.4%)	
Open reduction		105 (38.7%)	159 (55.2%)	**<0.001**
Use of cerclage wires		20 (7.4%)	45 (15.6%)	**0.004**
Post-op Mobilisation	FWB	150 (55.4%)	156 (54.2%)	
(first 6 weeks)	PWB	57 (21.0%)	65 (22.6%)	0.471
	TTWB	40 (14.8%)	33 (11.5%)	
	NWB	24 (8.9%)	34 (11.8%)	
Surgical time (min)		106.56 (43.60)	116.38 (45.77)	**0.010**
Anaesthetic Time (min)		48.07 (23.56)	48.83 (19.67)	0.677
Time from induction to recovery (min)		172.36 (46.28)	184.82 (52.35)	**0.003**
Level of First Surgeon				
	Registrar	158 (58.5%)	174 (60.4%)	0.711
	Consultant	112 (41.5%)	114 (39.6%)	
Level of Senior Surgeon Present				
	Registrar	152 (56.3%)	154 (53.5%)	0.559
	Consultant	118 (43.7%)	134 (46.5%)	
Complications		**No Tx**	**Tx within 48h**	**p-value**
Nail complications		44 (16.2%)	56 (19.4%)	0.380
Failure at lag screw junction		14 (5.2%)	10 (3.5%)	0.436
Self-dynamisation		6 (2.2%)	19 (6.6%)	**0.021**
Cut-out		8 (3.0%)	5 (1.7%)	0.501
Non-union		41 (15.1%)	42 (14.6%)	0.950
Nail infection		1 (0.4%)	4 (1.4%)	0.406
Peri-implant fracture		5 (1.8%)	9 (3.1%)	0.486
HAP / CAP		42 (15.5%)	63 (21.9%)	0.069
UTI		42 (15.5%)	36 (12.5%)	0.368
Wound infection	Superficial	10 (3.7%)	11 (3.8%)	**0.022**
	Deep	2 (0.7%)	13 (4.5%)	
Washout / Revision for Infection		1 (7.7%)	13 (43.3%)	0.053
CKD Stage pre-operatively				
	Mild	219 (83.3%)	164 (57.3%)	**<0.001**
	Moderate / Severe	44 (16.7%)	122 (42.7%)	
CKD Stage post-operatively				
	Mild	223 (86.4%)	173 (60.3%)	**<0.001**
	Moderate / Severe	35 (13.6%)	114 (39.7%)	
Pre-operative Transfusion		19 (7.0%)	29 (10.1%)	0.255

Complications		No Tx	Tx within 48h	p-value
Post-operative Transfusion (total)		65 (24.0%)	288 (100%)	**<0.001**
Hb Drop (g/L)		49.12 (16.44)	44.26 (20.16)	**0.002**
VTE	No	49 (87.5%)	58 (79.5%)	0.287
	DVT	5 (8.9%)	7 (9.6%)	
	PE	2 (3.6%)	8 (11%)	
Biochemistry		**No Tx**	**Tx within 48h**	**p-value**
Adjusted Calcium	Normal	164 (79.6%)	183 (72.9%)	0.119
	Low	42 (20.4%)	68 (27.1%)	
Albumin	Normal	94 (40.9%)	63 (23.2%)	**<0.001**
	Low	136 (59.1%)	208 (76.8%)	
Alkaline Phosphatase	High	46 (20.2%)	50 (18.5%)	0.785
	Normal	160 (70.2%)	197 (73%)	
	Low	22 (9.6%)	23 (8.5%)	
Phosphate	Normal / High	169 (81.6%)	203 (80.6%)	0.860
	Low	38 (18.4%)	49 (19.4%)	
TSH	High	16 (12.2%)	15 (10.1%)	0.817
	Normal	113 (86.3%)	131 (87.9%)	
	Low	2 (1.5%)	3 (2%)	
Free T4	High	15 (11.8%)	24 (16.4%)	0.166
	Normal	111 (87.4%)	117 (80.1%)	
	Low	1 (0.8%)	5 (3.4%)	
PTH	High	59 (50.9%)	64 (46.7%)	0.595
	Normal	57 (49.1%)	73 (53.3%)	
Total 25OH Vitamin D	Normal	18 (13.6%)	17 (11.7%)	0.766
	Low	114 (86.4%)	128 (88.3%)	
Radiographic measurements		**No Tx**	**Tx within 48h**	**p-value**
Femoral Neck Shaft Angle				0.813
	Normal	175 (65.3%)	190 (67.6%)	
	Coxa Valga	73 (27.2%)	73 (26%)	
	Coxa Vara	20 (7.5%)	18 (6.4%)	
Number of fragments (Comminution)	Simple	91 (33.6%)	72 (25.1%)	**0.048**
	Moderate	129 (47.6%)	143 (49.8%)	
	Severe	51 (18.8%)	72 (25.1%)	
Only Subtrochanteric Extension		60 (22.1%)	39 (13.6%)	**0.011**
Atypical		21 (7.7%)	9 (3.1%)	**0.026**
Pathological		17 (6.3%)	13 (4.5%)	0.469
Distal Extension		79 (29.2%)	107 (37.3%)	0.052
Greater Trochanter Fracture		28 (10.3%)	31 (10.8%)	0.966
Lesser Trochanter Fracture		150 (55.4%)	209 (72.8%)	**<0.001**
Medial Calcar Comminution		16 (5.9%)	15 (5.2%)	0.869
AO/ OTA Classification		-	-	0.601
Lateral Cortex Gap Size (mm)	≤4	174 (64.4%)	168 (58.7%)	0.384
	5-9	63 (23.3%)	78 (27.3%)	
	≥10	33 (12.2%)	40 (14%)	
Medial Cortex Gap Size (mm)	≤4	183 (67.8%)	187 (65.4%)	0.464
	5-9	66 (24.4%)	68 (23.8%)	
	≥10	21 (7.8%)	31 (10.8%)	
Anterior Cortex Gap Size (mm)	≤4	175 (64.8%)	179 (62.4%)	0.354
	5-9	53 (19.6%)	70 (24.4%)	
	≥10	42 (15.6%)	38 (13.2%)	
Posterior Cortex Gap Size (mm)	≤4	213 (78.9%)	213 (74.2%)	0.401
	5-9	44 (16.3%)	55 (19.2%)	
	≥10	13 (4.8%)	19 (6.6%)	

Radiographic measurements		No Tx	Tx within 48h	p-value
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	205 (75.9%)	198 (69.2%)	0.288
	Valgus >5	16 (5.9%)	21 (7.3%)	
	Varus 5-10	10 (3.7%)	18 (6.3%)	
	Varus >10	39 (14.4%)	49 (17.1%)	
Antirotation Screw		93 (35.4%)	119 (41.8%)	0.148
TAD (mm)	<25	224 (86.2%)	249 (87.7%)	0.690
	≥25	36 (13.8%)	35 (12.3%)	
Distal locking (Number of Screws)	1	12 (4.4%)	6 (2.1%)	0.189
	2	259 (95.6%)	280 (97.9%)	
Method of locking				0.601
Static Locking		168 (62.7%)	188 (66%)	
Secondary Dynamisation		99 (36.9%)	95 (33.3%)	
Dynamic		1 (0.4%)	2 (0.7%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	172 (64.4%)	181 (63.7%)	0.913
	Lateral ≥5	46 (17.2%)	47 (16.5%)	
	Medial ≥5	49 (18.4%)	56 (19.7%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	211 (78.4%)	222 (78.2%)	0.907
	Anterior ≥5	52 (19.3%)	54 (19%)	
	Posterior ≥5	6 (2.2%)	8 (2.8%)	
Touching Anterior Cortex		67 (25.0%)	69 (24.3%)	0.926
Distance of tip of the nail from knee (mm)	<10	2 (0.7%)	5 (1.8%)	0.619
	10 to 19	24 (9.0%)	24 (8.5%)	
	20-29	92 (34.5%)	88 (31.2%)	
	≥30	149 (55.8%)	165 (58.5%)	
Nail / Canal Ratio		0.83 (0.07)	0.82 (0.08)	**0.034**
Hospital stay / Mortality		**No Tx**	**Tx within 48h**	**p-value**
HDU / ICU stay		20 (7.4%)	46 (16%)	**0.003**
Total length of hospital stay (days)		20.79 (20.03)	24.07 (17.10)	**0.037**
Weekend admission		77 (28.4%)	101 (35.1%)	0.110
Died within a year		42 (15.5%)	72 (25%)	**0.007**

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.10 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the day of admission (weekday versus weekend)

Demographics		**Weekday admission**	**Weekend admission**	**p-value**
Total number		382	179	-
Bilateral		19 (5.0%)	7 (3.9%)	0.732
Age < 65 y.o.		91 (23.8%)	47 (26.3%)	0.604
Age 65 – 75 y.o		54 (14.1%)	36 (20.1%)	0.094
Age > 75 y.o.		237 (62.0%)	96 (53.6%)	0.072
Gender	Male	152 (39.8%)	68 (38.0%)	0.753
	Female	230 (60.2%)	111 (62.0%)	
Injury Characteristics		**Weekday admission**	**Weekend admission**	**p-value**
Mechanism of Injury	Low energy	295 (77.2%)	138 (77.1%)	0.100
	High energy	56 (14.7%)	34 (19.0%)	
	Pathological	31 (8.1%)	7 (3.9%)	
Isolated		327 (85.6%)	149 (83.2%)	0.548
ISS > 16		20 (5.2%)	16 (8.9%)	0.138
Side	Left	202 (52.9%)	93 (52.0%)	0.910
	Right	180 (47.1%)	86 (48.0%)	
Open fracture		2 (0.5%)	5 (2.8%)	0.064
Medical Comorbidities		**Weekday admission**	**Weekend admission**	**p-value**
ASA	1	35 (9.2%)	14 (7.8%)	0.849
	2	95 (24.9%)	50 (27.9%)	
	3	190 (49.7%)	88 (49.2%)	
	4	62 (16.2%)	27 (15.1%)	
Charlson Comorbidity Score		5.44 (3.15)	4.91 (3.04)	0.061
Diabetes		54 (14.1%)	23 (12.8%)	0.778
Steroids		21 (5.5%)	8 (4.5%)	0.758
Malignancy		102 (26.7%)	35 (19.6%)	0.083
Dementia		84 (22.0%)	41 (22.9%)	0.893
Osteoporosis		**Weekday admission**	**Weekend admission**	**p-value**
Bisphosphonates pre-admission		69 (18.1%)	25 (14.0%)	0.276
Bisphosphonates on discharge		96 (26.8%)	40 (24.2%)	0.606
Calcium / Vitamin D pre-admission		107 (28.0%)	51 (28.5%)	0.986
Calcium / Vitamin D on discharge		175 (48.9%)	76 (46.1%)	0.613
Vitamin D loading during admission		60 (16.8%)	27 (16.4%)	1.000
Fragility Fractures Before		86 (22.6%)	37 (20.7%)	0.691
Fragility Fractures After		59 (15.5%)	27 (15.1%)	1.000
DEXA Result	Normal	3 (8.3%)	3 (25%)	0.269
	Osteopenia	11 (30.6%)	4 (33.3%)	
	Osteoporosis	22 (61.1%)	5 (41.7%)	
Singh Index	1	32 (9.6%)	10 (6.0%)	0.693
	2	74 (22.2%)	40 (24.0%)	
	3	72 (21.6%)	35 (21.0%)	
	4	81 (24.3%)	37 (22.2%)	
	5	39 (11.7%)	23 (13.8%)	
	6	36 (10.8%)	22 (13.2%)	
Social History		**Weekday admission**	**Weekend admission**	**p-value**
Smoking		73 (19.1%)	40 (22.3%)	0.437

Social History		Weekday admission	Weekend admission	p-value
Alcohol >10 units / week		64 (16.8%)	41 (22.9%)	0.104
Pre-operative Mobility				
	Independent	194 (50.8%)	99 (55.3%)	
	Stick(s) / Crutch(es)	106 (27.7%)	40 (22.3%)	0.162
	Frame	60 (15.7%)	35 (19.6%)	
	Wheelchair / Hoisted	22 (5.8%)	5 (2.8%)	
Frequent falls		109 (28.5%)	44 (24.6%)	0.380
Operation Characteristics		**Weekday admission**	**Weekend admission**	**p-value**
Operation in less than 48 hours		301 (78.8%)	143 (79.9%)	0.853
Simultaneous procedures		21 (5.5%)	16 (8.9%)	0.178
Type of Nail	Long Affixus Nail	214 (56%)	105 (58.7%)	
	Long Gamma Nail	136 (35.6%)	62 (34.6%)	0.735
	Others	32 (8.4%)	12 (6.7%)	
Nail Diameter (mm)	9	19 (5%)	12 (6.7%)	
	10	10 (2.6%)	3 (1.7%)	
	11	225 (59.2%)	120 (67.4%)	0.178
	12	2 (0.5%)	0 (0.0%)	
	13	124 (32.6%)	43 (24.2%)	
Open reduction		178 (46.6%)	87 (48.6%)	0.724
Use of cerclage wires		41 (10.7%)	24 (13.4%)	0.435
Post-op Mobilisation (first 6 weeks)	FWB	219 (57.3%)	88 (49.2%)	
	PWB	83 (21.7%)	39 (21.8%)	0.164
	TTWB	43 (11.3%)	30 (16.8%)	
	NWB	37 (9.7%)	22 (12.3%)	
Surgical time (min)		108.25 (40.3)	118.91 (53.04)	**0.009**
Anaesthetic Time (min)		49.33 (21.98)	46.60 (20.79)	0.165
Time from induction to recovery (min)		176.00 (46.69)	184.79 (55.71)	0.052
Level of First Surgeon				
	Registrar	228 (59.8%)	104 (58.8%)	0.880
	Consultant	153 (40.2%)	73 (41.2%)	
Level of Senior Surgeon Present				
	Registrar	211 (55.4%)	95 (53.7%)	0.775
	Consultant	170 (44.6%)	82 (46.3%)	
Complications		**Weekday admission**	**Weekend admission**	**p-value**
Nail complications		69 (18.1%)	31 (17.3%)	0.923
Failure at lag screw junction		16 (4.2%)	8 (4.5%)	1.000
Self-dynamisation		20 (5.2%)	5 (2.8%)	0.277
Cut-out		9 (2.4%)	4 (2.2%)	1.000
Non-union		55 (14.4%)	29 (16.2%)	0.666
Nail infection		4 (1.0%)	1 (0.6%)	0.927
Peri-implant fracture		9 (2.4%)	5 (2.8%)	0.985
HAP / CAP		69 (18.1%)	37 (20.7%)	0.535
UTI		57 (14.9%)	21 (11.7%)	0.375
Wound infection	Superficial	18 (4.7%)	3 (1.7%)	0.210
	Deep	10 (2.6%)	5 (2.8%)	
Washout / Revision for Infection		4 (7.3%)	3 (9.1%)	1.000
CKD Stage pre-operatively				
	Mild	254 (68.1%)	130 (73.4%)	0.239
	Moderate / Severe	119 (31.9%)	47 (26.6%)	

Complications		Weekday admission	Weekend admission	p-value
CKD Stage post-operatively				
	Mild	270 (73.0%)	127 (72.2%)	0.923
	Moderate / Severe	100 (27.0%)	49 (27.8%)	
Pre-operative Transfusion		34 (8.9%)	14 (7.9%)	0.799
Post-operative Transfusion (48 hours)		187 (49.1%)	101 (56.7%)	0.110
Post-operative Transfusion (total)		232 (60.9%)	121 (68%)	0.128
Hb Drop (g/L)		46.79 (18.72)	46.03 (18.45)	0.657
VTE	No	69 (83.1%)	38 (82.6%)	0.946
	DVT	8 (9.6%)	4 (8.7%)	
	PE	6 (7.2%)	4 (8.7%)	
Biochemistry		**Weekday admission**	**Weekend admission**	**p-value**
Adjusted Calcium	Normal	235 (76.1%)	113 (75.8%)	1.000
	Low	74 (23.9%)	36 (24.2%)	
Albumin	Normal	108 (31.7%)	50 (31.1%)	0.972
	Low	233 (68.3%)	111 (68.9%)	
Alkaline Phosphatase	Low	33 (9.8%)	12 (7.5%)	0.581
	Normal	243 (71.9%)	115 (71.4%)	
	High	62 (18.3%)	34 (21.1%)	
Phosphate	Normal / High	255 (82.3%)	118 (78.7%)	0.427
	Low	55 (17.7%)	32 (21.3%)	
TSH	Low	3 (1.5%)	2 (2.4%)	0.778
	Normal	171 (86.8%)	74 (88.1%)	
	High	23 (11.7%)	8 (9.5%)	
Free T4	Low	4 (2.1%)	2 (2.5%)	0.767
	Normal	165 (84.6%)	64 (81.0%)	
	High	26 (13.3%)	13 (16.5%)	
PTH	High	93 (53.8%)	37 (46.2%)	0.329
	Normal	80 (46.2%)	43 (53.8%)	
Total 25OH Vitamin D	Normal	23 (12.0%)	12 (14.0%)	0.804
	Low	168 (88.0%)	74 (86.0%)	
Radiographic measurements		**Weekday admission**	**Weekend admission**	**p-value**
Femoral Neck Shaft Angle				
	Normal	252 (67.6%)	114 (64.4%)	0.388
	Coxa Valga	99 (26.5%)	47 (26.6%)	
	Coxa Vara	22 (5.9%)	16 (9.0%)	
Number of fragments (Comminution)	Simple	113 (29.7%)	50 (27.9%)	0.063
	Moderate	194 (51.1%)	79 (44.1%)	
	Severe	73 (19.2%)	50 (27.9%)	
Only Subtrochanteric Extension		68 (17.9%)	31 (17.3%)	0.962
Atypical		20 (5.3%)	10 (5.6%)	1.000
Pathological		24 (6.3%)	6 (3.4%)	0.211
Distal Extension		120 (31.6%)	66 (36.9%)	0.253
Greater Trochanter Fracture		43 (11.3%)	16 (8.9%)	0.480
Lesser Trochanter Fracture		244 (64.2%)	116 (64.8%)	0.966
Medial Calcar Comminution		23 (6.1%)	8 (4.5%)	0.572
AO/ OTA Classification		-	-	0.357
Lateral Cortex Gap Size (mm)	≤4	244 (64.2%)	99 (55.6%)	0.141
	5-9	91 (23.9%)	51 (28.7%)	
	≥10	45 (11.8%)	28 (15.7%)	

Radiographic measurements		Weekday admission	Weekend admission	p-value
Medial Cortex Gap Size (mm)	≤4	260 (68.4%)	111 (62.4%)	0.214
	5-9	83 (21.8%)	51 (28.7%)	
	≥10	37 (9.7%)	16 (9.0%)	
Anterior Cortex Gap Size (mm)	≤4	240 (63%)	115 (64.6%)	0.442
	5-9	89 (23.4%)	34 (19.1%)	
	≥10	52 (13.6%)	29 (16.3%)	
Posterior Cortex Gap Size (mm)	≤4	299 (78.5%)	128 (71.9%)	0.093
	5-9	59 (15.5%)	41 (23%)	
	≥10	23 (6%)	9 (5.1%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	28 (7.4%)	10 (5.6%)	0.483
	Valgus >5	279 (73.4%)	125 (70.2%)	
	Varus 5-10	57 (15%)	31 (17.4%)	
	Varus >10	16 (4.2%)	12 (6.7%)	
TAD (mm)	<25	329 (88%)	145 (84.3%)	0.298
	≥25	45 (12%)	27 (15.7%)	
Distal locking (Number of Screws)	1	12 (3.1%)	6 (3.4%)	1.000
	2	369 (96.9%)	172 (96.6%)	
Method of locking				0.285
Static Locking		248 (65.4%)	108 (61.4%)	
Secondary Dynamisation		128 (33.8%)	68 (38.6%)	
Dynamic		3 (0.8%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	237 (63%)	118 (66.7%)	0.300
	Lateral ≥5	61 (16.2%)	32 (18.1%)	
	Medial ≥5	78 (20.7%)	27 (15.3%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	301 (79.6%)	133 (75.1%)	0.393
	Anterior ≥5	67 (17.7%)	40 (22.6%)	
	Posterior ≥5	10 (2.6%)	4 (2.3%)	
Touching Anterior Cortex		93 (24.7%)	44 (24.9%)	1.000
Distance of tip of the nail from knee (mm)	<10	5 (1.3%)	2 (1.1%)	0.847
	10 to 19	31 (8.2%)	17 (9.7%)	
	20-29	120 (31.9%)	60 (34.3%)	
	≥30	220 (58.5%)	96 (54.9%)	
Nail / Canal Ratio		0.82 (0.08)	0.83 (0.07)	0.393
Hospital stay / Mortality		**Weekday admission**	**Weekend admission**	**p-value**
HDU / ICU stay		48 (12.6%)	19 (10.6%)	0.600
Total length of hospital stay (days)		23.43 (20.32)	20.56 (14.19)	0.089
Died within 30-days		21 (5.5%)	12 (6.7%)	0.709
Died within a year		74 (19.4%)	41 (22.9%)	0.393

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.11 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the presence of HAP

Demographics		**No HAP**	**HAP**	**p-value**
Total number		468	93	-
Bilateral		22 (4.7%)	4 (4.3%)	1.000
Age < 65 y.o.		130 (27.8%)	8 (8.6%)	**<0.001**
Age 65 – 75 y.o		73 (15.6%)	17 (18.3%)	0.625
Age > 75 y.o.		265 (56.6%)	68 (73.1%)	**0.004**
Gender	Male	180 (38.5%)	40 (43.0%)	0.481
	Female	288 (61.5%)	53 (57.0%)	
Injury Characteristics		**No HAP**	**HAP**	**p-value**
Mechanism of Injury	Low energy	350 (74.8%)	83 (89.2%)	**0.009**
	High energy	84 (17.9%)	6 (6.5%)	
	Pathological	34 (7.3%)	4 (4.3%)	
Isolated		393 (84%)	83 (89.2%)	0.256
ISS > 16		34 (7.3%)	2 (2.2%)	0.108
Side	Left	245 (52.4%)	50 (53.8)	0.892
	Right	223 (47.6%)	43 (46.2)	
Open fracture		6 (1.3%)	1 (1.1%)	1.000
Medical Comorbidities		**No HAP**	**HAP**	**p-value**
ASA	1	49 (10.5%)	0 (0.0%)	**<0.001**
	2	134 (28.6%)	11 (11.8%)	
	3	220 (47%)	58 (62.4%)	
	4	65 (13.9%)	24 (25.8%)	
Charlson Comorbidity Score		4.99 (3.13%)	6.67 (2.66%)	**<0.001**
Diabetes		57 (12.2%)	20 (21.5%)	**0.026**
Steroids		23 (4.9%)	6 (6.5%)	0.723
Malignancy		113 (24.1%)	24 (25.8%)	0.835
Dementia		101 (21.6%)	24 (25.8%)	0.448
Osteoporosis		**No HAP**	**HAP**	**p-value**
Bisphosphonates pre-admission		76 (16.2%)	18 (19.4%)	0.560
Bisphosphonates on discharge		114 (25.2%)	22 (31.0%)	0.377
Calcium / Vitamin D pre-admission		129 (27.6%)	29 (31.2%)	0.560
Calcium / Vitamin D on discharge		209 (46.2%)	42 (59.2%)	0.058
Vitamin D loading during admission		64 (14.2%)	23 (32.4%)	**<0.001**
Fragility Fractures Before		100 (21.4%)	23 (24.7%)	0.570
Fragility Fractures After		75 (16.1%)	11 (11.8%)	0.381
DEXA Result	Normal	6 (15.4%)	0 (0.0%)	0.274
	Osteopenia	13 (33.3%)	2 (22.2%)	
	Osteoporosis	20 (51.3%)	7 (77.8%)	
Singh Index	1	32 (7.7%)	10 (11.5%)	**0.006**
	2	37 (21.3%)	26 (29.9%)	
	3	87 (21%)	20 (23.0%)	
	4	99 (23.9%)	19 (21.8%)	
	5	50 (12.1%)	12 (13.8%)	
	6	58 (14%)	0 (0.0%)	
Social History		**No HAP**	**HAP**	**p-value**
Smoking		90 (19.2%)	23 (24.7%)	0.286
Alcohol >10 units / week		89 (19%)	16 (17.2%)	0.792

Social History		No HAP	HAP	p-value
Pre-operative Mobility				
	Independent	258 (55.1%)	35 (37.6%)	**0.019**
	Stick(s) / Crutch(es)	115 (24.6%)	31 (33.3%)	
	Frame	75 (16%)	20 (21.5%)	
	Wheelchair / Hoisted	20 (4.3%)	7 (7.5%)	
Frequent falls		122 (26.1%)	31 (33.3%)	0.190
Operation Characteristics		**No HAP**	**HAP**	**p-value**
Operation in less than 48 hours		373 (79.7%)	71 (76.3%)	0.557
Simultaneous procedures		34 (7.3%)	3 (3.2%)	0.228
Type of Nail	Long Affixus Nail	253 (54.1%)	66 (71.0%)	**0.005**
	Long Gamma Nail	173 (37%)	25 (26.9%)	
	Others	42 (9%)	2 (2.2%)	
Nail Diameter (mm)	9	29 (6.2%)	2 (2.2%)	0.092
	10	12 (2.6%)	1 (1.1%)	
	11	292 (62.8%)	53 (57.0%)	
	12	1 (0.2%)	1 (1.1%)	
	13	131 (28.2%)	36 (38.7%)	
Open reduction		222 (47.4%)	43 (46.2%)	0.922
Use of cerclage wires		52 (11.1%)	13 (14.0%)	0.541
Post-op Mobilisation (first 6 weeks)	FWB	253 (54.1%)	54 (58.1%)	0.719
	PWB	103 (22.0%)	19 (20.4%)	
	TTWB	60 (12.8%)	13 (14.0%)	
	NWB	52 (11.1%)	7 (7.5%)	
Surgical time (min)		113 (45.27)	104.77 (42.95)	0.107
Anaesthetic Time (min)		48.09 (21.69)	50.35 (21.33)	0.356
Time from induction to recovery (min)		179.63 (50.85)	174.58 (44.51)	0.373
Level of First Surgeon				
	Registrar	273 (58.7%)	59 (63.4%)	0.464
	Consultant	192 (41.3%)	34 (36.6%)	
Level of Senior Surgeon Present				
	Registrar	250 (53.8%)	56 (60.2%)	0.304
	Consultant	215 (46.2%)	37 (39.8%)	
Complications		**No HAP**	**HAP**	**p-value**
Nail complications		88 (18.8%)	12 (12.9%)	0.226
Failure at lag screw junction		23 (4.9%)	1 (1.1%)	0.164
Self-dynamisation		22 (4.7%)	3 (3.2%)	0.723
Cut-out		12 (2.6%)	1 (1.1%)	0.621
Non-union		75 (16.0%)	9 (9.7%)	0.159
Nail infection		4 (0.9%)	1 (1.1%)	1.000
Peri-implant fracture		12 (2.6%)	2 (2.2%)	1.000
UTI		63 (13.5%)	15 (16.1%)	0.607
Wound infection	Superficial	20 (4.3%)	1 (1.1%)	0.182
	Deep	14 (3%)	1 (1.1%)	
Washout / Revision for Infection		6 (7.7%)	1 (10.0%)	1.000
CKD Stage pre-operatively				
	Mild	329 (71.5%)	55 (61.1%)	0.065
	Moderate / Severe	131 (28.5%)	35 (38.9%)	
CKD Stage post-operatively				
	Mild	343 (75.2%)	54 (60.0%)	**0.005**
	Moderate / Severe	113 (24.8%)	36 (40.0%)	
Pre-operative Transfusion		38 (8.2%)	10 (10.8%)	0.539
Post-operative Transfusion (48 hours)		234 (50.2%)	54 (58.1)	0.204

Complications		No HAP	HAP	p-value
Post-operative Transfusion (total)		287 (61.6%)	66 (71.0%)	0.111
Hb Drop (g/L)		45.91 (18.48)	49.72 (19.09)	0.076
VTE	No	85 (82.5%)	22 (84.6%)	0.147
	DVT	8 (7.8%)	37 (15.4%)	
	PE	10 (9.7%)	0 (0.0%)	
Biochemistry		**No HAP**	**HAP**	**p-value**
Adjusted Calcium	Normal	280 (75.3%)	68 (79.1%)	0.546
	Low	92 (24.7%)	18 (20.9%)	
Albumin	Normal	137 (33.2%)	21 (23.6%)	0.101
	Low	276 (66.8%)	68 (76.4%)	
Alkaline Phosphatase	Low	36 (8.8%)	9 (10.1%)	0.924
	Normal	295 (72%)	63 (70.8%)	
	High	79 (19.3%)	17 (19.1%)	
Phosphate	Normal / High	304 (81.5%)	69 (79.3%)	0.751
	Low	69 (18.5%)	18 (20.7%)	
TSH	High	26 (11.6%)	37 (8.9%)	0.070
	Normal	197 (87.6%)	48 (85.7%)	
	Low	2 (0.9%)	3 (5.4%)	
Free T4	High	32 (14.5%)	7 (13.2%)	0.156
	Normal	186 (84.2%)	43 (81.1%)	
	Low	3 (1.4%)	3 (5.7%)	
PTH	High	99 (50%)	31 (56.4%)	0.495
	Normal	99 (50%)	24 (43.6%)	
Total 25OH Vitamin D	Normal	30 (13.7%)	37 (8.6%)	0.416
	Low	189 (86.3%)	53 (91.4%)	
Radiographic measurements		**No HAP**	**HAP**	**p-value**
Femoral Neck Shaft Angle				0.688
Normal		309 (67.3%)	57 (62.6%)	
Coxa Valga		119 (25.9%)	27 (29.7%)	
Coxa Vara		31 (6.8%)	7 (7.7%)	
Number of fragments (Comminution)	Simple	133 (28.5%)	30 (32.3%)	0.707
	Moderate	231 (49.6%)	42 (45.2%)	
	Severe	102 (21.9%)	21 (22. 6%)	
Only Subtrochanteric Extension		84 (18%)	15 (16.1%)	0.773
Atypical		26 (5.6%)	4 (4.3%)	0.805
Pathological		22 (4.7%)	8 (8.6%)	0.206
Distal Extension		158 (33.9%)	28 (30.1%)	0.556
Greater Trochanter Fracture		51 (10.9%)	8 (8.6%)	0.627
Lesser Trochanter Fracture		297 (63.7%)	63 (67.7%)	0.536
Medial Calcar Comminution		29 (6.2%)	2 (2.2%)	0.187
AO/ OTA Classification		-	-	**0.031**
Lateral Cortex Gap Size (mm)	≤4	281 (60.4%)	62 (66.7%)	0.115
	5-9	117 (25.2%)	25 (26.9%)	
	≥10	67 (14.4%)	6 (6.5%)	
Medial Cortex Gap Size (mm)	≤4	305 (65.6%)	66 (71.0%)	0.311
	5-9	112 (24.1%)	22 (23.7%)	
	≥10	48 (10.3%)	5 (5.4%)	
Anterior Cortex Gap Size (mm)	≤4	291 (62.4%)	64 (68.8%)	0.428
	5-9	104 (22.3%)	19 (20.4%)	
	≥10	71 (15.2%)	10 (10.8%)	
Posterior Cortex Gap Size (mm)	≤4	355 (76.2%)	72 (77.4%)	0.811
	5-9	83 (17.8%)	17 (18.3%)	
	≥10	28 (6%)	4 (4.3%)	

Radiographic measurements		No HAP	HAP	p-value
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	336 (72.3%)	68 (73.1)	0.203
	Valgus >5	28 (6.0%)	10 (10.8)	
	Varus 5-10	75 (16.1%)	13 (14.0%)	
	Varus >10	26 (5.6%)	2 (2.2%)	
TAD (mm)	<25	393 (86.6%)	81 (88.0%)	0.831
	≥25	61 (13.4%)	11 (12.0%)	
Distal locking (Number of Screws)	1	15 (3.2%)	37 (3.2)	1.000
	2	451 (96.8%)	90 (96.8)	
Method of locking				
Static Locking		297 (64.3%)	59 (63.4)	0.718
Secondary Dynamisation		162 (35.1%)	34 (36.6)	
Dynamic		3 (0.6%)	0 (0.0)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	297 (64.3%)	58 (63.7)	0.403
	Lateral ≥5	81 (17.5%)	12 (13.2)	
	Medial ≥5	84 (18.2%)	21 (23.1)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	360 (77.8%)	74 (80.4)	0.665
	Anterior ≥5	92 (19.9%)	15 (16.3)	
	Posterior ≥5	11 (2.4%)	3 (3.3)	
Touching Anterior Cortex		115 (24.9%)	22 (23.9)	0.947
Distance of tip of the nail from knee (mm)	<10	6 (1.3%)	1 (1.1)	0.670
	10 to 19	39 (8.5%)	9 (9.9)	
	20-29	146 (31.7%)	34 (37.4)	
	≥30	269 (58.5%)	47 (51.6)	
Nail / Canal Ratio		0.82 (0.08)	0.83 (0.08)	0.357
Hospital stay / Mortality		**No HAP**	**HAP**	**p-value**
HDU / ICU stay		45 (9.6%)	22 (23.7)	**<0.001**
Weekend admission		145 (31%)	34 (36.6)	0.351
Total length of hospital stay (days)		20.85 (17.64)	30.9 (21.10)	**<0.001**
Died within 30-days		14 (3%)	19 (20.4)	**<0.001**
Died within a year		78 (16.7%)	37 (39.8)	**<0.001**

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.12 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the presence of an MI / CVA

Demographics		**No MI / CVA**	**MI / CVA**	**p-value**
Total number		538	23	-
Bilateral		25 (4.6%)	1 (4.3%)	1.000
Age < 65 y.o.		137 (25.5%)	1 (4.3%)	**0.040**
Age 65 – 75 y.o		87 (16.2%)	3 (13.0%)	0.912
Age > 75 y.o.		314 (58.4%)	19 (82.6%)	**0.036**
Gender	Male	214 (39.8%)	6 (26.1%)	0.272
	Female	324 (60.2%)	17 (73.9%)	
Injury Characteristics		**No MI / CVA**	**MI / CVA**	**p-value**
Mechanism of Injury	Low energy	413 (76.8%)	20 (87.0%)	0.063
	High energy	90 (16.7%)	0 (0.0%)	
	Pathological	35 (6.5%)	3 (13.0%)	
Isolated		454 (84.4%)	22 (95.7%)	0.239
ISS > 16		36 (6.7%)	0 (0.0%)	0.396
Side	Left	282 (52.4%)	13 (56.5%)	0.863
	Right	256 (47.6%)	10 (43.5%)	
Open fracture		7 (1.3%)	0 (0.0%)	1.000
Medical Comorbidities		**No MI / CVA**	**MI / CVA**	**p-value**
ASA	1	49 (9.1%)	0 (0.0%)	**0.002**
	2	141 (26.2%)	4 (17.4%)	
	3	269 (50.0%)	9 (39.1%)	
	4	79 (14.7%)	10 (43.5%)	
Charlson Comorbidity Score		5.18 (3.12)	7.43 (2.25)	**0.001**
Diabetes		71 (13.2%)	6 (26.1%)	0.147
Steroids		27 (5%)	2 (8.7%)	0.765
Malignancy		130 (24.2%)	7 (30.4%)	0.662
Dementia		116 (21.6%)	9 (39.1%)	0.084
Osteoporosis		**No MI / CVA**	**MI / CVA**	**p-value**
Bisphosphonates pre-admission		89 (16.5%)	5 (21.7%)	0.713
Bisphosphonates on discharge		130 (25.6%)	6 (37.5%)	0.438
Calcium / Vitamin D pre-admission		151 (28.1%)	7 (30.4%)	0.992
Calcium / Vitamin D on discharge		243 (47.9%)	8 (50.0%)	1.000
Vitamin D loading during admission		84 (16.6%)	3 (18.8%)	1.000
Fragility Fractures Before		119 (22.2%)	4 (17.4%)	0.777
Fragility Fractures After		81 (15.1%)	5 (21.7%)	0.568
DEXA Result	Normal	6 (12.8%)	0 (0.0%)	0.325
	Osteopenia	14 (29.8%)	1 (100.0%)	
	Osteoporosis	27 (57.4%)	0 (0.0%)	
Singh Index	1	42 (8.8%)	0 (0.0%)	0.069
	2	104 (21.8%)	10 (43.5%)	
	3	102 (21.3%)	5 (21.7%)	
	4	114 (23.8%)	4 (17.4%)	
	5	58 (12.1%)	4 (17.4%)	
	6	58 (12.1%)	0 (0.0%)	
Social History		**No MI / CVA**	**MI / CVA**	**p-value**
Smoking		110 (20.4%)	3 (13.0%)	0.548
Alcohol >10 units / week		103 (19.1%)	2 (8.7%)	0.325

Social History		No MI / CVA	MI / CVA	p-value
Pre-operative Mobility				
	Independent	284 (52.8%)	9 (39.1%)	
	Stick(s) / Crutch(es)	137 (25.5%)	9 (39.1%)	0.343
	Frame	92 (17.1%)	3 (13.0%)	
	Wheelchair / Hoisted	25 (4.6%)	2 (8.7%)	
Frequent falls		146 (27.1%)	7 (30.4%)	0.913
Operation Characteristics		**No MI / CVA**	**MI / CVA**	**p-value**
Operation in less than 48 hours		427 (79.4%)	17 (73.9%)	0.712
Simultaneous procedures		37 (6.9%)	0 (0.0%)	0.383
Type of Nail	Long Affixus Nail	305 (56.7%)	14 (60.9%)	
	Long Gamma Nail	189 (35.1%)	9 (39.1%)	0.359
	Others	44 (8.2%)	0 (0.0%)	
Nail Diameter	9	31 (5.8%)	0 (0.0%)	
(mm)	10	13 (2.4%)	0 (0.0%)	
	11	329 (61.5%)	16 (69.6%)	0.697
	12	2 (0.4%)	0 (0.0%)	
	13	160 (29.9%)	7 (30.4%)	
Open reduction		259 (48.1%)	6 (26.1%)	0.063
Use of cerclage wires		64 (11.9%)	1 (4.3%)	0.438
Post-op Mobilisation	FWB	293 (54.5%)	14 (60.9%)	
(first 6 weeks)	PWB	118 (21.9%)	4 (17.4%)	0.660
	TTWB	69 (12.8%)	4 (17.4%)	
	NWB	58 (10.8%)	1 (4.3%)	
Surgical time (min)		112.41 (45.46)	93.43 (25.73)	**0.047**
Anaesthetic Time (min)		48.51 (21.52)	47.43 (24.44)	0.816
Time from induction to recovery (min)		179.62 (50.09)	159.48 (40.02)	0.058
Level of First Surgeon				
	Registrar	320 (59.8%)	12 (52.2%)	0.607
	Consultant	215 (40.2%)	11 (47.8%)	
Level of Senior Surgeon Present				
	Registrar	295 (55.1%)	11 (47.8%)	0.634
	Consultant	240 (44.9%)	12 (52.2%)	
Complications		**No MI / CVA**	**MI / CVA**	**p-value**
Nail complications		99 (18.4%)	1 (4.3%)	0.148
Failure at lag screw junction		24 (4.5%)	0 (0.0%)	0.611
Self-dynamisation		25 (4.6%)	0 (0.0%)	0.588
Cut-out		13 (2.4%)	0 (0.0%)	0.963
Non-union		82 (15.2%)	2 (8.7%)	0.573
Nail infection		5 (0.9%)	0 (0.0%)	1.000
Peri-implant fracture		14 (2.6%)	0 (0.0%)	0.920
HAP / CAP		102 (19%)	4 (17.4%)	1.000
UTI		74 (13.8%)	4 (17.4%)	0.852
Wound infection	Superficial	19 (3.5%)	2 (8.7%)	0.329
	Deep	15 (2.8%)	0 (0.0%)	
Washout / Revision for Infection		7 (8%)	0 (0%)	-
CKD Stage pre-operatively				
	Mild	373 (70.8%)	11 (47.8%)	**0.034**
	Moderate / Severe	154 (29.2%)	12 (52.2%)	
CKD Stage post-operatively				
	Mild	386 (73.8%)	11 (47.8%)	**0.012**
	Moderate / Severe	137 (26.2%)	12 (52.2%)	
Pre-operative Transfusion		45 (8.4%)	3 (13.0%)	0.690

Complications		No MI / CVA	MI / CVA	p-value
Post-operative Transfusion (48 hours)		274 (51.1%)	14 (60.9%)	0.482
Post-operative Transfusion (total)		337 (62.9%)	16 (69.6%)	0.667
Hb Drop (g/L)		46.24 (18.51)	53.39 (20.23)	0.071
VTE	No DVT PE	99 (82.5%) 12 (10%) 9 (7.5%)	8 (88.9%) 0 (0.0%) 1 (11.1%)	0.582
Biochemistry		**No MI / CVA**	**MI / CVA**	**p-value**
Adjusted Calcium	Normal Low	331 (75.7%) 106 (24.3%)	17 (81.0%) 4 (19.0%)	0.776
Albumin	Normal Low	152 (31.6%) 329 (68.4%)	6 (28.6%) 15 (71.4%)	0.958
Alkaline Phosphatase	Low Normal High	44 (9.2%) 341 (71.3%) 93 (19.5%)	1 (4.8%) 17 (81.0%) 3 (14.3%)	0.613
Phosphate	Normal / High Low	357 (81.3%) 82 (18.7%)	16 (76.2%) 5 (23.8%)	0.763
TSH	Low Normal High	5 (1.9%) 236 (87.4%) 29 (10.7%)	0 (0.0%) 9 (81.8%) 2 (18.2%)	0.680
Free T4	Low Normal High	6 (2.3%) 222 (84.4%) 35 (13.3%)	0 (0.0%) 7 (63.6%) 4 (36.4%)	0.094
PTH	High Normal	119 (49.4%) 122 (50.6%)	4 (33.3%) 8 (66.7%)	0.430
Total 25OH Vitamin D	Normal Low	33 (12.4%) 233 (87.6%)	2 (18.2%) 9 (81.8%)	0.919
Radiographic measurements		**No MI / CVA**	**MI / CVA**	**p-value**
Femoral Neck Shaft Angle	Normal Coxa Valga Coxa Vara	351 (66.6%) 140 (26.6%) 36 (6.8%)	15 (65.2%) 6 (26.1%) 2 (8.7%)	0.942
Number of fragments (Comminution)	Simple Moderate Severe	157 (29.3%) 259 (48.3%) 120 (22.4%)	6 (26.1%) 14 (60.9%) 3 (13.0%)	0.435
Only Subtrochanteric Extension		96 (17.9%)	3 (13.0%)	0.749
Atypical		29 (5.4%)	1 (4.3%)	1.000
Pathological		29 (5.4%)	1 (4.3%)	1.000
Distal Extension		175 (32.6%)	11 (47.8%)	0.198
Greater Trochanter Fracture		56 (10.4%)	3 (13.0%)	0.960
Lesser Trochanter Fracture		347 (64.7%)	13 (56.5%)	0.560
Medial Calcar Comminution		29 (5.4%)	2 (8.7%)	0.835
AO/ OTA Classification		-	-	0.354
Lateral Cortex Gap Size (mm)	≤4 5-9 ≥10	328 (61.3%) 135 (25.2%) 72 (13.5%)	15 (65.2%) 7 (30.4%) 1 (4.3%)	0.430
Medial Cortex Gap Size (mm)	≤4 5-9 ≥10	355 (66.4%) 127 (23.7%) 53 (9.9%)	16 (69.6%) 7 (30.4%) 0 (0.0%)	0.256
Anterior Cortex Gap Size (mm)	≤4 5-9 ≥10	338 (63.1%) 119 (22.2%) 79 (14.7%)	17 (73.9%) 4 (17.4%) 2 (8.7%)	0.550

Radiographic measurements		No MI / CVA	MI / CVA	p-value
Posterior Cortex Gap Size (mm)	≤4	407 (75.9%)	20 (87.0%)	0.361
	5-9	97 (18.1%)	3 (13.0%)	
	≥10	32 (6%)	0 (0.0%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	384 (71.8%)	20 (87.0%)	0.302
	Valgus >5	38 (7.1%)	0 (0.0%)	
	Varus 5-10	85 (15.9%)	3 (13.0%)	
	Varus >10	28 (5.2%)	0 (0.0%)	
TAD (mm)	<25	452 (86.4%)	22 (95.7%)	0.334
	≥25	71 (13.6%)	1 (4.3%)	
Distal locking (Number of Screws)	1	18 (3.4%)	0 (0.0%)	0.772
	2	518 (96.6%)	23 (100.0%)	
Method of locking				0.818
Static Locking		340 (63.9%)	16 (69.6%)	
Secondary Dynamisation		189 (35.5%)	7 (30.4%)	
Dynamic		3 (0.6%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	340 (64.2%)	15 (65.2%)	0.672
	Lateral ≥5	88 (16.6%)	5 (21.7%)	
	Medial ≥5	102 (19.2%)	3 (13.0%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	412 (77.4%)	22 (95.7%)	0.053
	Anterior ≥5	107 (20.1%)	0 (0.0%)	
	Posterior ≥5	13 (2.4%)	1 (4.3%)	
Touching Anterior Cortex		133 (25%)	4 (17.4%)	0.558
Distance of tip of the nail from knee (mm)	<10	7 (1.3%)	0 (0.0%)	0.470
	10 to 19	44 (8.3%)	4 (17.4%)	
	20-29	173 (32.8%)	7 (30.4%)	
	≥30	304 (57.6%)	12 (52.2%)	
Nail / Canal Ratio		0.82 (0.08)	0.85 (0.09)	0.155
Hospital stay / Mortality		**No MI / CVA**	**MI / CVA**	**p-value**
HDU / ICU stay		64 (11.9%)	3 (13.0%)	1.000
Total length of hospital stay (days)		22.39 (18.68)	25.39 (17.36)	0.450
Weekend admission		172 (32%)	7 (30.4%)	1.000
Died within 30-days		26 (4.8%)	7 (30.4%)	**<0.001**
Died within a year		104 (19.3%)	11 (47.8%)	**0.002**

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.13 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the presence of post-operative delirium

Demographics		**No Delirium**	**Delirium**	**p-value**
Total number		505	56	-
Bilateral		25 (5%)	1 (1.8%)	0.463
Age < 65 y.o.		134 (26.5%)	4 (7.1%)	**0.002**
Age 65 – 75 y.o		86 (17%)	4 (7.1%)	0.085
Age > 75 y.o.		285 (56.4%)	48 (85.7%)	**<0.001**
Gender	Male	204 (40.4%)	16 (28.6%)	0.115
	Female	301 (59.6%)	40 (71.4%)	
Injury Characteristics		**No Delirium**	**Delirium**	**p-value**
Mechanism of Injury	Low energy	381 (75.4%)	52 (92.9%)	**0.013**
	High energy	87 (17.2%)	3 (5.4%)	
	Pathological	37 (7.3%)	1 (1.8%)	
Isolated		421 (83.4%)	55 (98.2%)	**0.006**
ISS > 16		36 (7.1%)	0 (0.0%)	0.075
Side	Left	265 (52.5%)	30 (53.6%)	0.988
	Right	240 (47.5%)	26 (46.4%)	
Open fracture		7 (1.4%)	0 (0.0%)	0.801
Medical Comorbidities		**No Delirium**	**Delirium**	**p-value**
ASA	1	49 (9.7%)	0 (0.0%)	**0.048**
	2	132 (26.1%)	13 (23.2%)	
	3	248 (49.1%)	30 (53.6%)	
	4	76 (15%)	13 (23.2%)	
Charlson Comorbidity Score		5.1 (3.16)	6.82 (2.25)	**<0.001**
Diabetes		70 (13.9%)	7 (12.5%)	0.939
Steroids		29 (5.7%)	0 (0.0%)	0.128
Malignancy		127 (25.1%)	10 (17.9%)	0.298
Dementia		97 (19.2%)	28 (50.0%)	**<0.001**
Osteoporosis		**No Delirium**	**Delirium**	**p-value**
Bisphosphonates pre-admission		85 (16.8%)	9 (16.1%)	1.000
Bisphosphonates on discharge		123 (26.1%)	13 (25.5%)	1.000
Calcium / Vitamin D pre-admission		139 (27.5%)	19 (33.9%)	0.393
Calcium / Vitamin D on discharge		223 (47.2%)	28 (54.9%)	0.372
Vitamin D loading during admission		73 (15.5%)	14 (27.5%)	**0.047**
Fragility Fractures Before		106 (21%)	17 (30.4%)	0.153
Fragility Fractures After		75 (14.9%)	11 (19.6%)	0.458
DEXA Result	Normal	6 (13%)	0 (0.0%)	0.444
	Osteopenia	15 (32.6%)	0 (0.0%)	
	Osteoporosis	25 (54.3%)	2 (100.0%)	
Singh Index	1	33 (7.3%)	9 (18.4%)	**0.013**
	2	105 (23.2%)	9 (18.4%)	
	3	97 (21.5%)	10 (20.4%)	
	4	103 (22.8%)	15 (30.6%)	
	5	56 (12.4%)	6 (12.2%)	
	6	58 (12.8%)	0 (0.0%)	
Social History		**No Delirium**	**Delirium**	**p-value**
Smoking		106 (21%)	7 (12.5%)	0.184
Alcohol >10 units / week		95 (18.8%)	10 (17.9%)	1.000

Social History		No Delirium	Delirium	p-value
Pre-operative Mobility				
	Independent	276 (54.7%)	17 (30.4%)	
	Stick(s) / Crutch(es)	126 (25.0%)	20 (35.7%)	**0.007**
	Frame	80 (15.8%)	15 (26.8%)	
	Wheelchair / Hoisted	23 (4.6%)	4 (7.1%)	
Frequent falls		129 (25.5%)	24 (42.9%)	**0.009**
Operation Characteristics		**No Delirium**	**Delirium**	**p-value**
Operation in less than 48 hours		397 (78.6%)	47 (83.9%)	0.450
Simultaneous procedures		36 (7.1%)	1 (1.8%)	0.213
Type of Nail	Long Affixus Nail	276 (54.7%)	43 (76.8%)	
	Long Gamma Nail	186 (36.8%)	12 (21.4%)	**0.005**
	Others	43 (8.5%)	1 (1.8%)	
Nail Diameter (mm)	9	29 (5.8%)	2 (3.6%)	
	10	12 (2.4%)	1 (1.8%)	
	11	308 (61.4%)	37 (66.1%)	0.915
	12	2 (0.4%)	0 (0.0%)	
	13	151 (30.1%)	16 (28.6%)	
Open reduction		239 (47.3%)	26 (46.4%)	1.000
Use of cerclage wires		58 (11.5%)	7 (12.5%)	0.996
Post-op Mobilisation (first 6 weeks)	FWB	269 (53.3%)	38 (67.9%)	
	PWB	111 (22.0%)	11 (19.6%)	0.150
	TTWB	69 (13.7%)	4 (7.1%)	
	NWB	56 (11.1%)	3 (5.4%)	
Surgical time (min)		112.42 (45.55)	104.55 (38.92)	0.215
Anaesthetic Time (min)		48.27 (21.96)	50.2 (18.45)	0.528
Time from induction to recovery (min)		179.63 (50.38)	171.27 (44.55)	0.234
Level of First Surgeon				
	Registrar	300 (59.8%)	32 (57.1%)	0.814
	Consultant	202 (40.2%)	24 (42.9%)	
Level of Senior Surgeon Present				
	Registrar	258 (56.1%)	31 (56.4%)	1.000
	Consultant	202 (43.9%)	24 (43.6%)	
Complications		**No Delirium**	**Delirium**	**p-value**
Nail complications		90 (17.8%)	10 (17.9%)	1.000
Failure at lag screw junction		22 (4.4%)	2 (3.6%)	1.000
Self-dynamisation		23 (4.6%)	2 (3.6%)	1.000
Cut-out		11 (2.2%)	2 (3.6%)	0.850
Non-union		67 (14.5%)	7 (12.7%)	0.879
Nail infection		5 (1%)	0 (0.0%)	1.000
Peri-implant fracture		13 (2.6%)	1 (1.8%)	1.000
HAP / CAP		87 (17.2%)	19 (33.9%)	**0.004**
UTI		58 (11.5%)	20 (35.7%)	**<0.001**
Wound infection	Superficial	18 (3.6%)	3 (5.4%)	0.718
	Deep	13 (2.6%)	2 (3.6%)	
Washout / Revision for Infection		7 (8.4%)	0 (0.0%)	1.000
CKD Stage pre-operatively				
	Mild	354 (71.7%)	30 (53.6%)	**0.008**
	Moderate / Severe	140 (28.3%)	26 (46.4%)	
CKD Stage post-operatively				
	Mild	370 (75.4%)	27 (49.1%)	**<0.001**
	Moderate / Severe	121 (24.6%)	28 (50.9%)	
Pre-operative Transfusion		40 (8%)	8 (14.3%)	0.176

Complications		No Delirium	Delirium	p-value
Post-operative Transfusion (48 hours)		233 (50.5%)	36 (65.5%)	0.051
Post-operative Transfusion (total)		309 (61.4%)	44 (78.6%)	**0.017**
Hb Drop (g/L)		46.42 (18.24)	47.67 (21.84)	0.635
VTE	No DVT PE	96 (83.5%) 12 (10.4%) 7 (6.1%)	11 (78.6%) 0 (0.0%) 3 (21.4%)	0.071
Biochemistry		**No Delirium**	**Delirium**	**p-value**
Adjusted Calcium	Normal Low	306 (75.9%) 97 (24.1%)	42 (76.4%) 13 (23.6%)	1.000
Albumin	Normal Low	142 (31.8%) 304 (68.2%)	16 (28.6%) 40 (71.4%)	0.731
Alkaline Phosphatase	Low Normal High	38 (8.6%) 319 (72%) 86 (19.4%)	7 (12.5%) 39 (69.6%) 10 (17.9%)	0.622
Phosphate	Normal / High Low	326 (80.7%) 78 (19.3%)	47 (83.9%) 9 (16.1%)	0.691
TSH	Low Normal High	5 (2.1%) 210 (87.9%) 24 (10%)	0 (0.0%) 35 (83.3%) 7 (16.7%)	0.304
Free T4	Low Normal High	4 (1.7%) 198 (85%) 31 (13.3%)	2 (4.9%) 31 (75.6%) 8 (19.5%)	0.304
PTH	High Normal	106 (49.8%) 107 (50.2%)	16 (40.0%) 31 (75.6%)	0.234
Total 25OH Vitamin D	Normal Low	33 (13.9%) 204 (86.1%)	2 (5.0%) 38 (95.0%)	0.189
Radiographic measurements		**No Delirium**	**Delirium**	**p-value**
Femoral Neck Shaft Angle	Normal Coxa Valga Coxa Vara	326 (65.9%) 134 (27.1%) 35 (7.1%)	40 (72.7%) 12 (21.8%) 3 (5.5%)	0.591
Number of fragments (Comminution)	Simple Moderate Severe	150 (29.8%) 244 (48.5%) 109 (21.7%)	13 (23.2%) 29 (51.8%) 14 (25.0%)	0.571
Only Subtrochanteric Extension		93 (18.5%)	6 (10.7%)	0.207
Atypical		30 (6%)	0 (0.0%)	0.117
Pathological		28 (5.6%)	2 (3.6%)	0.752
Distal Extension		173 (34.4%)	13 (23.2%)	0.125
Greater Trochanter Fracture		50 (9.9%)	9 (16.1%)	0.235
Lesser Trochanter Fracture		318 (63.2%)	42 (75.0%)	0.110
Medial Calcar Comminution		27 (5.4%)	4 (7.1%)	0.808
AO/ OTA Classification		-	-	**0.694**
Lateral Cortex Gap Size (mm)	≤4 5-9 ≥10	312 (62.2%) 127 (25.3%) 63 (12.5%)	31 (55.4%) 15 (26.8%) 10 (17.9%)	0.471
Medial Cortex Gap Size (mm)	≤4 5-9 ≥10	331 (65.9%) 126 (25.1%) 45 (9.0%)	40 (71.4%) 8 (14.3%) 8 (14.3%)	0.123
Anterior Cortex Gap Size (mm)	≤4 5-9 ≥10	320 (63.6%) 108 (21.5%) 75 (14.9%)	35 (62.5%) 15 (26.8%) 6 (10.7%)	0.530

Radiographic measurements		No Delirium	Delirium	p-value
Posterior Cortex Gap Size (mm)	≤4	378 (75.1%)	49 (87.5%)	0.061
	5-9	93 (18.5%)	7 (12.5%)	
	≥10	32 (6.4%)	0 (0.0%)	
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	364 (72.5%)	40 (71.4%)	0.705
	Valgus >5	33 (6.6%)	5 (8.9%)	
	Varus 5-10	81 (16.1%)	7 (12.5%)	
	Varus >10	24 (4.8%)	4 (7.1%)	
TAD (mm)	<25	421 (85.9%)	53 (94.6%)	0.105
	≥25	69 (14.1%)	3 (5.4%)	
Distal locking (Number of Screws)	1	17 (3.4%)	1 (1.8%)	0.809
	2	486 (96.6%)	55 (98.2%)	
Method of locking				0.395
Static Locking		321 (64.2%)	35 (63.6%)	
Secondary Dynamisation		177 (35.4%)	19 (34.5%)	
Dynamic		2 (0.4%)	1 (1.8%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	323 (64.7%)	32 (59.3%)	0.593
	Lateral ≥5	84 (16.8%)	9 (16.7%)	
	Medial ≥5	92 (18.4%)	13 (24.1%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	392 (78.4%)	42 (76.4%)	0.338
	Anterior ≥5	94 (18.8%)	13 (23.6%)	
	Posterior ≥5	14 (2.8%)	0 (0.0%)	
Touching Anterior Cortex		124 (24.8%)	13 (23.6%)	0.973
Distance of tip of the nail from knee (mm)	<10	7 (1.4%)	0 (0.0%)	0.821
	10 to 19	43 (8.7%)	5 (9.1%)	
	20-29	163 (32.9%)	17 (30.9%)	
	≥30	283 (57.1%)	33 (60.0%)	
Nail / Canal Ratio		0.83 (0.08%)	0.79 (0.09%)	**0.001**
Hospital stay / Mortality		**No Delirium**	**Delirium**	**p-value**
HDU / ICU stay		54 (10.7%)	13 (23.2%)	**0.012**
Weekend admission		163 (32.3%)	16 (28.6%)	0.679
Total length of hospital stay (days)		21.34 (17.88)	33.07 (21.76)	**<0.001**
Died within 30-days		29 (5.7%)	4 (7.1%)	0.902
Died within a year		100 (19.8%)	15 (26.8%)	0.292

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.14 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to the development of VTE

Demographics		**No VTE**	**VTE**	**p-value**
Total number		539	22	-
Bilateral		25 (4.6%)	1 (4.5%)	1.000
Age < 65 y.o.		134 (24.9%)	4 (18.2%)	0.645
Age 65 – 75 y.o		87 (16.1%)	3 (13.6%)	0.986
Age > 75 y.o.		318 (59.0%)	15 (68.2%)	0.523
Gender	Male	212 (39.3%)	8 (36.4%)	0.955
	Female	327 (60.7%)	14 (63.6%)	
Injury Characteristics		**No VTE**	**VTE**	**p-value**
Mechanism of Injury	Low energy	418 (77.6%)	15 (68.2%)	
	High energy	85 (15.8%)	5 (22.7%)	0.589
	Pathological	36 (6.7%)	2 (9.1%)	
Isolated		459 (85.2%)	17 (77.3%)	0.479
ISS > 16		33 (6.1%)	3 (13.6%)	0.334
Side	Left	287 (53.2%)	8 (36.4%)	0.181
	Right	252 (46.8%)	14 (63.6%)	
Open fracture		5 (0.9%)	2 (9.1%)	**0.016**
Medical Comorbidities		**No VTE**	**VTE**	**p-value**
ASA	1	48 (8.9%)	1 (4.5%)	
	2	140 (26.0%)	5 (22.7%)	0.792
	3	265 (49.2%)	13 (59.1%)	
	4	86 (16.0%)	3 (13.6%)	
Charlson Comorbidity Score		5.25 (3.10)	5.77 (3.54)	0.442
Diabetes		75 (13.9%)	2 (9.1%)	0.743
Steroids		27 (5.0%)	2 (9.1%)	0.722
Malignancy		129 (23.9%)	8 (36.4%)	0.281
Dementia		122 (22.6%)	3 (13.6%)	0.464
Osteoporosis		**No VTE**	**VTE**	**p-value**
Bisphosphonates pre-admission		92 (17.1%)	2 (9.1%)	0.490
Bisphosphonates on discharge		133 (26.5%)	3 (14.3%)	0.319
Calcium / Vitamin D pre-admission		154 (28.6%)	4 (18.2%)	0.412
Calcium / Vitamin D on discharge		241 (48.0%)	10 (47.6%)	1.000
Vitamin D loading during admission		85 (16.9%)	2 (9.5%)	0.552
Fragility Fractures Before		121 (22.5%)	2 (9.1%)	0.220
Fragility Fractures After		82 (15.2%)	4 (18.2%)	0.942
DEXA Result	Normal	6 (13.0%)	0 (0.0%)	
	Osteopenia	15 (32.6%)	0 (0.0%)	0.444
	Osteoporosis	25 (54.3%)	2 (100.0%)	
Singh Index	1	40 (8.3%)	2 (9.5%)	
	2	106 (22.1%)	8 (38.1%)	
	3	102 (21.2%)	5 (23.8%)	0.327
	4	117 (24.4%)	1 (4.8%)	
	5	59 (12.3%)	3 (14.3%)	
	6	56 (11.7%)	2 (9.5%)	
Social History		**No VTE**	**VTE**	**p-value**
Smoking		110 (20.4%)	3 (13.6%)	0.613
Alcohol >10 units / week		103 (19.1%)	2 (9.1%)	0.367

Social History		No VTE	VTE	p-value
Pre-operative Mobility				
	Independent	279 (51.8%)	14 (63.6%)	
	Stick(s) / Crutch(es)	141 (26.2%)	5 (22.7%)	0.590
	Frame	92 (17.1%)	3 (13.6%)	
	Wheelchair / Hoisted	27 (5.0%)	0 (0.0%)	
Frequent falls		148 (27.5%)	5 (22.7%)	0.807
Operation Characteristics		**No VTE**	**VTE**	**p-value**
Operation in less than 48 hours		430 (79.8%)	14 (63.6%)	0.119
Simultaneous procedures		37 (6.9%)	0 (0.0%)	0.405
Type of Nail	Long Affixus Nail	310 (57.5%)	9 (40.9%)	
	Long Gamma Nail	188 (34.9%)	10 (45.5%)	0.263
	Others	41 (7.6%)	3 (13.6%)	
Nail Diameter	9	30 (5.6%)	1 (4.5%)	
(mm)	10	12 (2.2%)	1 (4.5%)	
	11	333 (62.1%)	12 (54.5%)	**0.018**
	12	1 (0.2%)	1 (4.5%)	
	13	160 (29.9%)	7 (31.8%)	
Open reduction		253 (46.9%)	12 (54.5%)	0.629
Use of cerclage wires		61 (11.3%)	4 (18.2%)	0.518
Post-op Mobilisation	FWB	296 (54.9%)	11 (50.0%)	
(first 6 weeks)	PWB	116 (21.5%)	6 (27.3%)	0.926
	TTWB	70 (13.0%)	3 (13.6%)	
	NWB	57 (10.6%)	2 (9.1%)	
Surgical time (min)		111.51 (44.88)	114.5 (47.84)	0.760
Anaesthetic Time (min)		48.56 (21.80)	46.23 (17.23)	0.621
Time from induction to recovery (min)		178.67 (49.94)	181.68 (48.75)	0.781
Level of First Surgeon				
	Registrar	323 (60.3%)	9 (40.9%)	0.112
	Consultant	213 (39.7%)	13 (59.1%)	
Level of Senior Surgeon Present				
	Registrar	258 (56.1)	31 (56.4)	1.000
	Consultant	202 (43.9)	24 (43.6)	
Complications		**No VTE**	**VTE**	**p-value**
Nail complications		98 (18.2%)	2 (9.1%)	0.419
Failure at lag screw junction		24 (4.5%)	0 (0.0%)	0.635
Self-dynamisation		25 (4.6%)	0 (0.0%)	0.613
Cut-out		13 (2.4%)	0 (0.0%)	0.989
Non-union		67 (14.5%)	7 (12.7%)	0.879
Nail infection		5 (0.9%)	0 (0.0%)	1.000
Peri-implant fracture		14 (2.6%)	0 (0.0%)	0.946
HAP / CAP		102 (18.9%)	4 (18.2%)	1.000
UTI		76 (14.1%)	2 (9.1%)	0.725
Wound infection	Superficial	18 (3.3%)	3 (13.6%)	**0.035**
	Deep	15 (2.8%)	0 (0.0%)	
Washout / Revision for Infection		7 (8.0%)	0 (0.0%)	1.000
CKD Stage pre-operatively				
	Mild	370 (70.1%)	14 (63.6%)	0.684
	Moderate / Severe	158 (29.9%)	8 (36.4%)	
CKD Stage post-operatively				
	Mild	382 (72.8%)	15 (71.4%)	1.000
	Moderate / Severe	143 (27.2%)	6 (28.6%)	
Pre-operative Transfusion		46 (8.6%)	2 (9.1%)	1.000

Complications		No VTE	VTE	p-value
Post-operative Transfusion (48 hours)		273 (50.8%)	15 (68.2%)	0.168
Post-operative Transfusion (total)		338 (62.9%)	15 (68.2%)	0.784
Hb Drop (g/L)		46.54 (18.55)	46.57 (20.85)	0.994
Biochemistry		**No VTE**	**VTE**	**p-value**
Adjusted Calcium	Normal	103 (23.5%)	7 (35.0%)	0.364
	Low	335 (76.5%)	13 (65.0%)	
Albumin	Normal	328 (68.0%)	16 (80.0%)	0.378
	Low	154 (32.0%)	4 (20.0%)	
Alkaline Phosphatase	Low	41 (8.6%)	4 (20.0%)	0.211
	Normal	345 (72.0%)	13 (65.0%)	
	High	93 (19.4%)	3 (15.0%)	
Phosphate	Normal / High	84 (19.1%)	3 (15.0%)	0.869
	Low	356 (80.9%)	17 (85.0%)	
TSH	Low	5 (1.8%)	0 (0.0%)	0.606
	Normal	237 (87.5%)	8 (80.0%)	
	High	29 (10.7%)	2 (20.0%)	
Free T4	Low	6 (2.3%)	0 (0.0%)	0.056
	Normal	223 (84.5%)	6 (60.0%)	
	High	35 (13.3%)	4 (40.0%)	
PTH	High	120 (48.6%)	3 (50.0%)	1.000
	Normal	127 (51.4%)	3 (50.0%)	
Total 25OH Vitamin D	Normal	236 (87.1%)	6 (100.0%)	0.748
	Low	35 (12.9%)	0 (0.0%)	
Radiographic measurements		**No VTE**	**VTE**	**p-value**
Femoral Neck Shaft Angle				
Normal		350 (66.2%)	16 (76.2%)	0.392
Coxa Valga		141 (26.7%)	5 (23.8%)	
Coxa Vara		38 (7.2%)	0 (0.0%)	
Number of fragments (Comminution)	Simple	157 (29.2%)	6 (27.3%)	0.831
	Moderate	263 (49.0%)	10 (45.5%)	
	Severe	117 (21.8%)	6 (27.3%)	
Only Subtrochanteric Extension		96 (17.9%)	3 (13.6%)	0.821
Atypical		29 (5.4%)	1 (4.5%)	1.000
Pathological		28 (5.2%)	2 (9.1%)	0.758
Distal Extension		179 (33.3%)	7 (31.8%)	1.000
Greater Trochanter Fracture		55 (10.2%)	4 (18.2%)	0.404
Lesser Trochanter Fracture		345 (64.2%)	15 (68.2%)	0.880
Medial Calcar Comminution		30 (5.6%)	1 (4.5%)	1.000
AO/ OTA Classification		-	-	0.985
Lateral Cortex Gap Size (mm)	≤4	329 (61.4%)	14 (63.6%)	0.956
	5-9	137 (25.6%)	5 (22.7%)	
	≥10	70 (13.1%)	3 (13.6%)	
Medial Cortex Gap Size (mm)	≤4	354 (66.0%)	17 (77.3%)	0.518
	5-9	130 (24.3%)	4 (18.2%)	
	≥10	52 (9.7%)	1 (4.5%)	
Anterior Cortex Gap Size (mm)	≤4	340 (63.3%)	15 (68.2%)	0.761
	5-9	118 (22.0%)	5 (22.7%)	
	≥10	79 (14.7%)	2 (9.1%)	
Posterior Cortex Gap Size (mm)	≤4	410 (76.4%)	17 (77.3%)	0.971
	5-9	96 (17.9%)	4 (18.2%)	
	≥10	31 (5.8%)	1 (4.5%)	

Radiographic measurements		No VTE	VTE	p-value
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	388 (72.4%)	16 (72.7%)	0.703
	Valgus >5	36 (6.7%)	2 (9.1%)	
	Varus 5-10	84 (15.7%)	4 (18.2%)	
	Varus >10	28 (5.2%)	0 (0.0%)	
TAD (mm)	<25	456 (87.0%)	18 (81.8%)	0.700
	≥25	68 (13.0%)	4 (18.2%)	
Distal locking (Number of Screws)	1	18 (3.3%)	0 (0.0%)	0.824
	2	520 (96.7%)	21 (100.0%)	
Method of locking				
Static Locking		339 (63.5%)	17 (81.0%)	0.257
Secondary Dynamisation		192 (36.0%)	4 (19.0%)	
Dynamic		3 (0.6%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	340 (63.9%)	15 (71.4%)	0.773
	Lateral ≥5	90 (16.9%)	3 (14.3%)	
	Medial ≥5	102 (19.2%)	3 (14.3%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	419 (78.5%)	15 (71.4%)	0.437
	Anterior ≥5	101 (18.9%)	6 (28.6%)	
	Posterior ≥5	14 (2.6%)	0 (0.0%)	
Touching Anterior Cortex		132 (24.8%)	5 (23.8%)	1.000
Distance of tip of the nail from knee (mm)	<10	7 (1.3%)	0 (0.0%)	0.441
	10 to 19	48 (9.0%)	0 (0.0%)	
	20-29	174 (32.8%)	6 (30.0%)	
	≥30	302 (56.9%)	14 (70.0%)	
Nail / Canal Ratio		0.82 (0.08)	0.85 (0.06)	0.148
Hospital stay / Mortality		**No VTE**	**VTE**	**p-value**
HDU / ICU stay		65 (12.1%)	2 (9.1%)	0.932
Total length of hospital stay (days)		22.43 (18.80)	24.5 (13.74)	0.610
Weekend admission		171 (31.7%)	8 (36.4%)	0.823
Died within 30-days		33 (6.1%)	0 (0.0%)	0.463
Died within a year		110 (20.4%)	5 (22.7%)	1.000

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Table C.15 Table presenting the demographics / characteristics of patients having their operation in LTH, stratified according to one-year mortality

Demographics		**Alive**	**Dead**	**p-value**
Total number		435	114	-
Bilateral		13 (3.0%)	1 (0.9%)	0.348
Age < 65 y.o.		127 (29.2%)	7 (6.1%)	**<0.001**
Age 65 – 75 y.o		71 (16.3%)	18 (15.8%)	1.000
Age > 75 y.o.		237 (54.5%)	89 (78.1%)	**<0.001**
Gender	Male	169 (38.9%)	47 (41.2%)	0.723
	Female	266 (61.1%)	67 (58.8%)	
Injury Characteristics		**Alive**	**Dead**	**p-value**
Mechanism of Injury	Low energy	334 (76.8%)	89 (78.1%)	**<0.001**
	High energy	16 (3.7%)	21 (18.4%)	
	Pathological	85 (19.5%)	4 (3.5%)	
Isolated		362 (83.2%)	103 (90.4%)	0.082
ISS > 16		34 (7.8%)	1 (0.9%)	**0.013**
Side	Left	226 (52.0%)	63 (55.3%)	0.600
	Right	209 (48.0%)	51 (44.7%)	
Open fracture		7 (1.6%)	0 (0.0%)	0.371
Medical Comorbidities		**Alive**	**Dead**	**p-value**
ASA	1	48 (11.0%)	0 (0.0%)	**<0.001**
	2	128 (29.4%)	11 (9.6%)	
	3	208 (47.8%)	66 (57.9%)	
	4	51 (11.7%)	37 (32.5%)	
Charlson Comorbidity Score		4.67 (3.00)	7.63 (2.40)	**<0.001**
Diabetes		56 (12.9%)	18 (15.8%)	0.511
Steroids		17 (3.9%)	10 (8.8%)	0.058
Malignancy		86 (19.8%)	47 (41.2%)	**<0.001**
Dementia		72 (16.6%)	51 (44.7%)	**<0.001**
Osteoporosis		**Alive**	**Dead**	**p-value**
Bisphosphonates pre-admission		76 (17.5%)	13 (11.4%)	0.155
Bisphosphonates on discharge		115 (26.4%)	18 (23.7%)	0.717
Calcium / Vitamin D pre-admission		122 (28.0%)	29 (25.4%)	0.662
Calcium / Vitamin D on discharge		205 (47.1%)	40 (52.6%)	0.446
Vitamin D loading on admission		71 (16.3%)	14 (18.4%)	0.775
Fragility Fractures Before		84 (19.4%)	30 (26.3%)	0.134
Fragility Fractures After		78 (18.0%)	6 (5.3%)	**0.001**
DEXA Result	Normal	6 (13.6%)	0 (0.0%)	0.664
	Osteopenia	14 (31.8%)	0 (0.0%)	
	Osteoporosis	24 (54.5%)	1 (100.0%)	
Singh Index	1	30 (7.7%)	11 (10.9%)	**0.006**
	2	86 (22.1%)	25 (24.8%)	
	3	78 (20.0%)	28 (27.7%)	
	4	92 (23.6%)	22 (21.8%)	
	5	47 (12.1%)	14 (13.9%)	
	6	57 (14.6%)	1 (1.0%)	
Social History		**Alive**	**Dead**	**p-value**
Smoking		93 (21.4%)	18 (15.8%)	0.233
Alcohol >10 units / week		88 (20.2%)	14 (12.3%)	0.071
Pre-operative Mobility				**0.029**
Independent		240 (55.2%)	49 (43.0%)	
Stick(s) / Crutch(es)		111 (25.5%)	29 (25.4%)	
Frame		66 (15.2%)	27 (23.7%)	
Wheelchair / Hoisted		18 (4.1%)	9 (7.9%)	

Social History		Alive	Dead	p-value
Frequent falls		108 (24.8%)	39 (34.2%)	0.058
Operation Characteristics		**Alive**	**Dead**	**p-value**
Operation in less than 48 hours		348 (80.0%)	85 (74.6%)	0.255
Simultaneous procedures		35 (8.0%)	2 (1.8%)	**0.030**
Type of Nail	Long Affixus Nail	240 (55.2%)	69 (60.5%)	0.536
	Long Gamma Nail	159 (36.6%)	38 (33.3%)	
	Others	36 (8.3%)	7 (6.1%)	
Nail Diameter (mm)	9	26 (6.0%)	4 (3.5%)	0.233
	10	9 (2.1%)	4 (3.5%)	
	11	275 (63.7%)	64 (56.1%)	
	12	1 (0.2%)	1 (0.9%)	
	13	121 (28.0%)	41 (36.0%)	
Open reduction		205 (47.1%)	57 (50.0%)	0.659
Use of cerclage wires		56 (12.9%)	7 (6.1%)	0.065
Post-op Mobilisation (first 6 weeks)	FWB	232 (53.3%)	69 (60.5%)	0.256
	PWB	95 (21.8%)	23 (20.2%)	
	TTWB	63 (14.5%)	9 (7.9%)	
	NWB	45 (10.3%)	13 (11.4%)	
Surgical time (min)		112.63 (45.71)	107.58 (43.46)	0.292
Anaesthetic Time (min)		48.24 (22.37)	49.19 (19.66)	0.681
Time from induction to recovery (min)		179.86 (50.33)	174.95 (49.19)	0.354
Level of First Surgeon				0.970
	Registrar	260 (60.0%)	67 (59.3%)	
	Consultant	173 (40.0%)	46 (40.7%)	
Level of Senior Surgeon Present				0.965
	Registrar	238 (55%)	63 (55.8%)	
	Consultant	195 (45%)	50 (44.2%)	
Complications		**Alive**	**Dead**	**p-value**
Nail complications		90 (20.7%)	7 (6.1%)	**<0.001**
Failure at lag screw junction		21 (4.8%)	2 (1.8%)	0.232
Self-dynamisation		22 (5.1%)	1 (0.9%)	0.085
Cut-out		13 (3.0%)	0 (0.0%)	0.128
Nail infection		5 (1.1%)	0 (0.0%)	0.551
Peri-implant fracture		11 (2.5%)	2 (1.8%)	0.890
HAP / CAP		62 (14.3%)	40 (35.1%)	**<0.001**
UTI		60 (13.8%)	18 (15.8%)	0.694
Wound infection	Superficial	16 (3.7%)	5 (4.4%)	0.376
	Deep	14 (3.2%)	1 (0.9%)	
Washout / Revision for Infection		13 (38.2%)	1 (11.1%)	0.253
CKD Stage pre-operatively				**0.005**
	Mild	311 (72.8%)	65 (58.6%)	
	Moderate / Severe	116 (27.2%)	46 (41.4%)	
CKD Stage post-operatively				**<0.001**
	Mild	322 (76.1%)	65 (58.6%)	
	Moderate / Severe	101 (23.9%)	46 (41.4%)	
Pre-operative Transfusion		37 (8.5%)	10 (8.8%)	1.000
Post-operative Transfusion (48 hours)		211 (48.6%)	71 (62.8%)	**0.010**
Post-operative Transfusion (total)		267 (61.5%)	79 (69.9%)	0.124
Hb Drop (g/L)		45.72 (18.20)	49.86 (20.05)	**0.037**

Complications		Alive	Dead	p-value
VTE	No	83 (83.0%)	23 (82.1%)	0.749
	DVT	10 (10.0%)	2 (7.1%)	
	PE	7 (7.0%)	3 (10.7%)	
Biochemistry		**Alive**	**Dead**	**p-value**
Adjusted Calcium	Normal	250 (72.7%)	88 (85.4%)	**0.012**
	Low	94 (27.3%)	15 (14.6%)	
Albumin	Normal	138 (36.1%)	14 (13.0%)	**<0.001**
	Low	244 (63.9%)	94 (87.0%)	
Alkaline Phosphatase	High	72 (19.0%)	24 (22.2%)	0.676
	Normal	272 (71.8%)	76 (70.4%)	
	Low	35 (9.2%)	8 (7.4%)	
Phosphate	Normal / High	280 (81.4%)	83 (79.0%)	0.694
	Low	64 (18.6%)	22 (21.0%)	
TSH	High	22 (10.3%)	9 (14.8%)	0.320
	Normal	186 (87.3%)	52 (85.2%)	
	Low	5 (2.3%)	0 (0.0%)	
Free T4	High	31 (14.9%)	8 (13.6%)	0.395
	Normal	171 (82.2%)	51 (86.4%)	
	Low	6 (2.9%)	0 (0.0%)	
PTH	High	98 (51.9%)	29 (53.7%)	0.932
	Normal	91 (48.1%)	25 (46.3%)	
Total 25OH Vitamin D	Normal	28 (13.3%)	5 (8.8%)	0.483
	Low	182 (86.7%)	52 (91.2%)	
Radiographic measurements		**Alive**	**Dead**	**p-value**
Femoral Neck Shaft Angle				**0.004**
	Normal	293 (68.6%)	62 (55.9%)	
	Coxa Valga	111 (26.0%)	34 (30.6%)	
	Coxa Vara	23 (5.4%)	15 (13.5%)	
Number of fragments (Comminution)	Simple	112 (25.9%)	45 (39.5%)	**0.017**
	Moderate	221 (51.0%)	48 (42.1%)	
	Severe	100 (23.1%)	21 (18.4%)	
Only Subtrochanteric Extension		67 (15.5%)	28 (24.6%)	**0.032**
Atypical		24 (5.5%)	2 (1.8%)	0.149
Pathological		9 (2.1%)	21 (18.4%)	**<0.001**
Distal Extension		152 (35.1%)	30 (26.3%)	0.097
Greater Trochanter Fracture		49 (11.3%)	10 (8.8%)	0.542
Lesser Trochanter Fracture		289 (66.7%)	67 (58.8%)	0.139
Medial Calcar Comminution		28 (6.5%)	3 (2.6%)	0.178
AO/ OTA Classification		-	-	0.178
Lateral Cortex Gap Size (mm)	≤4	254 (58.8%)	80 (70.2%)	0.083
	5-9	116 (26.9%)	23 (20.2%)	
	≥10	62 (14.4%)	11 (9.6%)	
Medial Cortex Gap Size (mm)	≤4	286 (66.2%)	77 (67.5%)	0.958
	5-9	104 (24.1%)	26 (22.8%)	
	≥10	42 (9.7%)	11 (9.6%)	
Anterior Cortex Gap Size (mm)	≤4	280 (64.7%)	69 (60.5%)	0.580
	5-9	93 (21.5%)	25 (21.9%)	
	≥10	60 (13.9%)	20 (17.5%)	
Posterior Cortex Gap Size (mm)	≤4	326 (75.3%)	93 (81.6%)	0.211
	5-9	79 (18.2%)	18 (15.8%)	
	≥10	28 (6.5%)	3 (2.6%)	

Radiographic measurements		Alive	Dead	p-value
Reduction Angle Grouped (degrees)	Valgus 5 – Varus 5	316 (73.1%)	80 (70.2%)	0.396
	Valgus >5	31 (7.2%)	5 (4.4%)	
	Varus 5-10	63 (14.6%)	23 (20.2%)	
	Varus >10	22 (5.1%)	6 (5.3%)	
Antirotation Screw		164 (38.7%)	44 (38.6%)	1.000
TAD (mm)	<25	362 (86.2%)	101 (88.6%)	0.606
	≥25	58 (13.8%)	13 (11.4%)	
Distal locking (Number of Screws)	1	13 (3.0%)	4 (3.5%)	1.000
	2	421 (97.0%)	109 (96.5%)	
Method of locking	Static Locking	284 (65.9%)	61 (54.5%)	**0.044**
	Secondary Dynamizationynamisation	144 (33.4%)	51 (45.5%)	
	Dynamic	3 (0.7%)	0 (0.0%)	
Distance of tip of the nail from centre (AP) (mm)	-4 to 4	269 (62.4%)	78 (70.9%)	**0.011**
	Lateral ≥5	83 (19.3%)	8 (7.3%)	
	Medial ≥5	79 (18.3%)	24 (21.8%)	
Distance of tip of the nail from centre (LAT) (mm)	-4 to 4	344 (79.6%)	83 (74.8%)	0.214
	Anterior ≥5	80 (18.5%)	23 (20.7%)	
	Posterior ≥5	8 (1.9%)	5 (4.5%)	
Touching Anterior Cortex		102 (23.7%)	31 (27.9%)	0.420
Distance of tip of the nail from knee (mm)	<10	5 (1.2%)	2 (1.8%)	0.347
	10 to 19	35 (8.2%)	13 (11.8%)	
	20-29	137 (31.9%)	40 (36.4%)	
	≥30	252 (58.7%)	55 (50.0%)	
Nail / Canal Ratio		0.83 (0.08%)	0.82 (0.09%)	0.581
Hospital stay / Mortality		**Alive**	**Dead**	**p-value**
HDU / ICU stay		44 (10.1%)	20 (17.5%)	**0.042**
Total length of hospital stay (days)		22.34 (18.67)	24 (19.00)	0.401
Weekend admission		134 (30.8%)	41 (36%)	0.347

Continuous variables are presented as mean (SD)
Significant parameters are presented in bold ($p < 0.05$)

Appendix D

Study Documents (Laboratory work)

D.1. Lab project ethics

NHS
Health Research Authority
NRES Committee Yorkshire & The Humber - Leeds East
Yorkshire and Humber REC Office
First Floor, Millside
Mill Pond Lane
Meanwood
Leeds
LS6 4RA

Tel: 0113 3050174
Fax: 0113 8556191

23 October 2012

Prof Peter Giannoudis
Consultant
Univeristy of Leeds
Department of Trauma and Orthopaedics
St James's University Hospital
LS9 7TF

Dear Prof Giannoudis

Study title:	**Biological properties of Mesenchymal Stem Cells in Fracture Healing**
REC reference:	**06/Q1206/127**

This study was given a favourable ethical opinion by the Committee on 31 August 2006.

Research Ethics Committees are required to keep a favourable opinion under review in the light of progress reports and any developments in the study. You should submit a progress report for the study 12 months after the date on which the favourable opinion was given, and then annually thereafter. Our records indicate that a progress report is overdue. It would be appreciated if you could complete and submit the report by no later than one month from the date of this letter.

Guidance on progress reports and a copy of the standard NRES progress report form is available from the National Research Ethics Service website.

The NRES website also provides guidance on declaring the end of the study.

Failure to submit progress reports may lead to the REC reviewing its opinion on the study.

06/Q1206/127:	**Please quote this number on all correspondence**

Yours sincerely

E. Rainford

Miss Emma Rainford
Committee Assistant Co-ordinator
E-mail: nrescommittee.yorkandhumber-leedseast@nhs.net

Copy to: *University of Leeds*

D.2. Patient Information Sheet

The Leeds Teaching Hospitals NHS
NHS Trust

PATIENT INFORMATION SHEET

Collection of Bone, Bone Marrow & Blood

You are being invited to take part in a RESEARCH study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends, relatives, and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled "medical Research and You". This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from your study doctor.

Thank you for reading this.

1. What is the purpose of the study?
Special cells in the body termed Mesenchymal Stem Cells (MSCs) can make bone, cartilage, bone, muscle, tendon and ligament. MSCs have been found in all tissues to date but just how they work is still not understood. There is interest in the use of MSCs as a way of repairing damaged joints. Our research is aimed at understanding how these cells work in health and in disease.

2. Why have I been chosen?
You suffer from a fractured bone. At surgery we would like to take a small sample of your bone and bone marrow from the fracture site. This tissue is sitting at the fracture site and is normally discarded during the operation when the fracture site is being cleaned and repaired.

Taking bone, bone marrow and blood at the time of surgery will not cause you further discomfort and will not delay healing in any way.

3. Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care that you receive.

4. What will happen to me if I take part?
If you decide to take part you will be asked to sign an informed consent sheet, and you will be given a copy of the information and the signed consent sheet to keep. You will be asked to donate a small amount of bone marrow normally discarded during your operation.

5. What do I have to do?
Apart from donating a small amount of bone, bone marrow and blood for research, there are no other requirements/tests.

It is very important, when giving your medical history to the doctor, that you tell him/her whether or not you are regularly taking any medicine. Taking certain types of medicines means we will not be able to recruit you into the study.

6. What are the side effects of taking part?
There are no side effects. The collection of the bone, bone marrow and blood is of limited quantity.

7. What are the possible benefits of taking part?
You will not benefit directly from taking part in this research and all other aspects of your care will be the same as if you did not take part.

8. What if something goes wrong?
If you are harmed by taking part in this research, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanism may be available to you.

9. Will my taking part in this study be kept confidential?
As soon as the bone, bone marrow and blood are taken from you, all information that identifies you will be removed so that you cannot be recognised.

10. What will happen to the results of the study?
At the end of the study, the results will be written into a scientific paper for publication in a scientific journal.

11. Who is organising the research?
This project is being organized by Doctors of Leeds General Infirmary and Leeds Institute Molecular Medicine under the supervision and support of Leeds University.

12. Who has reviewed this study?
This study has been reviewed by the independent ethics committee called the Leeds (East) ethics committee. This committee is appointed to determine that research studies are ethical and do not impair the rights or well-being of patients. We have received approval by this committee to be able to do this research study.

13. Contact for further information
Please do not hesitate to contact your GP or any other independent person if you need advice. For further information on the study please contact Professor Peter Giannoudis at 0113 3466460.

D.3. Patient Consent Form

The Leeds Teaching Hospitals NHS
NHS Trust

PATIENT CONSENT FORM

Collection of Bone, Bone Marrow & Blood

Prof Peter Giannoudis

Patient Name: ……..

Patient Identification Number: ……

Please circle as appropriate

1. I have read the patient information sheet for the above study. **Yes/No**

2. I have had the opportunity to ask questions about the study and to discuss it with family and friends if I so wish to. **Yes/No**

3. I understand the purpose of the study, and how I will be involved. **Yes/No**

4. I understand, and accept, that if I take part in the study I may not gain direct personal benefit from it. **Yes/No**

5. I understand that all information collected in the study will be held in confidence and that, if it is presented or published, all my personal details will be removed. **Yes/No**

6. I give permission for responsible individuals from regulatory authorities to have access to my medical notes where it is relevant to my taking part in the research. This is on the understanding that no personal details which might identify me will be presented or published without my permission. **Yes/No**

7. I confirm that I will be taking part in this study of my own free will, and I understand that I am free to withdraw from the study at any time without giving a reason and without affecting my future care or legal rights. **Yes/No**

8. I have spoken to Dr ……..

9. I agree to take part in this research study.

PATIENT:
Signed: …….. **Date:** ……

Name (BLOCK CAPITALS): ……..

Investigator/Sub-investigator

I have explained the study to the above named participant and he/she has indicated his/her willingness to participate

Signed: ……. **Date:** ……..

Name (BLOCK CAPITALS): ……..

Appendix E

Bone marrow harvesting

The MarrowStim consentration system (Biomet Biologics, INC) was used (reproduced from relevant brochure).

MARROWSTIM
concentration system

Marrowstim™ Concentration System Instructions

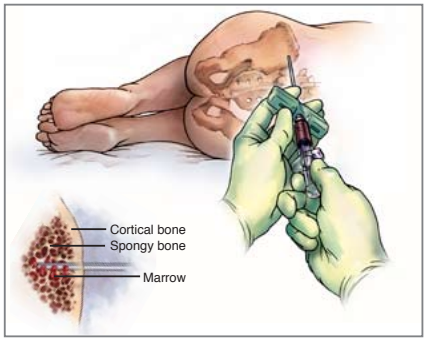


Figure 1

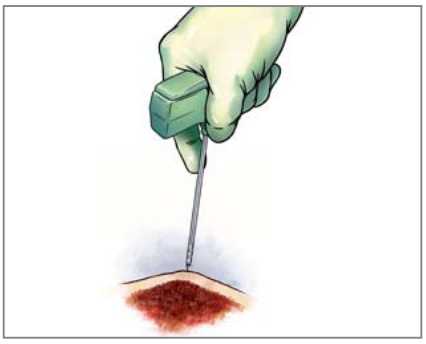


Figure 2

Step 1: Anticoagulation

Rinse MarrowStim™ bone marrow aspirate needle, disposable and two 30ml syringes with anticoagulant to ensure inner surfaces are coated. This will prevent clotting of bone marrow during aspiration. Perform **one** of the following techniques.

Method 1:
Heparin only technique (heparin not supplied in these kits):

Draw 3ml heparin solution (1000 U/ml) into a sterile 30 ml syringe; ensure the heparin coats the entire inner surface of the syringe and set aside. Draw 10ml heparin solution into a second sterile 30ml syringe; ensure the heparin coats the entire inner surface of the syringe. Remove inner trocar from BMA needle. Attach the second 30ml syringe to the BMA needle and prime with heparin, ensuring 3ml of heparin remains in the 30ml syringe. Remove BMA needle and replace the trocar.

Method 2:
ACD-A with heparin coating technique (heparin not supplied in these kits):

Heparin Coating:
Draw 10 ml heparin solution (1000U/ml) into a sterile 30ml syringe. Pull syringe plunger back completely, ensuring the heparin coats the entire inner surface of the syringe. After coating the syringe, push the plunger completely down on syringe to dispense all remaining heparin. Draw 10 ml heparin solution into a second sterile 30ml syringe. Pull syringe plunger back completely, ensuring the heparin coats the entire inner surface of the syringe. Remove inner trocar from BMA needle. Attach the second 30ml syringe to the BMA needle and prime with heparin, ensuring all heparin has been dispensed from the syringe through the needle. Remove BMA needle and replace the trocar.

ACD-A:
Draw 6ml ACD-A into each of the heparin coated 30ml syringes.

For the Marrowstim™ Mini System, only one 30ml syringe of anticoagulated marrow is utilised.

Step 2: Prepare Patient

After suitable anesthesia is achieved, place the patient in the lateral decubitus position. Using sterile technique, prepare the skin with antiseptic and drape. (Figure 1)

Step 3: Position Needle

Hold the needle with proximal end in palm and the index finger against the shaft toward the tip. (Figure 2)

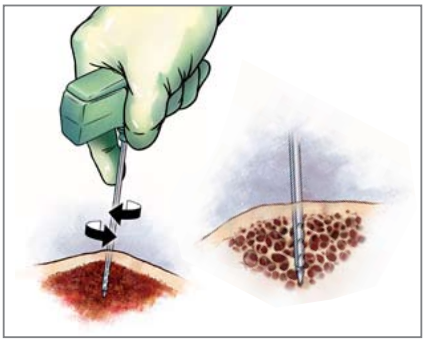


Figure 3

Step 4: Advance Needle

Using gentle but firm pressure, advance the needle, rotating it in an alternating clockwise/counterclockwise motion. Entrance into the marrow cavity is generally detected by decreased resistance. All of the side holes at the distal end of the needle must be introduced into the marrow cavity beyond the cortical bone, otherwise air with extra bony soft tissue may appear with the aspirated marrow. (Figure 3)

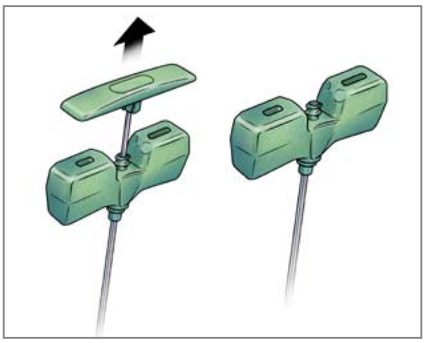


Figure 4

Step 5: Remove Stylet/Trocar

Once needle is in place, remove the stylet by pulling straight out. (Figure 4)

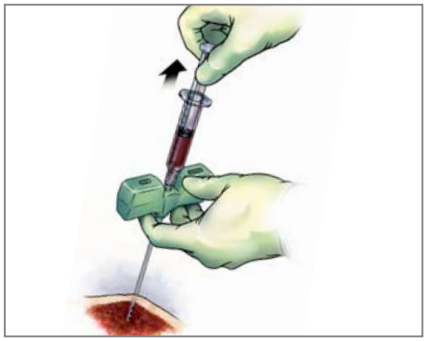


Figure 5

Step 6: Aspirate Marrow

Follow the BMA needle manufacturer package insert (steps 7–9) to obtain a total of 60ml anticoagulated bone marrow aspirate (3ml heparin with 27ml BMA per 30ml syringe **or** 6ml ACD-A with 24ml BMA per 30ml syringe). (Figure 5)

For the Marrowstim™ Mini System, only one 30ml syringe of anticoagulated marrow is utilised.

MARROWSTIM concentration system

Preparation of the Marrowstim™ and Mini Marrowstim™ Concentration Systems

Figure 1

Step 1: Load

Ensure BMA from only one patient is processed per spin.

Unscrew cap on centre port No. 1 and remove cap and green packaging post. (Figure 1)

Figure 2

Slowly load both aspirate filled 30ml syringes (6ml of ACD-A and 24ml of bone marrow aspirate per syringe or 3ml of heparin and 27ml of BMA per syringe), for a total of 60 ml of anticoagulated marrow into centre port No. 1. (Figure 2)

Mini Marrowstim™ Concentration System: Slowly load one 30ml syringe of anticoagulated marrow into centre port No. 1.

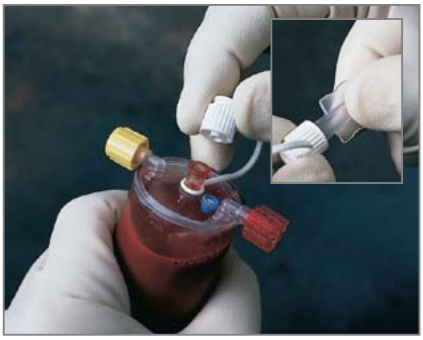


Figure 3

Remove protective cover on white tethered cap and discard. Screw white cap onto centre port No 1. (Figure 3)

Figure 4

Step 2: Balance

Press red button to release lid of centrifuge. Open and place the tube into the centrifuge. (Figure 4)

Mini Marrowstim™ Concentrate Kit: If using the mini kit, the purple mini buckets must be inserted into the centrifuge.

Figure 5

Insert Marrowstim™ Concentration System counterbalance with 60ml of sterile saline or a second Marrowstim™ disposable with BMA (when processing two tubes) into opposite side of centrifuge. (Figure 5)

Mini Marrowstim™ Concentrate Kit: Fill purple mini counterbalance with 30ml of sterile saline and place into opposite side of centrifuge.

Figure 6

Step 3: Spin

Close lid. Set speed at 3200 RPM and time to 15 minutes. Press green button to start spin. Once spin is completed, press red button to release lid and open. (Figure 6)

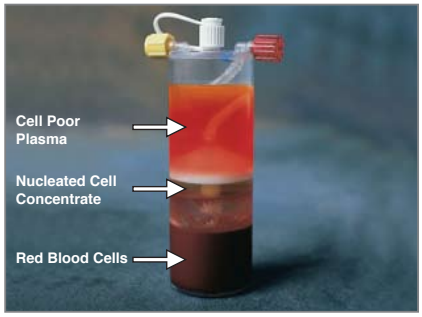


Figure 7: Nucleated cell concentrate (NCC) processed with the Marrowstim™ Concentration System

Remove Marrowstim™ tube from centrifuge and ensure BMA has separated into three distinct layers. (Figure 7).

Figure 8

Step 4: Cell Poor Plasma (CPP) Extraction

Remove yellow cap on side port No. 2 and connect a sterile 30ml syringe. Invert the tube and withdraw the cell poor plasma. (Figure 8)

Figure 9

Step 5: Suspend Nucleated Cell Concentrate (NCC)

While holding the tube in the upright position, shake vigorously for 30 seconds to suspend the cellular elements. (Figure 9)

Figure 10

Step 6: Nucleated Cell Concentrate (NCC) Extraction

Remove red cap from side port No. 3 and connect a sterile 10ml syringe to extract the nucleated cell concentrate. (Figure 10)

Appendix F
Standard Solutions

F.1. Cell freezing media

DMSO (10%), DMEM (45%) and FCS (45%) in this order.

F.2. Osteogenic media

Gibco, StemPro® Osteocyte/Chondrocyte Differentiation Basal Medium, #A10069-01; Gibco, StemPro® Osteogenesis Supplement, #A10066-01.

F.3. Standard MSC culture media

89% DMEM supplemented, with 10% FBS optimised for MSC cultures, and antibiotics (1%; penicillin and streptomycin).

F.4. Patient own serum MSC media

89% DMEM supplemented, with 10% serum derived from same patient, and antibiotics (1%; penicillin and streptomycin).

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Appendix G

Gene table

RT^2 Profiler^{TM} PCR Array Human Osteogenesis (PAHS-026Z)

Position	Symbol	Description
A01	ACVR1	Activin A receptor, type I
A02	AHSG	Alpha-2-HS-glycoprotein
A03	ALPL	Alkaline phosphatase, liver/bone/kidney
A04	ANXA5	Annexin A5
A05	BGLAP	Bone gamma-carboxyglutamate (gla) protein
A06	BGN	Biglycan
A07	BMP1	Bone morphogenetic protein 1
A08	BMP2	Bone morphogenetic protein 2
A09	BMP3	Bone morphogenetic protein 3
A10	BMP4	Bone morphogenetic protein 4
A11	BMP5	Bone morphogenetic protein 5
A12	BMP6	Bone morphogenetic protein 6
B01	BMP7	Bone morphogenetic protein 7
B02	BMPR1A	Bone morphogenetic protein receptor, type IA
B03	BMPR1B	Bone morphogenetic protein receptor, type IB
B04	BMPR2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
B05	CALCR	CALCITONIN RECEPTOR
B06	CD36	CD36 molecule (thrombospondin receptor)
B07	CDH11	Cadherin 11, type 2, OB-cadherin (osteoblast)
B08	CHRD	Chordin
B09	COL10A1	Collagen, type X, alpha 1
B10	COL14A1	Collagen, type XIV, alpha 1
B11	COL15A1	Collagen, type XV, alpha 1
B12	COL1A1	Collagen, type I, alpha 1
C01	COL1A2	Collagen, type I, alpha 2
C02	COL2A1	Collagen, type II, alpha 1
C03	COL3A1	Collagen, type III, alpha 1
C04	COL5A1	Collagen, type V, alpha 1
C05	COMP	Cartilage oligomeric matrix protein
C06	CSF1	Colony stimulating factor 1 (macrophage)
C07	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
C08	CSF3	Colony stimulating factor 3 (granulocyte)
C09	CTSK	Cathepsin K
C10	DLX5	Distal-less homeobox 5
C11	EGF	Epidermal growth factor
C12	EGFR	Epidermal growth factor receptor
D01	FGF1	Fibroblast growth factor 1 (acidic)
D02	FGF2	Fibroblast growth factor 2 (basic)
D03	FGFR1	Fibroblast growth factor receptor 1
D04	FGFR2	Fibroblast growth factor receptor 2
D05	FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
D06	FN1	Fibronectin 1

Position	Symbol	Description
D07	GDF10	Growth differentiation factor 10
D08	GLI1	GLI family zinc finger 1
D09	ICAM1	Intercellular adhesion molecule 1
D10	IGF1	Insulin-like growth factor 1 (somatomedin C)
D11	IGF1R	Insulin-like growth factor 1 receptor
D12	IGF2	Insulin-like growth factor 2 (somatomedin A)
E01	IHH	Indian hedgehog
E02	ITGA1	Integrin, alpha 1
E03	ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
E04	ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
E05	ITGAM	Integrin, alpha M (complement component 3 receptor 3 subunit)
E06	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
E07	MMP10	Matrix metallopeptidase 10 (stromelysin 2)
E08	MMP2	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
E09	MMP8	Matrix metallopeptidase 8 (neutrophil collagenase)
E10	MMP9	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
E11	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
E12	NOG	Noggin
F01	PDGFA	Platelet-derived growth factor alpha polypeptide
F02	PHEX	Phosphate regulating endopeptidase homolog, X-linked
F03	RUNX2	Runt-related transcription factor 2
F04	SERPINH1	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)
F05	SMAD1	SMAD family member 1
F06	SMAD2	SMAD family member 2
F07	SMAD3	SMAD family member 3
F08	SMAD4	SMAD family member 4
F09	SMAD5	SMAD family member 5
F10	SOX9	SRY (sex determining region Y)-box 9
F11	SP7	Sp7 transcription factor
F12	SPP1	Secreted phosphoprotein 1
G01	TGFB1	Transforming growth factor, beta 1
G02	TGFB2	Transforming growth factor, beta 2
G03	TGFB3	Transforming growth factor, beta 3
G04	TGFBR1	Transforming growth factor, beta receptor 1
G05	TGFBR2	Transforming growth factor, beta receptor II (70/80kDa)
G06	TNF	Tumor necrosis factor
G07	TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11
G08	TWIST1	Twist homolog 1 (Drosophila)
G09	VCAM1	Vascular cell adhesion molecule 1
G10	VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor
G11	VEGFA	Vascular endothelial growth factor A
G12	VEGFB	Vascular endothelial growth factor B
H01	ACTB	Actin, beta
H02	B2M	Beta-2-microglobulin

Position	Symbol	Description
H03	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	RPLP0	Ribosomal protein, large, P0
H06	HGDC	Human Genomic DNA Contamination
H07	RTC	Reverse Transcription Control
H08	RTC	Reverse Transcription Control
H09	RTC	Reverse Transcription Control
H10	PPC	Positive PCR Control
H11	PPC	Positive PCR Control
H12	PPC	Positive PCR Control