Teriparatide and Fracture Healing

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Abstract

Background: Ankle fractures are common injuries especially in the elderly that can cause morbidity. There is some evidence that Teriparatide, a drug used to treat osteoporosis, may accelerate fracture healing. To test any drug for its effect on fracture healing it is essential to assess fracture healing objectively. However, there is no optimum method for assessment of fracture healing, nor are there radiographic gold standard parameters of fracture healing.

Aims: 1-To determine if and how Teriparatide is being used by physicians to accelerate fracture healing. 2-To conduct a feasibility study in order to plan a possible future randomised control trial comparing the effect of Teriparatide treatment versus standard care on the healing of Weber B ankle fractures managed conservatively in older people.

Methods: A survey of physicians was carried out in the Kingdom of Bahrain, United Kingdom and United Arab Emirates from April 2016 to April 2017. A feasibility study of ankle fracture healing was carried out between 11th of Nov 2016 to 31st of April 2018 at Queen's Medical Centre, Nottingham. A total of 10 participants aged 50 years and above with ankle Weber B fractures managed conservatively were recruited. Five participants were randomised into a standard care group and five participants into a Teriparatide group and followed up every 2 weeks with CT scans for a total of 12 weeks. A fracture healing score table was developed using parameters from the ankle CT scans. Matlab^{TM} and ImageJ^{TM} software were used to quantify changes in trabecular bone region, mineralised callus and cortical bone. Patient reported outcome measures (PROMs) were recorded using Olerud Molander questionnaire for assessment of ankle function and quality of life EQ-5D-5L health status questionnaire. Additionally, an in-depth qualitative assessment of participants' experiences in the study was carried out on the last trial visit.

Results: Of the 104 included physicians responses, 45.2% (n=47) prescribed Teriparatide, of which six prescribed it to accelerate fracture healing. The reported barriers for Teriparatide usage were: the high cost of the drug as reported in 63%

(n=30); the fact that indication was off label in 36% (n=17); because the drug was only available in injection form in 19.1% (n=17). In the feasibility study, out of 81 patients screened ten participants were recruited and randomised 5 into standard care group and 5 into Teriparatide treatment group. All the ten participants completed the seven study visits without any reported drug side effects (n=5). The CT scan fracture healing score Kappa values (95% CI and P values), showed poor to moderate agreement with the percentage of agreement in the range of 34% to 78%. Internal consistency (Cronbach's alpha) for ankle fracture healing score table for rater AA was 0.804 and for rater WAW was 0.874. In the qualitative study participants were positive about participating in the study, but had some concerns about radiation exposure and the number of trial visits, which necessitated additional travel visits to the hospital.

Conclusion: Teriparatide is being used as an off-label prescription for acceleration of fracture healing. The cost of the drug, its off-label indication and its use in an injection form are the most common barriers for prescribing the drug. This feasibility study provides some evidence for supporting a future randomised clinical trial. However, for such a study to recruit enough patients to have statistical power, it would need to be a multicentre study with improvements in the method used for CT fracture scoring. The qualitative study provided information on the perspectives of the participants participating in the study, with recommendation to reduce the number of CT scans and thus hospital visits.

Declaration

The thesis submitted is an original work, significantly conducted by me with assistance from my supervisors Professor Brigitte Scammell, Emeritus Professor Angus Wallace, Dr Richard Pearson and Professor Opinder Sahota. The feasibility study in this thesis was funded by Nottingham University Hospital charity and sponsored by Research & Innovation, Nottingham University Hospitals NHS Trust. I confirm that, to my knowledge, there are no conflicts of interests in this thesis.

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Abbreviations

A	Standard care group
AA	Rater AA
B	Teriparatide group
BMC	Basic multicellular unit
BMD	Bone mineral density
BMPs	Bone morphogenetic proteins
BRC	Basic remodelling compartment
cAMP	Cyclic amino monoamine phosphate
CI	Confident interval
CT	Computerised tomography
DEXA	Dual energy X-ray absorptiometry
EU	European union
F	Female
FBC	Full blood count
FGF	Fibroblast growth factor
GCC countries	Gulf cooperation council (Kingdom of Saudi, Kingdom of Bahrain, Arabia, state of Kuwait, United Arab Emirates, State of Qatar, Sultanate of Oman)
ID	Identification number
IGF	Insulin like growth factor
IL-1	Interleukin 1
IL-11	Interleukin 11
IL-18	Interleukin 18
IL-6	Interleukin 6
IU	International unit
LFT	Liver function tests
Lt ankle	Left ankle
M	Male
Max	Maximum
Min	Minimum
MSC	Mesenchymal stem cells
no	Number
PDGF	Platelet derived growth factor
PMO	Post-menopausal osteoporosis

PROMS	Patient Reported Outcome Measures
PTH	Parathyroid hormone
RFT	Renal function test
RT ankle	Right ankle
SmPc	Summary of product characteristics
STDEV	Standard deviation
TFG	Transforming growth factor
TGF β	Transforming growth factor beta
TNF-α	Tumour necrotizing factor-alpha
TV	Trial visit
TV1	Trial visit 1
TV2	Trial visit 2
TV3	Trial visit 3
TV4	Trial visit 4
TV5	Trial visit 5
Tv6	Trial visit 6
Tv7	Trial visit 7
UK	United kingdom
VASP	Visual analogue scale for pain
WAW	Rater WAW
WHO	World health organisation
µg	Microgram
£	Sterling pound

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1 Chapter 1 Introduction

1.1 Rationale for the study

1.1.1 Fracture healing in older people

Our knowledge of bone cellular and molecular biology is continuously increasing and new anti-osteoporotic drugs for the prevention of fractures in the elderly are being developed. However despite this, the number of new cases of fractures in the older population is increasing (Ong, Sahota and Marshall, 2015). This is due to our ageing population. The Office for National Statistics reported that of those people who were living in 2014 to 2016, 65 year old men were expected to live a further 18.3 years, with an increase in age of 20.7 years in females of 65 years (Office for National Statistics, 2017).

Moreover, management of fractures in older people often includes surgical fixation, which adds to the cost and morbidity, but allows early mobilization to prevent the morbidity and mortality associated with bed rest (Farsetti *et al.*, 2009). Bone regeneration is a complex process, which is often compromised in older people. Age related changes in bones include, mechanical properties, bone morphology, gene expression and protein crosslinking (Boskey and Coleman, 2010). These changes have a potential to effect bone fracture healing. However, age *per se* has not been identified as a risk factor for non-union or delayed unions of fracture (Mills *et al.*, 2017). Diabetes, which is associated with aging and the severity of trauma have been identified as risk factors for non-union and delayed unions (Hernandez *et al.*, 2012). Additionally, fracture fixation is compromised due to screws not holding in osteoporotic bone. Therefore, fracture healing in older people can be very challenging (Giannoudis and Schneider, 2006).

1.1.2 Ankle fractures - the clinical problem

After hip fractures, ankle fractures are some of the most disabling fractures for patients to sustain (Thakore *et al.*, 2015). The majority of these fractures are treated non-surgically using either a plaster cast or a boot to support the fracture, while it is healing. This means patient's mobility is severely restricted because the patient is

either in a wheelchair or on crutches. Therefore, they have great difficulty getting around for the 6 to 12 weeks whilst their fracture heals, and they are often dependent on hospital care, family members or private carers.

Management of ankle fractures in older patients can be challenging as there is an increased risk of general complications (Varenne *et al.*, 2016). Although surgical management of ankle fracture is often recommended, for early mobilisation of patients, this increases the risk of general complications, as well as local or specific complications (Aigner *et al.*, 2016).

The incidence of ankle fractures in older people is steadily increasing. A study carried out in Finland reported a sharp and steady increase of ankle fracture incidence between the years 1970 (59 fracture per 100,000 persons) and 1997 (169 fracture per 100,000 persons), but then the incidence decreased in 2014 (126 fracture per 100,000 persons). The study concluded that although the incidence decreased, the rapid aging of the population is likely to increase the absolute number of these fractures in the near future (Kannus *et al.*, 2016). The same prediction of increasing number of new ankle fractures, given longevity and better health care of older people, has also been made for the UK (Court-Brown *et al.*, 2017).

1.1.3 Philosophy behind this research

Recombinant parathyroid hormone (rPTH 1-34 or Teriparatide) is one of the newer therapies used to treat osteoporosis. In the last ten years, evidence has emerged that suggests a role for Teriparatide in the management of fractures. Studies in both normal and delayed healing animal models have shown an improved rate of union of fractures (Babu et al 2015). The majority of research on humans has supported the animal research, but it is comprised of low levels of evidence, with few randomised controlled trials, which are not well designed, and many case reports and case series. This has led to a growing number of clinicians using Teriparatide “off license” to treat fractures and non-unions in their patients. Studies have shown that rPTH 1-34, treatment shortened the healing time of pelvic fractures (Peichl *et al.*, 2011), Colles (wrist) fractures (Aspenberg and Johansson, 2010), and

subtrochanteric and femoral fractures related to bisphosphonates usage (Yeh *et al.*, 2017).

This thesis includes a feasibility clinical trial to identify if the experimental design and implementation was feasible. In summary, Weber B ankle fracture patients were managed conservatively in a cast or walking boot with one arm of the trial being randomized to treatment with recombinant Parathyroid hormone (rPTH 1-34) in addition to standard care. Before carrying out a definitive study, a feasibility study is important to identify recruitment rate, retention rate and the best intervals for carrying out assessments such as imaging to document the extent of fracture healing. Currently the methods of measuring early fracture healing parameters have not been identified and this study provides new information on how to record early fracture healing parameters using CT scans.

1.2 Bone composition

1.2.1 Cellular composition

Osteoblasts are bone forming cells, which are located along the bone surfaces. They are derived from mesenchymal stem cells that are committed to the osteoprogenitor lineage by expression of specific genes. Osteoblast activity is regulated by many factors including parathyroid hormone, Vitamin D, glucocorticoids, prostaglandins, oestrogen, cytokines, insulin-like growth factors (IGFs), transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), bone morphogenic proteins (BMPs) and prostaglandins. Additionally mechanical loading is required for osteoblasts to differentiate from their precursors. They synthesise osteoid, the organic matrix and they are responsible for the mineralization of bone. Osteoblast cytoplasm is strongly basophilic containing alkaline phosphatase. Osteoblasts secrete osteocalcin, osteonectin, bone morphogenic protein and collagen type I. Osteoblast cells have receptors for oestrogen, 1,25(OH)2, Vitamin D3 and parathyroid hormone but do not have receptors for calcitonin, fluoride, strontium, and certain statins (Bartel *et al.*, 2007).

Osteocytes form from entrapped osteoblasts and are located in lacunae within the bone matrix. Osteocytes are extensively connected to the inner cambium layer

of the periosteum (osteogenic layer) located on the bone surface and to each other through cytoplasmic tubules running through canaliculi. Osteocytes do not only sense mineral changes and respond to such changes, but they also sense mechanical loading. The surface area of osteocytes is estimated to be 400 fold greater than the Harversian system (channels that transmit blood vessels parallel to the bone axis) and Volkmann canal (small channels that transmit blood vessels from periosteum) into the bone communicating with Harversian canals) areas and more than 100 fold the trabecular surface area, which thus has a huge potential to perform bone mineral homeostasis (Figure 1-1) (Noble, 2008).

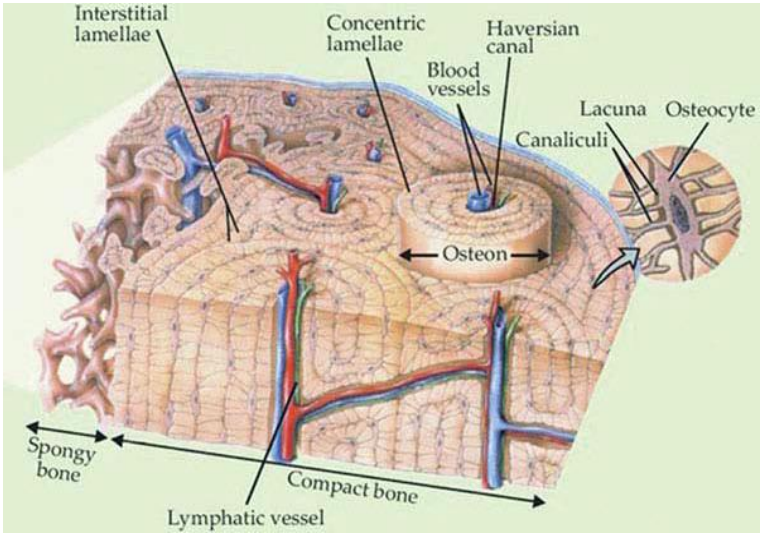


Figure 1-1 Cross section of a cortical bone showing an osteon (Heagerty, Sharma and Heagerty, 2017)

Osteoclasts are bone cells, but they differentiate from mononuclear phagocytes that originate from the haematopoietic system. They are multinucleated giant cells that have 2 to 20 nuclei in a single cell and a diameter of 20-100µm. Their cytoplasm is highly dense with Golgi apparati, mitochondria and lysosome vesicles. Their activity is bone matrix resorption, forming cavities (Howship's lacunae or resorption bays, pits, niches), which can take days to form. Their ruffled borders lies directly on the bone surface and osteoclasts are mobile cells, in common with their precursors. When they attach to bone they start secreting hydrogen ions lowering the surrounding pH hence dissolving hydroxyapatite crystals, which in turn activates metalloproteinases and cathepsin that digests the organic matrix. Tartrate resistance

acid phosphatase is one of the enzymes used as a marker for osteoclast activity (TRAP). Osteoclasts and osteoclast precursors have receptors for oestrogen, with oestrogen exerting its effect on bone by inhibition of osteoclast recruitment. Osteoclast stimulators include, RANK ligand, CSFs, IL-1, IL-6, IL-11, IL-17 and TNF α and β; whereas osteoclast inhibitors include IL-1 receptor antagonist, IL-4, IL-10, IL-12, IL-18, IFN ϒ and TGF β (Bellido, Plotkin and Bruzzaniti, 2014).

1.2.2 Non-cellular composition or bone matrix

The non-cellular component or bone matrix consists of an organic component, non-organic minerals and water (Boskey, 2013). The organic part consists of collagenous and non-collagenous proteins.

1.2.2.1 Collagen type 1

Collagen type 1 makes 90% of organic composition of bone. It is a triple helical molecule consisting of three amino acid chains. The cross linking of the collagen gives bone the property of viscoelasticity, and acts as a scaffold for mineral deposition and binding sites for other molecules. There are two types of crosslinks: 1-Crosslinks formed by enzymes, 2-Cross links formed by glycation. Both of these crosslinks increase with age and change with diseases, hence changing the mechanical properties of the collagen. Glycation crosslinks increase in diabetes and when exposed to oxidative stress making bones more brittle (Saito and Marumo, 2015).

1.2.2.2 Non collagenous proteins

Non-collagenous proteins, which make up 5% of the total bone weight, include osteonectin, osteopontin, fibronectin and bone sialoprotein II, bone morphogenetic proteins (BMPs), and growth factors (Aszódi *et al.*, 2000). These are: small integrin-binding N glycosylated (SIBLING), small leucine-rich proteoglycans (SLRP), y-carboxyglutamic acid protein (GLA protein), small secreted cysteine-rich protein CNN)) families (Sfeir *et al.*, 2005).

Proteins in the extracellular matrix varies with age, site, gender, ethnicity, health status and treatment (Gasser and Kneissel, 2017).

1.2.2.3 Non organic composition

Calcium hydroxyapatite (sometimes referred only as hydroxyapatite) is the principle inorganic or mineral component of the bone. The crystal structure of the hydroxyapatite in relation to size, micro-hardness, micro-strain and calcium to phosphorus ratio indicates the quality of the trabecular bone in normal and osteopenic or osteoporotic bones. In osteopenic and osteoporotic patients the hydroxyapatite crystal size, hardness, calcium/phosphorus ratio, trabecular numbers and bone density decreases compared to bones in normal people (Rollo *et al.*, 2015).

1.3 Bone turnover

1.3.1 Normal bone turnover

Bone is a highly specialised, metabolically active tissue, that provides a rigid structure to the human body, and which is able to remodel and repair itself without a scar. Bone provides protection for vital organs and in the centre of bone, the bone marrow produces new blood cells. Bone is a mineral reservoir for calcium homeostasis, and stores cytokine and growth factors, as well as being involved in acid base balance.

Bone constantly undergoes turnover/modelling throughout life to adapt to changing biomechanical forces, as well as remodelling to remove old, micro-fractured bone and replace it with new, mechanically stronger bone to help preserve bone strength. Remodelling is thus a process whereby bone maintains and replaces itself. The bone remodelling unit (BRU) or basic multicellular unit (BMU) is defined as a group of bone cells that remove and replace one bone structural unit or osteon (Parfitt, 1994). Basic multicellular units (BMU) are the basic building units, formed of local groups of osteoclasts, which are in the front and osteoblasts, which are at the rear of the unit, a vascular capillary in the centre, nerve supply and connective tissue. Each BMU unit is about is 1-2 mm long and 0.2-0.4 mm wide as shown in Figure 1-2 (Spencer, Mcgrath and Genever, 2007) .

The life span of a BMU is usually between 6 to 9 months, whilst an osteoclast's life span is 2 weeks and an osteoblast's life span is 3 months. BMU's operate at the speed of 25 µm per day with one BMU replacing 0.025 mm^3 in its life span. It is estimated that 10% of the skeleton is replaced each year (Manolagas, 2000).

Bone remodelling has six overlapping phases that starts with quiescence phase and ends with the cells going into the quiescence state or resting; 1-Quiescence phase, 2-Activation phase, 3-Resoprtion phase, 4-Reversal phase, 5-Formation phase.

1-Quiescence phase:

This is the resting phase (quiescence phase), where 80% to 90% of the normal bone surface is in this phase. The bone osteoblastogenesis and osteoclastogenesis are in equilibrium, the lining cells cover the bone surface and cells are in a resting/quiescence state for the wave of bone remodelling to start.

2-Activation phase:

In this phase (mononuclear /macrophages) osteoclast cell precursors from the circulation are recruited and migrate to their remodelling destination site. Multiple mononuclear cells fuse together forming multinucleated pre-osteoclasts. Pre-osteoclasts bind to the exposed bone matrix by an interaction between integrin receptors in the cell membrane and RGD (arginine, glycine, and asparagine)-containing peptides in the matrix proteins, to form an annular sealed zone with a bone-resorbing compartment beneath the multinucleated osteoclasts.

3-Resoprtion phase:

This is the third phase in remodelling which takes about 2 to 4 weeks during each remodelling cycle. Resorbing osteoclasts (Howship's lacunae in cancellous bone, or "cutting cones" in cortical bone) secrete hydrogen and chlorine ions into the sealed compartment thereby lowering its pH to as low as 4.5, that mobilises minerals from the bone matrix. Activated osteoclasts excrete: Tartrate resistant acid phosphatase, Cathepsin K, metalloproteinase 9 and gelatinase from cytoplasmic lysosomes that digest the bone matrix, eventually resulting in saucer shaped Howship's lacunae on the surface of trabecular bone and Harversian canal on the cortical bone. This phase ends by the multinucleated osteoclast entering apoptosis (Boyle, Simonet and Lacey, 2003).

4-Reversal phase:

This is the fourth phase in bone remodelling. It is a transitional phase from bone resorption to bone formation. Clefts at the end of resorption phase contain mononuclear cells including monocytes and osteocytes, which are released from bone matrix and pre-osteoblasts. Proposed coupling signals are bone matrix derived factors such as TGF-β, IGF-I, IGF-2, bone morphogenetic proteins (BMP) and platelet derived growth factor (PDGF) (Bonewald and Mundy, 1990).

TGF-β released from the matrix also inhibits RANKL production by osteoblast hence decreasing osteoclast resorption. Strain gradient may lead to serial activation of osteoclasts and osteoblasts where an increased gradient activates osteoblasts and reduced strain activates osteoclasts (Smit and Burger, 2000).

5-Bone formation phase:

This is the fifth phase in bone remodelling process, which takes 6 to 9 months to complete. Osteoblasts synthesise new collagenous matrix; they also secrete enzymes that destroy mineralization inhibitors such as pyrophosphates and proteoglycans. Additionally they secrete small membrane bound matrix vesicles that concentrate calcium and phosphate (Anderson, 2003). As matrix is secreted around the osteoblasts they get entrapped in it and become osteocytes, communicating with lining cells, osteoblasts and each other by cytoplasmic processes known as filopodia through a network of canaliculi. However 50 to 70% of the osteoblasts undergo apoptosis at this stage and therefore only the remaining ones become osteocytes or lining cells.

Equilibrium is reached between osteoblastogenesis and osteoclastogenesis and bone surface is covered with bone lining cells with phenotype indicative of the resting phase or quiescence phase being reached.

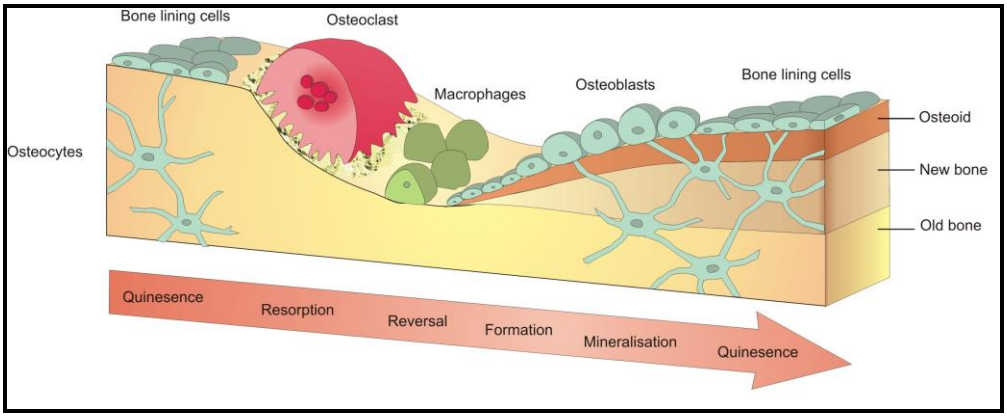


Figure 1-2 Bone remodelling unit (BRU) (Spencer, Mcgrath and Genever, 2007)

Bone remodelling (Figure 1-2) occurs at the periosteal and endosteal surfaces, and within cortical and trabecular bone. There are two ways of bone remodelling: 1-Stochastic remodelling: Occurs randomly, without any signals from cells determined by calcium homeostasis; and 2-Targeted remodelling: provoked by an external stress response to cellular signalling that there is local micro-damage and osteocyte apoptosis (Ruimerman, Hibers and Hulskes, 2005).

1.3.2 Regulation of bone remodelling

1.3.2.1 Systemic regulators of bone remodelling

A-Genetic factors

Genetic factors are important, determining 60 to 80% of bone mass, which explains some of the differences in bone mass between the different ethnic groups (Casman and Ginty, 2003). Experimental studies with animals and patients with certain skeletal conditions shown involvement of certain genes, which regulate the cellular and molecular bone modelling and remodelling. An example is osteopetrosis, where osteoclasts fail to resorb bone leading to excessive bone formation, where several genes have been discovered playing role in its pathogenesis (Baπiê-koretiê and Baπiê-jukiê, 2001). Genetic factors also partially explain why black people have greater bone mass than white people (Casman and Ginty, 2003). Low density Lipoprotein receptor protein (LRP)/Wnts (lipid modified glycoprotein)/Frizzeld receptor (FZD) also plays and important role in bone mass regulation. Genetic mutations leading to hypofunctional alleles of WNT1 causes autosomal-recessive

osteogenesis imperfecta, a congenital disorder characterized by reduced bone mass and recurrent fractures (Keupp *et al.*, 2013).

Anthropometric differences, and ethnic differences in gonadal hormone are some of the potential factors that contribute in ethnic variations in body mass (Leslie, 2012).

B-Mechanical factors

Bone adapts to mechanical loading. This can be recognized in vertebral bone where trabeculae, which are horizontal to the loading forces, tend to become thinner and disappear. Lack of muscular activity, weightlessness and rest have negative effects on bone mass. This has been observed in astronauts travelling in space (Shreyasee, 2010).

C-Nutritional factors

1-Calcium

A daily calcium intake of 1000 to 1200 mg is recommended in older people, either in the diet or as supplementary intake. This was shown in a meta-analysis conducted by Weaver et al; calcium with Vitamin D intake reduced the risk of fractures by 15%, with a 30% reduced risk of hip fractures, recommending the use of calcium and Vitamin D supplement as an intervention for fracture risk reduction (Weaver, Alexander and Boushey, 2016). But in another study conducted by Taj et al, they reported that increasing calcium intake from dietary sources, increased BMD by 0.6-1.0% at the total hip and total body at one year and by 0.7-1.8% at these sites and the lumbar spine and femoral neck at two years. There was no effect on BMD in the forearm. Calcium supplements increased BMD by 0.7-1.8% at all five skeletal sites at one, two, and over two and a half years, but the size of the increase in BMD at later time points was similar to the increase at one year. Their conclusion was increasing calcium dietary or supplementary intake results in a small non-progressive increase in BMD that is unlikely to lead to a clinically significant reduction in risk of fracture (Tai *et al.*, 2015).

2-Vitamin D

Gillespie et al reviewed forty-five trials of Vitamin D and Vitamin D analogues for preventing fractures associated with postmenopausal osteoporosis (Gillespie, Gillespie and O'Connell, 2009). They concluded that frail older people confined to institutions experience a reduction in hip and other non-vertebral fractures if given

Vitamin D with calcium supplements. The effectiveness in fracture prevention of administration of Vitamin D with calcium supplements to community-dwelling older people is unclear. Supplementation with Vitamin D and calcium, for fracture prevention, may be associated with a marginal reduction in mortality compatible with the reduction in hip fracture risk. Vitamin D alone, in the doses, which have been used, appears unlikely to be effective in fracture prevention in older people. There is no evidence that related Vitamin D compounds (analogues) have advantages in terms of effectiveness or reduced incidence of adverse effects compared with Vitamin D (Gillespie, Gillespie and O'Connell, 2009). A controversy exists on the level of Vitamin D in the blood that requires treatment. The National Osteoporosis Society (NOS), in agreement with the Institute for Medicine, has proposed these thresholds: serum 25OHD (25 hydroxy Vitamin D) < 30 nmol/L is deficient, Serum 25OHD 30-50 nmol/L may be inadequate in some people and Serum 25OHD > 50 nmol/L is sufficient for almost the whole population. The NOS recommend oral Vitamin D_3 as the treatment of choice for Vitamin D deficiency (Francis *et al.*, 2013).

Arabi et al recommend correction of PTH levels in Vitamin D hypovitaminosis in the elderly, as the Vitamin D low levels did not predict bone loss rates in the lumbar spine, hip, or forearm except in the trochanteric region. PTH negatively correlated with changes in bone mineral density at all skeletal sites, even after correction for age, changes in body mass index, serum creatinine, calcium intake and 25-OHD levels. PTH levels alone explained 3% of the variances in BMD changes at hip sub-regions (Arabi *et al.*, 2012).

Serum calcium, phosphorus and $1,25(OH)_2D_3$ all influence parathyroid glands in production and release of PTH. Calcium concentration changes are sensed in the blood by calcium sensing receptors (CaSR). These cell surface receptors are located in the parathyroid gland, kidney, gut and bone, where it regulates PTH secretion, vitamin D synthesis, and calcium absorption and resorption, respectively (Díaz-Soto *et al.*, 2016). Changes in serum calcium levels can alter PTH levels via three mechanisms: 1-PTH secretion, this response occurs within seconds, 2-Intracelluar PTH degradation, which occurs within 30 minutes and 3-Gene expression, which occurs within hours (Kumar and Thompson, 2011).

PTH secretion activates renal 1 α-hydroxylase increasing production of $1,25(OH)_2D_3$. Increase in $1,25(OH)_2D_3$ decreases PTH concentration by two feedback mechanisms 1- Increase in $1,25(OH)_2D_3$ influences on intestinal calcium absorption leading to increase in calcium serum levels and 2- $1,25(OH)_2D_3$ directly effects parathyroid glands. Thus rise in $1,25(OH)_2D_3$ levels decrease PTH levels (DiMeglio and Imel, 2013).

Primary hyperparathyroidism is the most common cause of raised PTH and calcium levels because around 85% of primary hyperparathyroidism are caused by isolated adenoma, 15% are diffused hyperplasia and <1% by parathyroid carcinoma. Other causes of hyperparathyroidism include multiple endocrine neoplasia, hyperparathyroidism jaw tumour syndrome and isolated familial hyperparathyroidism (Madkali *et al.*, 2016)

Thus even if vitamin D is corrected still the PTH level can be raised because the most common cause for PTH elevation is adenoma (Lips, 2001). A systematic review has investigated the determinants of the PTH level response to vitamin D supplementation and has confirmed that suppression of PTH level needs higher vitamin D intake (75 microgram/d) than the current recommendations and longer duration of 12 months treatment (Moslehi *et al.*, 2015).

D-Hormonal factors

1-Androgens

Androgens are secreted by the testes in men, ovaries in women and by adrenal glands in both men and women. They have a stimulatory effect on periosteal bone expansion that is greater in boys than girls during puberty. On the endocortical surface, oestrogens promote, and androgens suppress, bone formation during growth. Testosterone decreases bone resorption and increases bone formation. During growth and remodelling testosterone increases periosteal apposition combining with apposition of oestrogen to cause gender differences of bone size after puberty (Walsh, 2017). Figure 1-3 shows androgen effects on osteoblasts, osteoclasts and osteocytes, and the effects of oestrogens on osteoblasts, osteoclasts and osteocytes (Almeida *et al.*, 2017)

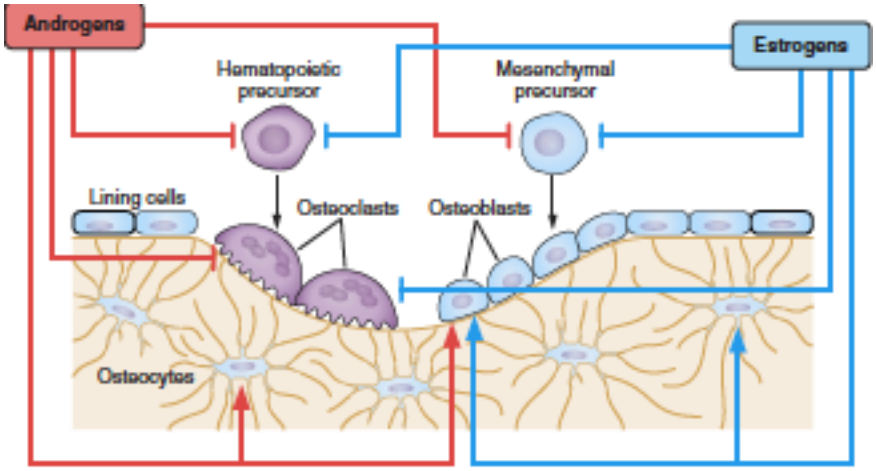


Figure 1-3 Action of Oestrogen and Androgens on osteoblasts, osteoclasts and osteocytes (Almeida *et al.*, 2017).

2-Leptin

Adipocyte hormone leptin is secreted locally by bone marrow adipocytes and has a direct and indirect effect on bone metabolism. Both chondrocytes and osteoblasts express leptin receptors suggesting a direct effect. Leptin regulates growth through activation of fibroblast growth factor 23 (FGF-23). It also impacts and regulates osteocalcin which regulates bone metabolism, insulin sensitivity and energy expenditure (Upadhyay, Farr and Mantzoros, 2015). The ventromedial hypothalamus is activated in response to leptin and activates noradrenergic signalling at osteoblasts (Takeda *et al.*, 2002).

3-Thyroid stimulating hormone

Thyroid stimulating hormone receptors are expressed on chondrocytes, osteoblasts and osteoclasts indicating a direct effect of thyroid stimulating hormone on these cells. Hypothyroidism and hyperthyroidism both affect bone remodelling. Hypothyroidism prolongs the length of the remodelling cycle, specifically the bone formation and mineralization phase resulting in low bone turnover and an increase in bone mass and mineralization. On the other hand hyperthyroidism (thyrotoxicosis) shortens the length of remodelling cycle and an increase in frequency of remodelling cycle initiation leading to high bone turnover, bone loss and decreased bone mineralization (Cardoso, Maciel and Paula, 2014).

4-Growth hormone

Growth hormone (GH), regulates bone remodelling and maintains bone mass through interaction of circulating growth hormone (GH), insulin like growth factors (IGFs), insulin like growth factors binding proteins (IGFBPs) and locally produced IGFs and IGFBPs. GH is a potent regulator of hepatic and skeletal IGF-1. IGF-1 is synthesised by the liver and circulates as an endocrine factor. IGF-1 is also synthesised by pre-osteoblasts, mature osteoblasts, osteocytes and osteoclasts and also acts as paracrine gland (Ueland, 2005). Most of the growth promoting effect of growth hormone (GH) is through Insulin like Growth Factor 1 (IGF-1). IGF-1 increases osteoblasts activity resulting in bone formation. It acts both on longitudinal bone growth that is important before puberty and periosteal growth, which is essential to maintain bone in the adult (Crane and Cao, 2014). Systemic up regulation of IGF-I in osteoblasts also occurs through the effects of parathyroid hormone, 17beta-estradiol and thyroid hormone (TH) (Lindsey and Mohan, 2016). There are also local regulators which influence the production of IGF-1; these are: fibroblast growth factor 2, transforming growth factor-b1, bone morphogenetic protein 7, and interleukin-1 which are locally produced growth factors (Oryan, Monazzah and Bigham-sadegh, 2015).

Calcitonin inhibits osteoclast action on bone remodelling by changing the ruffled border and cell immobility, thus arresting bone resorption. (Davey and Findlay, 2013).

1.3.2.2 Local regulators of bone remodelling

The discovery of the receptor activator NF-κB ligand (RANKL) / receptor activator NF-κB (RANK) and osteoprotegerin (OPG) system made a huge impact on understanding of bone remodelling. The system is composed of a ligand, RANKL (present as a transmembranous and secreted protein) and its two receptors RANK and OPG (present as a soluble molecule) that are expressed by mesenchymal cells, osteoblasts and osteocytes. Because OPG has a similar N-terminal to RANK this allows OPG to act as a decoy receptor to RANKL. Binding of RANKL to RANK drives differentiation of mononuclear precursors into multinucleated active osteoclasts and

it also promotes osteoclast survival. OPG counteracts RANKL activity and stops osteoclasts from excessive bone resorption (Figure 1-4) (Matsumoto *et al.*, 2016).

Macrophage colony stimulating factor (M-CSF) present as transmembranous and secreted forms that are produced by osteoblasts, precursors and osteocytes. M-CSF receptors are expressed by: multi-potent haematopoietic cells, mononuclear phagocytes progenitor cells, monocytes tissue macrophages and osteoclasts. Binding of M-CSF to its receptor activates pro-survival pathways, reorganization of the actin cytoskeleton and promotes transcription of RANK (fully committing the precursor to the osteoclast lineage). In mature osteoclasts, M-CSF with integrin remodels the actin cytoskeleton, forming an actin ring, a key structure for osteoclast activity (Bellido, Plotkin and Bruzzaniti, 2014).

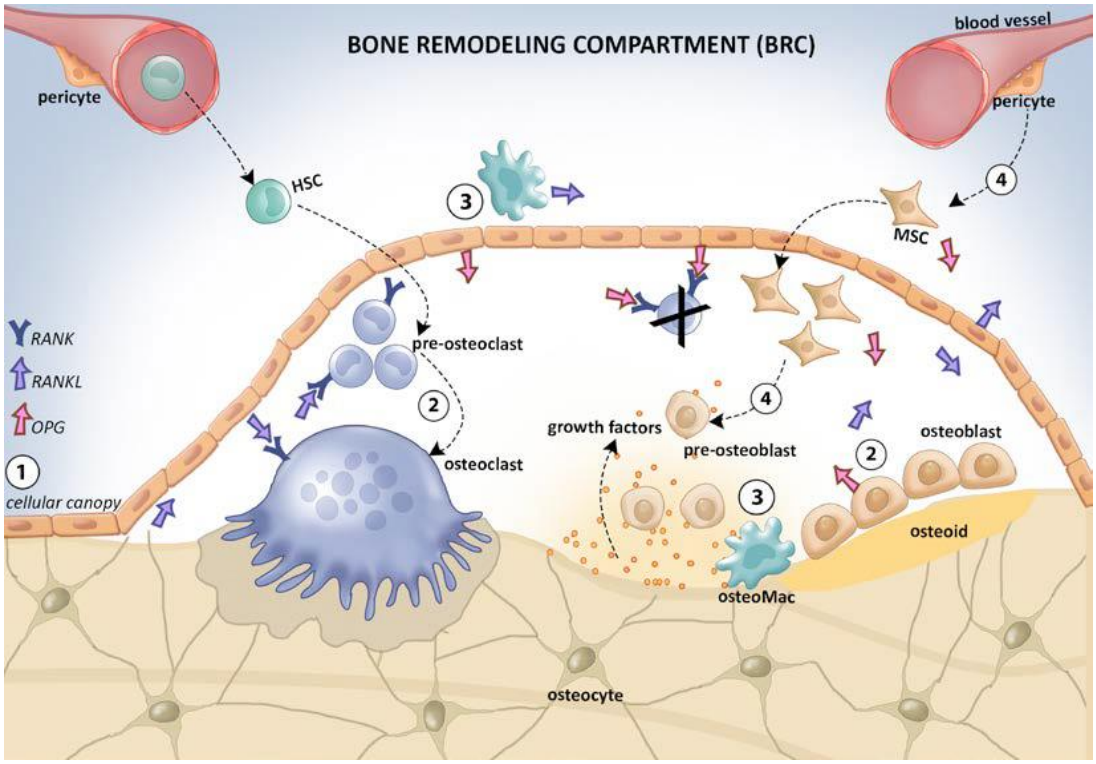


Figure 1-4 Bone remodelling compartment (BRC). 1) Osteocytes communicate with the lining cells of the endosteal surface, which work as a cellular canopy. 2) All osteoblastic lineage cells (canopy, pre-osteoblast and osteoblasts) can express regulatory markers of osteoclastogenesis (RANKL and OPG), according to the different stages of remodelling. 3) Also, OsteoMacs (macrophages) can express markers of osteoclastogenesis, such as RANKL and can remove the remaining debris

left from the resorbed bone. 4) MSC from pericites (perivascular cells) can differentiate into pre-osteoblasts and initiate new bone formation (Matsumoto *et al.*, 2016).

The sources of MSCs are widespread (Berebichez-Fridman and Montero-Olvera, 2018). The precise definition of these cells remains a matter of debate. A precursor cell is also known as progenitor cell, but progenitor cells are multipotent. Precursor cells are known as the intermediate cell before they become differentiated after a stem cell. Usually a precursor cell is a stem cell, which has the capacity to differentiate into only one cell type. Nevertheless, to date MSCs are widely defined as cell population that can be directed to differentiate *in vitro* into cells of osteogenic, chondrogenic, adipogenic, myogenic, and other lineages (Javazon, Beggs and Flake, 2004), (Prockop, 2009). As part of their nature MSCs proliferate and give rise to daughter cells that have the same pattern gene expression and phenotype, therefore, maintain the "stemness" of the original cells. Therefore, MSCs are progenitor cells as they can give rise to many cell types. A preosteoblast is a precursor cell of the osteoblasts.

1.3.3 Bone turnover in osteoporosis

1.3.3.1 Osteoporosis

It was John Hunter (1728-93) in the 18th century, who discovered the process of bone remodelling, whereby old bone is resorbed and new bone laid down (Meikle, 1997). The term osteoporosis is credited to a French pathologist Jean Lobstein (1777-1835) in 1830, who was the first to describe the 'holes' in bones of some of the patients to be larger than others. This was originally reported in an article published in French that was later translated to English by Stride (Stride, Patel and Kingston, 2013). Fuller Albright in 1941 presented the clinical features of post-menopausal osteoporosis. This was the first time clinical osteoporosis was described (Albright, Smith and M.A., 1941).

On plain x-rays of long bones in osteoporotic patients, cortices are thinned with minute concave like chippings at the endosteal surfaces of resorbed bone, and decreased trabecular bone is largely noticed at ends of the long bones. In lumbar

spine x-rays of an osteoporotic patient the vertebral bodies are demarcated by an outer line and empty box like inside, like a picture frame appearance. The trabeculae are verticulized due to rarefaction of the horizontal trabeculae and a compression fracture noted without discontinuation of the cortical line is very common. In a common osteoporotic wedge fracture, the anterior height of the vertebral body is reduced more than 4 mm comparing to the posterior height of the vertebral body (Patel *et al.*, 2015).

The World Health Organization (WHO) has defined osteoporosis "as a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures" (Ghannam, 1994). The WHO definition covers the clinical, diagnostic and pathological aspects of the disease. Most patients with osteoporosis do not present to clinic unless they sustained a low impact fracture, defined as a fracture occurring from a standing height or less, or a fragility fracture, defined as a fracture occurring spontaneously e.g. on coughing, sneezing or sudden movement.

WHO has proposed a clinical diagnosis of osteoporosis based on bone mineral density, stating that a patient is considered to have osteoporosis if the bone mineral density (BMD) T score is -2.5 or less standard deviations below the reference population mean, matched for a healthy 30 year old adult (man/woman).

The WHO study group has defined osteopenia and osteoporosis:

Normal: $T \geq -1.0$

Osteopenia: $-2.5 > T < -1.0$

Osteoporosis: $T \leq -2.5$

Established osteoporosis: $T \leq -2.5$ + one or more fractures.

The Z score is matched for age and gender reference group (Dimai, 2017).

1.3.3.2 Epidemiology of osteoporosis

Osteoporosis affects over 200 million people worldwide, and because it is a clinically silent disease of bone, patients often do not present until they have had a low impact fracture. Hence, osteoporosis is under-recognized and under-treated (Lin & Lane 2004). Osteoporosis has a huge impact globally. Currently it has been estimated that more than 200 million people are suffering from osteoporosis. The

prevalence of osteoporotic fractures in those over the age of 50 years is 1 in 3 for women and 1 in 5 for men (Sözen, Özışık and Başaran, 2017).

In a country specific report of osteoporosis in the European Union published in 2013, it was estimated that 5.5 million men and 22 million women had osteoporosis in 2010. There were 3.5 million new fragility fractures sustained, which were categorized into 610,000 hip fractures, 520,000 vertebral fractures, 560,000 forearm fractures and 1,800,000 as others (pelvis, humerus, ribs, tibia, fibula, clavicle, scapula, sternum, and femoral). The total cost of treatment in the EU was €37 billion annually (Svedbom *et al.*, 2013).

According to the office for National statistics, the United Kingdom population in mid 2018 was estimated to be 66,45 million with growth rate of 0.6% between mid 2017 and mid 2018. The composition of the population was determined by births, deaths and migrations that have been taken place in the previous years. However, the age groups are changing at different rates with the number of those aged 65 years and above growing faster than those at 65 years of age and below. The number of people aged 65 to 84 years had increased by 23.0% to 10.6 million between 2008 and 2018 indicating that UK elderly population size is increasing disproportionately (Office for National Statistics, 2019).

1.3.3.3 Classification of osteoporosis

Post-menopausal osteoporosis type 1

Post-menopausal osteoporosis (PMO) is the most common type of osteoporosis in women. It is estimated that there are 1.8 million women who have PMO in 2010 and this number will increase to 2.1 million in 2020; that is increase of +16.5% of women having PMO in UK (Gauthier *et al.*, 2011).

In postmenopausal type 1 osteoporosis the decrease in bone mineral density is within the first 15 to 20 years of menopause (Richelson *et al.*, 1984). Nordin et al (1990) reported that 11% of BMD lost is due to menopause and 18% is due to age in a 70 year old female (Nordin *et al.*, 1990)

Senile osteoporosis type 2

Senile osteoporosis as its name implies is in the older age group, 70 years old and above. There is a constant gradual loss of bone after the age of 70 years. As the

human body ages there is a shift from osteoblastogenesis to adipogenesis. This type of osteoporosis is equally distributed in men and women. The rate of bone demineralisation is equal in both men and women (Duque and Troen, 2008).

Secondary osteoporosis type 3

In this type of osteoporosis, there is decalcification of the bones due to an underlying cause. This was first noticed in 1932 associated with patients presenting with Cushing's syndrome (Sissons, 1956). High levels of corticosteroids, suppresses osteoblasts from forming new bones. Additionally corticosteroids inhibit intestinal calcium absorption, which raises parathyroid levels that increases bone resorption to maintain steady calcium levels in the blood. Glucocorticoids are known to increase calcium excretion in the urine (Canalis *et al.*, 2007).

There is wide range of secondary causes of osteoporosis related to endocrine disorder gastrointestinal diseases, bone marrow related disorders, organ transplantation, genetic disorders and miscellaneous causes (Fitzpatrick, 2002).

1.3.4 Risk factors of osteoporosis

1.3.4.1 Non-modifiable factors

Age:

A study carried out in Denmark at Aalborg University Hospital concluded that age is the highest predisposing risk for osteoporosis. Although the referrals for dual energy X-ray absorptiometry (DEXA) scan decreased for patients aged 70 years and above, the number of new cases of osteoporosis increased after the age of 80 years (Andersen and Laurberg, 2014).

Compston et al reported histomorphometric changes with ageing, namely a decrease in cortical bone and an increase in cortical porosity, which was qualitatively similar in men and women (Compston, 2011). Telomeres, which are short segments at the end of chromosomes, shorten in length with aging. This affects the cell's senses to responses to external forces by altering gene expression hence, altering morphological changes, abnormal protein expression and abnormal apoptosis (Boskey and Coleman, 2010).

Gender:

Osteoporosis starts at an earlier age in women than in men, menopause being the factor, which usually starts between mid-40s and early 50s. This oestrogen decline accelerates the rate of bone loss in women for the first two decades (Clarke and Khosla, 2010). The average decrease in trabecular volumetric BMD in women is around 55% and in men is around 46% at central sites ($p<0.001$) (Pinheiro *et al.*, 2010).

Ethnicity:

Contrary to general belief that osteoporosis is a white women's disease, Conner et al investigated a population of 197,484 women aged 50 years that constituted of 7784 (3.9%) black American (5.8% ≥80 years old), 6973 (3.5%) Hispanic American (7.0% ≥ 80 years old), 1912 (1.0%) Asian American (3.9% ≥ 80 years old), 1708 (0.9%) Native Americans (13.8% ≥ 80 years old), 179,470 (90.7%) White American (6.5% ≥ 80 years old). The percentage of osteoporosis in postmenopausal women was 11.9% in native Americans, 10% in Asian Americans, 9.8% in Hispanic Americans, 7.2% white Americans and 4.2% in black Americans. Additionally, osteopenia among different ethnic groups was as follows: 50.1% Asian, 46.5% Hispanic, 44.5% Native American, 39.6% white Americans and 28.1% blacks Americans (Barrett-Connor *et al.*, 2005)

1.3.4.2 Modifiable risk factors

Physical activity

It is well known that physical activity improves physical health and wellbeing of a person. Physical activity is also known to increase bone density. High impact exercise has been shown to increase BMD at the site of impact. For example, high impact exercise increased BMD at the hip in the exercise group but there was no effect on the radius were no forces impacted (Heinonen *et al.*, 1996). Exercise can stimulate osteocytes by mechanosensing either by direct stimulus through mechanical loading on these cells which are embedded in the bone, or stimulating them through channels, to signal increase osteoblastic activity and decrease osteoclastic activity, thus shifting the balance in favour of bone formation

(Bonewald, 2011). Figure 1-5 explains how mechanical loading is exerted on the osteocytes through hydrostatic pressure (Chen *et al.*, 2010)

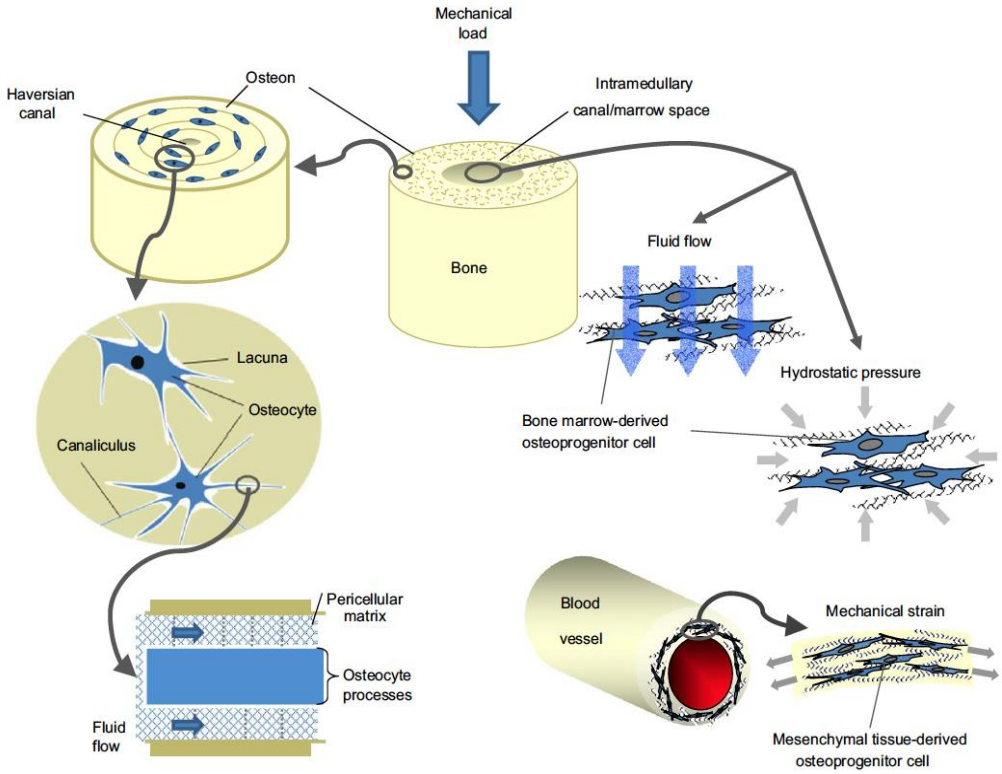


Figure 1-5 Hydrostatic pressure generated by fluid exerting its pressure on the osteocytes through canaliculi (Chen *et al.*, 2010).

Immobilization

Immobilization can be divided into temporary immobilization, when patients are placed in cast for a period of time or post-surgery were patient are immobilized in bed like post cardiac surgery; or permanent immobilization, when patients have a neurological injury that causes paralysis. A study of BMD differences between the paralysed and non-paralysed limbs in 48 patients (31 men and 17 postmenopausal women with lower limb hemiplegia, showed there was significant bone loss in the paralysed limb. Additional; analysis showed the degree of bone loss depended directly on the duration of immobilization, data was controlled for sex and age (R=0.193, p=0.034). But when time since menopause and length of immobilization were used as covariates, time since menopause was the only significant determinate of loss of bone density in post-menopausal women (R=0.312, p=0.039) (del Puente

et al., 1996). However, in another study conducted by Prince et al, the BMD of 74 patients was compared between the paralysed and non-paralysed upper limbs. Bone mineral content loss at the cortical and trabecular sites correlated significantly with duration of stroke and negatively correlated with reduction in forearm function. The authors concluded that bone mineral loss was also at least partly related to a prolonged period of immobilization (Prince, Price and Ho, 1988). Moreover, in a study conducted by Coupaud et al, they reported decrease in bone mineral density at trabecular and cortical bone compartment of the tibia and femur during the first year of spinal cord injury (Coupaud *et al.*, 2015).

Cigarette Smoking

Cigarette smoke contains more than 4,000 chemical compounds of which 42 compounds are known to be carcinogenic. The list is too long to mention all the compounds of cigarettes, however these are some of the chemical compounds in the smoke of the cigarette that are released nicotine, tar, carbon monoxide, formaldehyde, ammonia, hydrogen cyanide, and DDT.

Nicotine is the main compound that causes addiction, and it has a dose dependent effect on bone metabolism. In low doses it stimulates osteoblasts, whereas in high doses it has a deleterious effect on osteoblasts (Rothem *et al.*, 2009) (Kallala *et al.*, 2013). Kanis et al conducted a meta-analysis from 10 prospective cohort multicentre studies, which included 59,232 men and women, of which 18% were current smokers. For men and women combined, risk with current smoking was highest for hip fracture (RR= 1.84), lowest for fractures taken overall (RR=1.25) and intermediate for osteoporotic fracture (RR=1.29) (Kanis *et al.*, 2005),(Pearson *et al.*, 2016).

Alcohol consumption

There is a consensus that high intake of ethanol impairs a cell's ability to regenerate and grow (Michot and Gut, 1987).

In a study by Kim et al in a total of 36 subjects, 18 subjects consumed 40 g per day and 18 subjects consumed 20 g per day. BMDs at the femoral Wardels triangle and at the trochanteric region were significantly lower in the subjects who consumed 40 g of alcohol per day (Kim *et al.*, 2003).

Corticosteroid induced osteoporosis

Steroids are widely used in medical practice for the treatment of mixed connective tissue diseases like rheumatoid arthritis, systemic lupus erythematosus, asthma and others. Corticosteroids induce osteoporosis by decreasing osteoblast proliferation and biosynthesis activity. Sex steroid deficiency and hyperparathyroidism are some of the proposed mechanisms. High levels of corticosteroids have an inhibitory effect on osteoblastogenesis and promotes apoptosis of osteoblasts and osteocytes (Manolagas and Weinstein, 1999).

Parathyroid hormone

Parathyroid hormone is a major regulator of calcium homeostasis. The Food and Drug Administration (FDA) has approved the use of parathyroid hormone for the treatment of osteoporosis. There is growing evidence in the literature about parathyroid hormone (PTH) or Teriparatide (which is the first 1-34 amino acid of the parathyroid hormone) being used to accelerate fracture healing, although most of the trials are in animal models. There are a number of case reports and case series but very few trials of human subjects indicating the effect of Teriparatide in accelerating fracture union in a range of skeletal locations see Table 1-6.

Parathyroid hormone is an endogenous polypeptide hormone, which consists of 84 amino acids, secreted by the chief cells of the parathyroid glands. Parathyroid hormone 1-84 (PTH) initially is in the form of pre-pro PTH, a precursor, which consists of 115 polypeptide amino acids, cleaved within parathyroid cells at the N-terminal portion, first to pro-PTH (90 amino acids) and then to PTH (84 amino acids). PTH is the major form of the stored, secreted, and biologically active hormone (Slatopolsky, Martin and Hruska, 1980).

The parathyroid gland consists of two types of cells; chief and Oxyphil cells. Chief cells are the most abundant cells. When inactive, they are cuboidal in shape and contain few granules, whereas the active cells are larger, and contain acid phosphatase in a large number of secretory granules. Chief active cells are triggered to secrete PTH by low Ca^{2+} levels in the blood. Oxyphil cells are larger than the chief cells and tend to appear at the onset of puberty but their function is not known.

When Ca^{2+} sensing receptors (CaSR), present on the plasma membrane of the chief cells, sense low calcium levels in the blood, they trigger the cells to secrete PTH in the blood maintaining Ca^{2+} levels in a limited range. CaSR comprises of three segments; these are: 1) a large extracellular domain consists of 612 amino acids, which reacts to Ca^{+} in the blood; 2) an inter-membrane segment that has seven membrane spanning regions consisting of 250 amino acids; and 3) an intra-cellular domain consisting of approximately 200 amino acid. The calcium sensing receptor regulates the parathyroid gland by three mechanisms: 1) Secretion of parathyroid hormone, 2) Synthesis of parathyroid hormone, and 3) Cellular proliferation of parathyroid (Brown, 2007).

When a molecule is detected by the receptors outer part, the internal part activates the transduction signal pathway, thus activating a cellular response. There are two main intracellular signal transduction pathways: 1) the cAMP signal pathway, and 2) the phosphatidylinsitol signal pathway (Trzaskowski *et al.*, 2012).

Advancements in molecular biology have resulted in the synthesis of recombinant PTH. Recombinant parathyroid hormone (rhPTH (1-34)) has similar biological properties to PTH (1-84). The N-terminal portions of the hormone are inactive in blood, with only the initial 34 amino acids being essential for the hormone to function. However there are studies suggesting a role of the C-terminal (Osteostatin) in bone regeneration (Esbrit, 2013). There are three variants of Parathyroid hormone (1-84); human PTH (1-38) (Onyia *et al.*, 2000), Teriparatide (1-34) and Parathyroid related protein (hPTHrP 1-36). Currently only two forms of hormone are licenced for anti-osteoporosis treatment; PTH (1-84) and PTH (1-34**).**

There are two types of parathyroid hormone receptors. Type 1 (PTH1) was first cloned from opossum and bone tumour cells, and this is the common receptor for PTH and PTHrP referred as the PTH/PTHrP receptor. It is abundant in skeletal tissues and the kidneys with a uniform distribution in the tissues of these organs. Parathyroid hormone receptor type 2 (PTH2), has an amino acid sequence that matches 50% with PTH1 receptor. It is present in abundance in the brain, testes and in other tissues at low levels. The ligands for PTH1 and PTH2 receptors are parathyroid hormone (PTH), parathyroid hormone related protein (PTHrP) and the tuberoinfundibular peptide (TIP 39)(Suzuki, 2016). Ellegaard et al summarised the suggested effects of PTH on phases of fracture healing in Figure 1-6 (Ellegaard, Jørgensen and Schwarz, 2010).

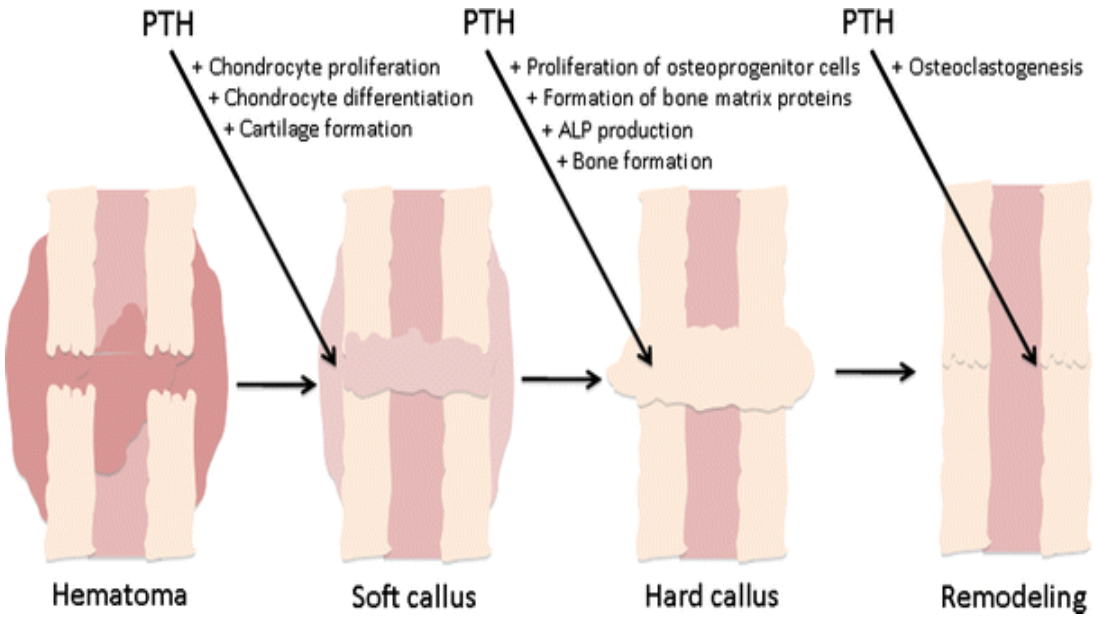


Figure 1-6 Role of PTH in phases of fracture healing (Ellegaard, Jørgensen and Schwarz, 2010)

Paradox of Parathyroid biological action:

Parathyroid hormone, when given in low, intermittent doses increases bone density. Paradoxically, if parathyroid hormone is given in high doses and for a prolonged period, it leads to osteoporosis, and parathyroid deficiency also leads to osteoporosis (Shimizu *et al.*, 2016).

Pre-osteoblast precursors and pre-osteoblasts have PTH receptors. PTH binds to the PTH receptor 1 and induces differentiation of pre-osteoblasts precursors to pre-osteoblasts and from pre-osteoblasts to osteoblasts. Osteoblasts indirectly stimulates pre-osteoclasts by generating IL-6, which in turn stimulates pre-osteoclasts to differentiate to osteoclast, osteoclasts resorb bone and liberate calcium but they lack PTH receptors so osteoclasts are influenced by osteoblasts indirectly (Kroll, 2000). Parathyroid glands show circadian and seasonal fluctuation. PTH levels decrease 20% below the annual mean in summer. PTH peaks in the early morning and drops in the late morning. A second lower PTH peak is in the afternoon. In a healthy adult 70% of the PTH is secreted in a tonic fashion, were as the remaining 30% is secreted in low amplitude and high frequency burst occurring every 10 to 20 minutes. Acute hypocalcaemia provokes a several fold increase in

burst and amplitude of PTH. Hypercalcemia supresses these pulsatile bursts of PTH as does calcitriol (Chiavistelli, Giustina and Mazziotti, 2015)

Studies have shown pulsatile PTH increases BMD, improves bone micro-architecture, reduce vertebral fractures and non-vertebral fractures also (Dempster *et al.*, 2001) (Neer *et al.*, 2001)

Homeostatic role of sustained elevation of PTH can maintain blood calcium against challenge of prolonged calcium deficiency by mobilizing calcium from bone (calcium bank), this reduces bone mass. On the other hand in the therapeutic role of deliberate short pulses of PTH dramatically builds bone mass (Kuo *et al.*, 2017).

1.3.4.3 Teriparatide

Teriparatide is a recombinant form of parathyroid hormone which consists of the first 34 amino acids of the parathyroid hormone 184 amino acids. In a study conducted by Neer et al, 1,637 post-menopausal women with prior vertebral fracture were randomly assigned to 20 or 40 μg teriparatide or placebo. In the placebo group there were 14% new vertebral fractures and 6% new non-vertebral fractures. In the Teriparatide 20 μg group there were 5% new vertebral fractures with relative risk of 0.35 (95% CI 0.25-0.88) and there were 3% new non-vertebral fractures with relative risk of 0.47 (95% CI 0.25-0.88). The BMD increased by 9% in the lumbar spine and by 3% in the femoral neck compared to placebo. In the Teriparatide 40 μg group there were 4% new vertebral fractures with relative risk 0.31 (95% CI 0.19-0.50) and there were 3% new non vertebral fractures with relative risk 0.46 (95% CI 0.25-0.86). The BMD increased by 13% in the lumbar spine and 6% in the femoral neck compared to placebo. But the BMD decreased by 2% in the radius. They concluded that Teriparatide increased the bone mineral density and decrease the risk of fractures in both 20 μg and 40 μg Teriparatide group but 40 μg Teriparatide group had higher side effects (Neer *et al.*, 2001).

In a randomised double blind study, conducted by Body et al, comparing efficacy of Teriparatide with alendronate, 146 postmenopausal women were randomised into oral alendronate 10 mg + placebo injection group (n=73) and 40 μg Teriparatide injection +oral placebo (n=73). Their median duration of the treatment was 14 months. However they reported an increase of BMD of the lumbar spine at 3

months of 12.2% in the Teriparatide group and 5.6% in the alendronate group (p=0.001). Furthermore the Teriparatide group increased BMD at femoral neck and total BMD compared to alendronate (p=0.001) but the BMD at the distal radial shaft decreased with 40 µg Teriparatide group comparing to alendronate group ($p \leq 0.001$)(Body *et al.*, 2002). Table 1-1 show pivotal clinical trials where the different classes of drugs have significant effect on bone density at different sites of skeleton. In the table Teriparatide had significant effect on the vertebral and non-vertebral bones except the hip bone no reports has been published (Hanley *et al.*, 2012).

Table 1-1 Pivotal clinical trials of anti-osteoporotic drugs and their indications in regards with their effects significant or non-significant on vertebral, non-vertebral and hip bones, adapted from (Hanley *et al.*, 2012).

Medications	Indication in PMO	Pivotal trial name	Statistically significant relative fracture risk reductions vs. control			Administration	Dose
			Vertebral	Non-vertebral	Hip		
Alendronate	Treatment and prevention of osteoporosis in postmenopausal women	FIT I FIT II	√ √	X X	√ X	Oral	5 mg daily for prevention of osteoporosis; 10 mg daily (alternatively 70 mg once weekly) for treatment
Risedronate	Treatment and prevention of osteoporosis in postmenopausal women	VERT NA HIPS	√ NR	√ NR	NR √	Oral	5 mg daily (alternatively 35 mg once weekly or 150 mg once monthly) for prevention and treatment
Zoledronic acid	Treatment of osteopenia/ osteoporosis in postmenopausal women, to reduce the incidence of hip, vertebral and non-vertebral fractures	HORIZON	√	√	√	Intravenous 5 mg as single 15–30 min	Intravenous 5 mg as single 15–30 min
Denosumab	Treatment of postmenopausal women with osteoporosis at high risk for fracture, defined as a history of osteoporotic fracture, or multiple risk factors for fracture; or patients who have failed or are intolerant to other available osteoporosis therapy	FREEDOM	√	√	√	Subcutaneous	60 mg every 6 months
Raloxifene	Treatment and prevention of osteoporosis in postmenopausal women	MORE	√	X	X	Oral	Oral 60 mg daily
Oestrogen replacement therapy*	Varies by formulation	WHI	√	NR	√	Oral or transdermal	Daily
Teriparatide	Treatment of postmenopausal women with severe osteoporosis who are at high risk of fracture or who have failed or are intolerant to previous osteoporosis therapy	FPT	√	√	NR	Subcutaneous	20 mcg daily

√ Significant benefit ($p < 0.05$) shown in pivotal trial; X, no significant effect; NR, not reported; PMO, postmenopausal osteoporosis.
*For menopausal women requiring treatment of osteoporosis in combination with treatment for vasomotor symptoms.

1.4 Biology of fracture healing

Fracture healing is a highly organized and timely process. Bone is the only tissue in the body, which heals to its original state without leaving any scar tissue. For a fracture to unite it must meet these settings: viable fragments, mechanical stability of the fragments, blood supply to the area and absence of infection, otherwise it may result in delayed or non-union, or a pseudoarthrosis.

In trauma, in addition to bone fracture, the soft tissue surrounding the bone i.e. blood vessels, muscles, tendons, periosteum, are also injured. Ruptured blood vessels cause local bleeding and the formation of blood clot containing platelets; these platelets release cytokines. Hypoxia and acidosis can develop with associated inflammation. Chemotactic factors may be released exacerbating the inflammation. For the bone fracture to heal it requires 4 elements to be present: 1-Osteogenic cells, 2-Osteconductive scaffolds, 3-Growth factors, and 4-A favourable mechanical environment (Figure 1-7) (Giannoudis, Einhorn and Marsh, 2007)).

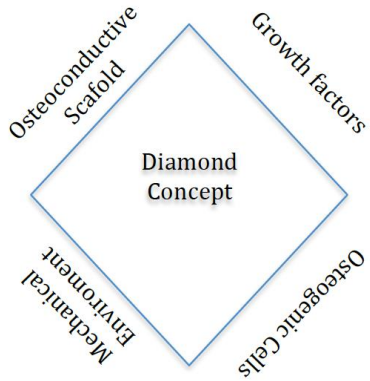


Figure 1-7 Diamond concept of fracture healing (Giannoudis, Einhorn and Marsh, 2007).

Fracture healing is categorized into two types 1-Direct fracture healing and 2-Indirect fracture healing.

1.4.1 Direct (primary) fracture healing

Direct healing occurs under certain conditions these are: absolute stability at the fracture site, fragments are aligned in absolute opposition, minimal gap or direct

contact, and no infection at the site of fracture (Dimitriou, Tsiridis and Giannoudis, 2005).

Direct fracture healing is further sub-classified into two subtypes 1-Contact healing and 2-Gap healing.

1.4.1.1 Contact healing

In contact healing the fragments are less than 0.01 mm apart and if the strain is less than 2% then primary contact healing can occur. Osteoclasts are at the tip of the cutting cones and osteoblasts follow at the tail of the cone. The osteoclast 'cut' across the fracture line forming longitudinal cavities at the rate of 50-100 µm/day and the osteoblasts, which are at the rear fill up the cavities formed by the osteoclasts with new bone. In the centre of the cutting cone, there are blood vessels. Lamellar bone is laid down as mature bridged osteons without the formation of an intermediary cartilaginous phase and without periosteal callus formation (Figure 1-8) (Marsell and Einhorn, 2011)). This is the same process as is seen in normal bone turnover.

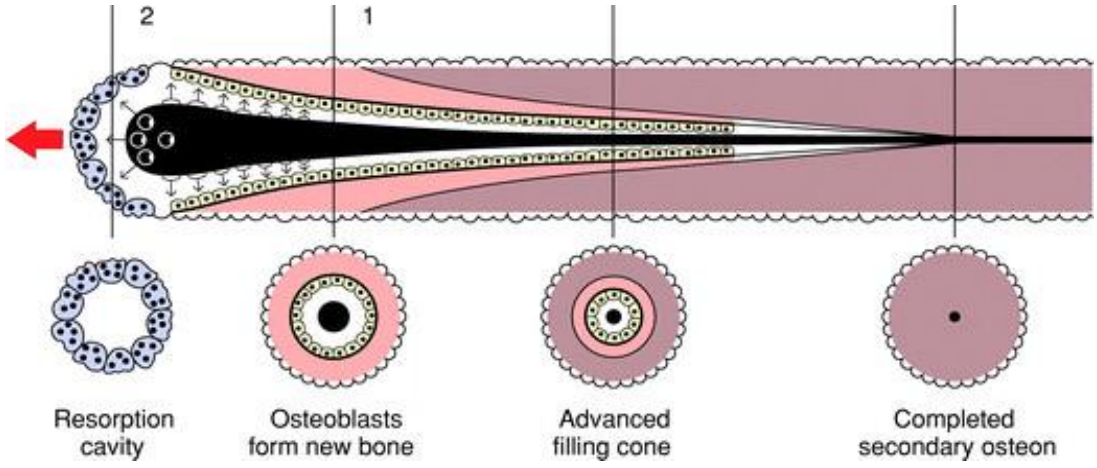


Figure 1-8 Cutting cone were osteoclasts are at the spear head of the cone and the osteoblast are at the rear end of the cone or the tail (Graber *et al.*, 2016).

1.4.1.2 Gap healing

In gap healing, the gap has to be less than 800 µm - 1 mm for healing to occur. The fracture site is primarily filled by lamellar bone oriented perpendicular to the long axis, requiring a secondary osteonal reconstruction unlike the process of contact healing. The primary bone structure is then gradually replaced by

longitudinal revascularised osteons carrying osteoprogenitor cells which differentiate into osteoblasts and produce lamellar bone on each surface of the gap This usually takes 3 to 8 weeks, which then starts the contact healing cascade with the cutting cone (Marsell and Einhorn, 2011).

1.4.2 Indirect (secondary) fracture healing

Indirect fracture healing is the commonest type of fracture healing. For indirect fracture healing to occur: anatomical reduction of bone fragments is not necessary, and micro-motion is present between the fragments at the fracture site. It can occur in surgically and non-surgically treated fractures and involves overlapping phases of healing (Sfeir *et al.*, 2005).

Fracture healing is not homogenous throughout the fractured bone. There are four areas that are important: the medullary canal, the inter-cortical area, the subperiosteal area and soft tissues surrounding the fracture; the extent of damage to these areas have an impact on fracture healing. For example, severe soft tissue damage and stripping of the periosteum will delay fracture healing.

Fracture healing involves different overlapping phases. There are no clear cut distinct lines between the phases, as it is a continuous process (Figure 1-9) (Westerman and Scammell, 2012).

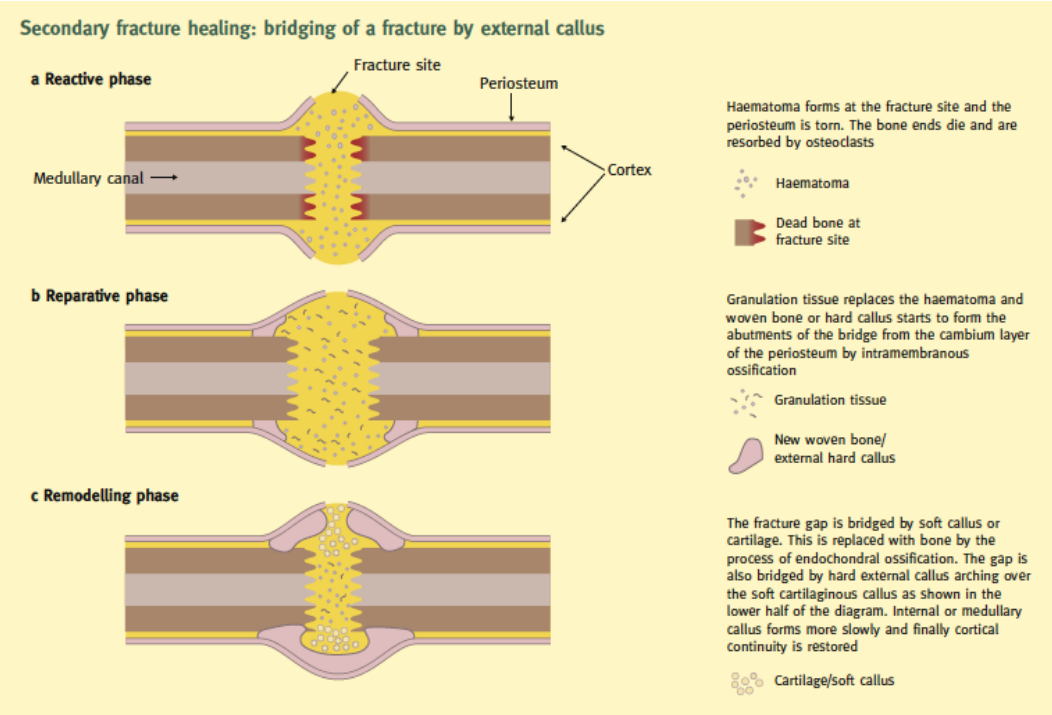


Figure 1-9 Stages of Indirect (secondary) fracture healing (Westerman and Scammell, 2012).

1.4.2.1 Phases of bone fracture healing:

Reactive phase

As a consequence of injury, damage to the soft tissues and bone, blood extravasates forming a local hematoma and clot. Platelets in the haematoma release various factors attracting neutrophils, macrophages, mast cells and fibroblasts to arrive at the fracture site. Damage to the tissues releases various cytokines. Chemotaxis mechanism starts signalling the inflammatory cells to respond and migrate to the site of haemorrhage (mesenchymal cells, endothelial cells and immune cells). A pro-inflammatory secretion of tumour necrosis factor-α (TNF-α), IL-1, IL-6, IL-11 and IL18 signals activation of inflammatory cells and angiogenesis (Phillips, 2005).

These are the major inflammatory signalling molecules that regulate inflammation process: Interleukin 1 (IL-1), Interleukin-6 (IL-6), transforming growth factor β (TGF β), insulin like growth factor (IGF), fibroblast growth factor (FGF),

platelet-derived growth factor (PDGF) and bone morphogenetic protein (BMPs) see Table 1-2 (Phillips, 2005).

Table 1-2 Cytokines, cells releasing them and their functions adapted (Phillips, 2005)

Cytokine	**Function**
TGFβ	Released from platelets, bone and cartilage extracellular matrix, is a pleiotropic growth factor responsible for stimulation of undifferentiated MSCs.
BMPs	Released from osteoprogenitor cells, osteoblasts and bone extracellular matrix, promote the differentiation of MSCs into chondrocytes and osteoblasts and osteoprogenitor cells into osteoblasts
FGF	Released from macrophages, MSCs, chondrocytes and osteoblasts, is mitogenic for MSCs, chondrocytes and osteoblasts
IGF	Released from bone matrix, osteoblasts and chondrocytes, promotes proliferation and differentiation of osteoprogenitor cells.
PDGF	Released from platelets and osteoblasts, is mitogenic for MSCs and osteoblasts and responsible for macrophage chemotaxis.

At the end of this phase a loose fibrin rich aggregation of tissues is formed, called granulation tissue composing of:

1-Extracelluar matrix formed by fibroblasts, consisting of collagens glycosaminoglycans, reticular and elastic fibres, glycoproteins and cytokines.

2-Immune cells, which are macrophages and neutrophils, are the main cells migrating to the injured site among other leukocytes. Macrophages phagocytose old and dead tissues, additionally defending against infection

3-Vascualriation: A network of vessels that are formed in the granulation tissue. This is essential for the transportation of nutrients and clearing the waste products also for oxygenation of the fibroblasts.

Soft callus formation

Chondroblasts appear at the fracture gap and convert the fibrovascular stroma into chondroid tissue. At this stage the fracture ends stabilize. In secondary healing there is endochondral and intramembranous ossification occurs at the same time. Formation of callus starts once recruitment of MSC cells from soft tissue, cortex, periosteum, bone marrow and from distal hematopoietic system are mobilised to fracture site. Seven to 10 days post fracture, chondrogenesis starts when

chondroprogenitor mesenchymal cells groups into pre-cartilage concentrations. Chondrogenesis depends on cell to cell, and cell to matrix interacting signals; it is also accompanied with augmented cell adhesion, formation of gap junctions and alterations in cytoskeletal structure. Histologically it has been shown that a few millimetres from the fracture site, an intramembranous woven bone is formed by the periosteum. Chondroblast synthesis and secrete cartilage specific matrix, collagen type II and proteoglycans. Once mechanical stability is reached, the cartilage undergoes hypertrophy and calcification. The calcified cartilage becomes vascularised and woven bone laid down onto the calcified cartilage matrix.

Hard callus formation (mineralisation)

As the stability of the fracture site is increased, the blood supply starts to increase to the site of fracture and osteoblast differentiation leads to woven bone formation. Chondrogenesis peaks at around 14 day. And by day 35 the cartilage tissue diminishes to ≈2.0% of the total tissue, reflecting chondrocyte hypertrophy and apoptosis. This is contrary to what is a general misconception that cartilaginous tissue makes up high percentage of the fracture callus. The osseous tissue is ≈75% of the total tissues and increases to ≈83% when callus remodels. At day 14 the osseous tissue is the original cortical bone but later on this tissue is from the end0chondral or secondary bone formation (Gerstenfeld *et al.*, 2006).

Remodelling

In this final phase of bone repair woven bone is substituted by lamellar bone and any remaining excess cartilage at the fracture site is removed. The process of remodelling bone follows Wolff's law, where new bone is formed gradually at sites of stress. This takes months to years depending on the bone. Figure 1-10 shows various overlapping phases of fracture healing chronologically from time of bone fracture to time of healing or completion of repair.

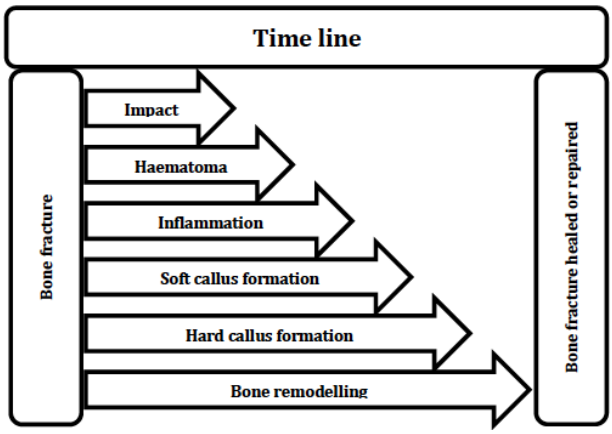


Figure 1-10 Phases of fracture healing with relation to time line

Wolff's law states "Every changes in the form and function of bone or of its function alone is followed by certain definite changes in its internal architecture, and equally definite alteration in its external conformation, in accordance with mathematical laws" (Wolff's original article was in German language this is a translation) (Frost, 1994). Frost proposed a "Mechanostat" model for bone regeneration. Mechanical mechanism controls the biological pathways that determine skeletal, size, shape, and distribution of bone tissue. There are separate molecular pathways that control the activities of woven bone, lamellar bone, fibrous tissue, hyaline cartilage and fibrocartilage and their repair mechanisms. Mechanical and no-mechanical mechanisms control partly and independently the biological activities of bone (Frost, 1996).

Converting external mechanical forces into biochemical signals inside the cells is termed as cellular mechanotransduction, that is divided into four distinct steps 1-mechanocoupling, transduction of mechanical forces applied to the bone into local mechanical signals perceived by sensor cell 2-biochemical coupling, transduction of a local mechanical signal into a biochemical signal, and hence gene expression 3-transmission of signal from a sensor cell to the effector cell, and 4-effector cell response, the end response of the tissue or the final action of the tissue (Duncan and Turner, 1995)

The limits of minimum effective strain (MES) for modelling are in the range of about ~ 1,500-3,000 micro strain (με). Strain is defined as percentage of change in length of the bone material in relation to original length. A new cortical bone is

formed in or above this strain. Repeated strains above around ~2,000 ± 30% με enhances increase of bone mass that the architect of the bone reduce the strain on the bone to ~2,000 με, this drift has an on and off property. Strains around 3,000 με range causes woven bone formation or anarchic resorption. Strains in the range of 25,000 ±30% με cause healthy lamellar bone to fracture.

The MES for remodelling is around ~100-300 με, under these strain thresholds increased bone remodelling occurs resulting in net bone loss (Tyrovola and Odont, 2015).

Deformation of cells in the fracture gap depends upon gap width and the amount of relative movement between fracture surfaces which is called strain (practically equals the amount of fracture gap movement (L) divided by the gap width (L), the movement is three dimensional)

When tissue is elongated beyond its limit it ruptures which is the upper limit of strain. On the other hand tissue differentiation or repair is not induced below certain limit of strain. Fracture heals between strain tolerance and strain induction (Perren, Fernandez and Regazzoni, 2015).

Bone modelling is the process defined by which bones change their overall shape in response to physiologic influences or mechanical forces, leading to gradual adjustment of the skeleton to the forces it encounters. Bone modelling is less frequent than remodelling in adults (Kobayashi *et al.*, 2003). Modelling may be increased in hypoparathyroidism (Ubara *et al.*, 2003), renal osteodystrophy (Ubara *et al.*, 2003), or treatment with anabolic agents (Lindsay *et al.*, 2006).

Bone remodelling is the process defined by a life long process wherein old bone is removed from the skeleton (a sub-process called bone resorption), and new bone is added (a sub-process called ossification or bone formation) (Hernandez-Gil *et al.*, 2006).

Table 1-3 shows comparison between remodelling and modelling of bone (Jee, 2001).

Table 1-3 Comparison of modelling and remodelling of bone (Jee, 2001)

	Remodelling	Modelling
Location	Different surfaces	Spatially related
Coupling	Activation ➔Resorption ➔Formation	Activation ➔Formation Activation ➔ Resorption
Timing	Cyclical	Continuous
Extent	Small (<20%)	Variable *
Apposition rate	Slow (0.3-1.0 μm/day)	Fast (2 -10 μm/day)
Balance	No changes, net loss or gain**	Net gain
Cement line	Scalloped	Smooth
Surfaces	Adjacent to marrow	All surfaces
Occurrence	Throughout life span	Prominent during growth: ineffective in adults
Function	Maintenance and repair	Skeletal adaptation of micro-damage to mechanical usage (shape and size)
MES threshold	<1000 micro-strain	>2000 micro-strain
MES=minimum effective strain. *Variable: large (>90%) in growing bone and small in adult skeletons. **Gain from treatment with bone anabolic agents.		

There are many bone self-regulation theories including degree of strain on bone modelling process, time dependent bone modelling, and remodelling processes. Influential factors include magnitude of strain/stress, number of loading cycles, number of loading occurrence, tensor of strain, and the strain energy density can lead to bone self regulation.

1.4.3 Factors affecting fracture healing

1.4.3.1 Age

Contrary to popular belief that non-union is commoner in the elderly, Mills et al, reported that the frequency of non-union was highest between the ages of 25 years to 54 years in male and female (Table 1-4) ((Mills *et al.*, 2017).

Table 1-4 frequency of non-union per 1.000 fractures, according to distribution of ages and sex (Mills *et al.*, 2017).

Age in (years)	15-24	25-34	35-44	45-54	55-64	65-74	75-84	≥85	All ages
Male	14.4	32.1	33.2	28.9	30.2	22.7	13.4	4.3	22.6
Female	12.4	21.5	24.0	21.4	16.1	15.5	11.9	5.9	15.4
Male and female	14.3	29.1	29.7	25.9	20.6	17.2	12.2	5.6	18.8

In the same study the different bone fractures had different peaks of non-union at different ages. For example, the radius and ulna showed higher non-union rates between the ages of 15 and 54 years. On the other hand pelvis and femur fractures showed higher non-union numbers between the ages of 65 to 84 years. For ankle fractures the higher non-unions were between the ages of 25 and 64 years old after the age of 64 the non-union numbers of cases dropped to almost half. This indicates that different bone types have different non-union rates at different ages. Age is not a risk factor for non-union for all the bones (Table 1-5) (Mills *et al.*, 2017).

Table 1-5 Total number of non-union patients in Scotland over 5 years between 2005 and 2010 according to age and ISD-10 (NHS Scotland Information Services Division 10) anatomical non-union site (Mills *et al.*, 2017)

Age (years)	15–24	25–34	35–44	45–54	55–64	65–74	75–84	≥ 85	Total
Pelvis and femur	18	31	65	64	98	121	112	63	572
Tibia and fibula	70	119	137	105	101	56	32	17	637
Ankle and foot	53	85	70	101	78	43	34	7	471
Clavicle and scapula	59	92	118	132	79	69	38	7	594
Humerus	21	44	69	121	137	154	147	31	724
Radius and ulna	264	287	243	127	95	73	49	9	1147
Hand	90	51	44	29	16	5	3	0	238
Axial skeleton	12	3	13	10	12	21	11	6	88
Site unspecified	1	1	1	2	3	0	2	0	10
Multiple sites	3	1	2	3	1	1	1	0	12
No additional details	27	42	39	26	35	27	21	5	222
Total	618	756	801	720	655	570	450	145	4175

1.4.3.2 Diabetes mellitus

In a study of patients with ankle fractures, those with a HbA1c of over 7%, were three times more likely to have at least one bone healing complication, than patients with ankle fractures with a HbA1c of less than 7%, with an overall complication rate in those patients with diabetes of 25.4% (Shibuya *et al.*, 2013). In a study conducted by Jones et al, they reported that patients with diabetes and comorbidity, had higher rates of complications than control patients or patients with diabetes but no co-comorbidity. Additionally, diabetic patients without comorbidity had an equal rate of complications compared with control patients. Patients with a history of Charcot neuropathy had the highest rate of complications. Risk factors for developing malunion, non-union, or Charcot neuroarthropathy were found in patients with a previous history of Charcot ($p = 0.005$), longer duration of diabetes ($p = 0.009$), use of insulin ($p = 0.009$), and the presence of nephropathy ($p = 0.031$) or neuropathy ($p = 0.038$) (Jones *et al.*, 2005).

1.4.4 Drugs affecting fracture healing

1.4.4.1 Corticosteroids

Corticosteroids inhibits osteoblastogenesis and also induce osteoblast and osteocytes apoptosis (Brien *et al.*, 2004). Corticosteroids are known to induce osteoporosis (Cherian, Kapoor and Paul, 2017). Further experimental studies on human subjects are needed to explore the effect of different types of corticosteroids and their different dosing regimens on the process of fracture healing.

1.4.4.2 Non-steroidal anti-inflammatory drugs (NSAID)

Non-steroidal anti-inflammatory drugs inhibit prostaglandins that affect blood vessels, nerves and inflammation. NSAID's inhibit the action of cyclooxygenase, thus inhibiting the synthesis of prostaglandins. Prostaglandins are very important in the initial phase of inflammation inducing chemotaxis and cell migration.

In a study conducted by Adolphson et al, there was a mean 7% bone mineral content decrease in the radius and 5% in the ulna among the patients treated with Piroxicam, versus 10% in the radius and 7% in the ulna in the placebo group. However, this difference was not statistically significant. Piroxicam did not decrease

the rate of fracture healing. The patients who received Piroxicam had significantly less pain during plaster treatment, but there was no difference in the rate of functional recovery between the groups.(Adolphson *et al.*, 1993).

In a study conducted by Glassman et al, they reported significant adverse effect on spinal fusion with more non-union cases in the Ketorolac than no drug group (Glassman *et al.*, 1998). Also in a study conducted by Reuben et al (2005), reported short use of low dose Cox2 inhibitors Rofecoxib, Celecoxib and Ketorolac did not have any effect on spinal fusion. But higher dose of Ketorolac (120-240 mg) had a higher number of cases of non-union in spinal fusion (Reuben, Ablett and Kaye, 2005).

Gianoudis (2000) reported Ibuprofen and Diclofenac increased risk of non-union in intramedullary nailed femoral fractures (Giannoudis *et al.*, 2000), but in a later review concluded no conclusive evidence that NSAID impaired stress fracture healing (Wheeler and Batt, 2005). A recent study reported no effects of Ibuprofen on fracture healing o the extremity in children (Depeter *et al.*, 2017)

In a systemic review on the quality of the studies by Marquez-lara et al, it was highlighted the great variability in the interpretation of the literature addressing the impact of NSAIDs on bone healing. Unfortunately, there was no consensus regarding the safety of NSAIDs following orthopaedic procedures, and recommended future studies for appropriate methodological designs to help to clarify existing discrepancies to improve the quality of care for orthopaedic patients (Marquez-lara *et al.*, 2016).

Taylor et al reported limited randomised clinical trials data indicate fracture healing is not impaired by non-steroidal anti-inflammatory drugs for short time usage for pain relief (Taylor, Lindblad and Kolber, 2014).

1.4.4.3 Teriparatide

The skeletal effect of Teriparatide (Forsteo) depends on the pattern of exposure to the bone. A once daily dose of 20 micrograms stimulates osteoblast activity over osteoclast activity, hence stimulating formation of new periosteal and endosteal bone (cortical and trabecular) leading to a positive net effect of bone formation. This results in increased bone mass and bone strength initially, but if exposure of teriparatide hormone continues, this will lead to bone resorption rather than bone formation as occurs in hyperparathyroidism. Teriparatide is a class IV drug prescribed for osteoporosis, however it may have a positive effect on fracture healing, i.e. stimulates osteoblasts activity over the osteoclasts activity, compared to patients who are not on teriparatide hormone (Peichl *et al.*, 2011) (Paridis and Karachalios, 2011).

Komatsubara et al used human parathyroid hormone intermittently in a rodent fracture model and reported earlier replacement of woven bone to lamellar bone (Komatsubara *et al.*, 2005). In a prospective randomised clinical trial of patients over 70 years of age with pubic or ischial rami fractures, 21 patients received parathyroid hormone 100 μg daily plus Vitamin D 800 IU with calcium 1000 mg, and 44 patients in the control group received Vitamin D 800 IU with calcium 1000 mg only. All patients had osteoporosis as defined by a DEXA scan T score below -2.5. Their radiological assessment was with a CT scan at week 0 (within 2 days), 4 weeks, 8 weeks and 12 weeks, and healing was defined as cortical bridging, which was reported by a trauma surgeon and an orthopaedic surgeon. Additionally clinical healing/ lack of pain was assessed with a visual analogue scale for pain at weeks 0, week 4, week 8, and week 12, and a timed get up and go test at week 8 (normal adults take 10 seconds to stand from chair walk 3 meters, and return back and sit in the chair). The findings were as follows. 7.8 weeks was the median time for healing in the treatment group and 12.6 weeks in the control group. At week 8 all the fractures in the treatment group healed (cortical bridging), whereas in the control group only 4 fractures healed. Visual analogue scale showed improvement from 7.6 ((95% CI, 7.0 to 8.1) to 3.2 (95% CI, 2.7 to 3.7) in the treatment group, whereas in the control group improvement was from 7.7 (95% CI, 7.1 to 8.3) to 6.5 (95% CI, 6.0 to 7.0). Time to get up to go was 22.9 seconds in the treatment group and 54.3 seconds

in the control group at week 8. At week 12 in the treatment group healing rate was 100% [95% CI, 86.7% to 100.0%], whereas in the control group it was 68.2% [95% CI, 52.4% to 81.4%]). However 14 fractures in the control group didn't heal by week 12; mean time was 14.9 weeks the range was 13 to 18. All patients in the treatment group continued PTH for 24 months, and no adverse events were reported (Peichl *et al.*, 2011).

In the study conducted by Peichl et al there were several deficiencies, the blinding process was not defined and how many CT slices, and at what spacing, where assessed to confirm union, is not given. For the VAS score (PTH 1-84 treatment group at week 0 7.6 ±1.1 and control group 7.7 ±1.1 p=0.73 and at week 8 PTH 1-84 treatment group 3.2 ± 1.0 and control group 6.5 ±0.9 p=0.001) The time up to go (TUG) test measures the time in which someone rises from a standard chair, walks 3m, turns around, walks back, and sits in the chair again. A normal adult should complete the test in less than ten seconds; those who are dependent for most activities of daily life take more than thirty seconds. TUG test values (PTH 1-84 treatment group at week 12 22.9 ±7.7 and control group 54.4 ±19.9 p=0.001) are given as means with standard deviations however upper and lower ranges are not mentioned with no significance difference of means at week 0 and significance difference of means at week 8 for the VAS score and significance difference of means at week 12 for the TUG score.

The age mentioned in the inclusion criteria as 70 years and above, but the two groups mean age or the range of the age is not mentioned, additionally, the PTH 1-84 treatment group is 21 patients and the control group without PTH 1-84 were 44 patients. There is a deficiency in the definition of fracture union. It is not clear whether it is when mineralised callus is formed between the opposing cortices, or cortical bone bridging or when the fracture line disappears. The fracture healing parameters were not defined objectively (Peichl *et al.*, 2011).

In another randomised control trial of 40 post-menopausal women with proximal humeral fractures, 39 patients were treated non-operatively. Nineteen patients were randomised to receive 20 μg Teriparatide for 4 weeks and physiotherapy plus analgesia and 20 patients in the control group received physiotherapy plus analgesia alone. Radiographic assessment was callus formation at

7 weeks by two radiologists who were blinded to treatment. Additionally, visual analogue scale and disability of the arm and hand score were measured at week seven and three months. In the article, 19 patients reported mild adverse events, three patients reported nausea within 2 hours after injection for 1-5 days, two patients reported sweating and one patient reported a slight headache the next morning. Their outcome was no statistical significant difference between the groups $P > 0.5$ (Johansson, 2015). A case study from Japan reported healing of femoral fracture in 8 weeks in a patient who had surgery twice for non-union fracture of femur (Tamai, Takamatsu and Kazuki, 2013).

Lindsey et al showed in a short treatment for 1 month with teriparatide resulted in significant stimulation in periosteal and endocortical and cancellous bone formation of the iliac bone in osteoporotic elderly ladies (Lindsay *et al.*, 2007). There have been many studies which suggest that Teriparatide shortens the duration of fracture healing (Raghavan and Christofides, 2012) (Moon *et al.*, 2012) (Aspenberg and Johansson, 2010) (Matalgia, Aguilar and Oliver, 2013).

A thorough online search of US national library of medicine (PubMed.gov), for published articles was carried out by the author and additionally references in the articles that matched our inclusion exclusion criteria were sought. In addition, Google scholar was used for searching any articles that were not found in the PubMed.gov. A research question was developed using the PICO approach

P= problem/patient/population=Adult patient with fracture

I=intervention/indicator=Teriparatide 1-34/Parathyroid hormone 1-84

C=comparison/comparator=placebo/standard care

O=outcome=healing/Union of fractures

And optional=time or type of study (case reports /case series/randomised clinical trials)

Does Teriparatide 1-34/Parathyroid 1-84 hormone leads to acceleration of fracture healing/union comparing to placebo or standard care in adults?

The inclusion criteria were:

English language texts

Human subjects

Publications in the last ten years from 31/Dec/2018

Case reports

Case series

Randomised control trials

Any bone and any type of fracture

The exclusion criteria were:

Not human subjects

Articles not written in English text

Study more than 10 years old

The following search details were used: (("fractures, bone"[MeSH Terms] OR ("fractures"[All Fields] AND "bone"[All Fields]) OR "bone fractures"[All Fields] OR "fracture"[All Fields]) OR ("fractures, bone"[MeSH Terms] OR ("fractures"[All Fields] AND "bone"[All Fields]) OR "bone fractures"[All Fields] OR "fractures"[All Fields]) OR "fractures, bone"[MeSH Terms] OR "Fracture Healing"[MeSH Terms]) AND (("teriparatide"[MeSH Terms] OR "teriparatide"[All Fields]) OR ("teriparatide"[MeSH Terms] OR "teriparatide"[All Fields] OR "parathyroid"[All Fields]) OR "teriparatide"[MeSH Terms]).

The search turned out 1552 articles of which 27 articles matched the inclusion exclusion criteria. Of the 27 articles there were 15 articles were case reports and seven articles were case series and 4 articles were randomised control clinical trials of which only 3 trials were completed and one was incomplete (not able to recruit patients into the trial) and one cohort observational study, see Table 1-6. In the case reports wide range of bones were reported with different types of fractures. Assessment varied using plain x-rays and CT scans union was reported subjectively. In the case reports Teriparatide 20 µg daily in an Injection form was used in most of the subjects, while some cases reported using Teriparatide 56.5 µg weekly in an injection forms, union was achieved.

In the randomised clinical trials Peichl et al used Parathyroid hormone 100 µg daily dose in the treatment elderly females with pubic rami fractures (Peichl *et al.*, 2011). Were Aspenberg et al, used both Teriparatide 20 µg daily and Teriparatide 40 µg daily doses in the treatment of Colles' fractures, they reported no differences in the healing time with Teriparatide 40 µg group comparing with no Teriparatide group, but they reported difference of 2 weeks with Teriparatide 20 µg group and no

Teriparatide group, but could not show statistical significant difference (Aspenberg *et al.*, 2010). The case reports, case series and randomised trials all lacked objective assessment of the fracture healing (Table 1-6).

There can be a bias of publishing studies that had only positive effect of Teriparatide. We do not know if there were any studies were Teriparatide was used and the fracture did not unite.

Table 1-6 Publications where Teriparatide 1-34/Parathyroid 1-84 used in the treatment of bone fractures

	Authors and year	Bone fracture	Assessment	Intervention	Comparator	Outcome
	Case reports					
1	(Resmini and Iolascon, 2007)	Proximal humerus	X-ray	Teriparatide 20 µg	N/A	+
2	(Yu *et al.*, 2008)	Neck of femure	X-ray	Teriparatide 20 µg	N/A	+
3	(Chintamaneni, Finzel and Gruber, 2010)	Sternum body	CT scan and MRI	Teriparatide 20 µg	N/A	+
4	(Zati *et al.*, 2011)	Prosthetic hip loosening	X-ray and CT scan	Teriparatide 20 µg	N/A	+
5	(Gomberg *et al.*, 2011)	Bilateral femur stress fracture	X-ray and MRI	Teriparatide 20 µg	N/A	+
6	(Borges, Freitas and Bilezikian, 2013)	Femur Trans-trochanteric	X-ray	Teriparatide 20 µg	N/A	+
7	(Mastalgia, G. and B, 2013)	Diaphyseal atypical femoral fracture	CT scan	Teriparatide 20 µg	N/A	+
8	(Tamai, Takamatsu and Kazuki, 2013)	Femur and Charcot ankle arthropathy	X-ray and CT scan	Teriparatide 20 µg	N/A	+
9	(Ochi *et al.*, 2013)	Peri-prosthetic femoral post TKR (K/C Rheumatoid arthritis)	X-ray and CT scan	Teriparatide 20 µg	N/A	+
10	(Giannotti *et al.*, 2013)	Distal femur post TKR	X-ray	Teriparatide 20 µg	N/A	+
11	(Oteo-álvaro and Marín, 2013)	Humeral multl-fragmented	X-ray	Teriparatide 20 µg	N/A	+
12	(Mitani, 2013)	Femur neck	X-ray and CT scan	Teriparatide 56.5 µg once weekly	N/A	+
13	(Fukuda, Kurinomaru and Hijioka, 2014)	Atypical subtrochanteric rt femur and Transverse subtrochanteric lt femur	X-ray and CT scan	Teriparatide 56.5 µg once weekly	N/A	+
14	(Kim *et al.*, 2015)	1-Femure supracondylar 2-Femur periprosthetics two	X-ray and CT scan	Teriparatide 20 µg	N/A	+
15	(Panagopoulos *et al.*,	Femur subtrochanteric and mid shaft	X-ray	Teriparatide 20	N/A	+

	2015)			µg		
	Case series					
1	(Brunnemann *et al.*, 2010)	1-Femur periprosthetics two 2-Radius non union one	X-ray	Teriparatide 20-60 µg	N/A	+
2	(Rubery and Bukata, 2010)	Odontoid process		Teriparatide 20 µg	N/A	+
3	(Moon *et al.*, 2012)	Pubic rami and sacrum	X-ray, SPECT and CT scan	Teriparatide 20 µg	N/A	+
4	(Raghavan and 5Christofides, 2012)	Metatarsal bone stress fracture two	X-ray	Teriparatide 20 µg	N/A	+
5	(Lee, Ha and Koo, 2012)	Femur distal, middle and neck	X-ray and CT scan	Teriparatide 20 µg	N/A	+
6	(Kundu, 2017)	Humerus	X-ray	Teriparatide 20 µg	N/A	+
7	(Chinoy, 2018)	1-Femur supracondylar 2-Humerus 3-Tibia nine cases 4-Femure subtrochanteric two 5-femur two	X-ray and Clinical assessment	Teriparatide 20 µg	N/A	+
	Randomised controlled clinical trials					
1	(Aspenberg *et al.*, 2010)	Colles'	X-ray and CT scan	Teriparatide 20 and 40 µg	Placebo	+
2	(Peichl *et al.*, 2011)	Pubic rami	CT scans	Parathyroid (PTH 1-84) 100 µg	Standard care	+
3	(Johansson, 2015)	Proximal humerus	X-ray	Teriparatide 20 µg	Standard care	
4	(Bhandari *et al.*, 2016)	Femoral neck	X-ray	Teriparatide 20 µg	Placebo	Not completed not able to recruit
Others						
1	(Carvalho *et al.*, 2011)	48 spine, 3 rib, 16 pelvis, 10 hip, 19 femur, 11 foot, 6 subtalar fusion, 10 humerus, 4 wrist	X-ray and CT scan	Teriparatide 20 µg	N/A	+

1.5 Assessment of bone fracture healing

Determining bone fracture healing is part of clinical care, which has significant impact on allowing patients to weight bear or non-weight bear and return to their normal activities. There is no gold standard for assessment of bone fracture healing more over there is a lack of consensus on bone fracture healing parameters, which is a big obstacle in conducting clinical trials of medicinal products on bone fracture healing. The available tools for determining bone fracture healing are 1-Clinical assessment 2-Mechanical assessment 3-Serological markers and 4-Radiographic assessment, the main two modalities used are plain X-rays and CT scans.

1.5.1 Clinical assessment of bone fracture healing

The most common technique used for clinical assessment of fracture healing is subjective evaluation of pain that is asking the patient if he/she has any pain at the fracture. Corrales et al, reported that the most common criteria used to clinically assess fracture union: in 77 articles that used clinical criteria to define fracture union, was no pain or tenderness on bearing weight and no pain or tenderness on palpation/examination 38(49%) and 30(39%) respectively, see Table 1-7 (Corrales *et al.*, 2008).

Table 1-7 Criteria used to define clinically fracture union (Corrales *et al.*, 2008).

Clinical criteria used to define fracture union*	Number of articles (N=77)
1-No pain/tenderness when bearing weight	38 (49%)
2-No pain/tenderness on palpation/examination	30 (39%)
3-Ability to bear weight	14 (18%)
4-Ability to walk /perform activities of daily living with no pain	11 (14%)
5-Ability to walk/perform activities of daily living	9 (12%)
6-No residual pain at fracture site	8 (10%)
7-No motion at fracture site on examination	4 (5%)
8-Full range of motion at adjacent joint	4 (5%)
9-Clinicaly stable /asymptomatic	2 (3%)
10-No residual warmth at fracture site	1 (1%)
11-Full range of motion at adjacent joint without pain	1 (1%)
12-Fracture stiffness measured mechanically[+]	1 (1%)

[*]The clinical criteria were grouped into twelve similar categories and were arranged in order of most to least common use.
[+]A fracture stiffness of >15 Nm/deg in two orthogonal planes was reported to indicate a sufficient healing for external fixator removed in the case of a tibial fracture.

1.5.2 Mechanical assessment of bone fracture healing

Stiffness and stability of the bone fracture are the two main properties assessed. The assessment can be divided into either direct or indirect assessment. A study conducted by Wade et al., measuring stiffness in a sagittal plane alone and in several planes on 103 unstable tibial fractures that were on external fixators. They removed the external fixator when the stiffness reached 15 Nm/degree in the sagittal plane only, the deformity occurred in 4 patients. But in further 27 patients the external fixator was removed when the stiffness reached 15 Nm/degree in at least two orthogonal planes there were no failures. Their recommendation was to measure fracture stiffness of 15 Nm/degree in at least in two orthogonal plane before removing the external fixator (Wade, Moorcroft and Thomas, 2001). Measuring stiffness can be a problematic when the long bone fractures are internally fixed with intramedullary nail and pin loosening is another problem.

1.5.3 Serological markers of bone fracture healing

Turnover of the bone can be measured by number of bone markers, which are divided in three categories 1-Bone resorption markers 2-Osteoclast regulatory proteins and 3-Bone formation markers. The levels of these markers varies during the process of fracture healing depending on the bone fractured and time it takes to heal and extent of the fracture. Level of bone markers varies in different bones(Cox *et al.*, 2010).

Bone formation activity markers include bone specific alkaline phosphate (ALP), procollagen type-I N-terminal propeptide (PINP), procollagen type-I C-terminal propetide (PICP), and osteocalcin (OC) (Coulibaly *et al.*, 2010).

Table 1-8 shows bone resorption and bone formation markers and their source, action and activity in fracture healing (Cox *et al.*, 2010)

Table 1-8 Markers of bone resorption osteoclast regulatory proteins and their activity in fracture healing and markers of bone formation with their activity in fracture healing (Cox *et al.*, 2010).

1-Markers of bone resorption , osteoclast regulatory proteins and their activity in fracture healing		
Bone resorption markers	Source / action	Markers activity in fracture healing
C-telopeptide of type-I collagen (CTX)	-8 amino-acid fragment from C-telopeptide of type-I collagen -Generated by cathepsin K activity	Rises first week after fracture of the tibial shaft and remains elevated throughout fracture healing
N-telopeptide of type-I collagen (NTX)	-8 amino-acid fragment from N-telopeptide of type-I collagen	Not been investigated in fracture healing
Carboxy-terminal telopeptide of type-I collagen (ICTP)	-Carboxyl-terminal of type-I collagen -Released by matrix metalloproteinase -Eradicated by cathepsin K activity	Rises first week after fracture of the tibial shaft and remains elevated throughout healing
Pyridinoline (PYD)	-Form cross links between mature collagen polypeptides	Peaks 1 to 4 weeks after fracture of the tibial shaft, 1 to 8 weeks after proximal femoral fracture
Deoxypyridinoline (DPD)	-Form cross-links between mature collagen polypeptides	Peaks 1 to 8 weeks after proximal femoral fracture
2-Osteoclast regulatory proteins		
RANKL	Member of tumour-necrosis family, produced by osteoblasts and activated by T lymphocytes	Not been investigated in fracture healing
OPG	Secreted by osteoblasts as a decoy receptor to bind RANKL. Down regulates osteoclasts activation and proliferation	Not been investigated in fracture healing
Tartrate-resistant acid phosphatase (TRAcP)	Glycoprotein produced by osteoclasts and activated by macrophages/dendritic cells. Acts as phosphatase and generator of oxygen free radicals	Peaks at 7 days after osteosynthesis in ankle fractures and 2 weeks in tibial fractures
Capthepsin-K	Cysteine protease produced	Not been investigated in

	by osteoclasts	fracture healing
3-Markers of bone formation and their activity in fracture healing		
Bone formation markers	Source/action	Marker activity in fracture healing
Procollagen type-III N-terminal Propetide (PIIINP)	-N-terminal peptide cleaved from type-III procollagen when it forms type-III collagen	Maximal levels at 2 weeks after ankle fracture and 12 weeks after fracture of the tibial shaft
Procollagen type-I C-terminal Propeptide (PICP)	-C-terminal peptide cleaved from type-I procollagen when forms type-I collagen	-Peaks 20 to 34 weeks after fracture of the tibial shaft -Peaks 2 weeks after distal radial fracture remaining elevated at 9 months
Procollagen type-I N-terminal Propetide (PINP)	N-terminal peptide cleaved from type-I procollagen when it forms type-I collagen	Maximal at 12 weeks after fracture of the tibial shaft remining elevated at 24 weeks. -Similar results with proximal femoral fractures
Osteocalcin (OC)	Main non-collagenous protein produced by osteoblasts	Elevated at 24 weeks after fracture of the tibial shaft -Elevated 1 week after distal radial fracture
Bone specific alkaline phosphatase (BSAP)	Isoenzyme produced by osteoblasts involved with calcification of skeleton and bone formation	Increased at 4 weeks after fracture of the tibial shaft remains elevated at 1 year

1.5.4 Radiographic assessment of bone fracture healing

1.5.4.1 Plain x-rays

Plain X-ray is an important tool used clinically to diagnose fractures. They are used in the assessment of fracture healing progress, alongside clinical assessment of local tenderness/movement on stressing the fracture site and pain on weight bearing. In the initial few weeks the fractured limb is often kept in a cast, which makes visualising the fracture line difficult as the cast consists of calcium carbonate, which is radiopaque on plain x-rays and overshadows the fracture lines.

Additionally the cast masks the trabecular bone, this makes assessment of trabecular bridging almost impossible. Precise geometrical alignment of the fractured bone in subsequent images of plain x-rays, in order to view the same exact

region of interest cannot be achieved. Furthermore in direct healing of fractures, the cortical bridging is impossible to visualise with the cast or metalwork overshadowing the fracture. A study conducted by Bartio et al on the reproducibility of radiographic alignment in total ankle replacement, indicated likely error measurements, which might lead to wrong inter and intra-observer reliability, they recommended further studies on the accuracy of plain x-ray parameters before concluding any outcomes after total ankle replacement (Braito *et al.*, 2015). This study shows that alignment of an ankle in subsequent plain x-rays are impossible to achieve, this results in an inconclusive outcome in comparing differences in the two images.

In a study conducted by Hammer et al reported that conventional X-ray examinations as a mean of assessing the stage of union are generally inconclusive (Hammer, Hammerby and Lindholm, 1985).

A survey conducted by Bhandari et al showed variability in the frequency of parameters used in the assessment of fracture healing among surgeons (Table 1-5). Callus formation was used in only 39.7% of surgeons on plain x-rays as a sign of fracture healing, whereas 45.8% of surgeons always used cortical continuity as a sign of fracture healing on plain x-rays, with 45.4% always using progressive loss of fracture line as sign of fracture healing on plain x-rays. Clinically 42.4% of surgeons always used ability to weight bear as a sign of fracture healing and 37.2% of surgeons always used pain at the fracture site on palpation as a sign of an non-united fracture, see Table 1-9 (Bhandari *et al.*, 2002).

Postacchini et al reported that in fractures of a diaphyseal long bone the first calcification foci are seen after 7 days of injury in the medullary canal. In the second week of injury the medullary callus showed numerous mesenchymal cells fibroblasts and new capillaries. In the periosteal callus new bone trabeculae is seen after 12 days of injury. New bone trabeculae appeared in the medullary callus in week 3, on biopsy of fractured long bone before going to ORIF in 1 to 21 days (Postacchini *et al.*, 1995).

The parameters used for assessment of fracture healing on plain x-rays in a prospective study to assess role of Teriparatide in fracture healing were callus formation, bony bridging, reduction of fracture line and complete bone union (Saraf and Munot, 2017). However the reproducibility of subsequent plain x-ray alignment

was not mentioned by the authors, which can have an effect on the parameters used for assessment of fracture healing.

Table 1-9 Frequency of parameters used in the assessment of fracture healing (Bhandari *et al.*, 2002).

	Always	Often	Sometimes	Never
Callus size on X-ray	39.7%	33.4%	20.9%	6.0%
Cortical continuity on X-ray	45.8%	33.5%	18.5%	2.2%
Progressive loss of fracture line on X-ray	45.4%	32.5%	18.0%	4.1%
Ability of patient to bear weight	42.4%	28.8%	19.0%	9.8%
Pain at the fracture site to palpation	37.2%	27.3%	19.7%	15.9%

Callus formation as an indicator of fracture healing progressing to union can be misleading, as excessive callus can be an indicator of hypertrophic non-union. Indeed, Salih et al, reported a callus fracture sign which predicts that the fracture is most likely going to hypertrophic non-union. They described it as a fracture line seen on the x-ray, that extends beyond the cortices but not the callus that is formed around the fracture, see Figure 1-12 (Salih *et al.*, 2015).

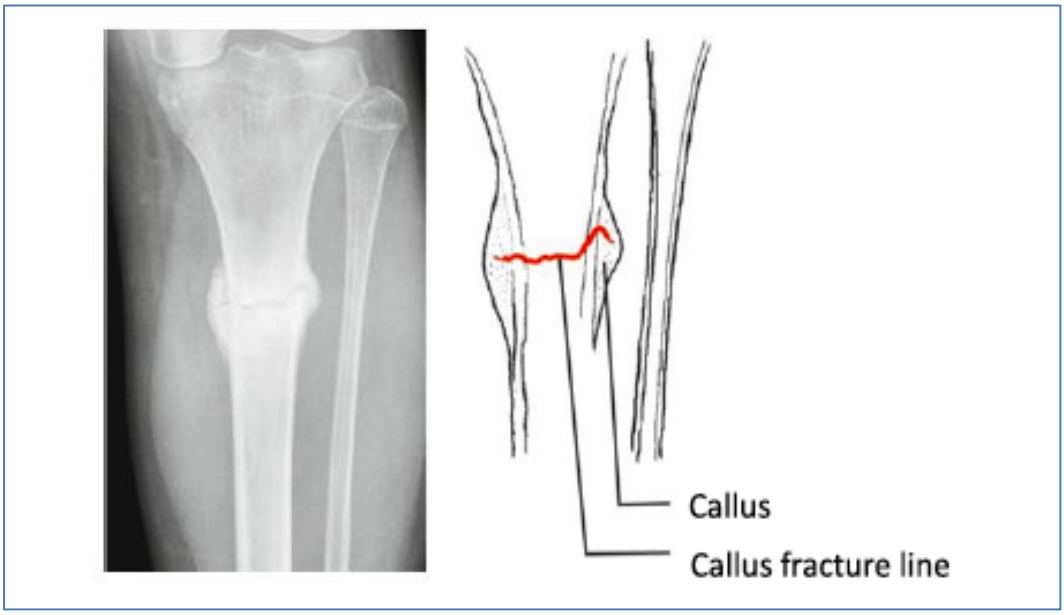


Figure 1-11 the callus fracture sign is the extension of the fracture line beyond the cortex into the callus but not to the edge of the callus. As shown above in the image, adopted from (Salih *et al.*, 2015).

In a systematic review conducted by Corrales et al looking at the most common fracture healing parameters used for defining union in 120 studies the outcome was 63 articles (53%) used bone bridging, callus and trabeculae, 32 articles (27%) used bridging at three cortices, 22 articles (18%) used obliteration of the fracture line, 7 articles (6%) used bridging of two cortices, 4 articles (3%) used bridging of fracture at 1 cortex, 4 articles (3%) used absences of fracture displacement, 3 articles (2.5) used absences of hardware failure/loosening, 2 articles (1.7%) used absences of osteonecrosis, 2 articles (1.7%) used calcification of callus, 1 article (1%) used bridging of fracture at 4 cortices, 1 article (1%) used presences of callus (Corrales *et al.*, 2008).

The Radiographic union score for tibia (RUST) scoring and modified radiographic union score for tibia (mRUST) scoring, uses cortical scoring system to quantify fracture union on radiographs. RUST provides indication of cortical healing on a continues scale from 4 to 12 points. The score is determined by visible callus with or without visible fracture line, presence of callus without visible fracture line =3 points, presence of callus with visible fracture line =2 points, absence of any callus =1 point. This is evaluated on each of the four cortices on anterior-posterior and lateral radiographic views (Whelan *et al.*, 2010).

The modified RUST score divided the presence of callus with visible fracture line into further two categories callus present and bridging callus making the total score scale ranges from 4 to 16 (Litrenta *et al.*, 2015).

1.5.4.2 Computerized tomography (CT) scanning

Computerized tomography scan acquires images of an object in all three dimension planes and by stacking the images, they are reconstructed into a three dimensional image that transforms a real representation, to a volumetric representation using “special” software. Because plain X-rays are two dimensional views of a three dimensional object the features of the object are stacked on top of each other, which can produce errors in reading the images. In a study conducted by Grigoryan et al, they observed the earliest signs of fracture healing was blurring of the fracture line margins (mean appearance was at 2.9 weeks on CT scan images and at 3.5 weeks on x-ray images). Sclerosis at the fracture line margins largely depended

on fracture type. Sclerosis at the fracture line margin could be seen in 71% of distal radial fracture at 4 weeks and the fracture line became invisible at 12 weeks. But in malleolar fractures the fracture line margins remained lucent in 60% of x-rays at week 4 and in 60% at 12 weeks the fracture line margins were sclerotic and in 45% of the x-rays the fracture line margins were absent at week 16. Malleolar fractures had the worst over all scoring agreement between x-rays and CT scan at only 49%. The tibial shaft fractures had 80% overall scoring agreement between x-rays and CT scans (Grigoryan *et al.*, 2003). In a study conducted by Ianni et al, the reported interrater agreement of healing in plain radiographs of scaphoid fractures was 57% (4 out 7 cases), whereas in CT scans it was 100% (7 out of 7 cases) although, sample size was small in the study (Ianni, 2012). These studies have shown that plain X-rays are not a good fracture healing assessment tools to use in trials for the investigation medicinal products. Additionally, CT scans have shown consistently that they can detect earlier signs of bone modelling/remodelling than plain X-rays.

A wide range of fracture healing parameters are used in assessing fracture healing radio-graphically, but still there is no consensus among orthopaedic trauma surgeons and among radiologists or between radiologist and orthopaedics on which is the gold standard parameter to use in assessing fracture healing. Although studies recommended use of CT scan for assessment of fracture healing in trials as they show the parameters of fracture healing much earlier than plain X-rays, not many studies have used CT scanning for assessment of normal fracture healing progression, hence this study investigated the parameters of healing in CT scans.

A study conducted by Bhattacharyya et al showed CT scans of tibial shaft fractures to determine non-union, had a very good diagnostic accuracy interclass correlation =0.89. The sensitivity for detecting non-union was 100% and overall accuracy was 89.0%. However the limitation was a low specificity of 62%, where three patients diagnosed with non-unions on CT scans and went for surgery but intra-operatively found out to have healed fractures (Bhattacharyya *et al.*, 2006).

Peichal et al, used CT scans in the assessment of accelerating pubic rami fracture healing with Parathyroid 1-84 in the elderly women. However, there were deficiencies in their method of reporting parameters of fracture healing in CT scans that was not clarified (this has been discussed on page 63) (Peichl *et al.*, 2011).

In a study conducted by Grigoryan et al, reported blurring of the line fracture margins and reactive sclerosis were the earliest signs of healing observed in both x-rays and CT scans. External callus formation was detected earlier with CT technique. Additionally, CT images permitted for more detailed visualization of healing compared with conventional X-rays, which were limited by cast and fixation hardware superimposition, especially in subjects with malleolar and distal radial fractures. The authors concluded that CT scans showed advantages over plain radiograph in the early detection of fracture healing (Grigoryan *et al.*, 2003).

1.6 Current National Institute for health and care excellence (NICE) guideline for ankle injury management

National institute for health and care excellence recommended guidelines for management of ankle fractures:

- Initial pain management and immobilisation

  -Regular assessment of pain using pain scale suitable for developmental stage and cognitive function

  -Continue using the same pain assessment scale in the hospital as used in the pre-hospital setting

- Initial pharmacological management of pain in adults (16 or over)

  -Oral Paracetamol for mild pain

  -Oral paracetamol and codeine for moderate pain

  -Intravenous paracetamol supplemented with intravenous morphine titrated to effect for severe pain

  -In the frail or older adults use intravenous opioids cautiously

  -Do not use non-steroidal anti-inflammatory drugs (NSAID) in frail or older adults with fractures

- Hot reporting

  -Do not discharge the patient without definitive written report from a radiologist, radiographer or trained reporter

- In the pre-hospital setting consider a vacuum splint for people with suspected long bone fractures

- Acute stage assessment and diagnostic imaging

  -Use the Ottawa ankle and foot clinical prediction rules for suspected ankle fractures to determine need of x-rays

- Non-surgical orthopaedic management of uni-malleolar ankle fractures

  -Advise immediate unrestricted weight bearing as tolerated

  -Orthopaedic follow up with in two 2 weeks if stability of the fracture uncertain

  -If not improving after 6 weeks of injury advise to return to review.

- Timing of surgery of ankle fractures

-If it is decided to treat surgically then operate the same day or next day following injury.

- Documentation

  -Consider developing and using standard documentation to prompt

  -Assessment of following visits from the first visit

  -Safeguarding

  -Comorbidities

  -Falls risk

  -Nature of fracture and classification where possible

  -Document all key communications with patients, family members and carers about the management

  -Follow structured process when handing over care to or from emergency and other departments

  -Ensure all documents are transferred with the patients when transferring

  -Produce a written summary giving diagnosis, management plan and expected outcome aiming to be sent to patient's GP within 24 hours, in plain English language understandable by patients, family members and carers and available in-patient record.

- Information and support for adults, family members and carers

  -Providing support

  -Manage expectation and avoid misinformation

  -Answer questions and provide information with your limits

  -Do not speculate and avoid being optimistic or pessimistic

  -Ask if there are any other questions

- Support for vulnerable adults

  -Allocate a dedicated member of staff to contact next of kin and provide support.

  -Work with family members or carers

- Providing information

  -Explain to patients, family members and carers, what is happening and why providing:

-Information on known injuries

-Details of immediate investigation and treatment and time schedules if possible

-Offer people with fractures the opportunity to see images of their injury taken before and after treatment

- Provide patients with fractures with both verbal and written information on the following when management plan is agreed or changed:

  -Expected outcome of treatment

  -Activities they can do

  -Home care options, if needed

  -Rehabilitation, including whom to contact and how

  -Mobilisation and weight bearing

  -Make sure all the information is accessible to all health care practitioner

  -Providing information about transfer from the emergency department

  -The reason for transfer

  -The location of the receiving centre and destination within receiving centre

  -Contact person responsible at the receiving centre

- Following are the recommendations for research

  -Imaging ankle fractures

  -Compare Clinical and cost effectiveness of CT with plain x-ray for planning surgical treatment

(NICE, 2018).

1.7 National institute for health research guidelines for feasibility and pilot studies

Clinical trials are scientific method of testing a new treatment. New treatment is not always better than an existing treatment. For a new drug to go from pre-clinical phase to clinical phase it has to go through a rigours process.

Before conducting any full-scale clinical trials it is advisable to lead with a feasibility study. Feasibility studies are small studies, which are critical for preparation to carry out further high quality statistically powered studies. Even some

of the funding sources will not consider application for research grants for full-scale studies unless feasibility studies have been conducted. Feasibility studies and pilot studies are explained and clarified in the National Institute for Health Research written guide see Table 1-10 (Williams, 2016).

Table 1-10 National Institute for Health Research difference in definitions of feasibility and pilot studies (Williams, 2016).

NIHR definitions of feasibility and pilot studies	
Feasibility study	Pilot study
Feasibility studies are principally conducted to establish whether large studies can be delivered parameter estimation is emphasised	Pilot studies are conducted to assess whether key study elements run smoothly there is no explicit mention of feasibility assessment in the definition of pilot studies
Little is said about the design of feasibility studies other than that they need not follow the design of the main study and may or may not be randomised	Pilot studies should be miniature versions of the main study, sharing many of its features
The focus of the analysis of feasibility studies is largely quantitative (parameters estimation) although some suggested activities will involve qualitative judgements (usefulness and limitations of a particular database) or development work (designing a suitable outcome measure)	No analysis methods are mentioned in relation to pilot studies
Feasibility study should not include evaluation of the main outcome that will be assessed in the full scale study and my not necessarily even measure that outcome	Outcome of interest should be measured in pilot study although any analysis should be set aside
The size of the feasibility study should be determined by the degree of accuracy needed to estimate parameters	No mention is made of how large pilot studies need to be
No mention is made of the possibility of multiple related feasibility studies	No mentioned about possible multiple related pilot studies

1.8 Aims and objectives

1.8.1 Aims

1-To determine if and how Teriparatide is being used by physicians to accelerate fracture healing.

2-To conduct a feasibility study to plan a future randomised control trial comparing the effect of Teriparatide treatment versus standard care on healing of ankle Weber B fractures managed conservatively in older people.

1.8.2 Objectives

Aim 1. In order to address the first aim, I shall conduct a survey to find out:

1.1. Is Teriparatide used among physicians?

1.2. What are the perceived reasons for off label usage of Teriparatide?

1.3. Is Teriparatide prescribed off label to accelerate fracture healing by the physicians?

1.4. If Teriparatide is being used to accelerate fracture healing in athletes?

1.5. What are the perceived barriers among physicians for using Teriparatide to aid fracture healing?

Aim 2. In order to address the second aim I shall:

2-1. Determine if recruitment is feasible by conducting an audit to estimate the number of patients with ankle Weber B fractures who are 50 years and above managed conservatively at Queen's Medical Centre Nottingham University Hospital in one year.

2-2. Ensure that we can we get the necessary approvals for viability of the study concept.

2-3. Assess the feasibility of administering Teriparatide self-injections and observe any side effects in the study group.

2-4. Assess the utility of CT scans to show progression of fracture healing

2-5. Assess any differences in fracture healing with Teriparatide treatment.

2-6. Determine the required frequency of CT scans, by studying the radiological parameters of fracture healing using CT scanning.

2-7. Identify the participant's pain during the treatment period as measured using a questionnaire (10-point visual analogue pain score on scale).

2-8. Compare ankle function between the Teriparatide group and standard care group using Olerud Molander questionnaire.

2-9. Measure health status of the participant using EQ-5D-5L quality of life questionnaire.

2-10. Carry out an in depth qualitative assessment of participants involvements in the study.

2 Chapter 2 Methods

2.1 Survey of off label usage of Teriparatide among physicians

2.1.1 Survey design

To address Aim 1, to determine if and how Teriparatide is being used by physicians, a survey was carried out in the Kingdom of Bahrain, the United Kingdom and the United Arab Emirates. An electronic link of the survey questions was sent via e-mail and/or by text message to physicians who were registered with appropriate medical societies (British Association of Sports Medicine, British Orthopaedic Association, National Osteoporosis Society and Bahrain Medical Society (Appendix A). Additionally, survey questionnaires in the form of a booklet were distributed and collected at the end of medical society meetings and conferences. The data was collected between April 2016 and April 2017.

The inclusion criterion was

1-All physicians willing to participate in the study

The exclusion criteria were

1-No email address or mobile contact number

2-Not a physician

The exclusion criteria of no email or mobile contact number, was not relevant when the questionnaire booklet was distributed in meetings and at conferences.

2.1.2 Peer review of the survey

The Nottingham University Hospital patient public involvement team was approached to assist with the writing of the information sheet and the questionnaire. Ethical approval for this study was obtained from the Faculty of Medicine & Health Science (FMHS) Research Ethics Committee, Ethics reference numbers M06052016 dated 27th of May 2016 and M06052016 dated 30th of August 2017 (please see Appendix B, Appendix C for the study approval letters).

2.1.3 Target group

We targeted physicians registered with relevant medical societies in the United Kingdom and the Kingdom of Bahrain. Initially we sent an electronic link of the survey (https://nottingham.onlinesurveys.ac.uk/survey-of-usage-of-teriparatide hormone-forsteoforteo-t) via email to the members of the Bahrain Medical Society and British Orthopaedic Society, but unfortunately the response rate was very low. Due to the low response rate to the electronic survey, we had to approach the target group, face to face and distribute the questionnaire in a booklet form. An amendment to the ethics approval with a modification to cover approaching the target group was made (Appendix C). We had to be pragmatic and opportunistic and targeted those doctors attending their society's annual conferences; the British Association of Sports and Exercise Medicine, the British Orthopaedic Association, the Bahrain Medical Association, and the National Osteoporosis Society annual conference. Additionally, we targeted orthopaedic surgeons attending local orthopaedic conferences in Nottingham and the International Conference for Joint Reconstruction Middle East held in the United Arab Emirates in 2016. The survey booklet consisted of three parts (Appendix A). The first part was an invitation letter to take part in the survey explaining the purpose of the survey, why they had been chosen, and an explanation that it was voluntary to take part in the survey and so on. The second part of the survey consisted of an introduction to Teriparatide and the third part of the study was the questionnaire of the study. We were looking for answers to the following questions:

- If Teriparatide is used among physicians?
- What are the indications of usage of Teriparatide?
- Is Teriparatide prescribed off label to accelerate fracture healing by physicians?
- What are the perceived barriers among physicians for using Teriparatide to aid fracture healing?
- Is Teriparatide being used to accelerate fracture healing in athletes

The questions were designed by an iterative process with my supervisors. The applicability of the questions was reviewed by asking three medical colleagues for feedback and their suggestions were incorporated, and the final version was collectively agreed upon.

2.2 Feasibility study of ankle fractures treated with Teriparatide

2.11 Our second aim was to conduct a feasibility study to plan a future randomised control trial comparing the effect of Teriparatide treatment versus standard care on the healing of ankle Weber B fractures managed conservatively in older people. Our main objectives were: 1) to determine if recruitment was feasible by conducting an audit to estimate the number of patients with ankle Weber B fractures who were 50 years and above managed conservatively at Queen's Medical Centre Nottingham University Hospital in one year; 2) to ensure that we can get the necessary approvals for viability of the study concept; 3) to assess the feasibility of administering Teriparatide self-injections and observe any side effects; in the study group; 4) to assess the utility of CT scans to show progression of fracture healing; 5) to assess any differences in fracture healing with Teriparatide treatment; and 6) to determine the required frequency of CT scans, by studying the radiological parameters of fracture healing using CT scanning.

2.2.1 Ankle fracture audit

To address objective 2.1, to determine if recruitment is feasible by conducting an audit to estimate the number of patients with ankle Weber B fractures who are 50 years and above managed conservatively at Queen's Medical Centre Nottingham University Hospital in one year, an audit of ankle fractures was carried out from 1st of January 2014 to 30th of December 2014 to estimate the number of patients that might be eligible to enter into a clinical trial. Before conducting the audit, a registration confirmation for the project and an approval from the clinical audit leader for trauma and orthopaedic research was obtained under project number 15-14 (Appendix D). The audit was set up to determine the number of patients aged 50 years old and above, with ankle fractures, and a DEXA scan report, confirming osteoporosis (T score ≤ -2.5 standard deviations below the mean of young adults of the same sex) attending the Fracture clinic at Queen's Medical Centre. The audit results are shown in Table 2-1. This information led us to exclude the T score criterion of ≤ -2.5 from our initial inclusion criteria as there were only 12 patients within a 12 month period who met all three entry criteria (age ≥ 50 years, Ankle

Weber B fracture managed conservatively and T score of ≤ -2.5), which would have made the feasibility study impossible to recruit to, and that was before the application of any further inclusion and exclusion criteria were applied.

Table 2-1 Audit of ankle fractures carried out at Queen's Medical Centre from 1st of January 2014 to 30th of December 2014

Total number of patient with ankle fractures treated conservatively, aged ≥50	Total number of patients =256 Male=67, Female=189
L ankle fracture	134
R ankle fracture	122
Patients with DEXA scan	152 (59%)
Patient without DEXA scan	104 (41%)
DEXA scan T score at spine ≤ -2.5	7 (5.4%)
DEXA scan T score at spine ≤ -1.5	24 (18.6%)
DEXA scan T score at spine >-2.5	98 (76.0%)
Missing results	23 (15.1%) from 152
DEXA scan T score at femoral neck ≤-2.5	7 (5.1%)
DEXA scan T score at femoral neck ≤-1.5	33 (23.9%)
DEXA scan T score at femoral neck >-2.5	98 (71.0%)
Missing results	14 (9.2%) from 152
DEXA scan T score at total hip ≤-2.5	9 (6.8%)
DEXA scan T score at total hip ≤-1.5	28 (21.2$)
DEXA scan T score at total hip >-2.5	95 (72.0%)
Missing results	20 (13.2%) from 152
DEXA scan T score of ≤-2.5 at any of the following sites spine, hip, total hip	12 (7.9%) from 152
-Only 152 (59%) of the 256 patient with ankle fracture had DEXA scan done in 2014 at Queen's Medical Centre -From 152 (59%) patient who had done DEXA scan only 12 (7.9%) patients had T score of ≤ -2.5 to diagnose them as having osteoporosis	

2.2.2 Patient Public involvement

Prior to ethical approval for the study, the patient public involvement officer at Nottingham University Hospitals NHS Trust organised a PPI group of people who were involved in writing the lay summary (Appendix E), participant information sheet (Appendix F), pre-consent form (Appendix H) and informed consent forms (Appendix G), to facilitate translation of scientific language to language understandable to a

layperson. They were also involved in the structuring of the qualitative questions used to interview the participants at the last clinical trial visit (Appendix I).

2.2.3 Feasibility study design

Initially the aim was to conduct a pilot double blind randomised controlled trial (RCT) that would result in proof of concept data that would be used to inform an application to National Institute for Health Research (NIHR), Research for Patient Benefit (RfPB) funding to conduct a multi-centre RCT. We wanted to answer the question, 'Does Teriparatide significantly accelerate fracture healing time?' Since the plan was to carry out a pilot study, but due to answers needed for the questions and limitation of time and limited funding, which was from a Nottingham University Hospital pump prime grant, the study was modified to a feasibility study with some overlap with a pilot study (Bowen *et al.*, 2009) (O'Cathain *et al.*, 2015).

The National Institute for Health Research defines feasibility studies as pieces of research done before a main study in order to answer the question "Can this study be done?" They are used to estimate important parameters that are needed to design the main study" (NIHR, 2018). Feasibility studies are designed to investigate uncertainties that will improve the precision of the main study, for example, are participants willing to be randomised, and what is the standard deviation of the main outcome measure, so that sample size estimate can be carried out for the main study. Feasibility studies are not necessarily the same design as a large study, and they are not designed to evaluate the main outcome of interest, which is the left to the main study. This differs from the pilot study, which is a miniature of the larger study: it assesses smoothness of the operation of different components of the study and the outcome of interest should be measured (Williams, 2016).

This study had complex components and interactions, which needed to be determined, including asking whether a future study would be viable and adequately powered to measure differences in fracture healing time between the Teriparatide and standard care groups of ankle Weber B fractures in older people. Ethical, technical and financial implications needed to be explored before proceeding to grant writing and a substantive study. Peichl et al reported mean fracture healing time of 4.8 weeks earlier in the Teriparatide treatment group (Peichl *et al.*, 2011). In

contrast, Johansson et al reported no difference in healing time or radiographic signs of fracture healing in the treatment group (Johansson, 2015). Additionally, there are many case reports of acceleration of fracture healing with use of Teriparatide, see Table 1-6, which have used different healing parameters. Thus one of the main areas of uncertainty in the design of a study to investigate accelerated fracture healing with Teriparatide was deciding when the fracture of interest has united sufficiently to allow return of function, as there is wide variability in reporting fracture healing parameters and healing time. Due to limitations in finance and time constrains, it was most appropriate to conduct a feasibility study to answer some of the questions to permit efficient planning of a future definitive randomised controlled trial.

2.2.4 Study population

We aimed to recruit 10 participants with conservatively treated Weber B ankle fractures into the study. Of these five participants were randomised into the intervention group (Teriparatide) and five participants were randomised into the control group (standard care). It was only feasible to recruit five to have Teriparatide group due to pharmacy costs of conducting this CTIMP study. As this was a feasibility study no formal sample size was calculated.

The inclusion criteria for recruitment were:

- Participants willing and able to give informed consent for participation in the study
- Male or female (women of non-child bearing potential only) aged 50 years and above
- Blood test results within normal range as defined by Nottingham University Hospitals NHS Trust (FBC, ESR, LFT, RFT, PTH, bone profile (calcium, albumin, total protein and alkaline phosphatase) and thyroid function, or clinically not significant as determined by Professor Sahota.
- Able and willing to comply with study requirements (frequent visits and CT scans)

The exclusion criteria were:

- Current Smokers (both tobacco and electronic cigarettes)
- Chronic renal disease
- Insulin dependent diabetes mellitus
- History of hypercalcemia
- End stage liver disease (liver cirrhosis)
- Patient with any current or past history of cancer
- Use of bisphosphonates, Zolendronic acid or fluorides in the past six months
- Any bone conditions other than osteoporosis
- Unable to get out of a chair or bed and walk without the help of another person pre ankle fracture walking aids were acceptable
- Contraindication to Teriparatide hormone:

  -Hypersensitivity

  -High risk of Osteosarcoma, (Paget's disease, chondromas, exposure to radiation, unexplained raised alkaline phosphatase, etc.)

  -Female participants of child-bearing potential, who are pregnant, lactating or planning pregnancy during course of study
- Scheduled elective surgery or other procedures requiring general anaesthesia during the study
- Terminally ill
- Planned blood donor donation during the study
- Participated in another research study involving an investigational product in the past 12 weeks
- Prior external beam of radiation or implant of radiation therapy to the skeleton.
- Any blood diseases leading to a bleeding tendency
- On Heparin, Warfarin or any Anticoagulants
- On Digoxin, Lanoxicaps and Lanoxin
- Already on Teriparatide or have had it prescribed previously
- Any other significant disease or disorder which, were in the opinion of the Investigator, may either put the participants at risk because of participation

in the study, may influence the results of the study, or the participant's ability to participate in the study.

All excluded participants were documented. The blood tests were done to rule out any underlying medical conditions that could have an effect on fracture healing or contraindications for receiving Teriparatide injections.

Age 50 years and above was taken as a cut off age for inclusion criteria. Menopause is a natural process occurs between the age of 45 and 55 years. In the UK, the average age for women to reach menopause is 51 years (Https://www.nhs.uk/conditions/menopause/, 2018). Hence, women age 50 years and above, are less likely to be child bearing potential. Due to the unknown side effects of Teriparatide in pregnancy, Medicines and Healthcare products Regulatory Agency (**MHRA**) conditioned the approval that female patients should be non-child bearing potential.

One in three women and one in twelve men older than 50 years will sustain an osteoporotic fracture at one of these most prone sites: wrist, spine and hip. An estimated 3 million people in the UK suffer from osteoporosis and more than 310,000 osteoporotic fractures are sustained each year (Rawlins, 2015)

2.2.5 Participants

A flow diagram shows the process of the participants flow in the study from point of identification until the end of their participation in the study (Figure 2-1).

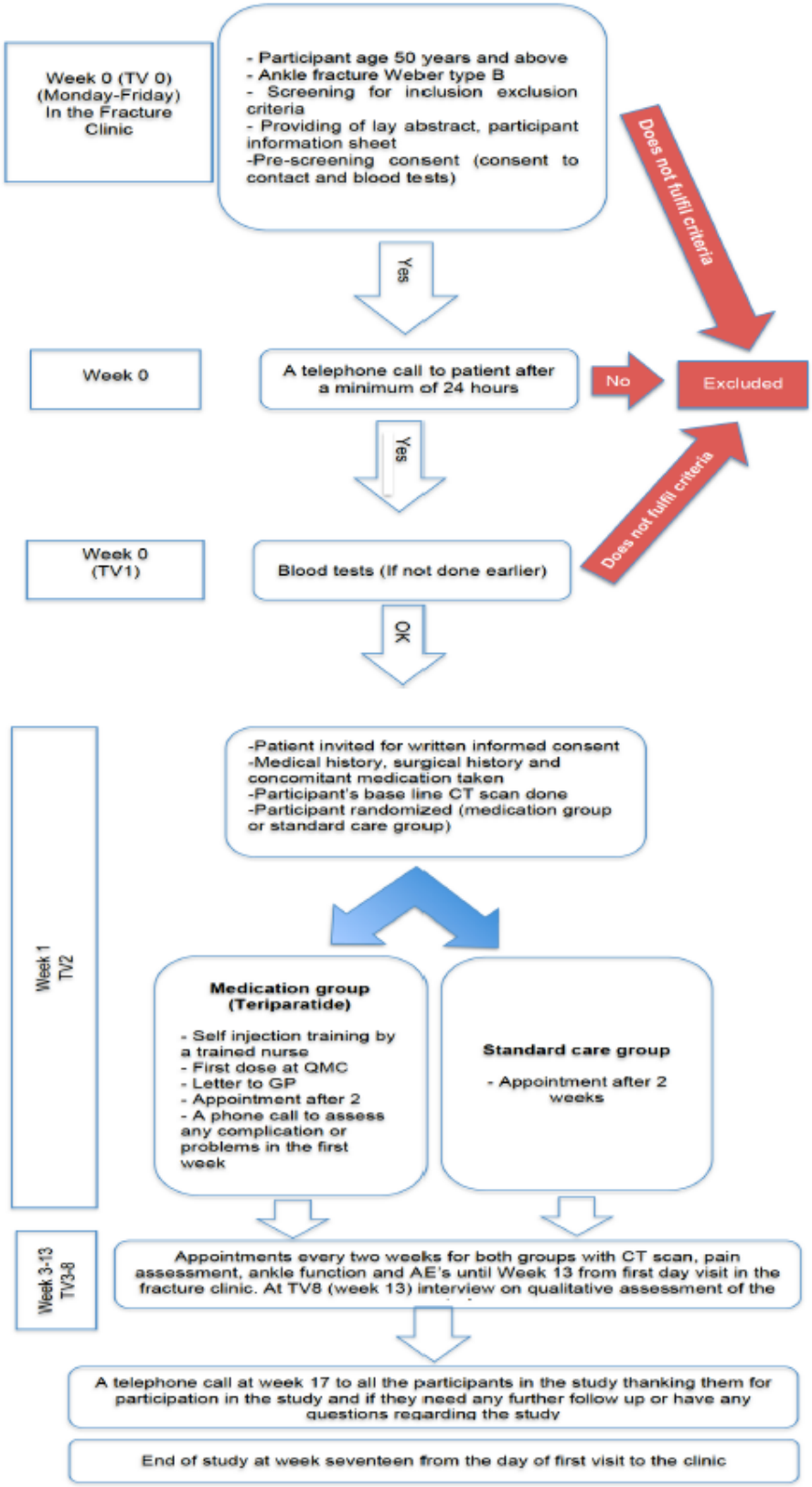


Figure 2-1 Patients flow in the clinic. TV=trial visit.

2.2.6 Randomization of participants

Randomisation was carried by an external provider called 'Simple randomization service', available from:

https://www.sealedenvelope.com/simplerandomiser/v1/
Randomization and online databases for clinical trials
Phone +44 2031764242 contact@sealedenvelope.com
Exmouth House, London EC1R 0JH, UK. Sealed Envelope Ltd 2015.

The study was not blinded and as such the participants were .aware if they were in the intervention group or the control group; thus, code breaking was not applicable. The blinding was not possible because there were no placebo injections available from the manufacturer, and it was considered unethical for the standard care group to self-inject placebo for 84 days. There was also a high cost involved in manufacturing a placebo injection that is similar to Teriparatide injection. The treatment group (intervention or control), were documented in the patient's medical records. However, the individuals analysing the CTs were blinded to the treatment group; as such a code was assigned to each participant (set of images). The participants were pre-randomized as there was a risk of not being able to carry out the randomization on the day of the participant enrolment visit (trial visit 1 or T) due to problems with the server and study drug availability.

2.2.7 Feasibility Study approvals

In order to assess objective 2-2, 'Can we get the necessary approvals for viability of study concept?' we applied for study approvals to the Midland-Nottingham 2 research ethics committee, Medicinal Heath Regulatory Authorisation and Health Research Authority.

This was a feasibility study of a clinical trial with an investigational medicinal product (CTIMP), sponsored by Nottingham University Hospital NHS Trust, protocol number 13OR006. This study received a favourable opinion from the East Midlands-Nottingham 2 research ethics committee dated 3rd of August 2016 REC reference number 16/EM/0299 (Appendix J). This study also received approval from Medicinal Health Regulatory Authorization to conduct the study on 17th of August 2016, reference number 19162/0226/001-0001 (Appendix K). Final approval of the study was from Health Research Authority on 6th of October 2016 (Appendix L). The

sponsor's green light was issued on 11th of October 2016 (Appendix M). Table 2-2 summarizes feasibility study governance.

Table 2-2 Summary of study approvals

Study title	Original title of the study: Feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people
Nottingham University Hospital sponsor number	13OR006
EurdraCT reference number	2015-005423-32
IRAS project ID number	143755
Clinical trial ID	NCT02955056
Nottingham 2 Research Ethics Committee reference number: 16/EM/0299	Approval date 3rd of August 2016
Medicines and Healthcare products Regulatory Agency (**MHRA**) reference number: 19162/0226/001-0001	Approval date18th of August 2016
Health Regulatory Authority (HRA)	Approval date 16th of October 2016
Nottingham University Hospital sponsor green light date	11th of October 2016

2.2.7.1 Study drug

Teriparatide hormone (FORSTEO Lilly France S.A.S, Rue du Colonel Lilly, F-67640 Fegersheim, France) was administered in a pre-filled pen as 20 micrograms/80 microliters solution. Teriparatide consists of rPTH (1-34), produced in *E. coli*, using recombinant DNA technology, homologous to the 34 N-terminal amino acid sequence of endogenous human parathyroid hormone.

2.2.7.2 Study drug administration

In order to assess objective 2-3, to assess the feasibility of administering Teriparatide self-injections and observing its side effects in the study group, those participants who were in the intervention (Teriparatide) group received training by a senior nurse specialising in osteoporosis to self-inject at the start of the study. The first dose was self-injected at Queen's Medical Centre following training and before they went home. The participants were contacted by telephone, by the nurse, within the first week of the study to check on any issues with the medication and administration of the medication. Also at each visit any side effects were recorded in the clinical trial folder. In addition to that a 24-hours contact number was provider to each participant in case of an emergency during the trial period. All participants were asked to ensure they had an appropriate storage facility for the drug (the study drug must be stored in a refrigerator between 2°C-8°C) before they were enrolled in the study. A drug information leaflet and pen user manual were provided with each pen device dispensed to the participants, which contained written information and questions and answers relating to the drug, in addition to that a cool bag was provided for transporting the drug home. Needles were supplied with a sharps box for needle disposal. The sharps box was collected with the used pens from the participant when they presented to collect the next pen at the clinical trials pharmacy at Queen's Medical Centre and at the end of the trial. The drug was administered subcutaneously in the abdomen or thigh.

2.2.8 Assessments of fracture healing

In order to assess objective 2-4, to assess the utility of CT scans to show differences in fracture healing with Teriparatide treatment and determine the required frequency of CT scans, by studying the radiological parameters of fracture healing using CT scanning a preliminary reproducibility study was performed.

2.2.8.1 Semi quantitative assessment of fracture healing using CT Scaphoid fracture reproducibility study

It was impossible, at the commencement of the study, to obtain images of CT scans of Weber type B ankle fractures managed conservatively to evaluate the parameters of fracture healing, as CT scans are not done routinely to monitor this fracture to union. Longitudinal CT scans were identified for patients having experienced a scaphoid fracture. CT scans of patients who had scaphoid fractures that were reported by a Nottingham University Hospitals radiologist were identified from the patient record system. Permission from the clinical director and the hospital Caldicott guardian was obtained to assess these CT scans for this purpose.

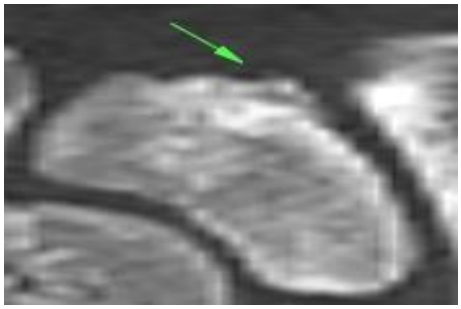
The CT scans were downloaded in DICOM format onto CDs after anonymising them and removing any identifiable information i.e. name, ID number, address or any other patient identifying information. A random code was assigned to each CD, Images of CT scans were reviewed on a desktop computer using the Philips DICOM viewer version R3.0-SP03. Fracture healing parameters were identified following review of the literature (see introduction section 1.5.3 and Table 2-3.

There was wide variability in assessing fracture healing radio-graphically in the systemic review carried out by Corrales et all that showed inconsistency of using fracture healing parameters. We combined fracture healing patterns primary and secondary fracture healing and fracture healing parameters, reported in the systemic review and choose the most relevant criteria to develop the fracture healing scoring table (Table 2-4),(Corrales *et al.*, 2008).

We identified the two ends of the fracture line in the scaphoid fracture CT scan images in both sagittal and coronal views of 10 scaphoid fractures. CT scans of different scaphoid fractures had different numbers of image slices depending on the

length of the fracture lines and orientation of the fracture. Image numbers were recorded of the scaphoid fracture healing onto a scoring table, so that all the raters were set up to report on the same images. We included all the image slices that showed a fracture line for both sagittal and coronal views. A meeting was held with the raters and the parameters were identified (Table 2-3). An exercise on scoring of the fracture healing parameters on some of the images using the scaphoid fracture healing scoring table was carried out. There were some difficulties if the fracture was comminuted or if there were two fracture lines. Additionally, for some of the images the slice thickness was 0.8 mm while for others it was 1.0 mm, these issues were resolved by choosing the same points to report for all the raters. Definitions of fracture healing parameters with example images are shown in Table 2-3. A fracture healing scoring table (Table 2-4) was developed using the parameters defined in Table 2-3. Four raters (Orthopaedic surgeon (BS), musculo-skeletal radiologist (RK), sports medicine specialist (AA) and senior research fellow (RP)) rated the images of the scaphoid CT scans independently.

Table 2-3 Definitions of radiological parameters of fracture healing using CT scans of scaphoid fractures

Callus present at cortical margins 0=Callus not present 1=Callus Present
 Arrow pointing to callus formed on the external margin of the cortical bone

Callus present within fracture line

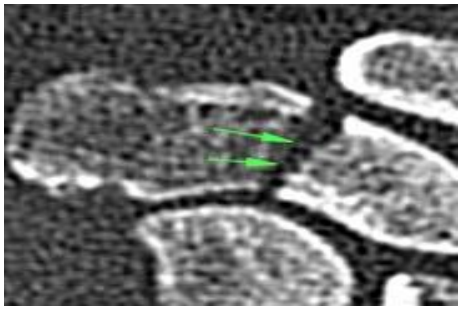
0=N0 callus formed

1=Callus formed=0%>≤25%

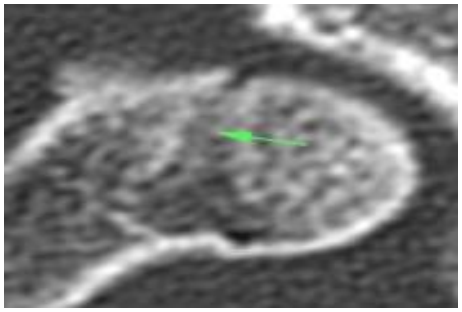
2=Callus formed=25%>≤50%

3=Callus formed=50%>≤75%

4=Callus formed=75% >≤100%



Arrows pointing along the fracture line showing no callus formation



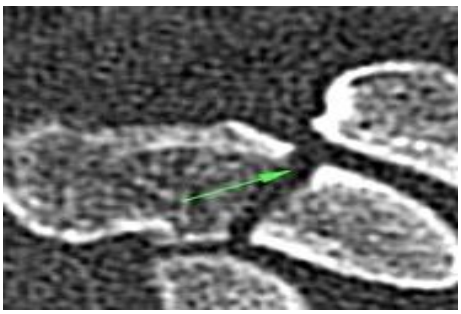
Arrow pointing along the fracture line showing callus formation

Cortical bridging

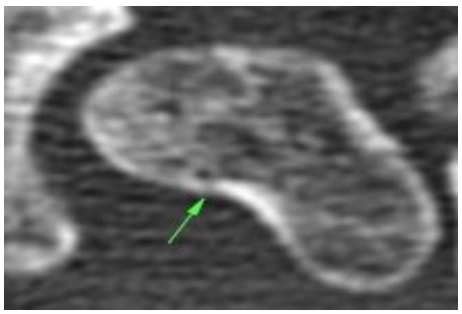
0=No cortical bone present

1=Partial bridging (some callus is present)

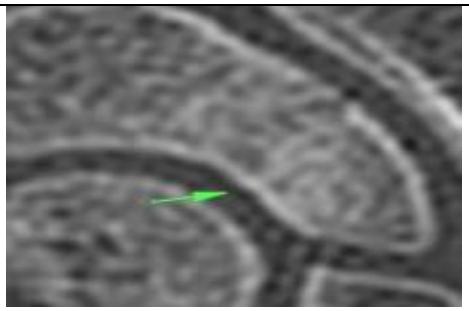
2=Complete bridging (cortical bone formed between the edges)



Arrow pointing to cortical edges showing no signs of cortical bridging



Arrow point to the development of callus between the cortical edges



Arrow pointing to development of complete bridging of cortical bone

Trabecular bridging

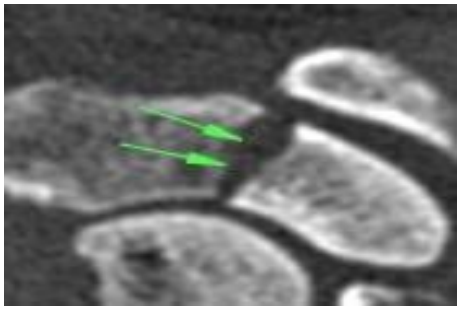
0=No trabecular bridging=0%

1=Trabecular bridging formed=0%>≤25%

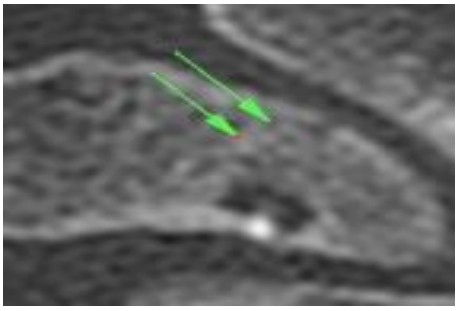
2-Trabecular bridging formed=25%>≤50%

3=Trabecular bridging formed=50%>≤75%

4=Trabecular bridging formed=75%>≤100%



Arrows pointing to no trabecular bridging between the edges of trabecular bone



Arrows pointing to formation of trabecular bridging across the trabecular bone

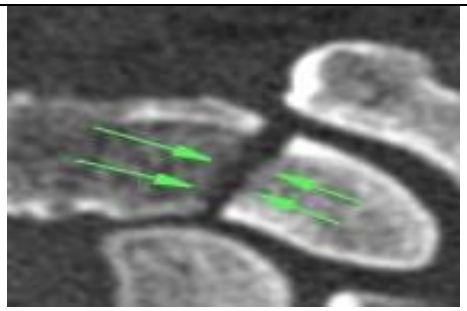
Fracture line margins

0=Fracture line margins sharp

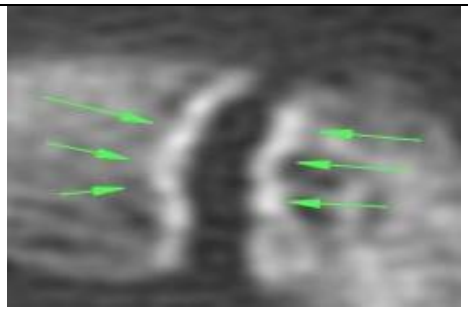
1=Fracture line margins not sharp

2=Fracture line margins sclerotic

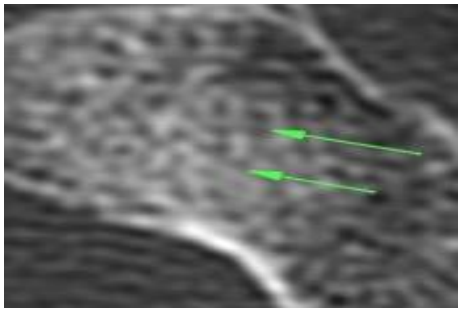
3=fracture line margins bridged



Arrows pointing to fracture line margins not sharp



Arrows pointing to sclerotic fracture line margins

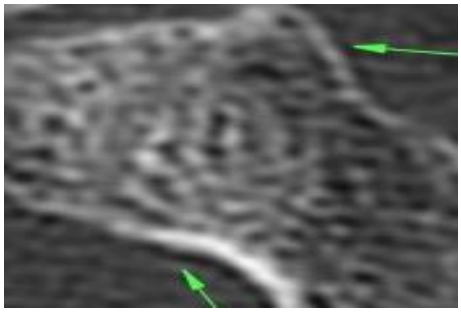


Arrows pointing to fracture line margins bridged

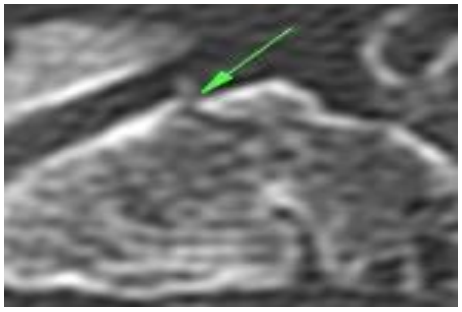
Fracture gap

0=Gap not present at cortical bon

1=Gap present at cortical bone



Arrows pointing to no gaps seen in the cortical edges



Arrow points to gap present between the cortical bone edges

Overall fracture healing percentage*

No fracture healing= 0,
Fracture healing =0%>≤25%=1,
Fracture healing =25%>≤50%=2
Fracture healing =50%>≤75%=3
Fracture healing =75%>≤100%=4

***This is a subjective summative assessment % of fracture healing by the rater**

Table 2-4 Fracture healing scoring table using CT scans of scaphoid fracture

CT scan fracture scoring table

CD No	
1-Age	
2-Sex	
3-Fracture	
4-Date of CT scan	
5-CT scan plane	
6-Slice thickness in mm	
7-Image number included	
8-Callus present at cortical margins externally	
Anterior margin Not present=0, Present=1	
Posterior margin Not present = 0, Present = 1	
9-Callus Present with in fracture line N0 callus=0, 0%>≤25%=1, 25%>≤50%=2, 50%>≤75%=3, 75% >≤100%=4	
10-Cortical bridging	
Anterior cortex Not present=0, [+]Partial bridging (Callus)=1, Complete bridging=2	
Posterior cortex Not present=0, [+]Partial bridging (callus)=1, Complete bridging=2	
11-Trabecular bridging No bridging=0, 0%>≤25%=1, 25%>≤50%=2, 50%>≤75%=3, 75%>≤100%=4	
12-Fracture line margins Sharp margins=0, Not sharp margins (resorption)=1, Sclerotic margins=2, Bridged margins=3	
13-★Fracture gap	
Anteriorly Not present=0, Present=1	
Posteriorly Not present=0, Present=1	
14-Over all fracture healing % No healing=0, 0%>≤25%=1, 25%>≤50%=2, 50%>≤75%=3, 75%>≤100%=4	

In sagittal plane Anterior margin=Volar of scaphoid, Posterior margin=Dorsal of scaphoid. In coronal plane Anterior margin=Proximal of scaphoid, Posterior margin=Lateral of scaphoid. [+]Partial bridging is when there is callus between the cortical margins. ★Fracture gap refer to gap between the cortical margins

IBM SPSS statistics version 22 was used for statistical analysis (inter-rater agreement). Table 2-6 shows inter-rater agreement using Kappa values with confidence intervals and P values for significance. The fracture healing parameters were: presence of callus, presence of callus within fracture line, cortical bridging, trabecular bridging, fracture line margins, fracture gap and overall fracture healing. It showed some of the raters had good agreement while others had fair to poor agreement for different healing parameters that were defined in Table 2-3 with the inter-rater agreement shown in Table 2-6. In Table 2-5 shown strengths of agreement and Kappa statistics (Gwet, 2014).

Table 2-5 Strengths of agreement and Kappa statistics (Gwet, 2014)

Landis and Koch (1977)		Altman's Kappa		Fleiss' Kappa	
Kappa Statistic	Strength of Agreement	Kappa Statistic	Strength of Agreement	Kappa Statistic	Strength of Agreement
< 0.0	Poor	< 0.20	Poor	< 0.40	Poor
0.0 to 0.20	Slight	0.21 to 0.40	Fair	0.40 to 0.75	Intermediate to Good
0.21 to 0.40	Fair	0.41 to 0.60	Moderate	More than 0.75	Excellent
0.41 to 0.60	Moderate	0.61 to 0.80	Good		
0.61 to 0.80	Substantial	0.81 to 1.00	Very Good		
0.81 to 1.00	Almost Perfect				

2.2.8.2 Parameters of fracture healing in computerized scan (CT scan)

The following parameters were used in the images of the CT scans to follow up changes in the progression of ankle fracture healing: the presence of callus at the cortical margins externally, the presences of callus within the fracture line, bridging of the cortices, bridging of the trabeculae, presence of fracture line margins, presence of fracture gap and subjective overall fracture healing. These fracture healing parameters were used in different studies (Corrales *et al.*, 2008) (Bhandari *et al.*, 2002) see also section 1.5.4. A table was constructed to score healing for each slice of CT scan image (Table 2-4).

2.2.8.3 Agreement among raters for fracture healing parameters scoring for CT scans of scaphoid fracture

Table 2-6 shows Kappa agreements with 95% confident intervals and P values also showing Standard errors and bias, of four inter raters for the fracture healing parameters that were assessed on CT scans of scaphoid fractures. These results were obtained by using a statistical software package IBM SPSS, version 22. Different raters showed agreement level ranges from good to fair for callus externally, callus within the fracture line, cortical bridging, trabecular bridging, at the fracture line margin, fracture gap and overall fracture healing. All of the mentioned parameters were included in the scoring table for the ankle fracture CT scan assessment.

Table 2-6 Agreement among raters for different parameters of fracture healing parameters seen on CT scans of scaphoid fracture

Fracture healing parameters	**Raters**	**N**	**Kappa Value**	**95% Confidence Interval of Kappa value**		**P value**
				Lower	upper	
Callus present at cortical margins externally anteriorly	AA vs RK	143	0.524	0.231	0.748	0.0005
	AA vs RP	139	0.227	0.059	0.417	0.004
	AA vs BS	140	0.082	-0.027	0.266	0.099
	RK vs RP	139	0.055	-0.074	0.208	0.377
	RK vs BS	140	-0.02	-0.048	0.000	0.402
	RP vs BS	138	0.029	-0.041	0.132	0.402
Callus present at cortical margins externally posteriorly	AA vs RK	142	0.394	0.183	0.573	0.0005
	AA vs RP	139	0.531	0.374	0.675	0.0005
	AA vs BS	140	0.633	0.448	0.782	0.0005
	RK vs RP	138	0.283	0.131	0.452	0.0005
	RK vs BS	139	0.447	0.213	0.640	0.0005
	RP vs BS	138	0.632	0.483	0.768	0.0005
Callus Present with in fracture line	AA vs RK	142	0.271	0.177	0.368	0.0005
	AA vs RP	139	0.134	0.057	0.211	0.001
	AA vs BS	140	0.177	0.081	0.272	0.0005
	RK vs RP	138	0.126	0.047	0.211	0.004
	RK vs BS	139	0.107	0.036	0.183	0.003
	RP vs BS	138	0.133	0.041	0.228	0.001
Cortical bridging anteriorly	AA vs RK	143	0.499	0.369	0.626	0.0005
	AA vs RP	138	0.330	0.193	0.457	0.0005
	AA vs BS	140	0.565	0.438	0.689	0.0005
	RK vs RP	138	0.310	0.178	0.435	0.0005
	RK vs BS	140	0.534	0.387	0.657	0.0005
	RP vs BS	138	0.400	0.263	0.523	0.0005
Cortical bridging posteriorly	AA vs RK	143	0.525	0.389	0.642	0.0005
	AA vs RP	139	0.415	0.287	0.538	0.0005
	AA vs BS	140	0.521	0.397	0.636	0.0005
	RK vs RP	139	0.385	0.247	0.516	0.0005
	RK vs BS	140	0.521	0.398	0.630	0.0005
	RP vs BS	138	0.344	0.233	0.470	0.0005

Fracture healing parameters	Raters	N	Value	95% confident interval of Kappa value		P value
				Lower	Upper	
Trabecular bridging	AA vs RK	143	0.596	0.498	0.697	0.0005
	AA vs RP	139	0.409	0.307	0.509	0.0005
	AA vs BS	139	0.438	0.337	0.545	0.0005
	RK vs RP	139	0.335	0.258	0.445	0.0005
	RK vs BS	139	0.475	0.371	0.575	0.0005
	RP vs BS	137	0.395	0.292	0.485	0.0005
Fracture line margins	AA vs RK	143	0.634	0.516	0.749	0.0005
	AA vs RP	138	0.647	0.520	0.761	0.0005
	AA vs BS	140	0.765	0.659	0.863	0.0005
	RK vs RP	138	0.477	0.310	0.579	0.0005
	RK vs BS	140	0.502	0.373	0.623	0.0005
	RP vs BS	137	0.656	0.537	0.778	0.0005
Fracture gap anteriorly	AA vs RK	143	0.671	0.528	0.788	0.0005
	AA vs RP	138	0.647	0.476	0.788	0.0005
	AA vs BS	140	0.604	0.455	0.744	0.0005
	RK vs RP	138	0.514	0.330	0.653	0.0005
	RK vs BS	140	0.574	0.430	0.713	0.0005
	RP vs BS	138	0.521	0.367	0.674	0.0005
Fracture gap posteriorly	AA vs RK	143	0.597	0.461	0.727	0.0005
	AA vs RP	138	0.652	0.479	0.795	0.0005
	AA vs BS	140	0.653	0.510	0.791	0.0005
	RK vs RP	138	0.423	0.281	0.562	0.0005
	RK vs BS	140	0.653	0.516	0.783	0.0005
	RP vs BS	138	0.540	0.396	0.676	0.0005
Overall fracture healing	AA vs RK	143	0.613	0.514	0.705	0.0005
	AA vs RP	139	0.249	0.154	0.336	0.0005
	AA vs BS	139	0.481	0.382	0.574	0.0005
	RK vs RP	139	0.187	0.100	0.278	0.0005
	RK vs BS	139	0.432	0.331	0.531	0.0005
	RP vs BS	137	0.350	0.253	0.441	0.0005

2.2.8.4 Ankle fractures

2.2.8.4.1 Standardisation of plain X-ray acquisitions

Standardized frontal and lateral radiographs were acquired together at baseline, at trial visit 2 and at trial visit 5, post injury (X-rays were part of standard care and as such the acquisition timing of the plain X-rays varied depending on the decision of the direct care team).

AP Mortise view*:*

- Patient on table with legs straight out in front of them

-The fractured ankle was placed on detector with foot dorsiflexed to 90°

-The leg a then Internally rotated approximately 15-20°, so that 5th metatarsal was perpendicular to the plate and the malleoli were equidistant from plate

-The centre of the beam was midway between the malleoli with the central ray at 90° to plate.

Lateral view:

-The patient was rotated towards the affected side so that lateral malleolus and lateral surface of foot was touching the plate

-The transverse axis of the patella was in a vertical position

-The plantar surface was perpendicular to the plate

-The foot was dorsiflexed to 90°

-The centre of the beam was directed to 1cm below the medial malleolus with the central ray at 90° to the plate

-The exposure was approximately 60kv/2mas FFD 110cms on wireless detector, Siemens digital.

2.2.8.4.2 Standardisation of computerized scan (CT scan) acquisitions

Ankle CT scans were performed using a standardised departmental protocol on a single Philips CT scanner (with provision for an identified alternate Philips scanner in case of equipment malfunction). The fracture ankle remained in a synthetic cast whilst being scanned; if a temporary boot used, this was removed for the scan. Axial CT sections span from 10 cm proximal to the ankle joint line through to below the malleoli and acquired at 1.0 mm nominal section thickness with 50% overlap using a

64 detectors or higher multi-detector clinical CT scanner. A phantom of known density was included within the scan field to allow for calibration and quantification. The images were reviewed on a clinical PACS workstation using standard tools including multi-planar reformats. Data from the CT scans after pseudo-anonymisation (and blinded by coding) were analysed off-line using custom programs on a workstation running Matlab™. Following a base-line assessment at TV1 (week 1), CT scan were performed at visits TV2 (week 3), TV3 (week 5), TV4 (week 7), TV5 (week 9), TV6 (week 11) and TV7 (week 13); that is every two weeks, with a total of 7 CT scans during the period of follow up in the study. Since this was a feasibility study and as there were no gold standard optimum timings to assess the fracture healing parameters and for obtaining a longitudinal series of CT with balancing the logistics of the number of participants visits, the dose of radiation and need to resolve the longitudinal differences of fracture healing/union between the two groups, it was decided to make the assessments every two weeks.

The definitions of the fracture healing parameters were revised after obtaining CT scans of trial participants with ankle Weber B fracture (Table 2-7). The scoring table was also revised with changes in the scoring scales for some of the parameters and removing the overall healing score from the total score (Table 2-8).

Radiation dose

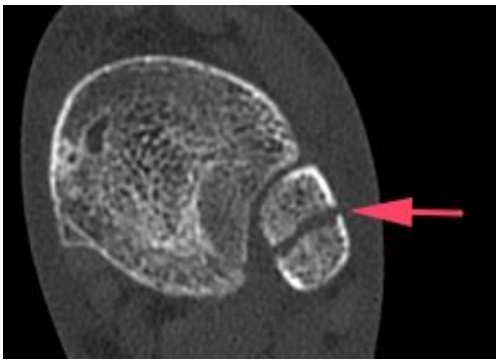
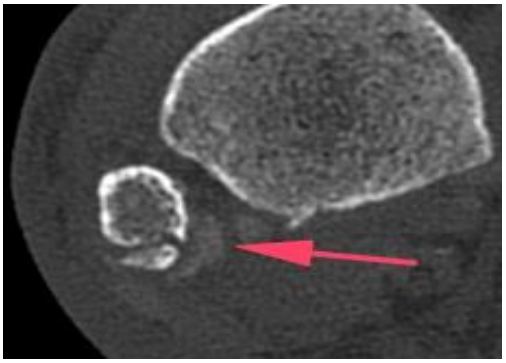
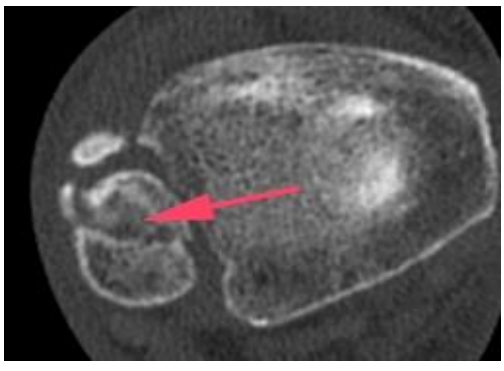
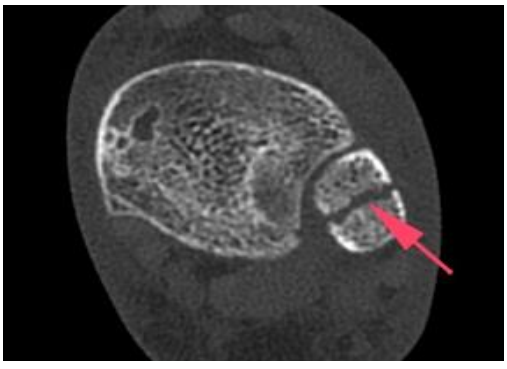
Our primary concern was the ionising radiation exposure to the participants in the study. Our Medical Physicist expert at Queen's Medical Centre calculated the radiation doses and assessed the risk of ionizing radiation exposure to the participants in the study. Millisievert (mSv) is the quantified unit of radiation absorbed by human tissue. It was estimated that for each routine AP and lateral x-ray views of the ankle, the participant would be exposed to 0.001 mSv per exam (Frequency and Collective Dose for Medical and Dental X-ray Examinations in the UK, 2008', HPA CRCE 012). And each CT scan of ankle exposed the participant to ≈0.07 mSv (Biswas *et al.*, 2009).

The trial involved approximately one to three AP and Lateral planar x-rays of the fractured ankle as part of a standard care depending on the judgment of the treating

physician and seven CT scans of the fractured ankle at weeks 1,3,5,7,9,11 and 13 which were part of the clinical trial.

Participation in this trial would have resulted in an ionized radiation exposure of approximately 0.50mSv. Of this, it was estimated that only the first planar x-rays would be as a part of standard care, therefore 99% of the exposure would be additional to routine standard care. The exposure of 0.50mSv is roughly equivalent to 12 weeks of background radiation received by a typical UK resident. The risk assessment for induction of fatal cancer this was based on a risk coefficient for developing fatal radiation induced cancer and heritable effects (adults) of 4.2%/Sv (ICRP103), a 0.50mSv effective dose would lead to a risk of about 1 in 48 000 for radiation exposure incurred as part of the trial.

Table 2-7 Definitions of radiological parameters of ankle fracture healing using CT scan of long bones (fibula/Lateral malleolus of ankle)

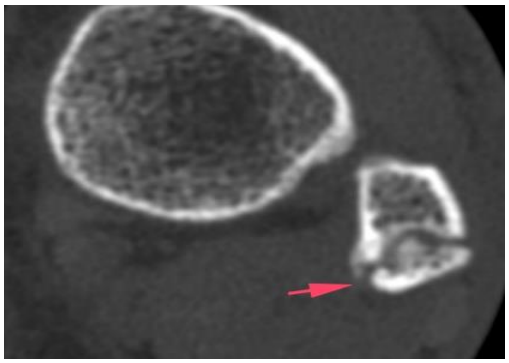
<table>
<tr><td colspan="2">Fracture healing parameters</td></tr>
<tr><td colspan="2">Callus/Woven bone present at cortical margins externally
0=No presence of callus or woven bone
1=Presence of callus or woven bone</td></tr>
<tr><td> 
In the image the arrow is pointing to cortical bone external margin were no callus/woven bone is formed</td><td> 
In the image the arrow is pointing to callus/woven bone on the external margin of the cortical bone</td></tr>
<tr><td colspan="2">Callus/woven bone present within the fracture line (trabecular bone)
1= No callus formation along within fracture line
2= callus formation more 0% and less than 25% along within fracture line
3= callus formation more than 25% and less than 50% along within fracture line
4= callus formation more than 50% and less than 75% along within fracture line
5= callus formation more than 75% to 100% along within fracture line</td></tr>
<tr><td> 
In the image the arrow is pointing to the area along the fracture line where callus is formed</td><td> 
In the image the arrow is pointing to the area along the fracture line margins where no callus formed</td></tr>
</table>

Cortical bridging

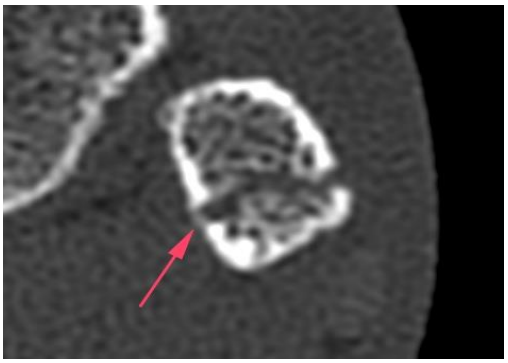
0=no cortical bridging

1=Partial cortical bridging

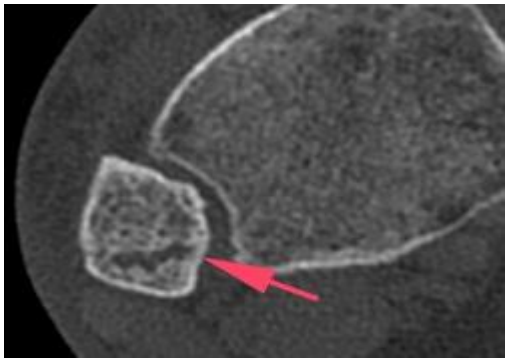
2=Complete cortical bridging



In the image the arrow is pointing to formation of callus/woven bone at the cortical edges but no cortical bridging



In the image the arrow is pointing to the cortical edges with formation of callus/woven bone classified as partial bridging



In the image the arrow is pointing to the cortical edges where complete bridging has occurred

Trabecular bridging

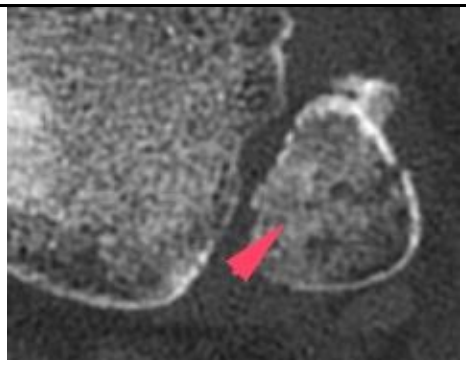
1= no trabecular bone formed along the fracture line bridging the edges of the cancellous bone

2= more than zero less than 25% formation of trabecular bone along the edges of cancellous bone

3= more than 25% less than 50% formation of trabecular bone along the edges of cancellous bone

4= more than 50% less than 75% formation of trabecular bone along the edges of cancellous bone

5= more than 75% formation of trabecular bone along the edges of cancellous bone



In the image the arrow is pointing to the bridged trabecular

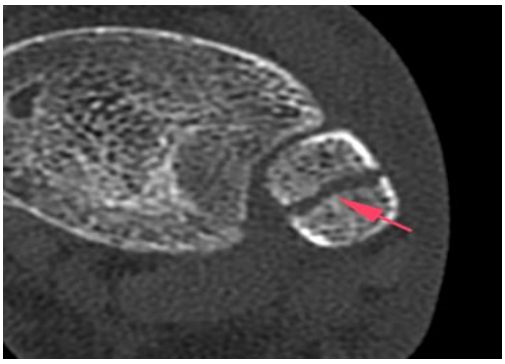
Fracture line margins

0=Sharp margins

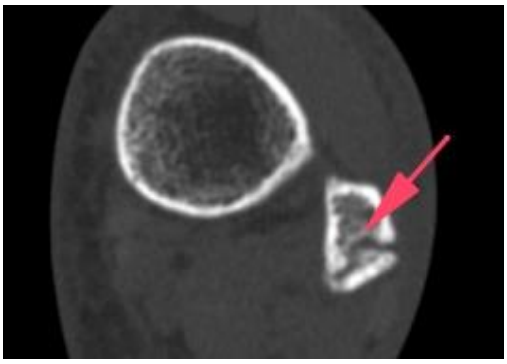
1=Not sharp margins

2=Sclerotic margins

3=Bridged margins



In the image the arrow is pointing to fracture line margin showing not sharp margins



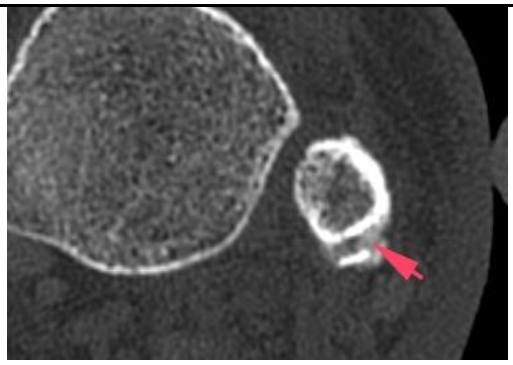
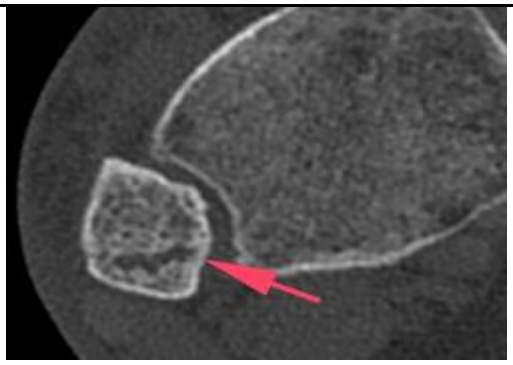
In the image the arrow is pointing to fracture line margins showing sclerosis

Fracture gap

1=gap present,

2=Partly healed

3= Not present

	
In the image the arrow is pointing to cortical margins where callus/woven bone is formed but fracture gap is still present	In the image the arrow is pointing to the cortical edge were complete bridging occurred showing no gap
14-Overall fracture healing* 1= no healing of fracture at all 2= Fracture healing more than 0% but equals or less than 25% 3= Fracture healing more than 25% but equals or less than 50% 4=Fracture healing more than 50% but equals or less than 75% 5=Fracture healing more than 75% up to 100% ***This is a subjective summative assessment % of fracture healing by the rater**	

Ankle fracture healing scoring table was modified after scoring 50% of the 70 ankle CT scans, as using scaphoid fracture CT scans did not seem appropriate for developing a long bone fracture healing scoring table (Table 2-8). Since scaphoid CT scans were not in axial views and scaphoid fractures do not heal with callus formation externally hence we used cortical bridging. Additionally, the CT scans were taken for delayed unions and non-union of scaphoid fractures and did not have the full spectrum of fracture healing parameters. Total fracture healing was added in the table (which is the sum of the scores of fracture healing parameters with maximum of 25 and minimum of 4). Subjective overall fracture healing score was moved to the bottom of the table, as it was not rational to add the overall fracture score in calculation of the total fracture healing score. Also, the scoring table was revised with changes in the scoring scales for some of the parameters. Some of the parameters score were changed to start at 1 instead of 0 this was because of the SPSS software issues.

Table 2-8 Revised ankle fracture healing scoring table

CD No																				
1-Age																				
2-Sex																				
3-Fracture																				
4-Date of CT scan																				
5-CT scan plane																				
6-Slice thickness in mm																				
7-Image number assessed																				**Max**
8-Callus/woven bone present at cortical margins externally																				
Anterior margin Not present = 0, Present=1																				**1**
Posterior margin Not present = 0, Present = 1																				**1**
9-Callus/woven bone present within fracture line (trabecular bone region) No callus=1; 0%>≤25%=2; 25%>≤50%=3; 50%>≤75%=4; 75% >≤100%=5																				**5**
10-Cortical bridging																				
Anterior cortex Not present=0; *Partial bridging (Callus/woven bone)=1; Complete bridging=2																				**2**
Posterior cortex Not present=0; *Partial bridging (callus/woven bone)=1; Complete bridging=2																				**2**
11-Trabecular bone bridging No bridging=1; 0%>≤25%=2; 25%>≤50%=3; 50%>≤75%=4; 75%>≤100%=5																				**5**
12-Fracture line (trabecular bone) margins Sharp margins=0; Not sharp margins (resorption)=1; Sclerotic margins=2; Bridged margins=3																				**3**
13-★Fracture gap (cortical bone)																				
Anteriorly Present=1; Partly healed =2; Not present=3																				**3**
Posteriorly Present=1; Partly healed =2; Not present=3																				**3**
14-Total fracture healing score	**0**	**0**	**0**	**0**	**0**		**0**	**0**	**0**	**0**	**0**		**0**	**0**	**0**	**0**	**0**			**25**
15-Overall fracture healing %[+] No healing=1; 0%>≤25%=2; 25%>≤50%=3; 50%>≤75%=4; 75%>≤100%=5																				**5**

In axial plane if fracture line is lateral then it is = Anterior margin. If fracture line is medial then it is = Posterior margin

***** Partial cortical bridging is, when there is callus/woven bone between the cortical margins. Partial cortical bridging includes cortical bone is bridged by trabecular bone

★ Fracture gap refer to gap between the cortical margins of the fracture. [+]**This is a subjective summative assessment % of fracture healing by the rater**

2.2.9 CT scan analysis using revised ankle fracture scoring table

The CT scans were pseudo-anonymised and downloaded onto cd's. The pseudo-anonymised CT scans were viewed on desktop using Philips DICOM viewer 3.0. Image slices were magnified up to 300% to have a better view of the features of the bone. Both raters (AA and WAW) were blinded to the participants allocation group (standard care group or Teriparatide treatment group). Table 2-8 was used to score the changes in fracture healing parameters of each image slice of the CT scans. The fracture line was divided into five regions and each region had five image slices assessed Figure 2-2. Total of 25 slice images for each CT scan, were scored by the two raters AA and WA independently with a grand total of 1750 slice images from 70 CT scans for 10 participants were viewed and assessed. The data was transferred on an excel spread sheet, for statistical analysis.

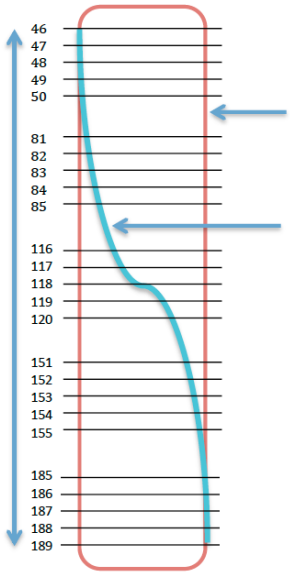


Figure 2-2 Regions of the fracture on the image slice from the CT scan scored

2.2.10 Quantitative CT scan method development

2.2.10.1 Effect of cast

An experimental CT scan was carried out on a cylinder of water phantom to assess the effect of the cast on the readings of water phantom densities using a Hounsfield unit (HU) scale. A cylindrical water phantom filled with water and a cast placed around it was scanned using the ankle protocol (see section 2.2.8.2.2) and then the scan was repeated the on the water cylinder without the cast. The images

were analysed using IQWorks (automated image analysis software for use with DICOM images). A region of interest (ROI) was drawn in the centre of the cylindrical water phantom covering approximately 80% of the water-filled region. The mean density in Hounsfield units of the voxels in the (region of interest (ROI) calculated for each slice. The central 40 slices from both scans were then selected, since this was the range over which the density in Hounsfield unit was deemed to be relatively stable this was decided by the medical physics experts. The mean voxel densities in Hounsfield units from the image slices with and without the cast are shown in systems.

Table 2-9. The hypothesis that the voxel density of the water phantom was not affected by the presence of a cast was tested using a paired two-tailed t-test in excel, giving a p-value of <0.000, which indicated a significant difference.

Although the measurements of density of the water phantom (Hounsfield units) showed a statistically significant difference, between the phantom alone or and the phantom within the cast, it is worth pointing out that the magnitude of the difference is around 5 HU, which the Institute of Physics and Engineering in Medicine (IPEM) report 91, recommended standard acceptable tolerance used to calibrate the scanners for routine performance of diagnostic x-ray systems.

Table 2-9 Density in HU measured using phantom cylinder of water with cast and without cast

	Cylinder of water without cast	Cylinder of water within cast
Number of cases	41	41
Mean	7.03 ± 0.19 Std.	8.43 ± 0.69 Std.
Median	7.05	8,35
Minimum	6.60	7.41
Maximum	7.43	10.34
A paired sample T test was conducted to measure the difference in densities in HU on CT scan between the water cylinder in cast (mean=8.43 Std deviation= ±0.69) and the water cylinder without cast (mean=7.03 Std deviation=± 0.19) there was a significant difference in densities measured (p<0.000)		

2.2.11 CT scan analysis using Matlab™ and ImageJ™ software

The images were separated into individual series using a DICOM viewer (3DSlicer v4.6.2 r25516, National Alliance for Medical Image Computing, Boston, US) and registered using in-house code written for Matlab™ (MATLAB Release 2017a, The MathWorks, Inc., Natick, Massachusetts, US). The registered volumes were saved in niftii format, so that they could then be analysed using ImageJ™ (ImageJ 1.51 j8, National Institutes of Health, US). For each CT scan raw data for densities with their corresponding number of voxels using ImageJ™ software were extrapolated on excel sheets. Number of voxels for each pre-specified range of densities for the callus, trabecular bone and cortical bone were calculated.

Two of the CT scans were not acquired as per protocol due the radiographer error in the settings of CT scanners which lead to the images densities being different and could therefore not be standardised in reference to the reference phantom placed on ankle of the participants.

2.2.11.1 Image registration

Image registration is a process of superimposing of two images exactly on each other, i.e. the same scene taken at two different time points and different orientations, with references to particular points. In order to assess the accuracy of registering two images three dimensionally on each other we carried out an experiment on a frozen lamb leg. We placed small metal balls (fiducial points) in the frozen lamb leg. Fiducial points are fixed reference points placed on or in an object in the field of an image that can be used as reference points. An initial CT scan of the lamb leg with the fiducial points was taken and referred as a reference image. Then a subsequent second CT scan of the same lamb leg was taken after moving it around and this is referred to as a transformation CT scan. Bony points on the CT scan of the lamb leg were chosen to compare to the reference CT scan and we tried to identify the same bony points in the transformation CT of the lamb leg. The transformation CT scan was registered to the reference CT scan by using the identified bony points. The difference (that is the offset in all three directions) between the reference CT scan and the transformation CT scan was measured by as the difference between the

fiducial points in the two CT scans (Figure 2-3). There was a 2 mm difference in either direction of the pixels and the maximum geometric error was 2.3 mm in any direction of a voxel in a 3D image (Table 2-10). This means the offset of the lamb bone is 2.3 mm in any direction of an image registration using this software (Matlab^{TM}).

Table 2-10 Differences between the registration of transformation scan points to reference scan points on lamb leg CT scan

Co-ordinates	**x**	**y**	**z**	**Difference**
Reference 00	308	308	70	(1, 0, 0)
00 Reg to 01	309	308	70	
Reference 00	206	322	180	(-2, 1, 2)
00 Reg to 01	204	323	182	
Reference 02	177	323	106	(-1, -1, 1)
06 Reg to 01	176	322	107	
Max difference in any direction: 2 mm-Max geometric error: $(2^2 + 2^2 + 2^2)^{⅓}$ = 2.3 mm				

Image 01 registered to Image 00:

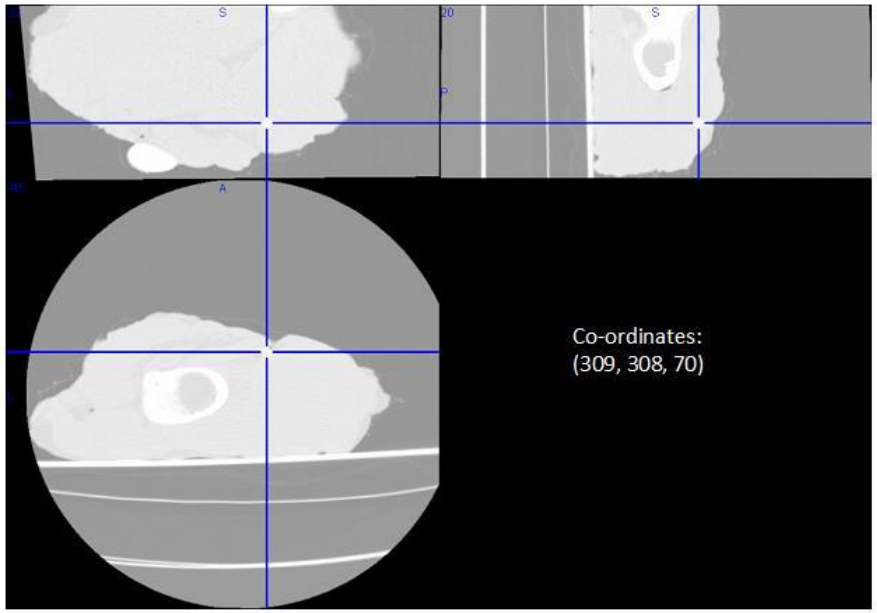
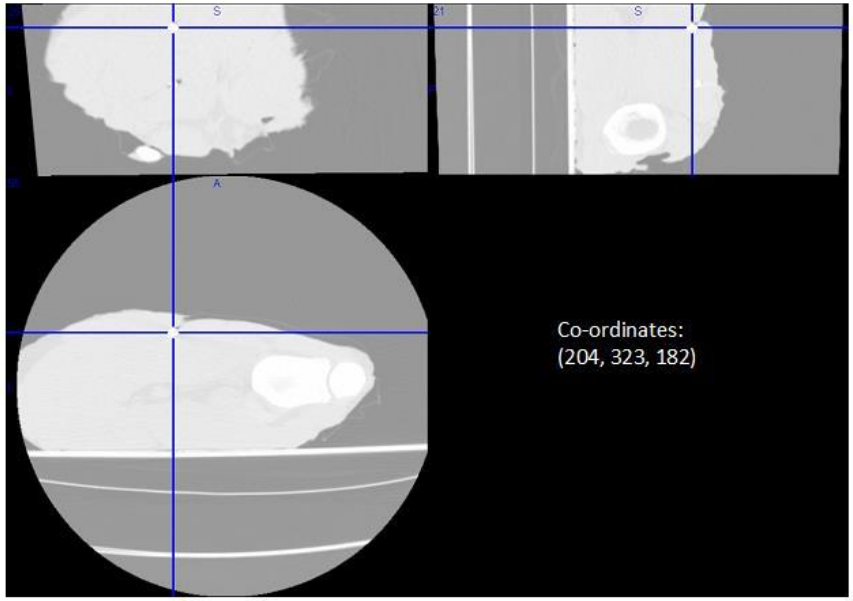
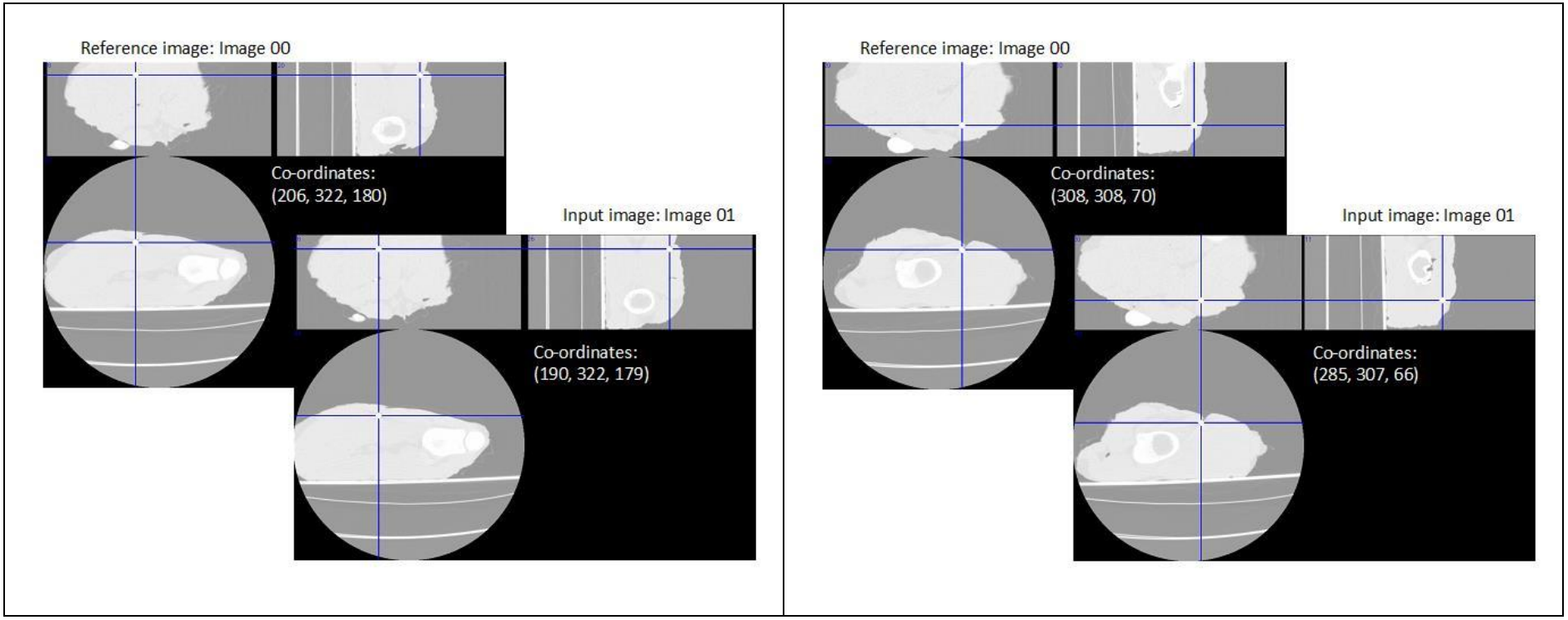


Image 01 registered to Image 00:




Reference image: Image 00
Co-ordinates:
(206, 322, 180)
Input image: Image 01
Co-ordinates:
(190, 322, 179)
Reference image: Image 00
Co-ordinates:
(308, 308, 70)
Input image: Image 01
Co-ordinates:
(285, 307, 66)

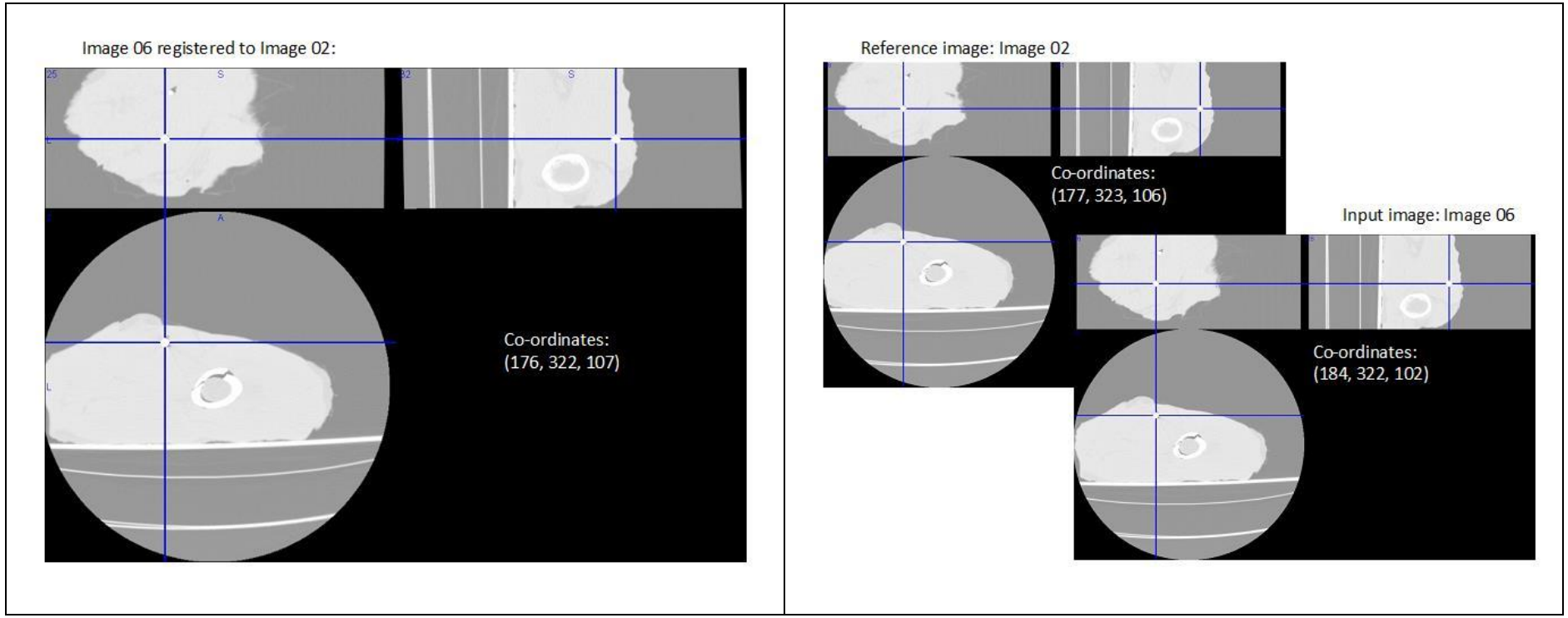


Figure 2-3 CT scan images of lamb leg showing registrations of fiducial point and matching them with reference points

2.2.11.2 Image segmentation and determining tissue densities

We identified these tissues on a CT scan image of an ankle fracture: fat, muscle, trabecular bone region, callus and cortical bone (regions of interest) Figure 2-4. By using IMAGJ^{TM} software, density ranges in each region of interest were identified that is for the different tissues showing the image. These densities are measured in Hounsfield units. From Table 2-11 we can see there is some overlap of densities between the tissues e.g. fat tissue and muscle tissue densities overlap to some extent and trabecular bone region overlaps with fat tissue densities. This is due to that muscle tissues contain fat in between the muscle fibres. Also, trabecular bone contains fatty marrow.

Table 2-11 Density ranges in Hounsfield unit of different tissue

	Min	Max	Mean	Std dev	Mode
Density range in HU for cortical bone, trabecular bone, soft tissue **Image 2-1**	-122	1803	383.678	548.758	78(374)
Density range in HU for fat and muscle **Image 2-2**	-133	170	26.655	65.801	73 (327)
Density range in HU for muscle **Image 2-3**	-34	143	77.729	21.591	70 (60)
Density range in HU for fat **Image 2-4**	-133	33	-68.481	25.719	-80 (33)
Density range in HU for trabecular bone **Image 2-5**	-102	641	109.498	130.500	109 (29)
Density range in HU for callus **Image 2-6**	522	970	790.354	88.265	860 (3)
Density range in HU for cortical bone **Image 2-7**	1093	1803	1623	99.932	1587 (10)

Image 2-1

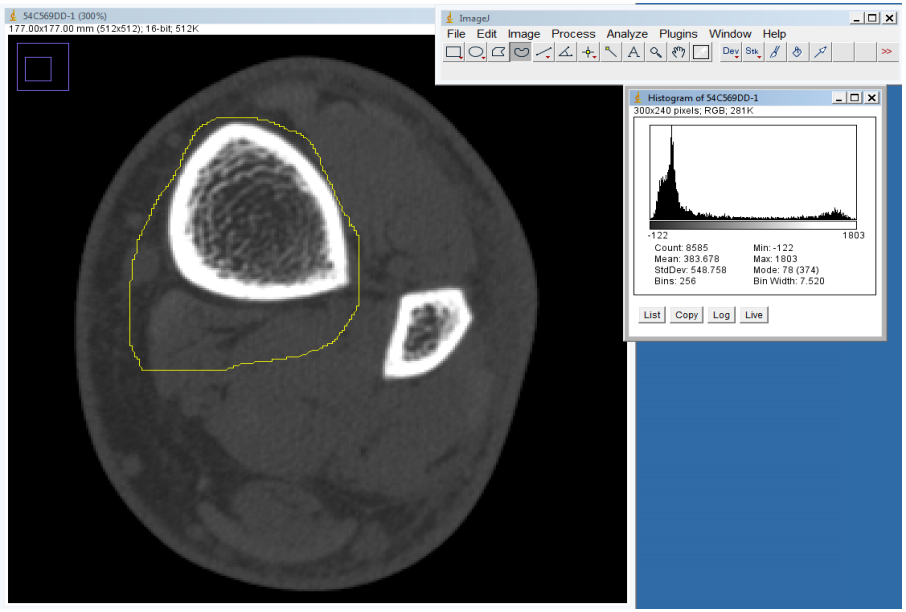


Image 2-2

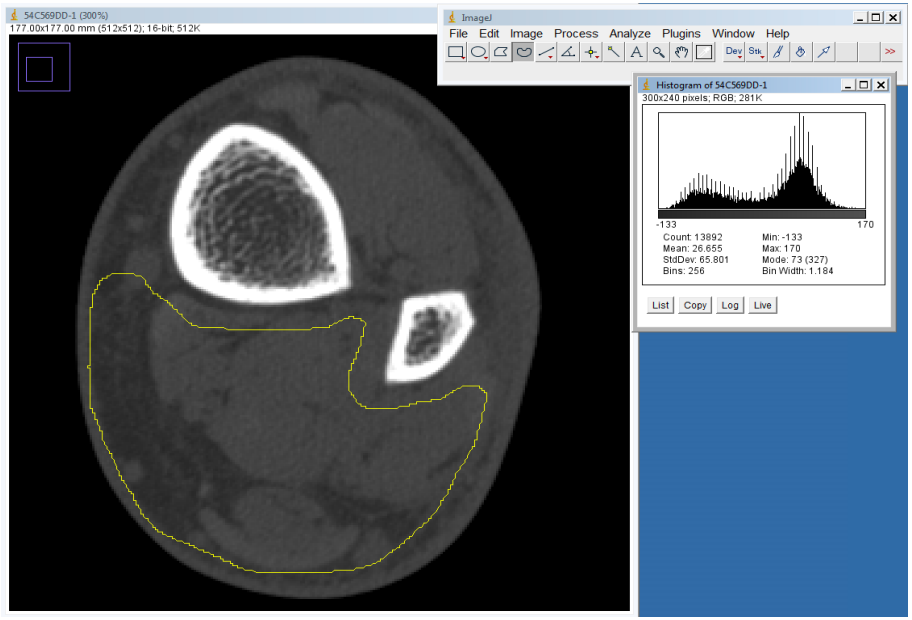


Image 2-3

Image 2-4

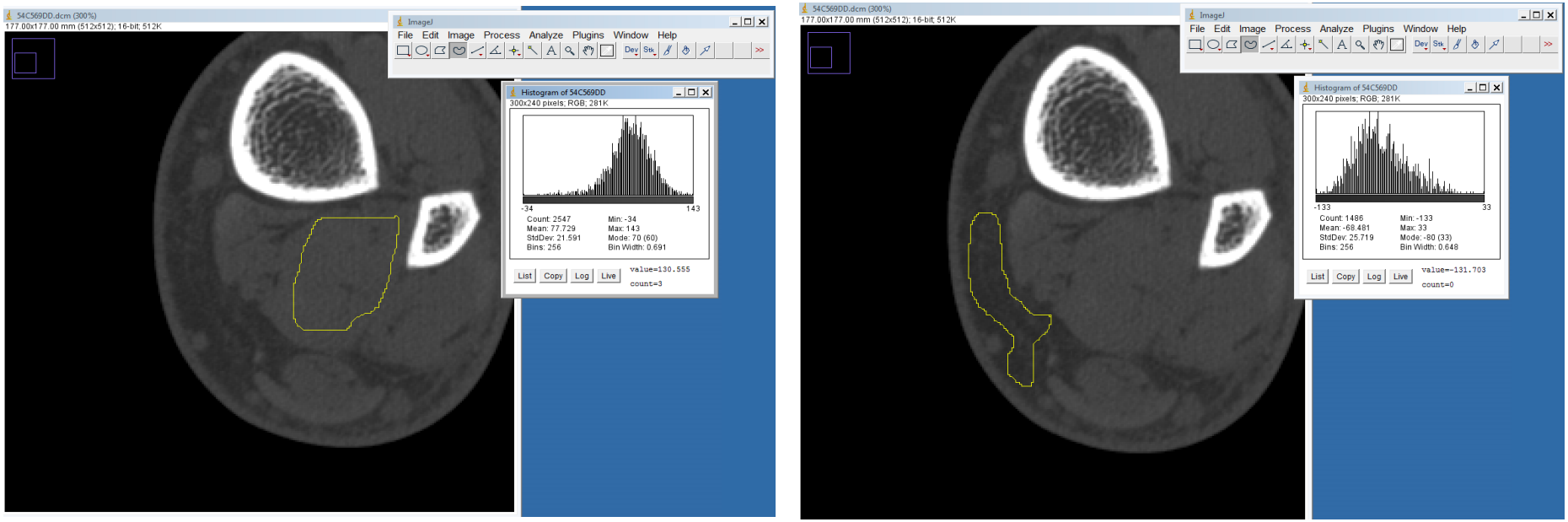


Figure 2-4 Identification of densities (Hounsfield units) of segments containing bone and soft tissue, soft tissues muscle and fat

Image 2-5

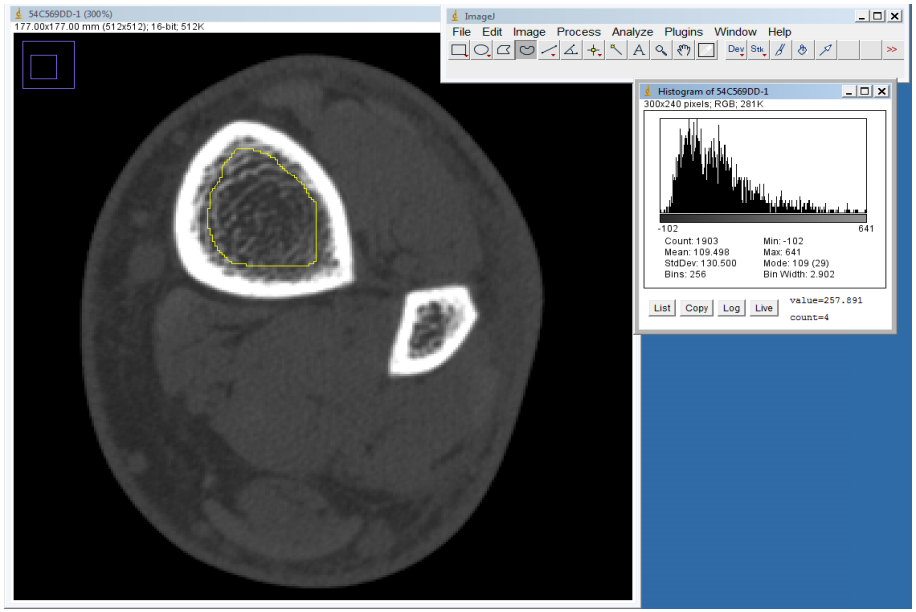


Image 2-6

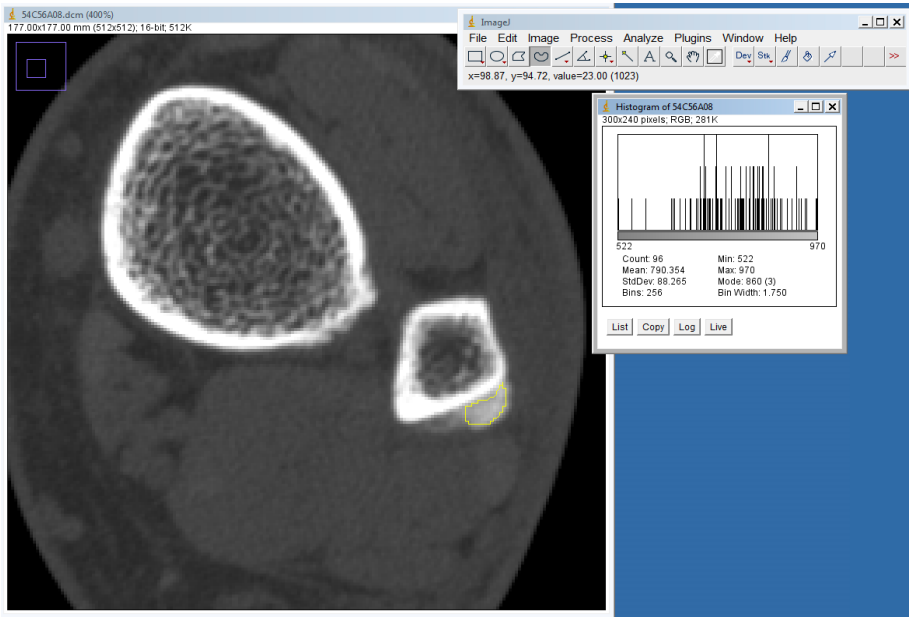


Image 2-7

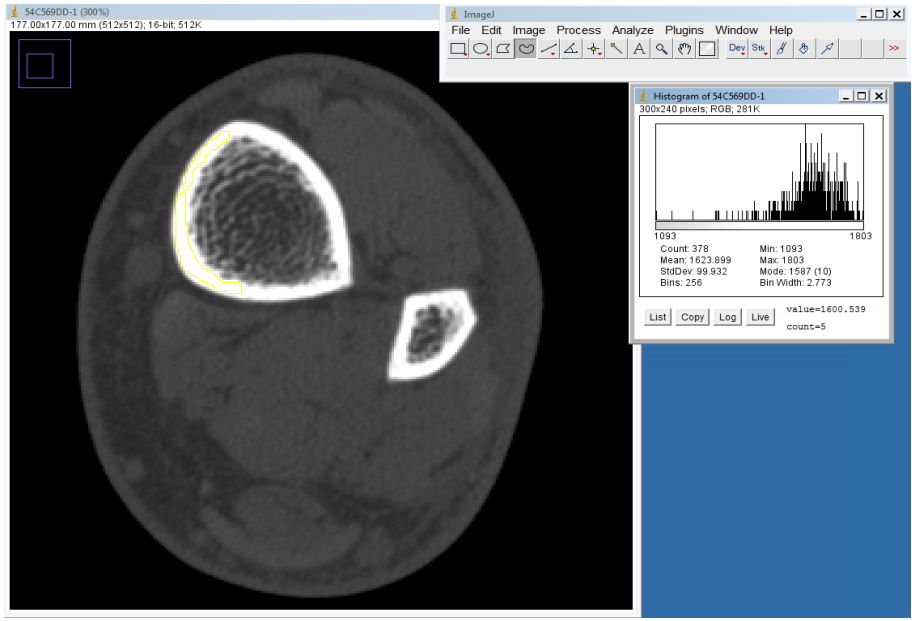


Figure 2-5 Identification cortical bone, trabecular bone and callus in the CT scan images and their corresponding range of densities in HU.

2.2.11.3 Quantitative CT scan of ankle fractures

A reference CT scan (base line CT scan at TV1) was identified for each participant and subsequent CT scans (CT scans at TV2, TV3, TV4, TV5, TV6, TV7 these were two weeks apart) were registered and transformed using Matlab™ and ImageJ™. A fixed volume on a 3D CT scan was calculated in the reference CT scan and this volume was fixed for the subsequent CT scans for each of the participants CT scans. The number of voxels for range of densities for cortical bone, trabecular region and callus in a prefixed volume were calculated for each CT scan of each participant from the fixed volume (this was carried out by the medical physicist). The counted number of voxels was normalised to the range 0.0 to 1.0 (normalisation of data to the range 0.0 to 1.0, this was done by taking the greatest value in the column and dividing it by each value). A table was made for cortical bone, trabecular region and callus for each participant in time sequence (the time sequence was the trial visit order) with normalised values of the participant CT scans of the ankle fracture. A line graph was plotted for each of the participant for cortical bone, trabecular region and callus, showing increase or decrease in the number of voxels for the specified range of densities as the fracture progressed toward healing (Figure 3-15) (Figure 3-16) (Figure 3-16) (Figure 3-17).

2.2.12 Patient reported outcome measures (PROM)

2.2.12.1 Visual analogue pain score

In order to assess objective 2-5, to identify the participant's pain during the treatment period as measured using a questionnaire (a 10 point visual analogue pain score scale), a 10-point visual analogue pain score (VAPS) was used for assessing participant pain at each visit see Appendix N.

Visual analogue pain score is the most common pain scoring scale used. It is easy to administer as paper and pencil measure, no previous training is required, it takes 1 minute to complete. Self-completed by respondent, available for public use with no cost and It has been used in diverse populations (Hawker *et al.*, 2011)

2.2.12.2 Olerud and Molander questionnaire for assessment of ankle function

In order to assess objective 2-6, to compare ankle function between the Teriparatide group and standard care group using Olerud and Molander questionnaire, a functional assessment of the ankle was carried out using the Olerud and Molander questionnaire at trial visit 5 when the cast or walking boot was taken off.

Olerud and Molander questionnaire is widely used for assessment of ankle function it has been validated and reliable tool(Button and Pinney, 2004). It is easily self-administered does not take much time in the clinic it covers, it has been recommended for scientific investigations and for minor subjective differences (Olerud and Molander, 1984)

2.2.12.3 Quality of life questionnaire EQ-5D-5L

In order to assess objective 2-7, to measure health status of the participant using EQ-5D-5L quality of life questionnaire, a health status questionnaire using EQ-5D-5L questionnaire was carried out at each trial visits.

For a treatment to succeed it must be perceived by patient to have an effect on daily living functions or quality of life functions. EQ-5D-5L quality of life questionnaire is a country specific validated tool that has been used in clinical trials. It covers areas of mobility, self-care, usual activities, pain/discomfort, anxiety/

depression and perceived health status of the patient with 5 level answers for each domain. It gives a single index value for health status that can used to compare between patients (Rabin and de Charro, 2001).

2.3 Qualitative study

In order to assess objective 2-8, to carry out an in depth qualitative assessment of participant's involvement in the study, at the last clinical trial visit for each participant in the study, a qualitative assessment of the patients' experiences of participating in the study was carried out, in the form of a semi-structured interview. Open-ended questions were developed with input from the patient public liaison officer to facilitate the interviews with participants taking in consideration broad aspect research areas involved and how to improve it in future. These were as following:

1 What did you think of the quality of care you received whilst taking part in this research study?
2 How was your experience with self-injection (specific for treatment group)?
3 How did you feel about visiting hospital every 2 weeks?
4 What did you think about having computerized scans (CT scans) every 2 weeks?
5 How important do you think this study is?
6 Would you consider taking part in any future research and if so why?
7 Do you have any further comments or suggestions that might improve this study?

The audio-recorded interviews were transcribed for analysis using Nvivo™ software. The information gathered from this qualitative part of the study aimed to give us an in depth understanding of participants' views going through the trial, which could be used to improve the planning for future definitive studies. Phenomenological method was used for understanding the participants live experience going through the clinical trial. The methodology used in phenomenology differs than most other qualitative research methodology. "Phenomenology is interested in the activities of consciousness and the objects that present themselves to consciousness" (Giorgi, 2012)

There are other methods e.g. grounded theory (The name "grounded theory" mirrors its fundamental premise that researchers can and should develop theory from rigorous analyses of empirical data) (Charmaz and Belgrave, 2015).

In the researcher's opinion, phenomenological method was the most suitable for underpinning this study. Purposeful sampling technique was used (other type of sampling snowballing, quota sampling), as all the participants enrolled in the study were interviewed at the last visit of the trial. Each interview lasted 15 to 20 minutes. The transcripts were not discussed with the participants. The data was collected in the fracture clinic at Queen's Medical Centre, Nottingham. Some of the participants had their partners accompany them in the interviews.

2.3.1 Phases for thematic synthesis

Thematic synthesis has 6 phases as mentioned by Braun and Clarke (Braun and Clarke, 2006).

Phases for thematic analysis	Description of the phase
1-Familarizing data	After audio-recording the interviews they were transcribed into paper format. Transcriptions of the interviews were read and reread again by the researcher until he had a comprehensive understanding of the contents of transcripts. The ideas were noted initially as the transcripts were read.
2-Initial codes were generated	The data was coded in a systemic approach and preliminary codes were assigned to the themes. After going through the data again, the context of the conversation was postulated, and the relative data with the codes assigned was combined into a collective code. Nvivo™ software was used for assigning codes.
3-Searching for themes	The combined codes were analysed and interpreted. The data was split or combined according to the relativeness. The codes, themes and subthemes were examined for relationships.
4-Reviewing themes	In depth review of themes, whether to combine, refine separate or discard was performed. The data was checked again for coherences within themes. A thematic map was generated
5-Defining and naming the themes	Themes and subthemes were defined and refined within data. The themes were named. Further analysis was carried out to enhance the developed theme
6-Report producing	Analysis of the data was transformed into an interpretative report were example to themes, questions and literature were used (Maguire and Delahunt, 2017)

In phase one of the thematic synthesis where the researcher becomes familiar with the data, is very important. It would have been more beneficial if the audio-recorded interviews, were transcribed by the researcher himself, but as this is a very time-consuming task, the transcribing was outsourced to a professional typist. In this study, the interviews were carried out by a single interviewer, which was the researcher. This was an advantage, as the researcher was able to be familiar with the data.

In phase two of the thematic synthesis, where codes are initially generated, in this study we used Nvivo™ software to identify the themes. The software highlighted different colours to the themes on the transcripts, which were in word documents and assigned codes accordingly (Figure 2-6).

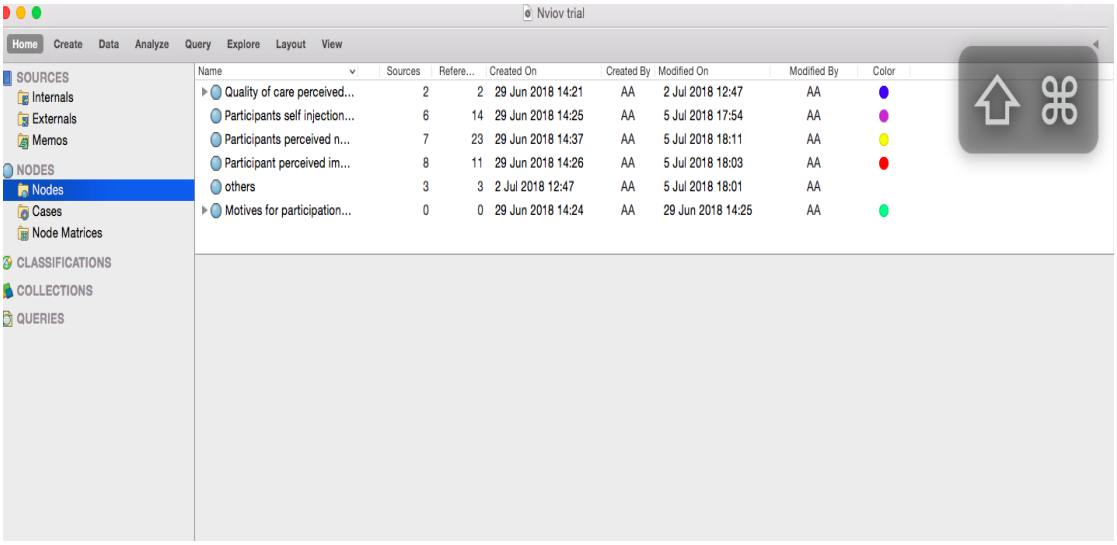


Figure 2-6 themes with high lighted colours using Nvivo™

In phase three of the thematic synthesis, a more in-depth relationship of the themes was looked at. The related themes were combined, whilst others were split into different themes. Additionally, subthemes were extracted from the themes. The aim was to evaluate which themes are relevant and which of the themes overlap with each other Again, Nvivo™ software was used to carry out this task. Coding was applied to the themes and sub coding was applied to the subthemes (Braun and Clarke, 2006).

2.3.2 Statistics

IBM SPSS version 3.0 and GraphPad PRISM 7.0d, were used for statistical analysis. Additionally Nvivo^{TM} software was used for qualitative thematic synthesis.

Cohen's Kappa coefficient (k) is a robust measure of agreement, which takes into account the possibility of agreement occurring by chance. We used k for qualitative items that were categorised, whilst for categorical scale items, the weighted Cohen's Kappa was used (Gwet, 2014).

Bland Altman plots are used to evaluate agreement among two different instruments or two measurements techniques, to allow for an assess any systematic differences between measurement (Giavarina, 2015).

The P value is the probability of rejecting the null hypothesis when it is true. Significance level of 0.05 was used for the P value (Dorey, 2010).

95% confidence interval, we would expect the true population parameter to fall within the interval estimates 95% of the time.

Standard deviation: estimate of the variability of the population from which the sample was drawn about 95% of the individuals will have values with 2 standard deviation of the mean. The standard error of the sample mean depends on both standard deviation and sample size. The formula for standard error is SE=SD/√sample size (Altman and Bland, 2005).

3 Chapter 3 - Results

3.1 Survey of off label usage of Teriparatide among physicians - results

The process of data collection for the survey of the usage of Teriparatide among physicians is summarised in Figure 3.1. A total of 700 questionnaires were circulated, 109 (15.6%) responded, 5 (4.5%) responses were excluded (reasons for exclusion of the 5 responses were: 4 responses were from medical students and one was an impolite response) resulting in 104 (95.4%) returned responses being included.

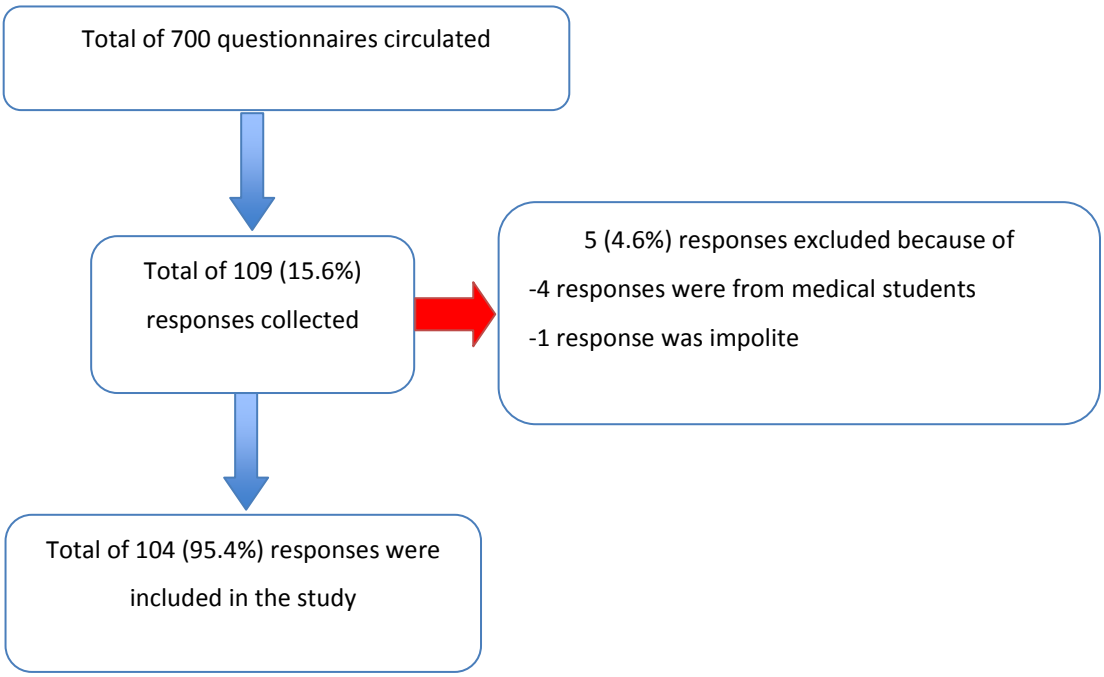


Figure 3-1 Process of data collection of survey of usage of Teriparatide among physicians

Because the response of the physicians may be influenced by their experience, Figure 3-2 describes how long the physicians had been practicing medicine (years). The Mode for years practicing medicine was 10 to 15 years.

Figure 3-2 Number of physicians practicing medicine in years, total of number of physicians included in the survey study n=104.

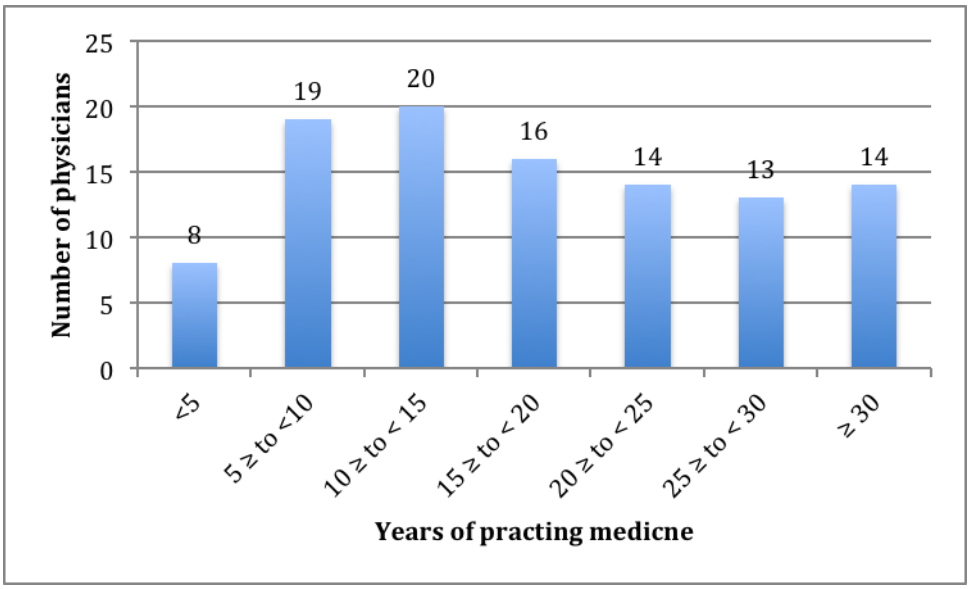


Figure 3-3 describes the country where the physicians are practicing and significantly reflects where the survey was carried out. This survey was carried out in the Kingdom of Bahrain, United Arab Emirates and United Kingdom. In the international conferences, the attendees were more likely to have an international background, such as the United Arab Emirate conference, where the delegates were from Iran, India, Syria, Spain and Turkey. Figure 3-4 reports Teriparatide prescription by countries. This result is obviously biased as the survey sample was taken from those countries.

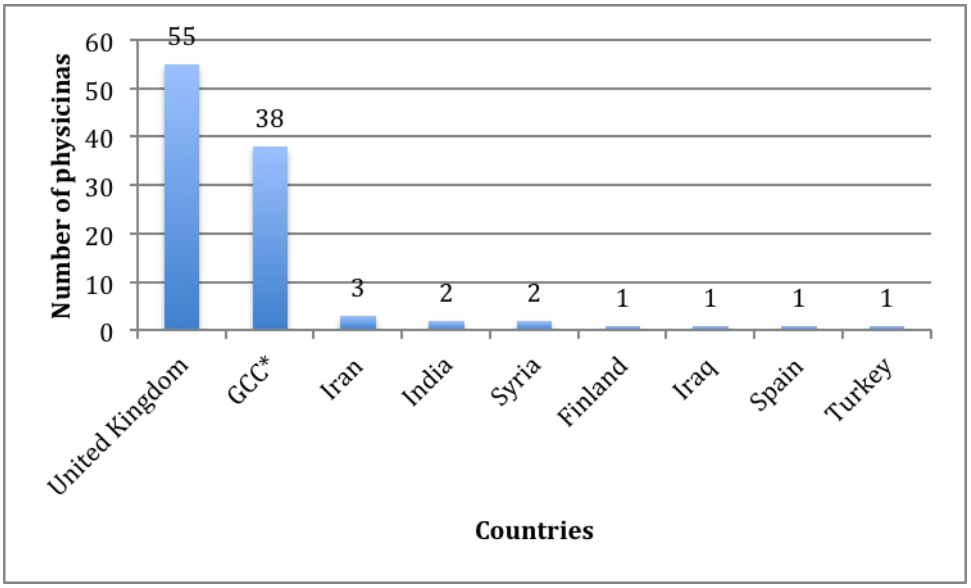


Figure 3-3 Physicians practicing medicine according to country. Total number of physicians included survey study n=104). *GCC countries are Kingdom of Bahrain, Kingdom of Saudi Arabia, State of Kuwait, State of Qatar, State of Emirates and Sultanate of Oman.

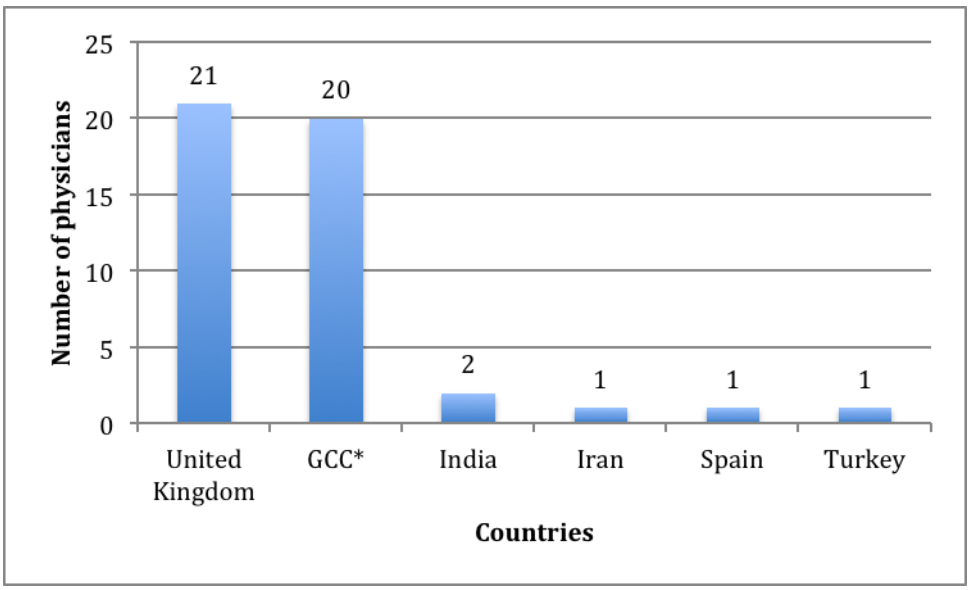


Figure 3-4 Teriparatide prescription by countries in the survey. Number of physicians prescribing Teriparatide n=47). *GCC countries are Kingdom of Bahrain, Kingdom of Saudi Arabia, State of Kuwait, State of Qatar, State of Emirates and Sultanate of Oman.

Figure 3-5 describes the percentage of physicians prescribing Teriparatide in the survey. It was surprising that 45% had prescribed Teriparatide but this probably reflects the type of physicians surveyed. There is an element of respondent bias that can be divided into three forms of respondent bias 1-Inability of the respondent to answer questions correctly (due to respondent fatigue, unfamiliarity, faulty recall and question format etc.) or 2-Unwillingnes of the respondent to answer the question honestly (known as social desirability bias). This is usually seen in sensitive subjects e.g. alcohol consumption, sexual behaviour and monetary income etc. in order to deal with respondent bias an opt-out question can minimise the bias and 3-confirmation bias, which is a cognitive bias that involves favouring information that confirms your previously existing beliefs or biases.

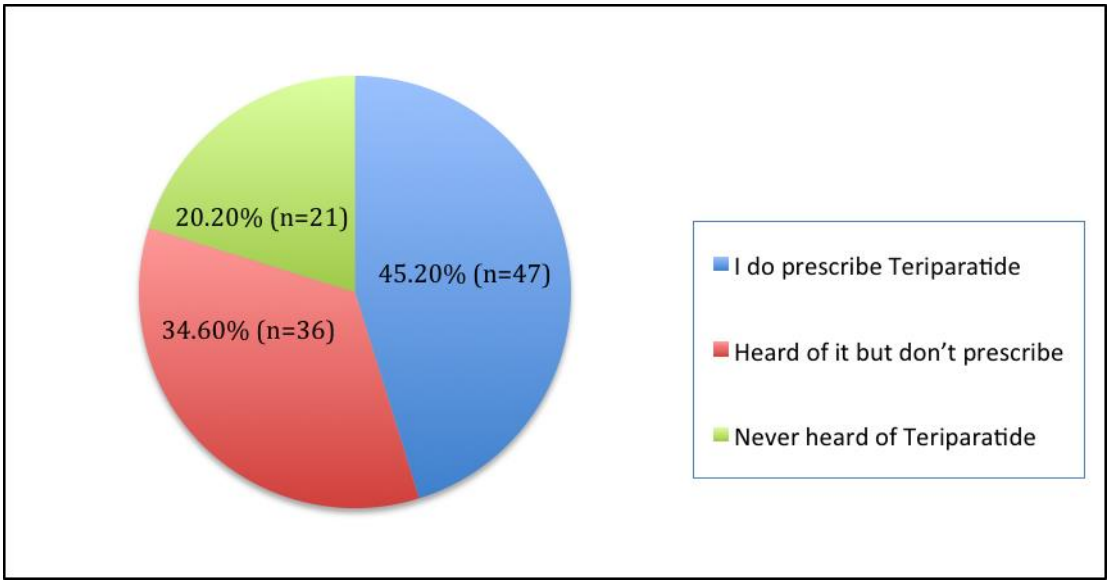


Figure 3-5 Percentage of physicians prescribing Teriparatide in the survey (total number of physicians included in the survey study n=104).

In Figure 3-6 showing indications of Teriparatide usage among physicians. For this question physicians could respond to treating more than one patient group. Although Teriparatide is indicated as second line treatment in osteoporosis we see that it is used as off label for fragility fractures and stress fractures. Again, there could be a respondent bias and more likely of inability of the respondent to answer correctly due to question format.

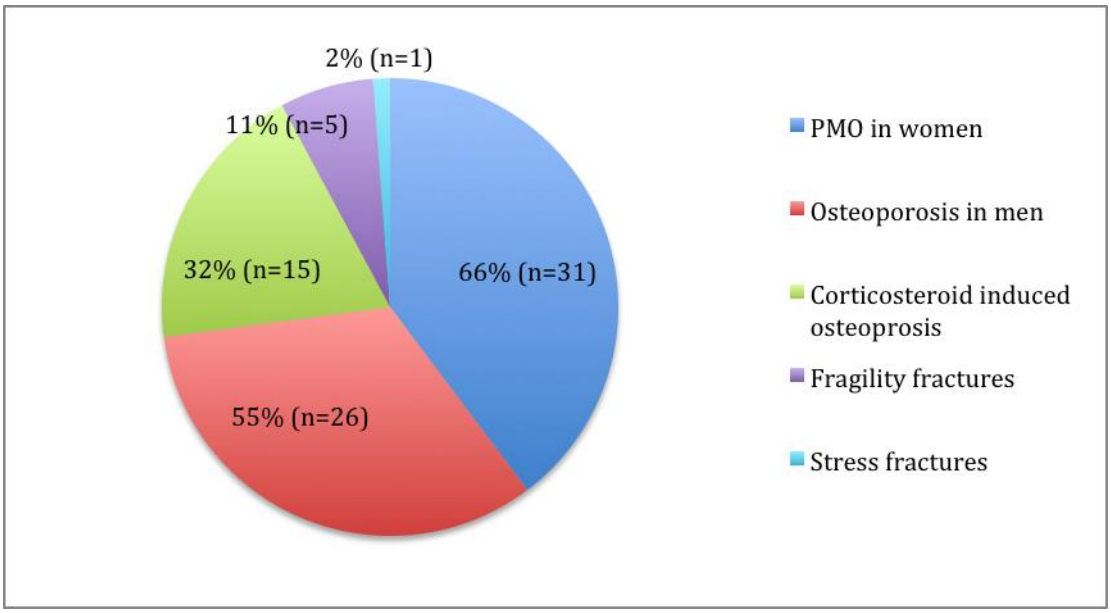


Figure 3-6 Indications of Teriparatide usage among physicians. Number of physicians prescribing Teriparatide n=47.

Figure 3-7 shows the speciality of the physicians prescribing Teriparatide who responded to the survey. This again reflects where the survey was conducted. The intention was to survey the relevant physicians who are most probably use Teriparatide. The conferences that we targeted to carry out the survey were for the relevant to speciality. There was no point of targeting specialities, which are not likely to use Teriparatide in their practice e.g. general surgery or obstetrics.

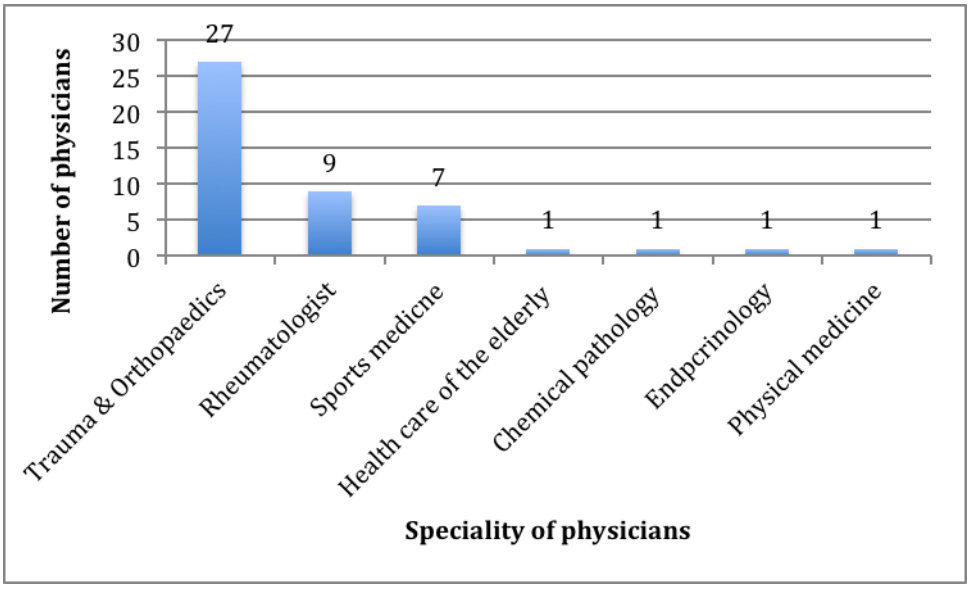


Figure 3-7 Specialty of physicians prescribing Teriparatide (total number of physicians prescribing Teriparatide in the survey n=47).

Figure 3-8 reports the indications for off-label prescription of Teriparatide by physicians for fracture healing. Nine physicians were using it for other reasons, which included hyperphosphatemia, insufficiency fractures and stress fractures. For this question the physicians had more than one choice to answer. The responses for this question, gives an indication that Teriparatide is used as off-label for fracture healing.

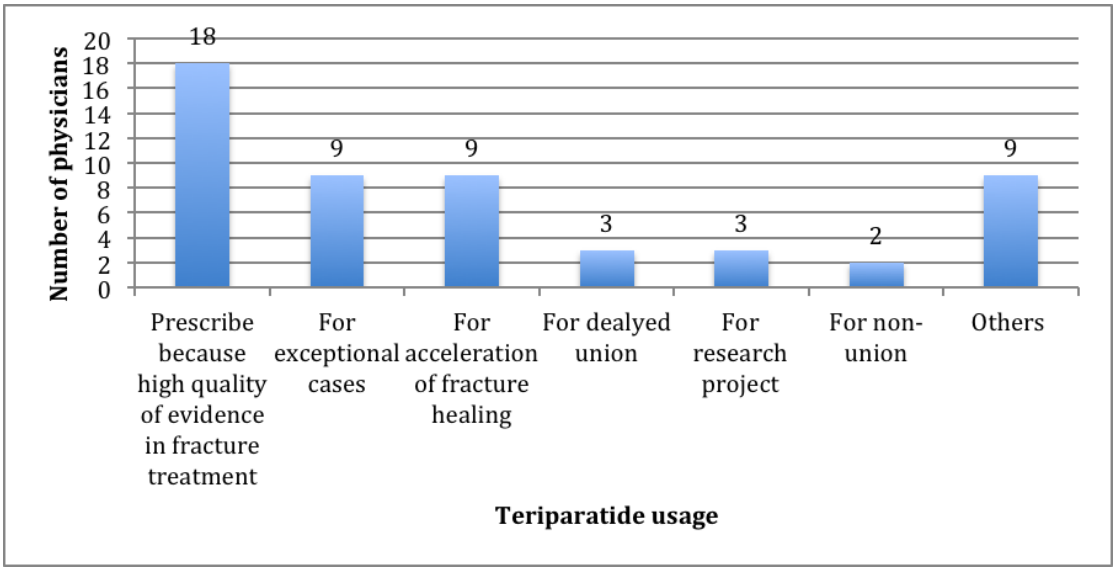


Figure 3-8 Perceived reasons for off label prescription of Teriparatide among physicians for fracture healing. Number of physicians prescribing Teriparatide n=47

Figure 3-9 describes the prescribing of Teriparatide with regard to the age of the patients (Elderly (age ≥ 65 years), Adult (age ≥ 21 years < 65) and Athlete (a person who does sport regularly)). This also indicates that Teriparatide is prescribed off-label, because it is unlikely that athletes and adults are osteoporotic.

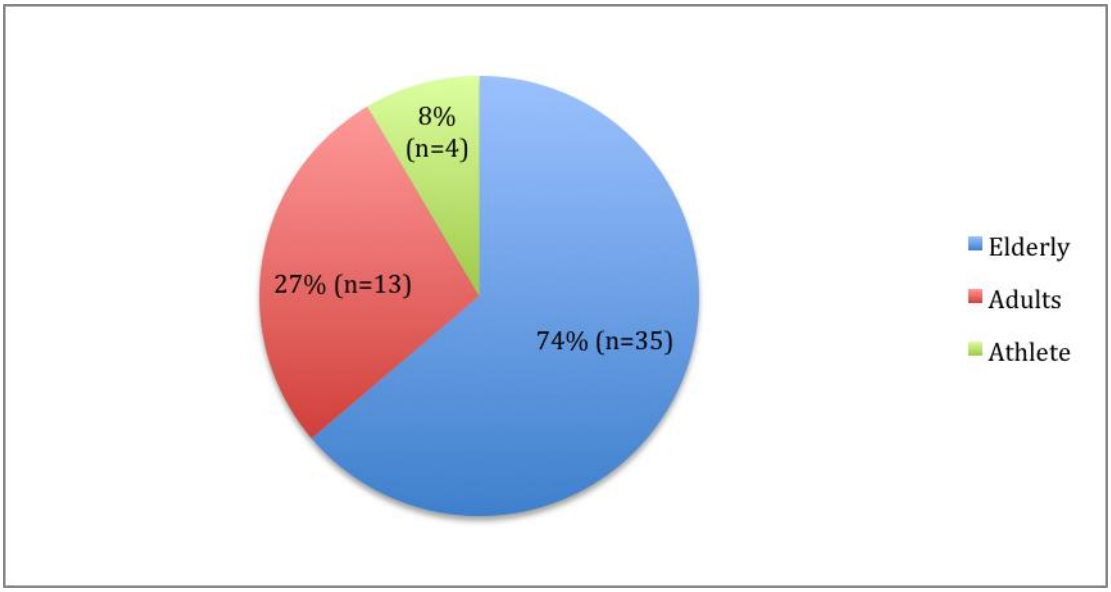


Figure 3-9 Prescription of Teriparatide with regards to the age of patient. Number of physicians prescribing Teriparatide n=47.

Figure 3-10 shows barriers reported by the physicians for not prescribing Teriparatide for acceleration of fracture healing are presented. The cost was the main barrier for prescribing Teriparatide, reported by 30 physicians. Regarding this question, physicians had the choice of answering more than once.

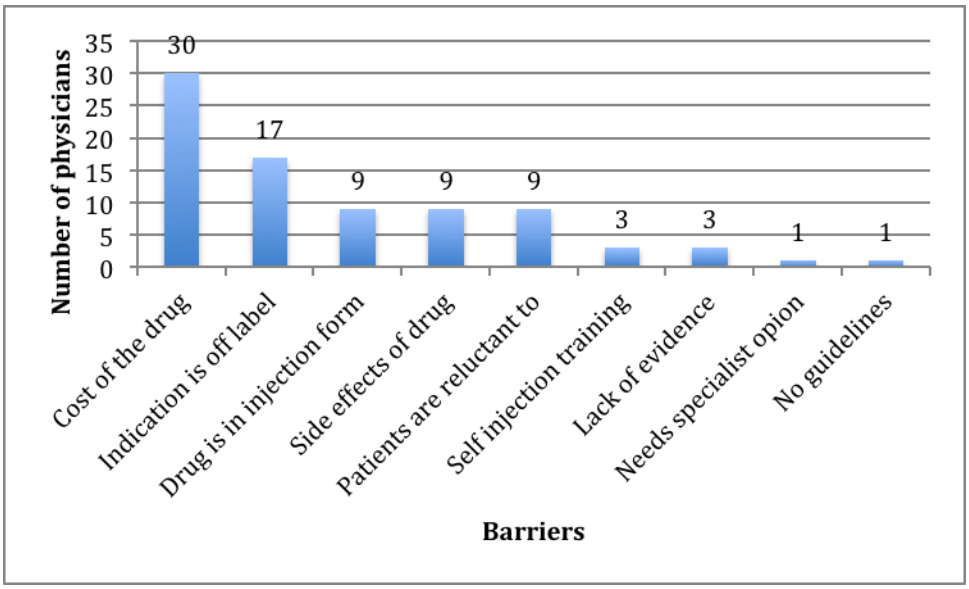


Figure 3-10 Barriers reported by the physicians for prescribing Teriparatide. Number of physicians prescribing Teriparatide n=47

Figure 3-11 shows the speciality of physicians who responded to the survey who had heard of Teriparatide but do not prescribe it. There were 36 physicians out of 104 physicians who responded that they had heard of Teriparatide but do not prescribe it. Most of the responders (29 physicians) were Trauma and orthopaedic surgeons. The survey was carried out at conferences mostly attended by the Trauma and orthopaedic surgeons, which could explain that most of the responses are from Trauma and orthopaedics surgeons.

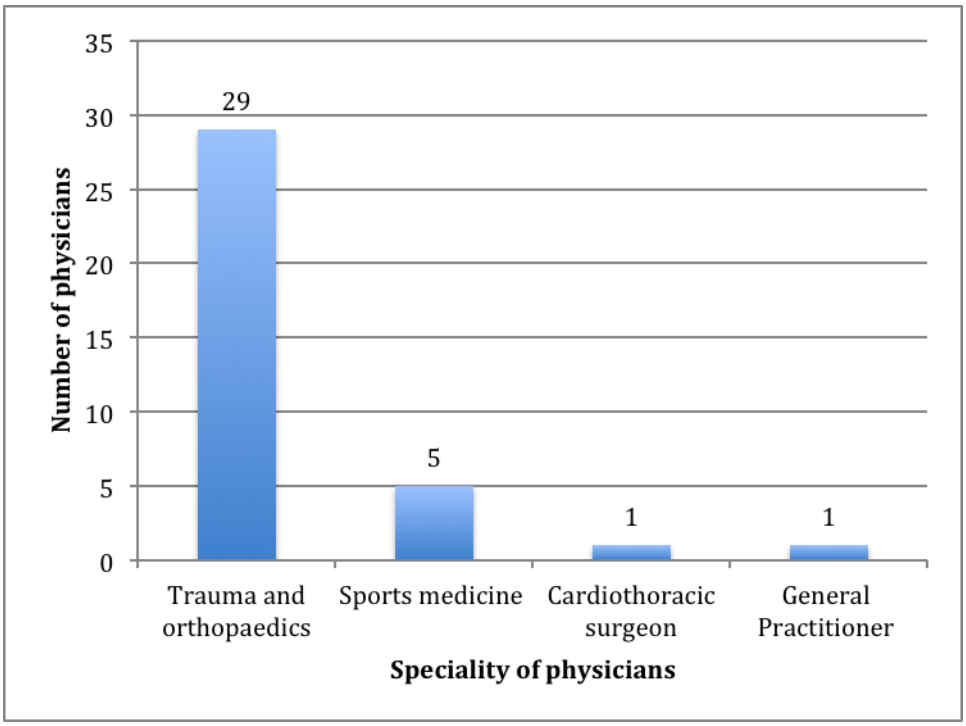


Figure 3-11 Speciality of physicians who had heard of Teriparatide but do not prescribe it (n=36).

Figure 3-12 shows barriers perceived by the physicians who had heard of Teriparatide but do not prescribe it. The cost of the drug was the main barrier were 19 (53%) responses. The cost of the drug was also preceived as a brarrier by the physicians who prescribed Teriparatide. The second barrier with most responses was the side effect of the of the drug 16 (44%) responses. Side effect of the drug was to a lesser extent percievd in the responses by physicians who prescribed Teriparatide. Regarding this question, physicians had the choice of answering more than once.

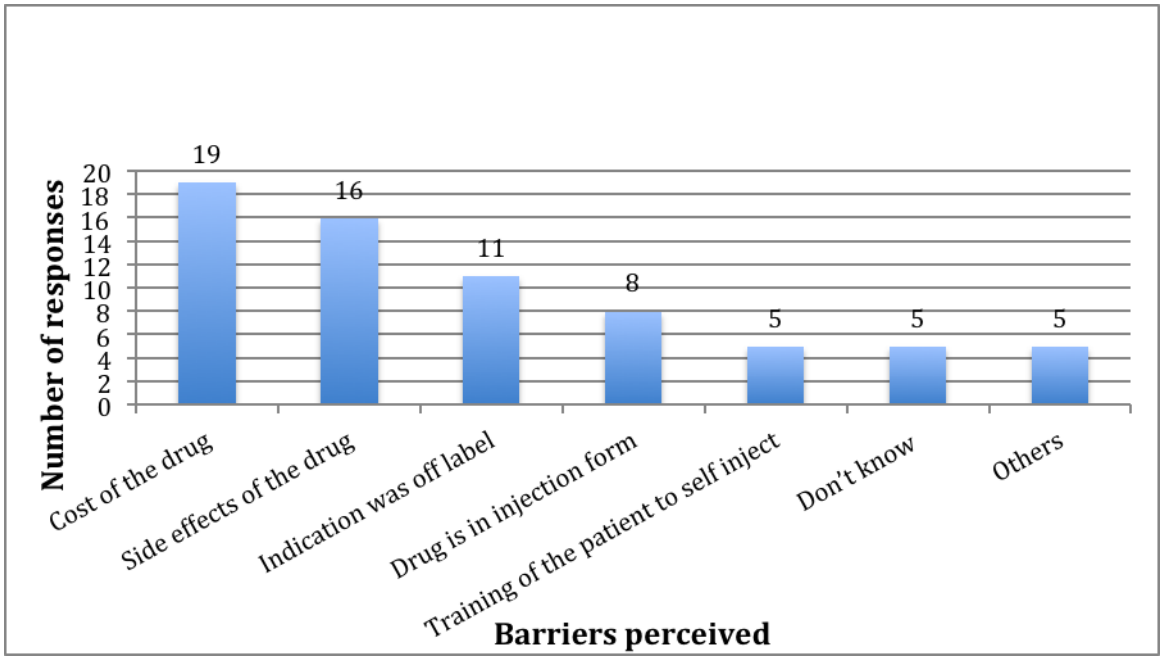


Figure 3-12 Barriers perceived by physicians who have heard of Teriparatide but do not prescribe it. In the others category, there were three responses it is prescribed by endocrinologist and two responses it is prescribed by general practitioner.

3.2 Feasibility study of ankle fractures treated with Teriparatide

Figure 3-13 show the flow of patients in the study from the point of screening in the clinic until the end of the study. We screened a total of 81 patients from 11th of November 2016 to 22nd of December 2017. Reasons for exclusion are mentioned in Figure 3-13.

From the 81 potential eligible patients, 19 (23%) of them said they did not want to participate in the study, this indicated that 62 (77%) of the patents were willing to participate in the study. Unfortunately for those patients who refused to participate in the study, we could not aske them their reason for not participating in the study nor could we assess their comorbidities once they said no to participation in study because for ethical reasons. Almost all the clinicians showed willingness to recruit patients into the trial once information was sent to them about the trial and it was confirmed that there was a formal approval from the health regulatory agency to conduct the trial in the hospital.

In our feasibility study we did not have any dropouts from the study. Patients adherence and compliance with taking the drug was 100%, and also compliance to the drug was 100% according to the self-injecting log no patient missed any of the injections. We did not had any issues with the participants randomisation in our study.

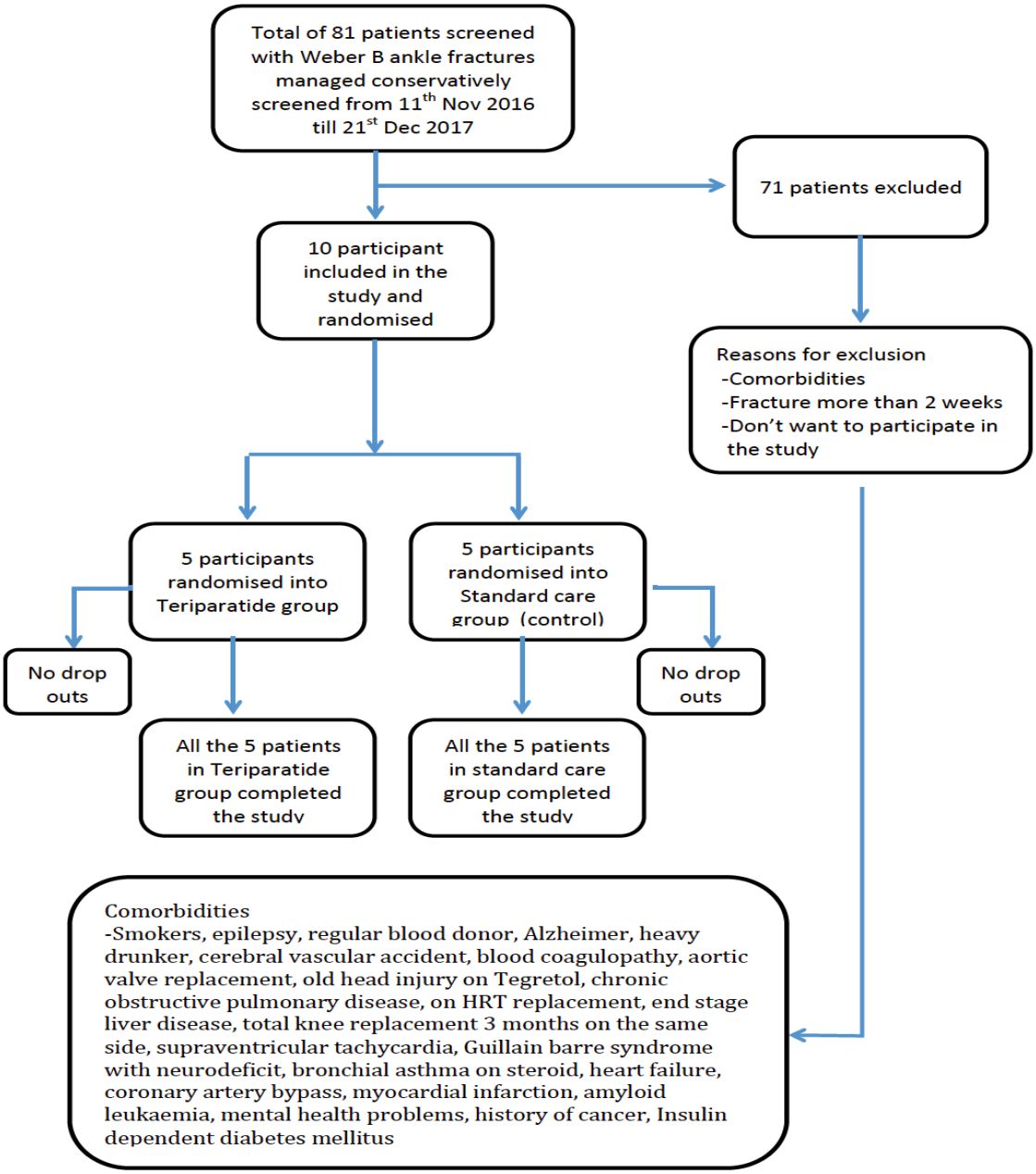


Figure 3-13 Flow chart of patient recruitment in the ankle fracture feasibility study

3.2.1 Demographics of study participants

In Table 3-1, demographic data are presented. The mean age of the participants recruited in the standard care group and Teriparatide group was 65 ±13 years and 64 ±5 years respectively. The majority of participants were female (n=8). In the Teriparatide group all 5 participants were females. All the participants completed their follow up appointments within ±3 days. The table also highlights the dates of each patient's attendance for the study. There were no dropouts or missing appointments in the follow up. This is an achievement and reflects the forward planning and commitment of all those who contributed to this study.

Table 3-1 Participants treatment groups, sex, age, side of the ankle fracture and their dates of clinical trial visits of standard care group (A) and Teriparatide group (B)

Demographic results **A=Standard care, B=Teriparatide group**										
Treatment group	**Sex**	**Age in years**	**Ankle**	**TV1**	**TV2**	**TV3**	**TV4**	**TV5**	**TV6**	**TV7**
B	F	61	RT ankle	16/12/2016	30/12/2016	13/01/2017	27/01/2017	10/02/2017	24/02/2017	10/03/2017
A	M	80	Lt ankle	16/12/2016	30/12/2016	13/01/2017	27/01/2017	10/02/2017	24/02/2017	10/03/2017
B	F	57	Lt ankle	03/02/2017	17/02/2017	3/03/2017	16/03/2017	03/04/2017	13/04/2017	28/04/2017
A	M	54	Lt ankle	17/03/2017	31/03/2017	13/04/2017	28/04/2017	12/05/2017	26/05/2017	09/06/2017
A	F	78	Lt ankle	27/03/2017	07/04/2017	21/04/2017	05/05/2017	19/05/2017	02/06/2017	16/06/2017
B	F	68	Lt ankle	22/09/2017	01/09/2017	15/09/2017	29/09/2017	13/10/2017	27/10/2017	17/11/2107
B	F	69	RT ankle	10/11/2017	23/11/2017	08/12/2017	22/12/2017	05/01/2018	19/01/2018	02/02/2018
A	F	61	RT ankle	17/11/2017	01/12/2017	15/12/2017	29/12/2017	12/01/2018	26/01/2018	09/02/2018
B	F	65	RT ankle	18/12/2017	29/12/2018	12/01/2018	26/01/2018	09/02/2018	23/02/2018	12/03/2018
A	M	52	Lt ankle	22/12/2017	05/01/2018	19/01/2018	02/02/2018	16/02/2018	06/03/2018	16/03/2018
Standard care group=A Mean age 65 years old 40% female, 60% male 20% Rt ankle, 80% Lt ankle						Teriparatide group =B Mean age 64 years old 100% female, 0% male 40% Rt ankle, 60% Lt ankle				

In Table 3-2 baseline blood test results of study participants, some of the participants had abnormal blood test results but these test results were clinically not sufficiently abnormal to exclude them from the study.

Some variations in laboratory test results are expected in patients because of age, biological rhythms of the hormones, physiological changes, individual variations that could be due to nutritional status, ethnicity or gender. Timing of the tests can have effect on the tests results this can be du to the stage of illness, also if the patient is on certain medications and to analytical variations due to collection, storage and transport of the blood samples (Kyle, 2015).

Consultation with Professor Opinder Sahota (Professor of Orthogeriatric Medicine & Consultant Physician) was made for any out of the range blood tests results and his approval was taken before enrolment of any patients into the study.

Table 3-2 Baseline blood test results of study participants

Treatment group	A	A	A	A	A		B	B	B	B	B
I.D	1362	1047	1180	1375	1034		1448	1230	1226	1164	1276
Blood test (reference range values)											
Full blood count (FBC)											
Haemoglobin (115-165 g/L)	138	136	116	144	148		137	136	130	134	150
White cell count (4.00-11.00 x10^g/L)	7.40	9.60	*11.50	9.00	6.40		9.90	7.70	5.90	7.30	8.50
Platelet count (150.00-450.00 x10^g/L)	219.00	286.00	268.00	251.00	164.00		301.00	345.00	199.00	256.00	242.00
Red cell count (3.80-5.80 x10^12g/L)	4.40	4.59	3.85	4.35	4.98		4.49	4.92	4.41	4.72	4.65
Haematocrit (0.370-0.470 L/L)	0.394	0.430	0.354	0.421	0.460		0.391	0.44	0.411	0.423	0.446
MCV (84.00-102.00 fL)	90.00	94.00	92.00	97.00	92.00		87.00	89.00	93.00	90.00	96.00
MCH (28.00-33.00 pg)	31.40	29.60	30.10	*33.10	29.70		30.50	27.60	29.50	28.40	32.30
MCHC (300-350 g/L)	350	316	328	342	322		350	310	316	317	336
White cell differential											
Neutrophils (2.0-7.5 x10^g/L)	4.4	6.5	*8.1	6.4	4.5		6.1	4.7	4.0	5.3	5.5
Lymphocytes (1.00-4.00 x10^g/L)	1.60	1.80	2.50	1.70	1.20		2.60	2.20	1.50	1.30	2.00
Monocytes (0.10-1.50 x10^g/L)	0.80	0.60	0.90	0.50	0.50		0.90	0.50	0.40	0.60	0.90
Eosinophils (0.04.40 x10^g/L)	0.40	0.70	0.00	0.40	0.20		0.10	0.30	0.10	0.10	0.20

Basophils (0.01-0.15 x10^g/L)	0.10	0.10	0.00	0.10	0.10		0.00	0.10	0.00	0.00	0.10
Erythrocyte sedimentation rate (ESR) (1-20 mm/1Hr)	17	*37	*32	*35	15		6	*47	*26	*26	2
Liver function tests (LFT)											
ALT (0.00-35.00 U/L)	18.00	23.00	19.00	31.00	31.00		20.00	28.00	12.00	23.00	19.00
AST (0.00-30.00 U/L)	18.00	25.00	*34.00	29.0	30.00		22.00	*129.00	*42.00	26.00	26.00
Total bilirubin (Up to 21 umol/L)	19	19	9	10	17		9	9	10	10	20
Bone profile											
Total alkaline phosphate (40-130 U/L)	63	89	77	129	88		78	190	70	106	60
Adjusted calcium (2.20-2.60 mmol/L)	2.30	2.39	2.32	2.39	2.31		2.27	2.34	2.27	2.27	2.32
Albumin (35-52 g/L)	44	40	42	41	44		45	40	45	46	45
Phosphate (0.80-1.45 mmol/L)	*0.75	1.09	0.96	*0.67	0.89		1.14	1.30	1.22	0.93	1.20
Total protein (60-80 g/L)	76	70	74	74	74		71	72	72	79	68
Renal function											
Sodium (134-145 mmol/L)	138	140	142	140	141		140	142	140	140	139
Potassium (3.5-5.3 mmol/L)	4.1	4.6	4.0	4.5	4.5		4.2	4.6	4.4	4.4	4.4
Urea (2.9-7.0 mmol/L)	4.6	5.8	6.1	3.4	4.6		4.5	4.0	6.0	7.7	6.6
Creatinine (45-84 umol/L)	*98	*87	75	55	69		62	67	65	57	72
eGFR by CKDEPI/1.73m2 (>60-200 mL/min)	88	87	66	90	90		90	88	84	90	76
PTH (18-80 ng/L)	54	68	67	67	82		60	52	66	79	*88
Thyroid function test TSH (0.30-5.50 mU/L)	*7.60	3.50	0.60	3.00	1.50		3.50	1.10	3.60	0.98	0.88

* Abnormal but clinically not significant
MCV=Mean Corpuscular Volume. MCH=Mean Corpuscular haemoglobin. MCHC=Mean Corpuscular Haemoglobin Concentration
ALT=Alanine Aminotransferase, AST=Aspartate Aminotransferase.
g/L= gram per Litre. L/L=litre of cells per litre of blood. fL=femtolitre (US femtoliter) is the metric unit of volume equal to 10^{-15}. pg=picogram

Table 3-3 show the past medical, surgical and medication history. Although some of the participants had some conditions but they were not reasons to exclude them. Standard care group appear to have more comorbidities, clearly this is a problem of small numbers in the study.

Table 3-3 Past medical, surgical and medication history of participants in standard care group (A) and Teriparatide group (B)

Past medical and surgical history of participants **Standard care=A, Teriparatide group=B**				
I.D.	**Treatment group**	**Medical history**	**Surgical history**	**Medications**
1362	A	-Hypertension, Transient ischemic attack, Labyrinthitis	Non	Amlodipine 10 mg, Simvastatin 20mg, Clopidogrel 25 mg
1047	A	Allergic to hey and dust mite	Nasal septal deviation correction, Circumcision	Cetirizine 10 mg OD, Prednisolone Inhaler, 200 mg, Beconase PRN, Bricanyl 2 puffs BD
1180	A	High cholesterol	Spinal fusion, Rt shoulder scopy RT carpal tunnel release, Eye surgery	Furosemide 10 mg, Statin 10 mg
1375	A	Non	Non	Non
1034	A	Mild depression	Leg ulcer excised 2015	Fluoxetine 20 mg
1448	B	Rheumatic fever	Tonsillectomy, Caesarean section, Varicose veins, Appendectomy	Non
1230	B	Non	Cholecystectomy	Non
1226	B	Non	Tubal ligation	Calcium supplement
1164	B	Hypertension controlled, High cholesterol controlled	Hysterectomy, Oophorectomy	Simvastatin 40 mg, Lisiropril 20 mg
1276	B	Non	Caesarean section twice	Non

3.3 Ankle fracture healing parameters on CT scans

3.3.1 Callus/woven bone present at cortical margins externally

Table 3-4 shows the agreement between two raters (AA and WA) for callus/woven bone anteriorly. At cortical margins externally on the anterior side of the fracture there was agreement of 77.6%. Cohen's Kappa was run to determine if there was an agreement between the two rates beyond chance. There was a moderate agreement between the two raters Kappa=0.55 (95% CI 0.51 to 0.38), P<0.001.

Table 3-4 Cross tabulation of agreement between rater AA and WAW of presence of callus/woven bone anteriorly at cortical margin externally

Callus/woven bone anteriorly Not present=0, Present=1							
			WAW ant callus		Total	Agreement %	Kappa value (95% CI) P
			0	1			
AA ant callus	.0	Count	558	250	808	77.6%	0.55 (0.51-0.58) P<0.001
		% of Total	31.9%	14.3%	46.2%		
	1.0	Count	142	798	940		
		% of Total	8.1%	45.7%	53.8%		
Total		Count	700	1048	1748		
		% of Total	40.0%	60.0%	100.0%		

In Table 3-5 Callus/woven bone posteriorly, at cortical margins externally in the posterior side of the fracture there was agreement of 74.9%. Cohen's Kappa was run to determine if there was an agreement between the two raters beyond chance. There was a moderate agreement between the two raters Kappa=0.46 (95% CI 0.41 to 0.50), P<0.001

Table 3-5 Cross tabulation of agreement between rater AA and WAW of presence of callus/woven bone posteriorly at cortical margin externally

Callus/woven bone posteriorly Not present=0, present=1							
			WA post callus		Total	Agreement%	Kappa value (95% CI) P value
			0	1			
AA post callus	.0	Count	388	323	711	74.9%	0.46 (0.412-0.496) P<0.001
		% of Total	22.2%	18.5%	40.7%		
	1.0	Count	115	920	1035		
		% of Total	6.6%	52.7%	59.3%		
Total		Count	503	1243	1746		
		% of Total	28.8%	71.2%	100.0%		

3.3.2 Callus/woven bone present within fracture line (trabecular bone region)

In Table 3-6 callus/woven bone present within the fracture line (trabecular region), there was an agreement of 35.7%. Cohen's Kappa was run to determine if there was an agreement between the two raters beyond chance. There was a poor agreement between the two raters Kappa=0.13 (95% CI 0.11 to 0.0.16), P<0.001.

Table 3-6 Cross tabulation of agreement between rater AA and WAW of presence of callus/woven bone within fracture line (trabecular bone region)

Callus/woven bone within fracture line
No callus/woven bone present=1
Callus/woven bone present 0%>≤25%=2
Callus/woven bone present 25%>≤50%=3
Callus/woven bone present 50%>≤75%=4
Callus/woven bone present 75% >≤100%=5

			WAW callus within						Agreement%	W. Kappa value (95% CI) P value
			1	2	3	4	5	Total		
AA callus within	1	Count	21	18	21	20	7	87	35.7%	0.13 (0.11-0.16) P<0.001
		% of Total	1.2%	1.0%	1.2%	1.1%	0.4%	5.0%		
	2	Count	29	50	52	48	21	200		
		% of Total	1.7%	2.9%	3.0%	2.7%	1.2%	11.4%		
	3	Count	12	40	58	70	34	214		
		% of Total	0.7%	2.3%	3.3%	4.0%	1.9%	12.2%		
	4	Count	12	41	107	106	72	338		
		% of Total	0.7%	2.3%	6.1%	6.1%	4.1%	19.3%		
	5	Count	28	49	128	315	388	908		
		% of Total	1.6%	2.8%	7.3%	18.0%	22.2%	52.0%		
Total		Count	102	198	366	559	522	1747		
		% of Total	5.8%	11.3%	21.0%	32.0%	29.9%	100.0%		

3.3.3 Cortical bone bridging

In Table 3-7 cortical bone bridging anteriorly, there was an agreement of 68.3%. Cohen's Kappa was run to determine if there was an agreement between the two raters beyond chance. There was a moderate agreement between the two raters Kappa=0.45 (95% CI 0.42 to 0.49), P<0.001

Table 3-7 cross tabulation of agreement between raters AA and WAW of cortical bone bridging anteriorly

Cortical bone bridging anteriorly Cortical (Callus/woven bone) bridging not present=0 Partial cortical bridging (Callus/woven bone)=1 Complete cortical bridging=2							
		WAW ant cortical			Total	Agreement%	Kappa value (95% CI) P value
		0	1	2			
AA ant cortical	0 Count	816	43	7	866	68.3%	0.45 (0.42-0.49) P<0.001
	% of Total	46.7%	2.5%	0.4%	49.6%		
	1 Count	313	250	122	685		
	% of Total	17.9%	14.3%	7.0%	39.2%		
	2 Count	15	54	127	196		
	% of Total	0.9%	3.1%	7.3%	11.2%		
Total	Count	1144	347	256	1747		
	% of Total	65.5%	19.9%	14.7%	100.0%		

In Table 3-8 cortical bone bridging posteriorly, there was an agreement of 61.2%. Cohen's Kappa was run to determine if there was an agreement between the two rates beyond chance. There was a moderate agreement between the two raters Kappa=0.41 (95% CI 0.37 to 0.44), P<0.001

Table 3-8 cross tabulation of agreement between raters AA and WAW of cortical bone bridging posteriorly

Cortical bone bridging posteriorly Cortical (Callus/woven bone) bridging not present=0 Partial cortical bridging (Callus/woven bone)=1 Complete cortical bridging=2							
		WAW post cortical			Total	Agreement%	Kappa value (95% CI) P value
		0	1	2			
AA post cortical	0 Count	494	43	5	542	61.2%	0.41 (0.37-0.44) P<0.001
	% of Total	28.3%	2.5%	0.3%	31.0%		
	1 Count	307	397	173	877		
	% of Total	17.6%	22.7%	9.9%	50.2%		
	2 Count	42	107	179	328		
	% of Total	2.4%	6.1%	10.2%	18.8%		
Total	Count	843	547	357	1747		
	% of Total	48.3%	31.3%	20.4%	100.0%		

3.3.4 Trabecular bone bridging

In Table 3-9 trabecular bone bridging, there was an agreement of 34.3%. Cohen's weighted Kappa was run to determine if there was an agreement between the two rates beyond chance. There was a poor agreement between the two raters Kappa=0.18 (95% CI 0.15 to 0.20), P<0.001

Table 3-9 Cross tabulation of agreement between raters AA and WAW of trabecular bone bridging

Trabecular bone bridging
No trabecular bridging=1
Trabecular bridging 0%>≤25%=2
Trabecular bridging 25%>≤50%=3
Trabecular bridging 50%>≤75%=4
Trabecular bridging 75%>≤100%=5

			WA trabecular bone					Total	Agreement%	W Kappa (95%CI) P value
			1	2	3	4	5			
AA trabecular bone	1	Count	128	139	69	47	17	400	34.3%	0.18 (0.15-0.20) P<0.001
		% of Total	7.3%	8.0%	3.9%	2.7%	1.0%	22.9%		
	2	Count	46	178	182	99	27	532		
		% of Total	2.6%	10.2%	10.4%	5.7%	1.5%	30.5%		
	3	Count	16	74	105	67	33	295		
		% of Total	0.9%	4.2%	6.0%	3.8%	1.9%	16.9%		
	4	Count	12	49	86	67	67	281		
		% of Total	0.7%	2.8%	4.9%	3.8%	3.8%	16.1%		
	5	Count	5	8	44	59	123	239		
		% of Total	0.3%	0.5%	2.5%	3.4%	7.0%	13.7%		
Total		Count	207	448	486	339	267	1747		
		% of Total	11.8%	25.6%	27.8%	19.4%	15.3%	100.0%		

3.3.5 Fracture line (Trabecular bone) margins

In Table 3-10 Fracture line (trabecular bone) margins, there was agreement of 47.5%. Cohen's Kappa was run to determine if there was an agreement between the two rates beyond chance. There was a fair agreement between the two raters Kappa=0.28 (95% CI 0.25 to 0.31), P<0.001.

Table 3-10 cross tabulation of agreement between raters AA and WAW of fracture line (trabecular bone) margins

Fracture line (trabecular bone) margins
Sharp fracture line margins=0
Not sharp fracture line margins (resorption)=1
Sclerotic fracture line margins=2
Bridged fracture line margins=3

			WAW fracture margin				Total	Agreement%	Kappa value (95% CI) P value
			0	1	2	3			
AA fracture margin	0	Count	10	0	0	0	10	47.5%	0.28 (0.25-0.31) P<0.001
		% of Total	0.6%	0.0%	0.0%	0.0%	0.6%		
	1	Count	187	288	83	70	628		
		% of Total	10.7%	16.5%	4.8%	4.0%	35.9%		
	2	Count	43	255	293	221	812		
		% of Total	2.5%	14.6%	16.8%	12.7%	46.5%		
	3	Count	21	32	6	238	297		
		% of Total	1.2%	1.8%	0.3%	13.6%	17.0%		
Total		Count	261	575	382	529	1747		
		% of Total	14.9%	32.9%	21.9%	30.3%	100.0%		

3.3.6 Fracture gap (Cortical bone)

In Table 3-11Fracture gap (cortical bone) anteriorly, there was agreement of 69.5%. Cohen's Kappa was run to determine if there was an agreement between the two rates beyond chance. There was a moderate agreement between the two raters Kappa=0.45 (95% CI 0.41 to 0.48), P<0.001.

Table 3-11 Cross tabulation of agreement between raters AA and WAW of fracture gap (Cortical bone) anteriorly

Fracture gap (cortical bone) anteriorly
Fracture gap present=1
Fracture gap partially healed=2
Fracture gap bot present=3

			WAW ant gap					Agreement%	Kappa value (95% CI) P value
			0	1	2	3	Total		
AA ant gap	1	Count	9	889	66	9	973	69.5%	0.45 (0.41-0.48) P<0.001
		% of Total	0.5%	50.9%	3.8%	0.5%	55.7%		
	2	Count	4	270	206	120	600		
		% of Total	0.2%	15.5%	11.8%	6.9%	34.3%		
	3	Count	1	17	37	119	174		
		% of Total	0.1%	1.0%	2.1%	6.8%	10.0%		
Total		Count	14	1176	309	248	1747		
		% of Total	0.8%	67.3%	17.7%	14.2%	100.0%		

In Table 3-12 Fracture gap (cortical bone) posteriorly, there was an agreement of 0.44%. Cohen's Kappa was run to determine if there was an agreement between the two rates beyond chance. There was a moderate agreement between the two raters Kappa=0.44 (95% CI 0.40 to 0.47), P<0.001.

Table 3-12 Cross tabulation of agreement between raters AA and WAW of fracture gap (Cortical bone) posteriorly

Fracture gap (cortical bone) posteriorly Fracture gap present=1 Fracture gap partially healed=2 Fracture gap not present=3								
		WAW post gap				Total	Agreement%	Kappa value (95% CI) P value
		0	1	2	3			
AA post gap	1 Count	0	595	64	8	667	64.3%	0.44 (0.40-0.47) P<0.001
	% of Total	0.0%	34.1%	3.7%	0.5%	38.2%		
	2 Count	2	293	361	136	792		
	% of Total	0.1%	16.8%	20.7%	7.8%	45.4%		
	3 Count	0	39	82	166	287		
	% of Total	0.0%	2.2%	4.7%	9.5%	16.4%		
Total	Count	2	927	507	310	1746		
	% of Total	0.1%	53.1%	29.0%	17.8%	100.0%		

3.3.7 Total fracture healing score

Total fracture healing score is the sum of all the parameters scored on the revised ankle CT scan fracture score table. In the revised ankle fracture healing score table the minimum score is 4 and maximum score is 25.

In Figure 3-14 Bland Altman plot representing the difference between the raters. The rater agreement is bounded by the 95% CI. (±2 standard deviations). The difference between the rater exhibited cone and funnel effects (low and high scores). The difference between the rater exhibited cone and funnel effects (low and high scores) this reflects that the differences for the total fracture healing score between the raters AA and WAW are small in the beginning and later in the assessment of fracture healing.

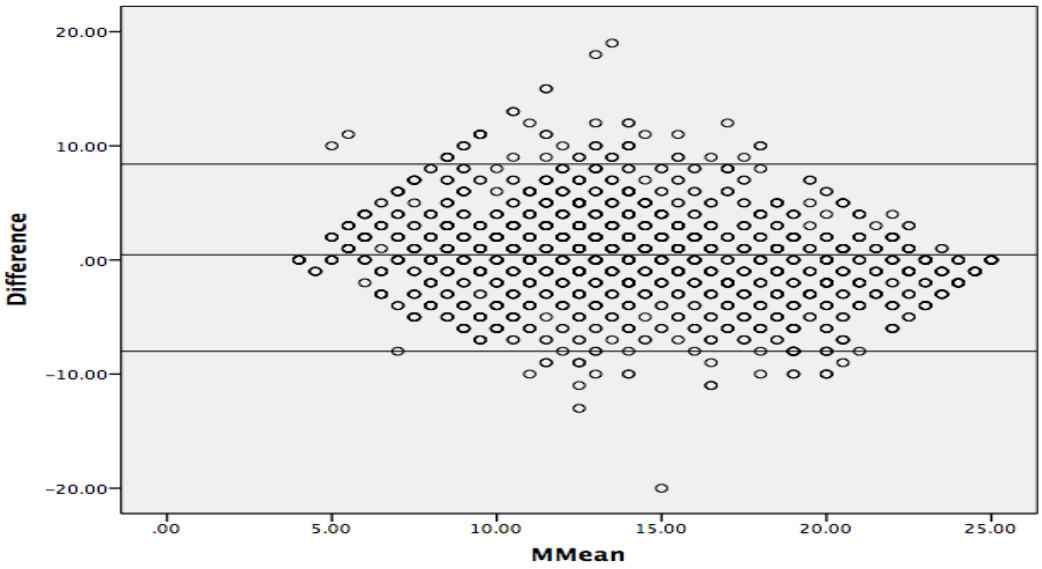


Figure 3-14 Bland Altman plot showing the agreement between the two raters

In Table 3-13 Reliability statistics of internal consistency of ankle fracture score table for rater AA, for 9 items that are used to assess fracture healing total score, Cronbach's Alpha is 0.804 and Cronbach's Alpha based on standardised items is 0.817 showing that all the fracture healing parameters are consistently measuring fracture healing.

Table 3-13 Reliability statistics of internal consistency measurement of ankle fracture healing score table for rater AA

Reliability Statistics of internal consistency measurement of fracture healing parameters of ankle fracture healing score table for rater AA		
Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.804	.817	9

Table 3-14 Fracture healing parameters for internal consistency measurement, all the parameters Cronbach's Alpha ranges between 0.70 and 0.95 even if each parameter is deleted one by one, it still does not go out of the range (Tavakol and Dennick, 2011).

Table 3-14 Internal consistency of fracture healing parameters of ankle fracture healing score table for rater AA

Fracture healing parameters internal consistency measurement in ankle fracture score table					
	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation	Cronbach's Alpha if Item Deleted
AA ant callus (Rater AA callus/woven bone anteriorly at cortical margin externally)	13.898	20.458	.278	.250	.807
AA post callus (Rater AA callus/woven bone posteriorly at cortical margin externally)	13.842	20.666	.236	.181	.810
AA callus within (Rater AA callus/woven bone within fracture line (trabecular bone region))	10.417	15.003	.562	.447	.784
AA ant cortical (Rater AA cortical bridging anteriorly)	13.819	18.022	.603	.815	.775
AA post cortical (Rater AA cortical bridging posteriorly)	13.558	17.817	.624	.786	.772
AA trabecular bone (Rater AA trabecular bone bridging)	11.764	13.626	.655	.565	.771
AA fracture margin (Rater AA fracture line (trabecular bone) margins)	12.636	18.485	.482	.303	.788
AA ant gap (Rater AA fracture gap (Cortical bone) anteriorly)	12.894	18.026	.614	.811	.774
AA post gap (Rater AA fracture gap (Cortical bone) posteriorly)	12.653	17.891	.598	.782	.775

In Table 3-15, reliability statistics of internal consistency of ankle fracture score table for rater WAW, for 9 items that are used to assess fracture healing, total Cronbach's Alpha is 0.874 and Cronbach's Alpha based on standardised items is 0.868 showing that all the fracture healing parameters are consistently measuring fracture healing. Additionally in Table 3-16, fracture healing parameters for internal consistency measurement of all the parameters, Cronbach's Alpha ranges between 0.70 and 0.95 even if each parameter is deleted one by one still it does not go out of range (Tavakol and Dennick, 2011).

Table 3-15 Reliability statistics of internal consistency of ankle fracture healing score table for rater WAW

Reliability Statistics of internal consistency of fracture healing parameters of ankle fracture healing score table for rater WAW		
Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.874	.868	9

Table 3-16 Internal consistency of fracture healing parameters of ankle fracture healing score table for rater WAW

Fracture healing parameters internal consistency in ankle fracture score table					
	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation	Cronbach's Alpha if Item Deleted
WAW ant callus (Rater WAW callus/woven bone anteriorly at cortical margin externally)	13.38	28.802	.272	.109	.883
WAW post callus (Rater WAW callus/woven bone posteriorly at cortical margin externally)	13.27	28.949	.271	.127	.883
WAW callus within ((Rater WAW callus/woven bone within fracture line (trabecular bone region))	10.30	21.520	.691	.728	.856
WAW ant cortical (Rater WAW cortical bone bridging anteriorly)	13.49	24.991	.669	.829	.857
WAW post cortical (Rater WAW cortical bone bridging anteriorly	13.26	24.552	.686	.752	.855
WAW trabecular bone (Rater WAW trabecular bone bridging)	10.98	20.047	.800	.806	.843
WAW fracture margin (Rater WAW fracture line (trabecular bone) margins)	12.31	21.601	.785	.643	.843
WAW ant gap (Rater WA fracture gap (Cortical bone) anteriorly)	12.53	25.038	.659	.829	.858
WAW post gap (Rater WAW fracture gap (Cortical bone) posteriorly)	12.34	24.609	.695	.754	.855

In the fracture healing scoring table parameters for internal consistency measurements, for all the parameters, Cronbach's Alpha ranges between 0.70 and 0.95 even if each parameter is deleted one by one, it still does not go out of range. Both the raters AA and WAW have shown similar consistency measurement of total score using the revised ankle fracture healing score table.

3.3.8 Overall fracture healing

Overall fracture healing is a subjective evaluation of the fracture healing, this is the way fracture healing is looked at in the clinic. We divided the overall fracture healing scores into five categories: No fracture healing=1, Fracture healed 0%>≤25%=2, Fracture healed 25%>≤50%=3, Fracture healed 50%>≤75%=4, and Fracture healed 75%>≤100%=5. In Table 3-17 Overall fracture healing, there was an agreement of 42.2%. Cohen's weighted Kappa was run to determine if there was an agreement between the two raters beyond chance. There was a moderate agreement between the two raters Kappa=0.42 (95% CI 0.39 to 0.45), P<0.001.

Table 3-17 Cross tabulation of agreement between raters AA and WAW of overall fracture healing percentage

Overall fracture healing
No fracture healing=1
Fracture healed 0%>≤25%=2
Fracture healed 25%>≤50%=3
Fracture healed 50%>≤75%=4
Fracture healed 75%>≤100%=5

			WAW overall score					Total	Agreement%	W Kappa (95% CI) P value
			1	2	3	4	5			
AA overall score	1	Count	18	13	13	2	0	46	42.2%ß	0.42 (0.39-0.45) P=0.001
		% of Total	1.1%	0.8%	0.8%	0.1%	0.0%	2.7%		
	2	Count	100	146	122	60	12	440		
		% of Total	5.9%	8.6%	7.2%	3.5%	0.7%	25.8%		
	3	Count	45	88	251	147	58	589		
		% of Total	2.6%	5.2%	14.7%	8.6%	3.4%	34.5%		
	4	Count	9	54	80	107	110	360		
		% of Total	0.5%	3.2%	4.7%	6.3%	6.4%	21.1%		
	5	Count	5	10	10	46	200	271		
		% of Total	0.3%	0.6%	0.6%	2.7%	11.7%	15.9%		
Total		Count	177	311	476	362	380	1706		
		% of Total	10.4%	18.2%	27.9%	21.2%	22.3%	100.0%		

3.3.9 Quantitative CT scan - Results

The quantitative CT scans were analysed as in the methods section 2.2.11 using CT scan analysis using Matlab™ and ImageJ software. Table 3-18 shows changes in the mean standardised number of voxels density ranges of -102 to 64 (trabecular bone region) in fracture line of CT scans at each trial visit, in standard care group and Teriparatide treatment group. The results indicate a trend towards a better outcome in the Teriparatide group with a statistical significance (P=0.014), (Figure 3-15).

Table 3-18 Mean standardised number of voxels of density ranges of -102 to 644 (Trabecular bone region) in the fracture line (Trabecular bone) of CT scans

	Participant I.D.	TV1	TV2	TV3	TV4	Tv5	TV6	TV7
Standard care Group A	1362	0.96	0.99	0.99	1.00	0.96	0.94	0.92
	1375	0.95	0.97	0.95	0.91	0.91	0.85	0.86
	1047	-	-	-	-	-	-	-
	1180	0.99	1.00	0.97	0.94	0.93	0.94	0.94
	1034	0.97	0.96	0.99	1.00	0.99	0.98	0.97
Mean		0.97	0.98	0.98	0.96	0.95	0.93	0.92
STDEV		0.02	0.02	0.02	0.05	0.04	0.06	0.05
Teriparatide treatment Group B	1230	0.96	0.98	0.96	0.97	0.95	0.94	0.94
	1448	0.96	0.95	0.95	0.97	0.96	0.96	0.95
	1226	0.93	0.95	0.96	0.96	0.99	0.95	0.94
	1276	0.94	0.97	0.97	0.98	1.00	0.97	0.97
	1164	0.98	0.99	0.98	0.98	0.95	0.94	0.91
Mean		0.95	0.97	0.96	0.97	0.97	0.95	0.94
STDEV		0.02	0.02	0.01	0.01	0.02	0.01	0.02

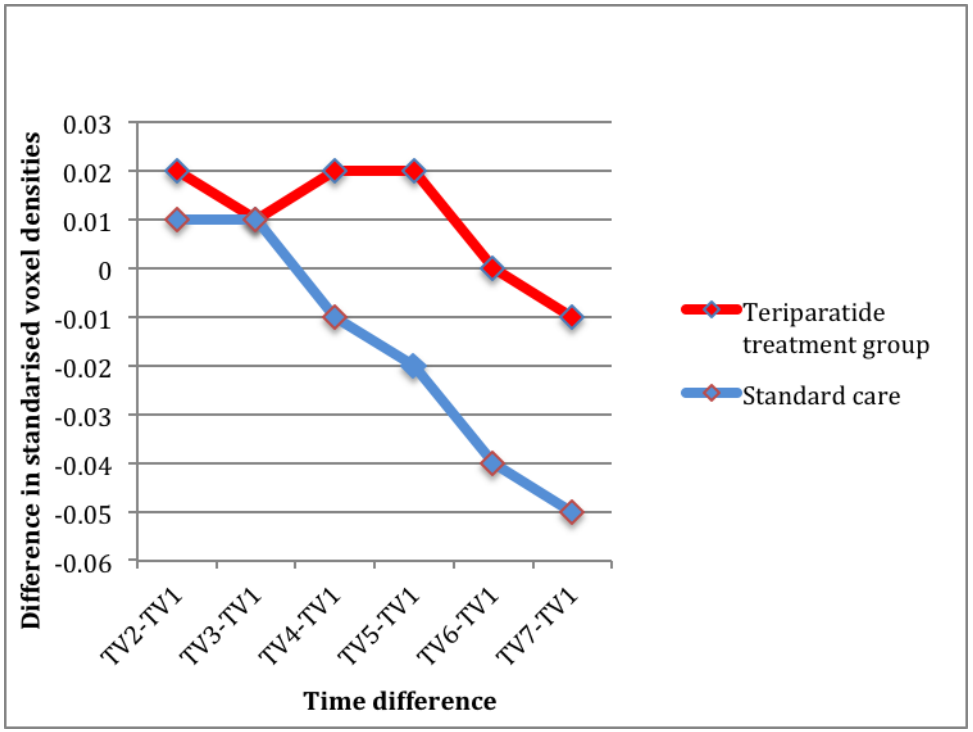


Figure 3-15 Mean differences in standardised voxel densities of trabecular bone region (-102 to 644) at each visit against baseline

Table 3-19 shows changes in the mean standardised number of voxels of density ranges of 522 to 970 (Callus) in the fracture line of CT scans at each trial visit, in the standard care group and Teriparatide treatment group. The results indicate a trend towards a better outcome in the Teriparatide group with a statistical significance (P<0.001), (Figure 3-16)

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Table 3-19 Mean standardised number of voxels of density ranges of 522 to 970 (Callus) in fracture line of CT scans

	Participant I.D.	TV1	TV2	TV3	TV4	Tv5	TV6	TV7
Standard care Group A	1362	0.72	0.74	0.67	0.67	0.75	0.80	0.87
	1375	0.58	0.62	0.75	0.85	0.89	0.94	0.95
	1047	-	-	-	-	-	-	-
	1180	0.60	0.67	0.80	0.90	0.99	0.95	0.93
	1034	0.75	0.91	0.83	0.79	0.81	0.85	0.89
Mean		0.66	0.74	0.76	0.80	0.86	0.89	0.91
STDEV		0.08	0.13	0.07	0.10	0.10	0.07	0.04
Teriparatide treatment Group B	1230	0.73	0.67	0.79	0.77	0.83	0.83	0.87
	1448	0.93	0.75	0.89	0.84	0.89	0.87	0.86
	1226	0.81	0.76	0.78	0.81	0.81	0.80	0.86
	1276	0.69	0.58	0.59	0.61	0.62	0.76	0.82
	1164	0.60	0.63	0.65	0.69	0.81	0.82	0.97
Mean		0.75	0.68	0.74	0.74	0.79	0.82	0.88
STDEV		0.12	0.08	0.12	0.09	0.10	0.04	0.06

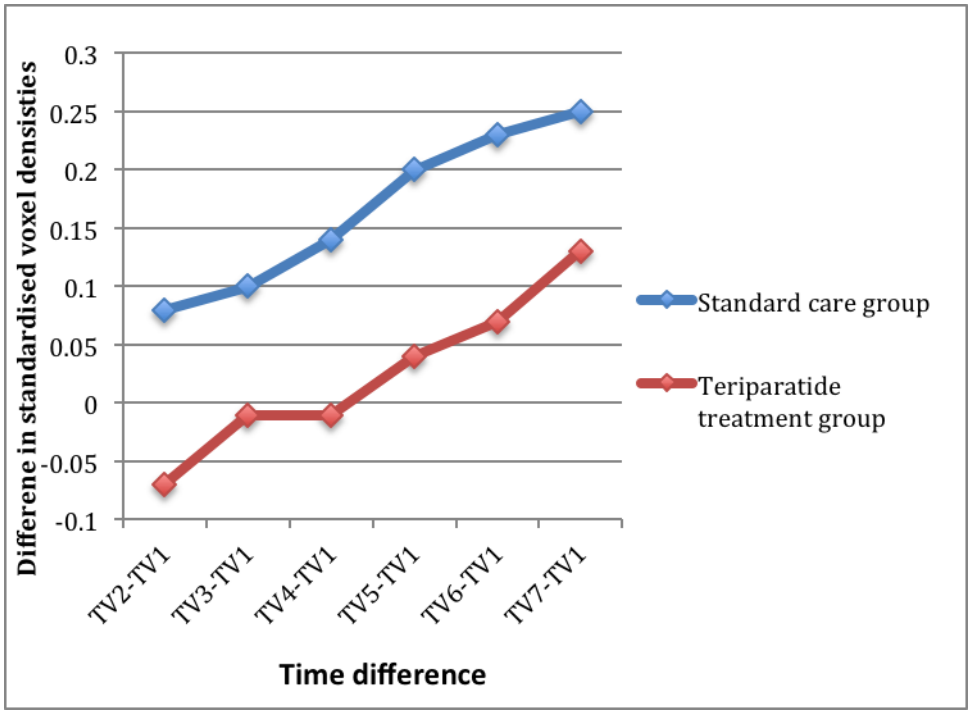

Figure 3-16 Mean difference in standardised voxel densities of callus (552 to 970) at each visit against baseline

Table 3-20 shows changes in the mean standardised number of voxels of density ranges of 1093 (Cortical bone) to end in fracture line (Cortical bone) of CT scans at each trial visit, in standard care group and Teriparatide treatment group. The results did not indicate any trend or statistical significance (*P*=0.291) (Figure 3-17).

Table 3-20 Mean standardised number of voxels of density ranges of 1093 (Cortical bone) to end in fracture line (Cortical bone) of CT scans

	Participant I.D.	TV1	TV2	TV3	TV4	Tv5	TV6	TV7
Standard care Group A	1362	1.00		0.61	0.52	0.61	0.55	0.63
	1375	1.00	0.74	0.61	0.61	0.51	0.68	0.72
	1047	-	-	-	-	-	-	-
	1180	0.97	0.08	0.28	0.49	0.58	0.52	0.58
	1034	0.95	0.99	0.84	0.81	0.82	0.77	0.82
Mean		0.98	0.45	0.59	0.61	0.63	0.63	0.69
STDEV		0.02	0.47	0.23	0.14	0.13	0.12	0.11
Teriparatide treatment Group B	1230	0.86	0.82	0.78	0.61	0.69	0.68	0.61
	1448	0.77	0.99	0.87	0.79	0.78	0.73	0.75
	1226	0.98	0.92	0.73	0.69	0.51	0.64	0.65
	1276	1.00	0.66	0.41	0.30	0.11	0.16	0.09
	1164	0.86	0.84	0.45	0.37	0.49	0.37	0.72
Mean		0.89	0.85	0.65	0.55	0.52	0.52	0.56
STDEV		0.10	0.12	0.21	0.21	0.26	0.24	0.27

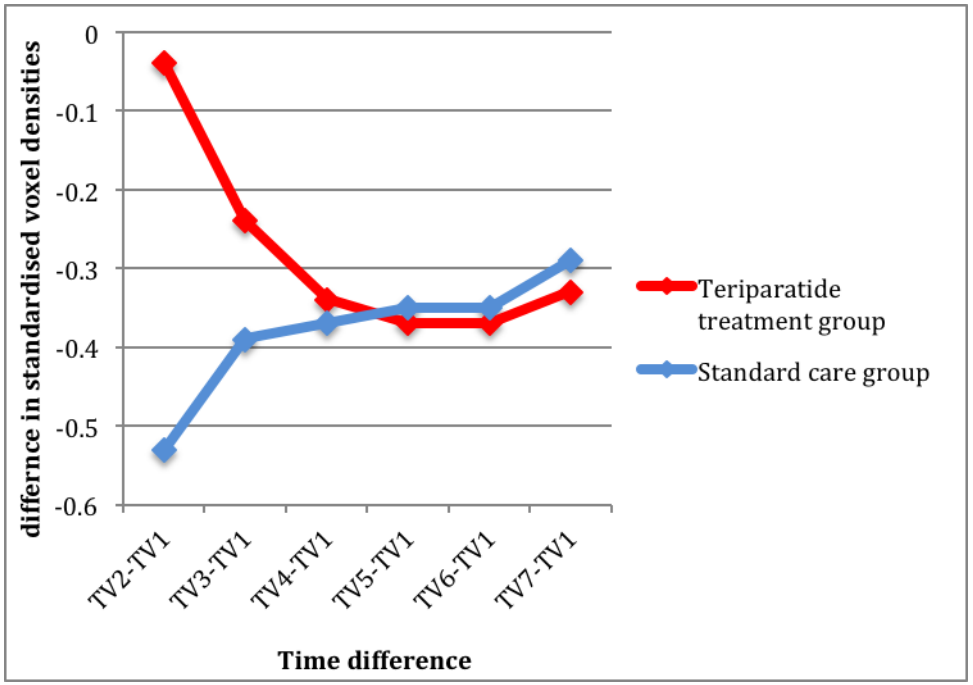

Figure 3-17 Difference of standardised voxel densities of cortical bone (1093 to end) at each visit against baseline

Table 3-21 shows the changes in standardised no of voxels of density ranges -102 to 64 (Trabecular bone) in no fracture zone of CT scans at each trial visit, in standard care group and Teriparatide treatment group. The differences in the number of voxels in the density range are very minute. Differences in changes are not significant (P=0.124) (Figure 3-18).

Table 3-21 Standardised no of voxels of density ranges -102 to 644 (Trabecular bone) in no fracture zone of CT scans

	Participant I.D.	TV1	TV2	TV3	TV4	Tv5	TV6	TV7
Standard care Group A	1362	0.95	0.94	0.98	0.99	0.98	1.00	0.99
	1375	0.96	0.97	0.99	0.98	1.00	0.97	0.93
	1047	-	-	-	-	-	-	-
	1180	1.00			0.92	0.90	0.92	0.93
	1034	0.97	1.00	0.99	0.99	0.97	0.98	0.96
Mean		0.97	0.73	0.74	0.97	0.96	0.97	0.95
STDEV		0.02	0.03	0.01	0.03	0.04	0.03	0.03
Teriparatide treatment Group B	1230	1.00	0.96	0.94	1.00	0.93	0.95	0.97
	1448	-	-	-	-	-	-	-
	1226	0.91	0.93	0.93	0.94	1.00	0.93	0.93
	1276	0.93	0.94	0.96	0.97	1.00	0.98	0.97
	1164	0.99	1.00	0.99	0.98	0.98	0.99	1.00
Mean		0.77	0.77	0.76	0.78	0.78	0.77	0.77
STDEV		0.04	0.03	0.03	0.03	0.03	0.03	0.03

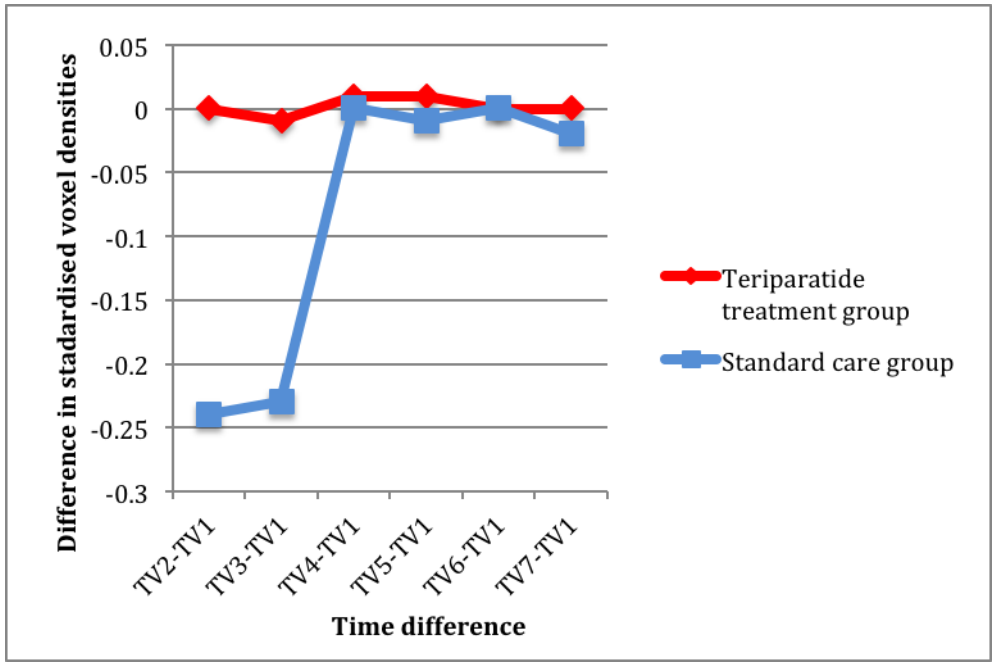

Figure 3-18 Mean difference in the standardised voxel densities of trabecular bone region (-102 to 644) at each visit against baseline in no fracture zone

Table 3-22 shows the changes in standardised number of voxels of density ranges 522 to 970 (Callus) in no fracture zone of CT scans at each trial visit, in standard care group and Teriparatide treatment group. The differences in the number of voxels in the density range are very minute. Differences in changes are not significant (P=0.213), (Figure 3-19).

Table 3-22 Standardised number of voxels of density ranges 522 to 970 (Callus) in no fracture zone

	Participant I.D.	TV1	TV2	TV3	TV4	Tv5	TV6	TV7
Standard care Group A	1362	0.80	1.00	0.87	0.89	0.85	0.80	0.81
	1375	0.82	0.84	0.85	0.84	1.00	0.82	0.98
	1047	-	-	-	-	-	-	-
	1180	0.98			1.00	0.97	0.90	0.91
	1034	0.92	0.87	0.94	1.00	0.95	0.96	0.95
Mean		0.88	0.68	0.67	0.93	0.94	0.87	0.91
STDEV		0.08	0.09	0.05	0.08	0.07	0.07	0.07
Teriparatide treatment Group B	1230	1.00	0.88	0.97	0.96	0.89	0.89	0.98
	1448	-	-	-	-	-	-	-
	1226	0.78	0.69	0.74	0.72	1.00	0.69	0.76
	1276	0.89	0.89	0.91	0.89	1.00	0.92	0.99
	1164	0.83	0.82	0.87	0.94	0.91	1.00	1.00
Mean		0.88	0.82	0.87	0.88	0.95	0.88	0.93
STDEV		0.09	0.09	0.10	0.11	0.06	0.13	0.12

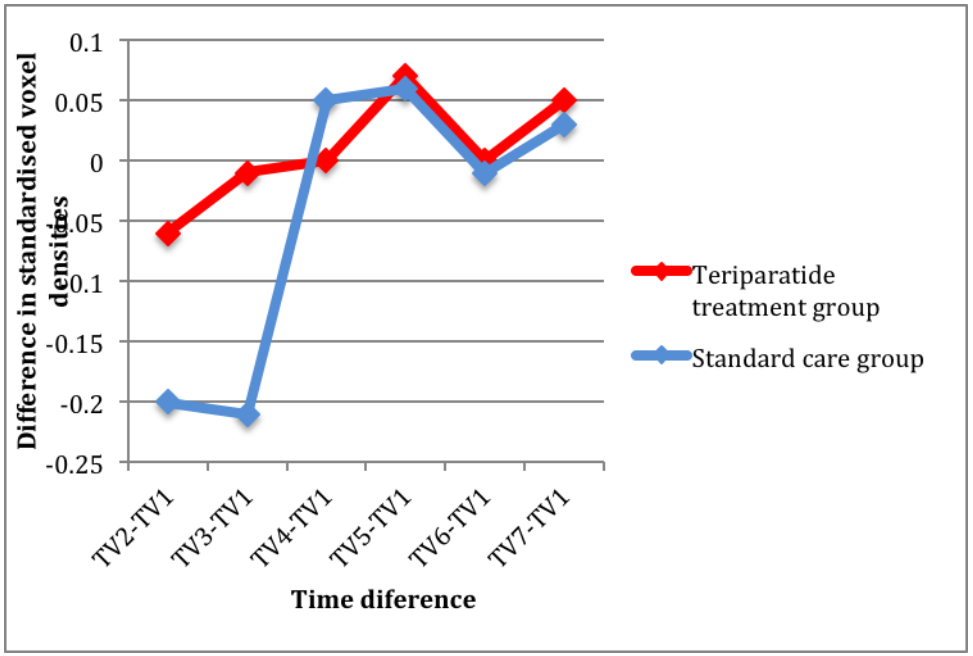

Figure 3-19 Mean differences in standardised voxel densities of callus (522 to 970) at each visit against baseline in no fracture zone

Table 3-23 shows the changes in standardized no of voxels of density ranges 1093 to end (Cortical) in no fracture zone of CT scans at each trial visit, in standard care group and Teriparatide treatment group. The differences in the number of voxels in the density range are very minute. Differences in changes are not significant (P=0.189) (Figure 3-20).

Table 3-23 Standardised number of voxels of density ranges 1093 to end (Cortical) in no fracture zone

	Participant I.D.	TV1	TV2	TV3	TV4	Tv5	TV6	TV7
Standard care Group A	1362	1.00	0.86	0.87	0.81	0.92	0.88	0.90
	1375	0.99	1.00	0.97	0.96	0.88	0.95	0.87
	1047	-	-	-	-	-	-	-
	1180	0.97			0.99	1.00	0.98	0.97
	1034	1.00	1.00	0.99	0.98	0.98	0.96	0.99
Mean		0.99	0.72	0.71	0.94	0.95	0.94	0.93
STDEV		0.01	0.08	0.06	0.08	0.06	0.04	0.06
Teriparatide treatment Group B	1230	0.97	0.97	0.99	0.98	1.00	0.99	0.99
	1448	-	-	-	-	-	-	-
	1226	0.98	0.99	1.00	0.98	0.93	0.99	0.97
	1276	1.00	0.98	0.95	0.92	0.86	0.88	0.71
	1164	0.99	0.98	0.98	0.94	1.00	0.92	0.89
Mean		0.99	0.98	0.98	0.96	0.95	0.95	0.89
STDEV		0.01	0.01	0.02	0.03	0.07	0.05	0.13

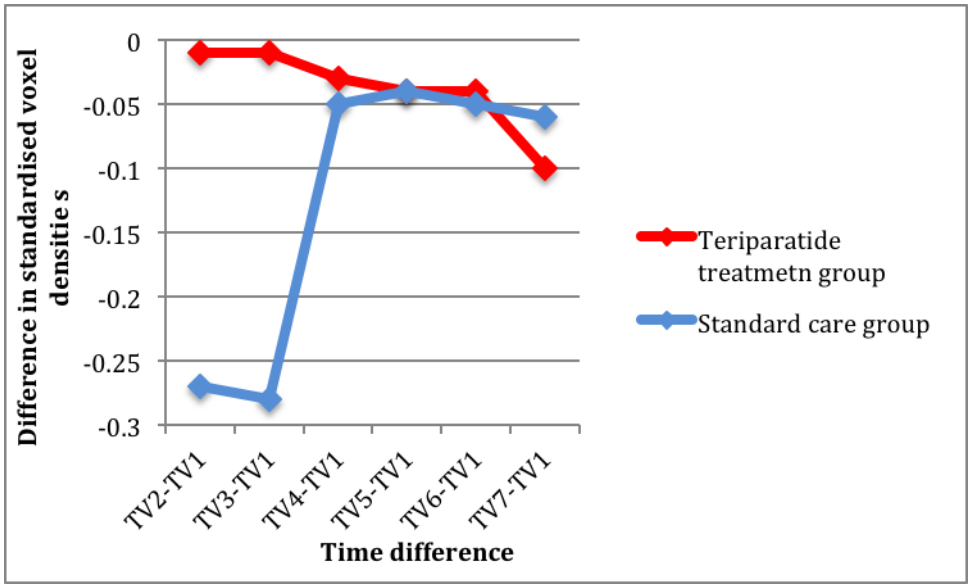

Figure 3-20 Mean difference in standardised voxel densities of cortical bone (1093 to end) at each visit against baseline in no fracture zone

3.3.10 Results-Power calculation for a follow-on substantive study

We carried out a statistical calculation for number of participant needed to enrol into the study in order to have statistical power. Table 3-24 and Table 3-25 show the sample size required to detect a minimum effect between the standard care group and Teriparatide treatment group for the different parameters. Sample size depends on acceptable significance level, study power, effect size, event rate in the population and standard deviation. There are other factors also which determine the sample size like drop out rate and allocation ratio in each arm of the study (Kadam and Bhalerao, 2010).

Table 3-24 Sample size comparing two means at 6 weeks (TV4) for different parameters

Confidence Interval (2-sided) 95% Power 80% Ratio of sample size is 1 (Group 1/Group 2)					
Parameters	Group 1 mean st dev	Group 2 mean st dev	Difference	Sample size in each group	Total sample required to detect minimum effects between groups (+20% for dropouts)
Callus rater AA	4.056 ±0.82	3.768 ±0.38	0.288	77	185
Trabecular bone AA	2.616 ±0.79	2.264 ±0.90	0.352	91	218
Total fracture healing AA	14.568 ±2.71	13.136 ±2.51	1.432	53	174
Overall fracture healing AA	3.264 ±0.66	2.96 ±0.69	0.304	78	187
Callus rater WAW	3.96 ±0.46	3.592 ±0.76	0.368	46	110
Trabecular bone WAW	3.112 ±0.56	2.8 ±0.81	0.312	79	190
Total fracture healing WAW	14.64 ±2.69	13.376 ±3.9	1.264	111	266
Overall fracture healing score WAW	3.472 ±0.62	3.224 ±0.77	0.248	124	298

Table 3-25 Sample comparing two means at 6 weeks for different parameters assessed quantitatively in CT scans.

Confidence Interval (2-sided) 95% Power 80% Ratio of sample size (Group 1/Group 2) 1					
Parameters	Group 1 mean stdev	Group 2 mean stdev	Difference	Sample size in each group	Total sample required to detect minimum effects between groups (+20% for dropouts)
Quantitative callus	0.8036 ±0.10	0.7459 ±0.09	0.058	46	110
Quantitative trabecular bone region	0.961 ±0.04	0.9731 ±0.01	0.021	113	271
Quantitative cortical bone	0.636 ±0.16	0.5528 ±0.21	0.083	80	192

3.3.11 Results-Differences in total fracture healing score between trial visit one (TV1) and subsequent trial visits for standard care group and Teriparatide group

Table 3-26 shows the mean total fracture healing score for standard care group and Teriparatide treatment group from TV1 to TV7 for rater AA.

Table 3-26 mean of total fracture healing score at each trial visit for standard care group and Teriparatide treatment group

Rater AA		
	Standard care group	Teriparatide treatment group
TV1	13.464	12.16
TV2	13.584	12.84
TV3	13.44	17.48
TV4	14.568	17.04
TV5	16.536	17.72
TV6	16.184	18.2
TV7	17.592	19.00

Table 3-27 shows the difference in the total fracture healing score at each visit against baseline, (*P*=0.05) for rater AA.

Table 3-27 Difference in mean of total fracture healing score at each visit against baseline in the standard care group and Teriparatide treatment group

Rater AA		
Parameter	Standard care group	Teriparatide treatment group
TV2-TV1	0.12	0.68
TV3-TV1	-0.024	5.32
TV4-TV1	0.984	4.88
TV5-TV1	3.096	5.56
TV6-TV1	1.616	6.04
TV7-TV1	1.056	6.84

Table 3-28 shows the mean total fracture healing score for standard care group and Teriparatide treatment group at each visit against baseline for rater WAW

Table 3-28 Mean of total fracture healing score at each visit against baseline in standard care group and Teriparatide treatment group

Rater WAW		
	Standard care group	Teriparatide treatment group
TV1	14.64	8.312
TV2	12.736	7.776
TV3	11.088	15.048
TV4	14.64	13.376
TV5	17.144	15.208
TV6	16.864	16.2
TV7	18.336	17.816

Table 3-29 shows the difference in the total fracture healing score at each visit against baseline (P=0.05) for rater WAW.

Table 3-29 Difference in mean of total fracture healing score at each visit against baseline in the standard care group and Teriparatide treatment group

Rater WAW		
Parameter	Standard care group	Teriparatide treatment group
TV2-TV1	-1.904	-0.536
TV3-TV1	-3.552	6.736
TV4-TV1	1.904	5.064
TV5-TV1	6.056	6.896
TV6-TV1	2.224	7.888
TV7-TV1	1.192	9.504

Table 3-30 shows the mean of total fracture healing score for standard care group and Teriparatide treatment group at each visit against baseline. (The mean is mean of two raters AA and WAW)

Table 3-30 Mean of total fracture healing score at each visit against baseline in standard care group and Teriparatide treatment group (The mean is the mean of the two raters AA and WAW)

Mean of total fracture healing score		
	Standard care group	Teriparatide treatment group
TV1	14.052	10.236
TV2	13.16	10.308
TV3	12.264	16.264
TV4	14.604	15.208
TV5	16.84	16.464
TV6	16.524	17.2
TV7	17.964	18.408

Figure 3-21 shows the difference in the total fracture healing score at each visit against baseline (P =0.005).

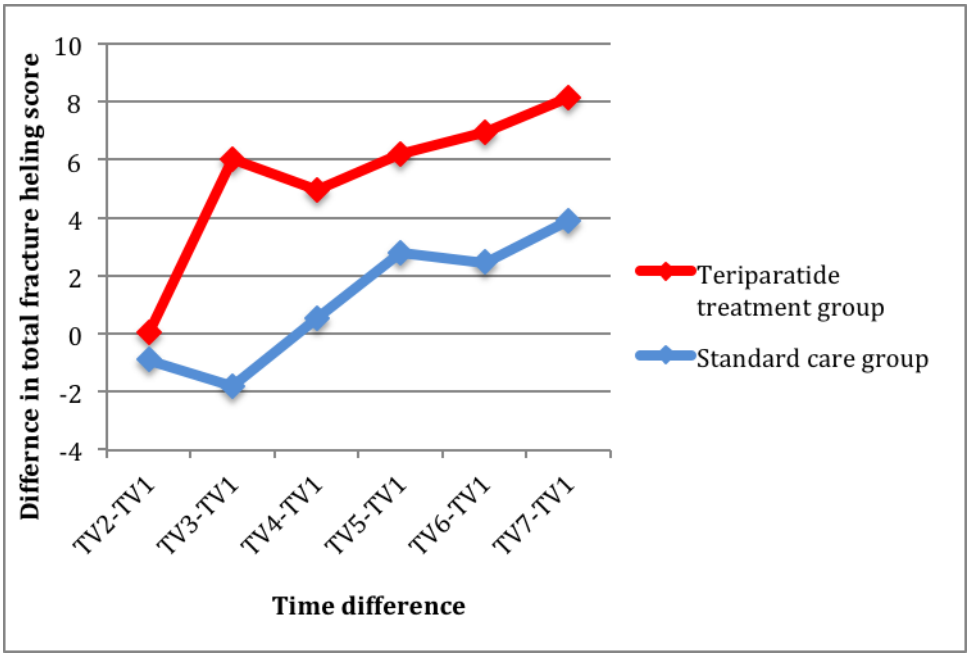

Figure 3-21 Difference in mean of total fracture healing score at each visit against baseline in the standard care group and Teriparatide treatment group

From the results shown in Table 3-27 and Table 3-29 we can conclude that for a definitive study, the best timing of CT scans from the feasibility study are at baseline or TV1, and at TV3, which are at the four week time points and at TV4, which is at the six week time points. This would reduce the total number of CT scans from seven CT scans to three CT scans. Additionally, the trial visits would also be reduced to three visits. Moreover the Teriparatide injections will be reduced to six weeks.

Figures below showing CT scans of Weber B ankle fractures of a participant in the standard care group and a participant in the Teriparatide treatment group at baseline TV1 (scan1) and at six weeks TV4 (scan 4) and at 12 weeks TV7 (scan 7) showing the differences in healing of fractures (see Figure 3-22, Figure 3-23, Figure 3-24, Figure 3-25, Figure 3-26, Figure 3-27)

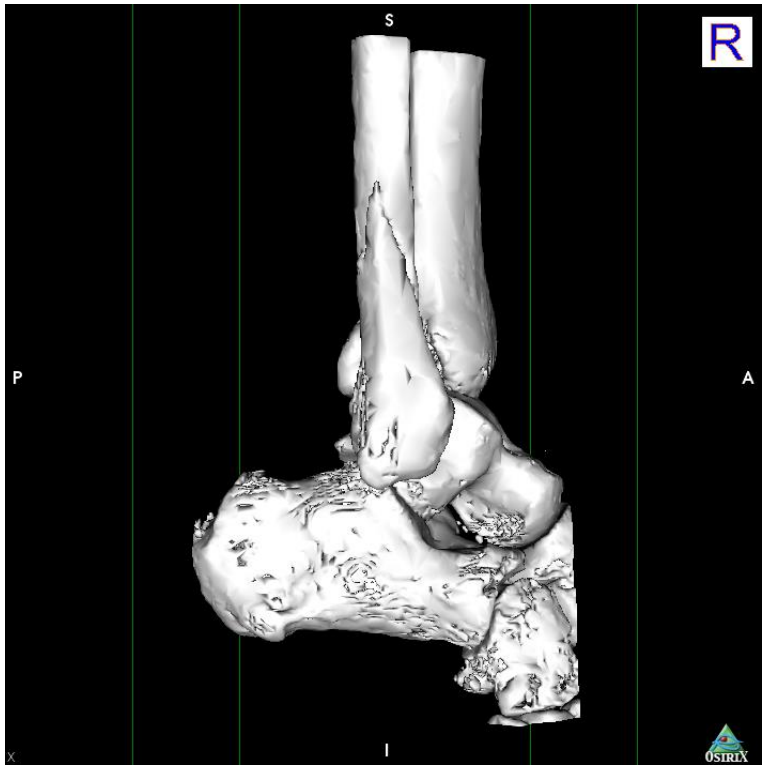

Figure 3-22 TV1 (Scan1) participant 1375 standard care group

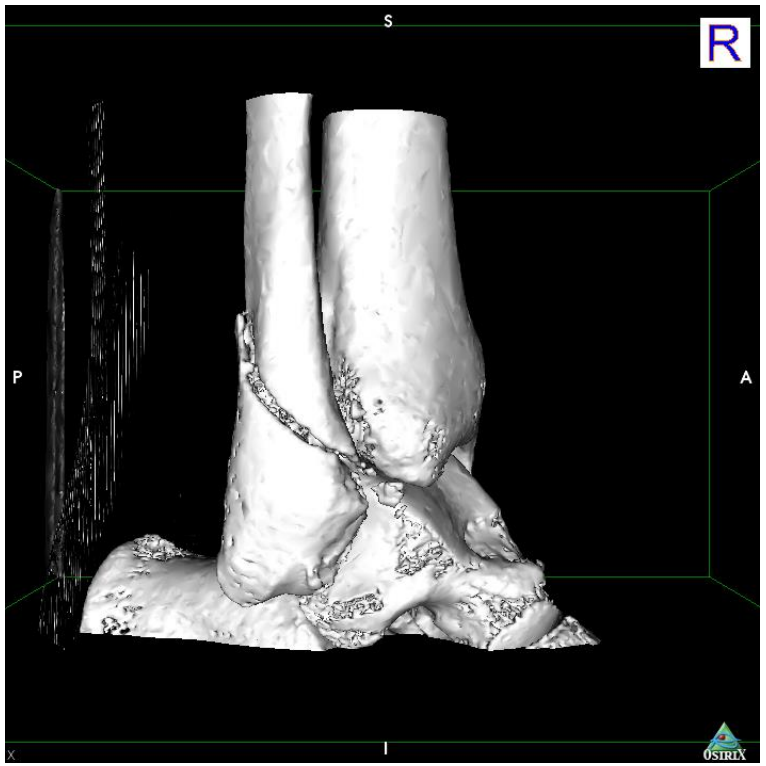

Figure 3-23 TV1 (Scan1) participant 1164 Teriparatide treatment group

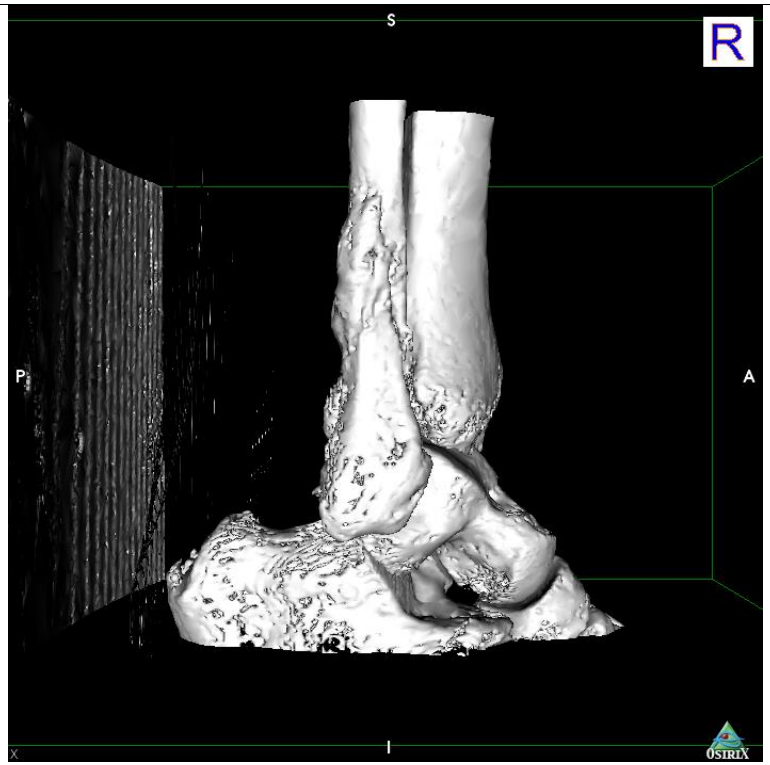

Figure 3-24 TV4 (Scan4) participant 1375 standard care group

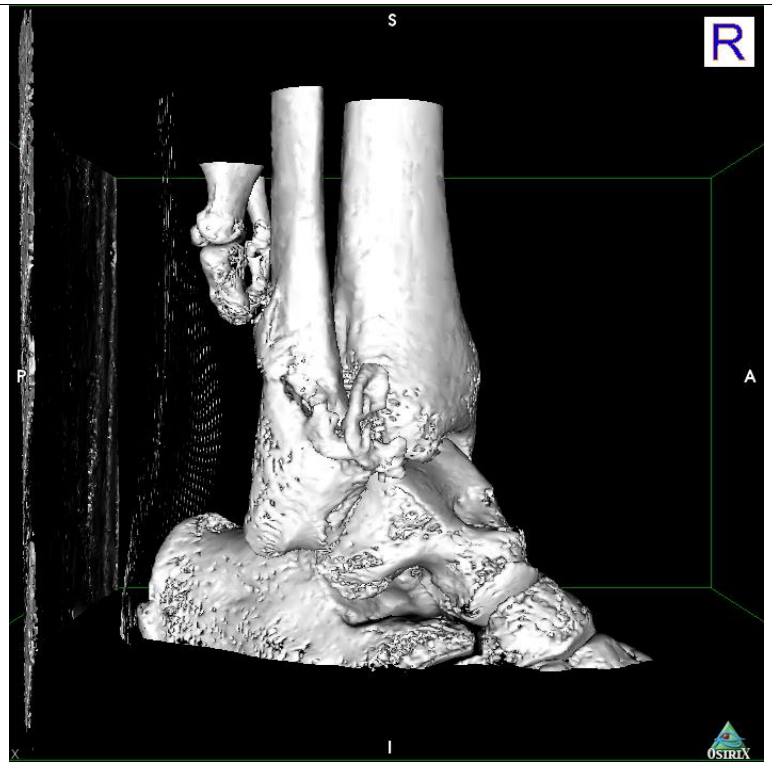

Figure 3-25 TV4 (Scan4) participant 1164 Teriparatide treatment group

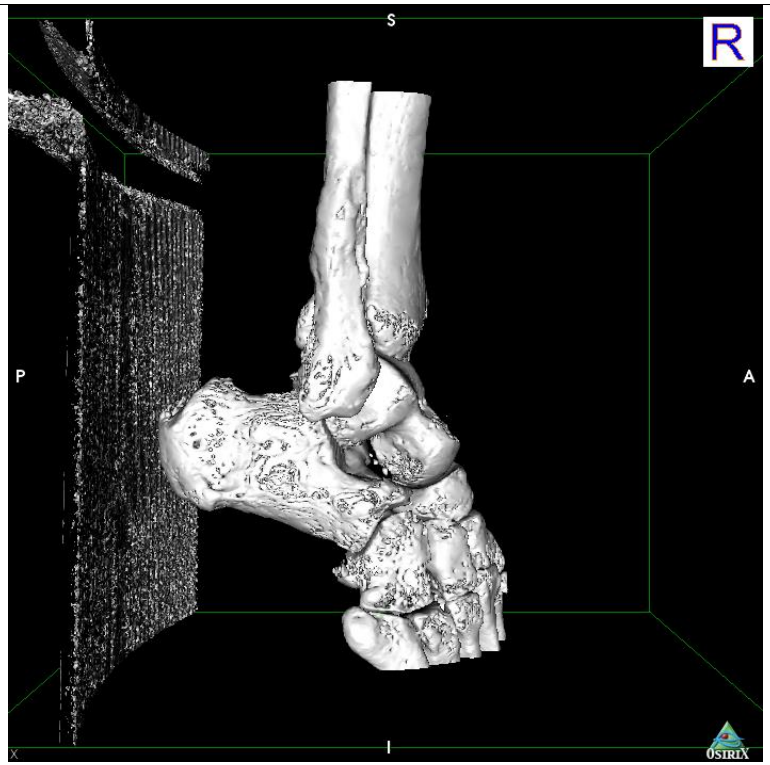

Figure 3-26 TV7 (Scan7) participant 1375 standard care group

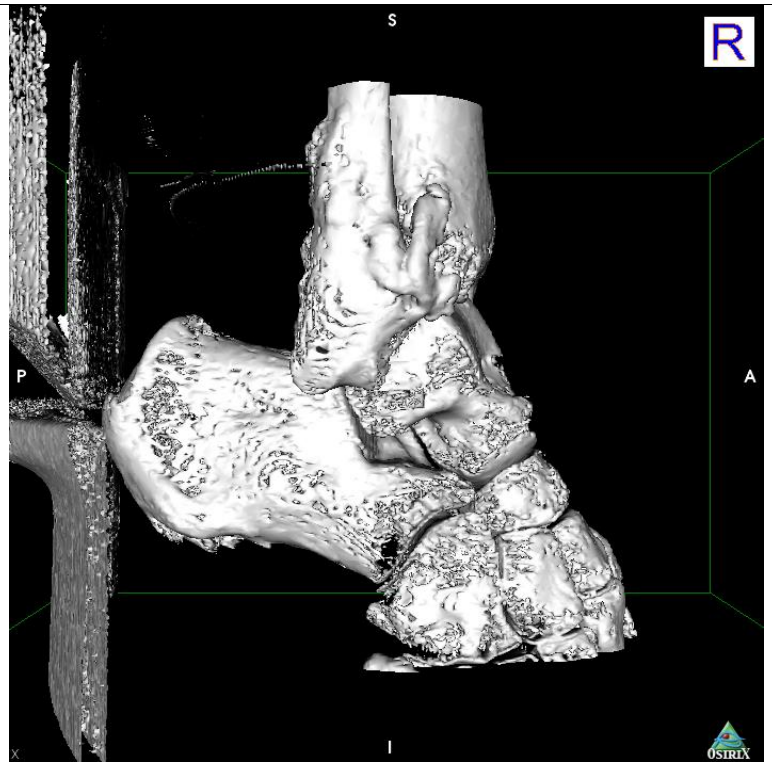

Figure 3-27 TV7 (Scan7) participant 1164 Teriparatide treatment group

3.3.12 Patient reported outcome measures

3.3.12.1 Olerud and Molander ankle function scores of participants in standard care group A and Teriparatide group (B)

In Table 3-31 Olerud Molander ankle function-sum points for all the 9 parameters score shows that mean sum points for all the 9 parameters score for standard care group was 36 ± 19.81 and the Teriparatide group was 47 ± 13.01 at TV4. At TV7 the score for Standard care group increased to 61 ± 27.25 and for the Teriparatide group increased to 61 ± 16.73. It was not practical to assess the ankle function prior to TV4 as the participants were in the cast.

Table 3-31 Olerud and Molander ankle function scores of participants in standard care group (A) and Teriparatide group (B), for sum points for all the 9 parameters (pain, stiffness, swelling, stair climbing, running, jumping, squatting, supports, works and activity of daily living)

Olerud and Molander ankle function-Sum points for all the 9 parameters Minimum=0, Maximum=100. A=Standard care, B=Teriparatide group									
	Treatment group	Patient ID	TV1	TV2	TV3	TV4	TV5	TV6	TV7
1	A	1362	X	X	X	40	55	50	65
2	A	1047	X	X	X	30	45	70	70
3	A	1180	X	X	X	50	30	40	55
4	A	1375	X	X	X	55	75	95	95
5	A	1034	X	X	X	5	0	45	20
					Median	40	45	50	65
					Mean	36	41	60	61
					Min	5	0	40	20
					Max	55	75	95	95
					STDEV	19.81	28.15	22.64	27.25
1	B	1448	X	X	X	55	45	45	60
2	B	1230	X	X	X	25	30	60	70
3	B	1226	X	X	X	45	50	60	95
4	B	1164	X	X	X	55	50	40	70
5	B	1276	X	X	X	55	40	45	50
					Median	55	45	45	70
					Mean	47	43	50	69
					Min	25	30	40	50
					Max	55	50	60	95
					STDEV	13.01	8.37	9.35	16.73

3.3.12.2 EQ-5D-5L questionnaire UK version results

In Table 3-32 EQ-5D-5L questionnaire-Over all self-rated health status score shows that mean over all self-rated health status score for standard care group was 68 ± 16.43 and the Teriparatide group was 66 ±31.3 at TV1. At TV7 the score for Standard care group increased to 83.4 ± 13.39 and for the Teriparatide group increased to 94.2 ± 8.17.

Table 3-32 EQ-5D-5L questionnaire scores of participants in standard care group (A) and Teriparatide group (B), for overall self-rated health status

EQ-5D-5L questionnaire-Overall self-rated health status
A=Standard care, B=Teriparatide group

	Treatment group	Patient ID	TV1	TV2	TV3	TV4	TV5	TV6	TV7
1	A	1362	70	90	95	85	90	85	90
2	A	1047	55	77	79	81	84	85	88
3	A	1180	65	55	80	56	58	55	65
4	A	1375	95	97	97	98	98	98	99
5	A	1034	55	65	60	75	75	85	75
		Median	65	77	80	81	84	85	88
		Mean	68	76.8	82.2	79	81	81.6	83.4
		Min	55	55	60	56	58	55	65
		Max	95	97	97	98	98	98	99
		STDEV	16.43	17.30	14.92	15.38	15.36	15.90	13.39
1	B	1448	80	80	80	87	90	87	95
2	B	1230	20	80	95	95	95	95	97
3	B	1226	80	85	90	96	93	94	99
4	B	1164	100	100	100	100	100	100	100
5	B	1276	50	40	80	79	80	80	80
		Median	80	80	90	95	93	94	97
		Mean	66	77	89	91.4	91.6	91.2	94.2
		Min	20	40	80	79	80	80	80
		Max	100	100	100	100	100	100	100
		STDEV	31.31	22.25	8.94	8.38	7.44	7.79	8.17

In Table 3-33 EQ-5D-5L questionnaire profile, index values and mean index value shows that mean index value for standard care group was 0.646 ± 0.07 and for the Teriparatide group was 0.6144 ± 0.17 at TV1. And TV7 the mean index value for standard care group increased to 0.758 ± 0.11 and for the Teriparatide group increased to 0.7926 ± 0.03.

Table 3-33 EQ-5D-5L questionnaires profile with index values means and standard deviations of participants in clinical trial visits

EQ-5D-5L questionnaire profiles, index values and index mean values for standard care group and Teriparatide group from TV1 to TV7									
Treatment group	I.D		TV1	TV2	TV3	TV4	TV5	TV6	TV7
A	1362	Profile	31321	32321	21221	21221	21221	21221	21221
		Index	0.747	0.671	0.819	0.766	0.766	0.766	0.766
A	1047	Profile	32321	32421	31421	21321	31331	21221	21221
		Index	0.671	0.641	0.717	0.757	0.734	0.766	0.766
A	1180	Profile	42421	21531	31521	31521	31221	31321	21221
		Index	0.63	0.652	0.655	0.655	0.756	0.747	0.766
A	1375	Profile	33332	32331	22221	21221	11121	11111	11111
		Index	0.606	0.657	0.689	0.766	0.846	0.9	0.9
A	1034	Profile	33432	42533	32533	22523	33333	22223	33333
		Index	0.575	0.509	0.519	0.543	0.596	0.643	0.596
Mean index value for standard care group A			0.6458	0.626	0.6798	0.6974	0.7396	0.7644	0.7588
STDEV of index value for standard care group A			0.07	0.07	0.11	0.10	0.09	0.09	0.11
B	1448	Profile	43531	32431	32321	21221	21221	21221	21121
		Index	0.539	0.627	0.671	0.766	0.766	0.766	0.8
B	1230	Profile	53531	43521	32411	21221	31421	21431	11221
		Index	0.402	0.553	0.694	0.766	0.717	0.714	0.812
B	1226	Profile	41131	31221	21221	21221	21221	21221	41131
		Index	0.766	0.756	0.766	0.766	0.766	0.766	0.766
B	1164	Profile	21121	11211	21211	21221	21221	21221	21211
		Index	0.8	0.866	0.819	0.766	0.766	0.766	0.819
B	1276	Profile	32531	33431	31321	41442	31332	31332	21221
		Index	0.565	0.612	0.747	0.563	0.698	0.698	0.766
Mean index value for standard care group A			0.6144	0.6828	0.7394	0.7254	0.7426	0.742	0.7926
STDEV of index value for standard care group A			0.17	0.13	0.059	0.091	0.033	0.033	0.03

3.3.12.3 Visual analogue scale for pain (VAS Pain)

In Table 3-34, visual analogue pain scores are reported. The mean visual analogue pain score of standard care group was 4.2 ± 2.28 and for the Teriparatide group was 4.6 ± 2.61 at TV1. At TV7 the mean visual analogue pain score for the standard care group decreased to 1.4 ± 0.89 and for the Teriparatide group it decreased to 0.8 ± 1.09 at TV7. The visual analogue pain scale, see Figure 3-28

Table 3-34 Visual analogue pain (VAS Pain) score of participants in standard care group (A) and Teriparatide group (B)

Visual analogue pain score A=Standard care group, B=Teriparatide group=B								
Patient ID	Treatment group	TV1	TV2	TV3	TV4	TV5	TV6	TV7
1362	A	3	2	0.5	3	2	3	1
1047	A	2	2	2	2	3	2	2
1180	A	4	4	2	1	5	4	2
1375	A	4	2	2	2	2	0	0
1034	A	8	4	4	2	4	2	2
Mean VAS pain score of participants in standard care group (A)		4.2	2.8	2.1	2	3.2	2.2	1.4
STDEV of mean VAS pain score of participants in standard care group (A)		2.28	1.10	1.25	0.71	1.30	1.48	0.89
1448	B	8	7	1	2	3	4	2
1230	B	6	2	0	2	2	2	0
1226	B	4	3	2	1	2	1	0
1164	B	1	0	0	0	0	0	0
1276	B	4	4	2	4	3	3	2
Mean VAS pain score in of participants in Teriparatide group (B)		4.6	3.2	1	1.8	2	2	0.8
STDEV of mean VAS pain score of participants in Teriparatide group (B)		2.61	2.59	1	1.48	1.22	1.58	1.09

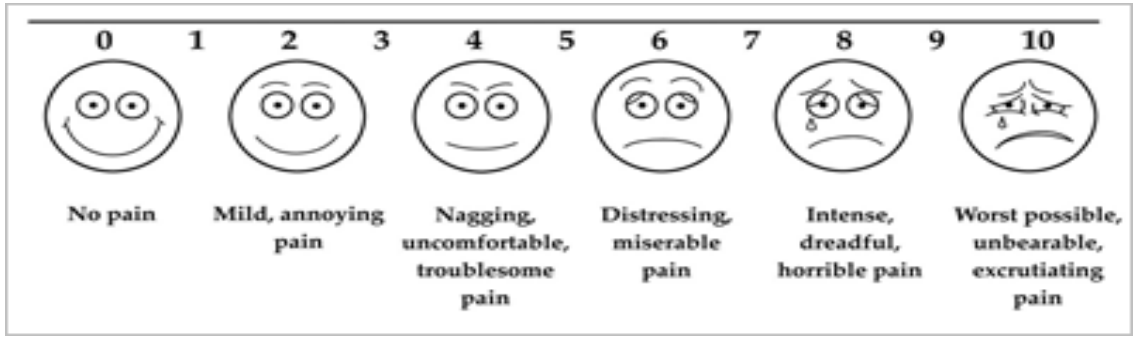

Figure 3-28 visual analogue pain scale

3.3.13 Qualitative study results

The demographic characteristics of participants interviewed in this study were shown earlier in Table 3-1. All the participants completed their clinical trial visits, within a visiting window of ±3 days. The length of the interviews were between 20 to 25 minutes so as not to exhaust the participants because they were also having CT scan, in addition to their clinical visits. The interviews were conducted at Queen's Medical Centre in one of the rooms in the fracture clinic and were recorded and then transcribed. All the transcribed interviews were allocated a participant unique identifier number. The following questions used to facilitate the dialogue in the interviews and the themes were also evolved around them. However, the subthemes evolved from the transcribed interviews.

1. What did you think of the quality of care you received whilst taking part in this research study?
2. How was your experience with self-injection (specific for treatment group)?
3. How did you feel about visiting hospital every 2 weeks?
4. What did you think about having computerized scans (CT scans) every 2 weeks?
5. How important do you think this study is?
6. Would you consider taking part in any future research and if so why?
7. Do you have any further comments or suggestions that might improve this study?

3.3.13.1 Themes identified from the transcribed interviews and the questions

Table 3-35 refined themes and subthemes extracted from the transcripts of the interviews

Refined themes	Subthemes
1-Quality of care perceived by the participants in the clinical trial	1.1 Number of trial visits for the study 1.2 Punctuality of the researchers in seeing the participants 1.3 Trial visits of participant for CT scans 1.4 Communications channels between the participant and the researcher team
2-Particpants self-injection experience during the study	2.1 Perceived fear of injection 2.2 Fears of side effects of medication
3-Motives of the participants for participation in the clinical trial	3.1 Perceived benefits of drug (Teriparatide injection) 3.2 Monitoring of participants medical condition 3.3 Contribution to the society
4-Perceived negative experience by the participants in the clinical trial	4.1 Hospital staff awareness about ongoing studies 4.2 Length of the study period 4.3 Researchers eminent background

1-Quality of care perceived by the participants in the clinical trial

The first sub-theme was quality of care, which has tangible and intangible aspects; both can have a considerable effect on quality of care of the patients or participants in a clinical trial. Usually what is perceived by the participant depending on his background knowledge and experience, has the most effect on satisfaction on the participants.

1.1 Number of trial visits for the study

The number of trial visits are crucial, as increasing the number of visits places an unnecessary burden on participants, who may refuse to participate or may dropout during the course of trial, which increases the overall cost of the trial trying to replace the dropouts. We asked the question of how convenient the number of trial visits were to the participants, looking for the potential window of increasing or

reducing the trial visits without missing any scientific information in the number and between duration of successive visits.

Example quote from participant

"Think two weeks is sufficient because if you did it weekly it would be too much to try and, I think two weeks is sufficient because it gives you time to rest in between each visit".

Example quote from another participant

"That's not really one for me because you're doing the study and I'm not. You need the data to make a decision about what's good and what's not with your stuff. Obviously I'd like them to be less because it's less inconvenience for me."

1.2 Punctuality of the researchers in seeing the participants

Punctuality for the researcher to see the participant in the clinical trial visits is an essential factor to retain participants in trials. It gives them the attention expected from their involvement. It also gives the participant the confidence to continue in the trials.

Example quote from participant

"You were there every time and that was our biggest worry, we didn't want to be coming on a regular basis and then sat here waiting and you were always there. It was faultless. Really good"

Example quote from another participant

"I think it's been excellent, it's been very, very good indeed. You've made sure that I've been able to keep the appointment, you've made sure that when I was in the hospital that I've not been left or had to wait too long, it's been very good.

1.3 Trial visits of participants for CT scans

Doing CT scans every two weeks can be worrying to the participants from being over exposed to radiation and the consequences of radiation. On the other hand, it was a sort of reassurance to the participants that fracture healing was progressing in right direction. Confidence of participants in the research procedures is very important.

Example quote from participants

"It's in the back of your mind that you might have had too much radiation. I think that having them every two weeks was quite reassuring, that things were healing as they should have been".

Example quote from another participant

"I'm not an expert, I don't know how much radiation my body should be taking and things like that. I'm just trusting medical science that the radiation I've taken isn't too much".

1.4 Communication channels between the participant and research team

Communication channels between the research team and participant, at least with a designated member of a research, are an important factor as this can deter potential participants to take part in the research or drop out of the study. Participants' concerns should be addressed immediately and an open line of communication kept 24 hours a day to solve issues that can arise during the conduct of trial.

Example quote from participant

"No, I don't think so, as I said I everybody I've encountered has been very helpful, you know, explained what is happening, no I wouldn't say there's any negatives at all. I mean, you've been very explicit in terms of how you've explained things, what's happening and so on. And so from that point of view I've quite enjoyed being part of the trial."

Example quote from another participant,

"Sometimes when I gave my name, they didn't know that I was coming or they said that the appointment had been cancelled but I hadn't received anything to say that it had been cancelled. I think that happened on a couple of occasions but once we'd contacted yourself that was quickly sorted out."

Others example quotes from participants that could be the reason that we did not have any drop outs in the study and completing the visits within the specified period,

"You come in and answer the questionnaires and have the scans. All the medication that I've needed for the injections I've had there's not been any issues with that. I

was taught how to use the injection and there are a lot of numbers to call if I've needed any advice."

"I think it's been excellent, it's been very, very good indeed. You've made sure that I've been able to keep the appointment, you've made sure that when I was in the hospital that I've not been left or had to wait too long, it's been very good."

2.0 Participants self-injection experience during the study

There are people who have a fear of injections, once this fear becomes persistent excessive and unreasonable it becomes a phobia. Self-injection can cause a specific type of anxiety (fear of giving injection to self) to some of the participants especially when injecting for the first time. Fear of injection can discourage some of the potential participants to take part in the study involving taking injections. But with proper explanation and training of self-injection some of the anxiety can be alleviated. Also there is always a fear of side effects of a drug which can stop the participant from joining or continuing in the trial.

2.1 Perceived fear of injection

Example quotes from participant

"Usually I'm not bothered about needles but I think it was just the doing it myself. I'm thinking "oh dear" but it was fine. Anybody can give me an injection and I wouldn't worry but it was trying to and thinking."

2.2 Fears of side effects of medication

Example quote from another participant

"Maybe if I had got the side effects maybe I would have had to stop it if I was feeling that there were any problems but there hasn't been."

3.0 Motives of participants for participation in the clinical trial

For a patient to take part in the study there must be something that drives them. These can internal drives (internal motivation) or some externally drives

(external motivation). Knowing these motives can increase recruitment rate and retention of participants in the any study.

3.1 Perceived benefits of drug (Teriparatide injection)

Example quote from participant,

"That was one of the main reasons that I volunteered to take part in the feasibility study because I was hoping to have the injections".

Monitoring of participants medical condition

Example quote from another participant

"I think in the later weeks it's just been monitoring me. In the earlier weeks it was just monitoring the plaster."

3.3 Contribution to the society

Example quote from another participant,

"No not really. I think there was a question about will I do another study. Not for a while, I've done my bit for medical science. I think these things when you're retired it's not so bad, but when you've got a job it can get in the way."

4.0 Perceived negative experience by the participants while going through the clinical trial

Participants can experience a lot of issues while going through the period of the clinical trials. These issues can be detrimental to the retention rate or recruitment rate of participants. Solving these issues improves participant's recruitment and retention rates for future studies.

4.1 Hospital staffs are awareness about on going studies

Example quote from participant

"It is sort of a notorious hospital parking and getting here and then finding it and then going to reception and then reception haven't got your notes and they don't know what's happening and they'll send you to the cubicles and then they'll find out

that you've been looking for me in the waiting room, so the logistics can be a bit frustrating sometimes."

4.2 Length of the study period

Example quote from another participant

"I wasn't expecting it to take this long to recover."

4.3 Researchers eminent background

Example quote from another participant

"I think it probably does, I think I would have taken part in the trial in any case, but I think when someone who is running the trial has got an eminent background then I think you would be more likely to want to be part of something."

4 Chapter 4 Discussion

4.1 Summary of principal findings in the study

Teriparatide was prescribed by 45.2% (n=47) of the physicians who responded to the survey. Perceived reasons for off label usage of Teriparatide were high quality evidence in fracture treatment 38% (n=18), being used for exceptional cases 19% (n=9), using it for acceleration of fracture healing 18%, using it for delayed union and research projects 6% (n=3), while 4% (n=2) were using it for non-unions and 19% (n=9) using it for other reasons, including hyperphosphatemia, insufficiency fractures and stress fractures. Teriparatide was used to accelerate fracture healing in the athletes in by 8% (n=4) of physicians surveyed. .

The most common barrier for usage of Teriparatide was the high cost of the drug 63% (n=30) of responses. Other perceived barriers for prescribing the drug to accelerate fracture healing were; that the indication was off label 36.20% (n=17), the drug was only in injection form 19.1% (n=17), the side effects profile of the drug 19.10% (n=9), self-injection training 6.80% (n=3), lack of evidence 6.83% (n=3), needs specialist opinion 2.1% (n=1) and the absence of guidelines 2.1% (n=1). The physicians who have heard of Teriparartide but do not prescribe it, the most common barriers were cost of the drug 19 (53%) and side off the drug 16 (44%).

An audit for a 12 months period at the Queen's Medical Centre Nottingham University Hospital was carried out to estimate the number of patients with conservatively managed ankle Weber B fractures who were ≥50 years and showed there were sufficient fractures occurring in patients.

We were able to assess feasibility of administering Teriparatide self-injections and observe side effects in the study group through keeping a clinical record file. The common side effects that were mentioned in the Summary of Product characteristics (SmPc) were not observed or reported by the participants in their regular trial visits in this study. The risks remain consistent as mentioned in the SmPC although we did not encounter any risks in this study. There were no dropouts in the follow up visits for this study.

Fracture healing parameters on CT scans of ankle fractures showing percentage of agreement, Kappa value, 95% confident interval and P value. The Kappa values showed poor to moderate agreement although the percentage of agreement was in the range of 34% to 78%. Internal consistency (Cronbach's alpha) for ankle fracture healing score table for rater AA was 0.804 and for rater WAW was 0.874. Total fracture healing score showed better outcome in the Teriparatide treatment group (P=0.05), however the study was a feasibility study and thus fracture healing was not the focus of the study. Additionally, the study was not powered to investigate outcomes.

Quantitative assessment of fracture healing parameters which are mainly in the trabecular bone region and callus, showed a better outcome in the Teriparatide treatment group (for trabecular bone region P=0.014 and for callus P=<0.001), however for cortical bone no trend could not be shown towards any of the groups (P=0.291).

Comparing ankle functions using the Olerud-Molander ankle function score, showed the standard care group mean at TV4 was 36 ±19.81 and the mean for the Teriparatide group was 47 ±13.01. At TV7 for standard group the mean increased to 61 ±27.25 and for the Teriparatide group to 69 ±16.73.

The participant's pain was identified for the standard care group and the Teriparatide group during the study period using a visual analogue pain scale (VAS), that showed a mean VAS score at TV1 of was 4.2 ± 2.28 for the standard care group, and for the Teriparatide it was 4.6 ± 2.61. At TV7 for the standard care group mean VAS score decreased to 1.4 ±0.89 and for the Teriparatide the VAS score decreased to 0.8 ±1.09.

For the EQ-5D-5L overall self-rated health status, at TV1 the standard care group mean was 68 ± 16.43 and for the Teriparatide group the mean was 66 ± 31.31. At TV7 the standard care health status mean increased 83.4 ±13.39 and for the Teriparatide group the mean health status increased 94.2 ±8.17 (*P*=0.018).

The EQ-5D-5L health status index value, at TV1 for the standard care mean was 0.65 ±0.07 and for the Teriparatide group was 0.61 ± 0.17. At TV7 the health status index means value for the standard care group increased to 0.76 ±0.11 and for the Teriparatide the health status mean index value increased to 0.79 ±0.03.

The participants' interviews gave, an insight into what participants were experiencing while going through the clinical trial. Patients were positive regarding the number of trial visits in the study and for the periods between each visit. If the trial visits were closer, this could have resulted in recruitment problems or drop outs from the study. Visits further apart would lead to loss of some of the vital information. Researchers being punctual when meeting with participants reassured them that they were being well looked after, and we surmise that this made them less likely to dropout. Participants did have concerns about the number of CT scans and the amount of radiation exposure. Participants appreciated open communication channels with the research team enabling administrative issues arising in the period of the research to be resolved. Self-injecting medication was seen as a barrier by some patients into participating in the study and created fear, however, the first experience of self-injecting, determined whether the fear was relieved or enhanced, and a patient dropped out or not. Motives that drive the patients into participating in a study can be either internal motives or external. In this study their motive was they might benefit from the injection and they would be looked after more closely regarding their fracture. Negative experience can make the participants not wish to continue, for example travelling to and from hospital, parking issues and extra costs they have to bear just to participate in the study.

4.2 Survey of off label usage of Teriparatide among physicians

There is a huge wealth of medical knowledge and experience, which rests with the physicians that can have an impact on the management of patients. Unfortunately, most of this knowledge is not published in journals or presented at conferences. Approaching professionals like physicians with questionnaires and asking them to spend their precious time answering the questionnaires is more potentially difficult than approaching the general public. Additionally, physicians are an elite group of people and their time costs money. Because of their highly valued responses to the questions they are frequently approached and their in reaction is to be more cautious to respond to surveys or even reluctant to answer any questions. In our study the response rate of physicians to emails were very low which made us change our approach. Nicholls et al reported no responses to the electronic version of their survey (Nicholls *et al.*, 2011). Cunningham et al reported an overall response rate of 35% which varied according to the speciality of the physicians (Cunningham *et al.*, 2015). Not having an incentive can be one of the reasons for a low response (Cook *et al.*, 2016). In our survey the combined response rate from the electronic and hardcopy survey was 15.6% although this droped to 14.9% for those who completed eligible responses that could be included in the study were even though we did not provide any incentives for responding to the survey. The excluded responses were due to bad language and not fulfilling the inclusion criteria such as not being a physician (Figure 3-1). Cunningham et al reported that duration of practicing medicine did have an impact on response rate with length of practicing medicine ≥15 years of 44.6% and quarter of the response rate of 18.3% with a duration of practicing medicine ≤ 5 years (Cunningham *et al.*, 2015). In our survey the response rate was 55% for length of practicing medicine ≥ 15 years and 45.9% response rate for length of practicing medicine < 15 years. It seems the value of responding to research surveys increases with length of practicing medicine by physicians (Figure 3-2).

Surveys can provide knowledge to the responders and at the same time knowledge can be collected from the responders. For any new drug that is launched onto the market the feedback responses from the physicians are very important.

Drug usage indications are always written in the certified product information leaflet. Off label prescriptions of antibiotics, anti-psychiatric and for rare clinical conditions have been reported. In study published by Radley et al, off-label prescription of drugs in the USA, showed that there were 150 million prescriptions that were off label (95% confidence interval, 127-173 millions), which accounted for 21% of all medication usage (Radley, Finkelstein and Stafford, 2006).

Off-label prescription is defined as prescription of a licensed drug for an indication not listed in the drug information leaflet. There are three broad categories into which off label usage of drugs fall into. The first category is that if there is high quality evidence supporting its usage for the intended case. The second category is its usage for research purposes, and the third category is its usage for exceptional individual cases as a last resort of treatment (Gazarian *et al.*, 2006).

Teriparatide 1-34 and Parathyroid hormone have been licensed for the treatment of osteoporosis as a second line drug. It has been prescribed as an off-label drug to accelerate fracture healing in randomized clinical trials (Peichl *et al.*, 2011), (Johansson, 2015) and case series and case reports (Table 1-6). Additionally, in the survey physicians have reported its usage as off label to accelerate fracture healing (Figure 3-8).

The most common barrier perceived by the physicians for prescribing Teriparatide was the cost of the medication. Yap et al reported the cost of the drug as a barrier for prescribing among other barriers (Yap, Thirumoorthy and Kwan, 2016).

4.3 Feasibility study Ankle fracture treated with Teriparatide

4.3.1 Ankle fracture audit

An audit of ankle fractures managed conservatively at Queen's Medical Centre in Nottingham was an essential initial step in the process of conducting the feasibility trial. The viability and predicted success of the trial was very much dependent on this initial step, as it made us change the inclusion criteria in the protocol of the trial. The trial would not have succeeded in recruiting participants in the specified time if the criteria of T score equal or below -2.5 with ankle Weber B fracture included in the protocol. Contrary to the general perception of ankle fractures in elderly patients being osteoporotic fractures, most are not osteoporotic. Greenfield and Eastell reported that ankle fractures are not typical osteoporotic fractures as ankle fracture in the elderly did not show a decrease in the BMD (Greenfield and Eastell, 2001). Also, the comorbidities associated with elderly patients with ankle fractures were un-predicable as the information for the audit was not detailed to the level to inform us about comorbidities.

4.3.2 Can we carry out this study?

Kurar reported that >50% of ankle fractures were Weber B ankle fractures (Kurar, 2016). With a recruitment ratio of 1 in 5, we would require at least 50 patients per year for our anticipated recruitment rate in a year. However, our study showed there were 81 patients above 50 years of age, with Weber B ankle fractures who presented to the trauma clinic at Queen's Medical Centre which were managed conservatively, from 11th Nov 2016 till 21st Dec 2017 from which we were able to recruit 10 patients for the study, that is recruitment rate of 1 in 8 which is 12% of Weber B ankle fractures. Potential participants were excluded because of their associated comorbidities, time at presentation to the clinic were more than 14 days and simply because they did not want to participate in the study. The recruitment rate is one of the main reasons clinical studies are delayed or terminated, as the cost increases to the pre-planned time scale of recruitment. In our study the recruitment was bimodal two peaks are shown in Table 3-1. The weather seemed to be the cause of this bimodal occurrence. This could be problematic if the enrolment started at the

start of summer and a time scale of 3 months was identified for the first patient recruitment onto the study as we did not had any recruitment between end of March 2016 and late Sept 2017. Simon et al reported an increase in ankle fractures during the winter season in the UK (Simons *et al.*, 2012). Another study by Jantzen et al., reported that a decrease in road temperature increased the daily number of all fractures including ankle fractures in Denmark (Jantzen *et al.*, 2014). In summary, it took 13 months to recruit 10 patients in a single centre study. Any future RCT would have to be multicentre and with dedicated recruitment staff in high volume trauma centres. It is likely that if the trial had an additional placebo controlled group, the recruitment would have been even slower, and this would need to be factored into the time recruitment might take.

4.3.3 Sample size of the study

For a study to be statistically powered sample size is very important. In a study conducted by Billingham et al, an audit of sample size of pilot and feasibility randomised control trials registered in United Kingdom research data base, showed from a sample size of 79 trials, 63.3% were pilot studies and 25% feasibility studies. 14 studies in the audit were both pilot and feasibility studies. In the audit, pilot trials tended to have smaller sample size than feasibility trials, per arm. Pilot trial sample size was a median of 30 participants (range = 8 to 114), whereas feasibility trials sample size was a median of 36 (range = 10 to 300). The sample size differed with regards to dichotomous or continuous endpoints; the median sample size per arm was 36 (range = 10 to 300 participants) for trials with a dichotomous endpoint, and 30 (range = 8 to 114 participants) for trials with a continuous endpoint over both feasibility and pilot trials. Additionally, publicly funded pilot trials had larger sample sizes than industrial funded pilot trials (Billingham, Whitehead and Julious, 2013). Our sample size of 10 participants does not seem to be different from the sample of feasibility/pilot randomised clinical trials conducted in the UK, remembering of course that in a feasibility study, sample size is not of prime importance.

4.3.4 What is the retention rate of participants in the study?

Retaining patients in the study is another limiting factor for completing the study with pre-calculated power. There are usually dropouts in all studies, so depending on the length of follow up, this can have enormous impact on the costs and outcome of the studies. We had a recruitment rate of 1 in 8 patients over a period of 1 year for the sample size of 10 participants and retention rate of 100% at Queen's Medical Centre Nottingham. The retention rate in our study could be because of the nature of the fracture healing period of 3 months which is not a lengthy period for the patients to become exhausted of attending appointments. The other reasons could be due to internal motivation of the patient to seek attention for their fractured ankle, also the provision of the transport to and from hospital alleviated some travelling stresses of the participants. A further reason could be there were no major side effects of Teriparatide self-injection in the study (Gul and Ali, 2010).

4.3.5 Will all the complex components of the study process work together?

Having complex components integrated smoothly, aiming for the outcome of participants completing the study taking the injections, investigations and visits, are cumbersome and time-consuming processes.

Having laboratory tests done at least 24 hours before consenting the patient was not an obstacle. However, making a decision on out of normal range blood results and classify them as clinically not significant results takes some time and this was not anticipated when the protocol of the study was written, and a consultation had to be done with a consultant physician. When patients did their screening blood tests it was a good sign that they most likely wanted to participate in the study.

Components may work independently or interdependently. There were occasions when patients were delayed for CT scans, because of either emergency cases brought in which obviously took priority, and on one occasion the CT scanner was malfunctioning and all the inpatients were shifted to the study planned second CT scanner, which again resulted in urgent patients taking priority over the study patients, rescheduling an appointment for the CT scan had to be made with an extra

transport cost and was inconvenient to the patient especially if the patients were working and had to take time off work. This can be a problematic issue if the next day is an official holiday or falls on a weekend, therefore one can miss the window period of ±3 days of the planned study visit. The number of CT scan visits by the participants coincided with the clinical visits, which participants did not mind, as long as the cost for transport was covered by the study group (Campbell *et al.*, 2007).

4.3.6 Will the participants accept randomisation into the standard care group or Teriparatide group?

The participants in our study group preferred to be randomised into the Teriparatide treatment group, because of the patient information sheet stating that Teriparatide increases calcium in bone and may strengthen your bone leading to faster recovery from fracture. But they all continued in the study when they were randomised into the standard care group. Randomisation was carried out in advance as we were informed that the server would be down intermittently during our study for routine maintenance, so in order to avoid being held up with a patient in the clinic and not able to randomise, we carried out randomisation of all the expected participants in advance (Watson and Torgerson, 2006). One of the flaws of this feasibility study is that we did not know if patients would be happy to be randomised to a standard care group and placebo injections.

4.3.7 What are the characteristics of participants in the study?

All the patients recruited in the study were motivated to be in the study and the demographic characteristics are summarised in Table 3-1. 70% of the participants were female and 30% were male in the study. Most of the participants were above 60 years old, which is 10 years older than the 50 years old inclusion criteria.

4.3.8 Willingness of clinicians to recruit participants?

Most of the clinicians showed willingness to participate in the study. However, there were some clinicians who showed some concerns about the study and who

asked for evidence of ethical approval from the regulatory authorities, which was presented to them.

4.3.9 What are the issues of self-injection with participants to Teriparatide?

Keeping medication in a temperature control environment was a concern especially when travelling abroad in the summer-time. We did have an issue with one of the patients who stored the needles instead of the syringes containing the medication in the fridge but this issue was resolved within 48 hours as the patient had an emergency contact number and was advised to replace the medication syringes, and no adverse effects were observed. The official website for Forteo recommendation was for daily use "Forteo should be refrigerated, when traveling and if refrigeration in was not available, the Forteo drug can remain at room temperature conditions (77^{o} [$25^{o\ C}$]) for up to a total of 36 hours per device" (https/www.forteo.com/taking-foteo/how-to-inject).

4.3.10 Fracture healing parameters

Plain radiographs are still most widely acceptable technique for assessment of bone fracture healing, because they are the cheapest and fastest imaging modality. Plain radiographs are a two-dimensional image assessment of a three-dimensional object, thus much of the information can be lost in the process. Also getting a 3D fracture alignment in subsequent radiographs is not possible, which leads to a dilemma of "Are we assessing changes accurately of the same point over time". Corrales et al, in their systemic review of radiographic criteria of fracture union reported the most common to least common criteria used to define fracture union in the articles published (Corrales et al. 2008). We used the most common fracture healing criteria and defined them on the images in Table 2-7, as we could not find any standard universal definitions of radiographic criteria. The continuous parameters were given the scale from 1 to 5 and the dichotomous were reported as yes or no (0= no and 1 =yes). The agreement varied, Kappa ranged from poor to very good with different raters (Table 2-6), although scaphoid bone fracture healing is different from long bone fracture healing as the scaphoid is an intra-articular bone and does not have the same periosteum coverage, hence it is not likely to form external callus. Subject to variations in Kappa agreements we did not exclude any parameters in the ankle fracture healing scoring table. Once ankle fracture CT scans were available, we were able to define different fracture healing parameters for ankle fractures. Our readings were continuous over time scale where the difference from one point to the next point could be very small or even none at all. The raters were blinded to the treatment allocation when assessing the CT scans.

The difference between the means at the beginning and at the end of each time point was very small as can be seen in the Bland Altman graph (Figure 3-14).

Defining different parameters of the fracture healing process radio-graphically is a challenging task, e.g. soft callus is difficult to distinguish from haematoma, trabecular bridging from callus formation is another problem as we found in this study, where we found poor inter-rater correlation for this parameter. These parameters become more challenging to quantify as the fracture line becomes more

complex and the healing varies at different points along the fracture line adding complexity.

The RUSH (radiological union scoring of hip) scoring system measures the end points of cortical bone union on two radio-graphical views (Morshed, 2014). The issue is, are the same points on the fracture line being assessed constantly over time?. Also, if the effect of the drug on fracture healing is being evaluated then, where does it act, is it on cortical bone, trabecular bone or callus formation?.

Callus formation does not necessarily mean that a fracture is uniting, e.g. in hypertrophic non-unions there is callus but the bones are not united (Panagiotis, 2005). Thus if in a scoring system, callus is given a numerical value, and say for example that more callus is a higher value than less callus, then it is possible, that healing could be over-scored, when in fact the excessive callus formation is a sign of delayed union.

Regular follow up of fracture healing over time using CT scans, has not been studied or reported in the literature, so we could not compare our results to others. The fracture healing scoring table that we devised, examines the fracture healing process, and it is a three-dimensional assessment along the fracture line. The table had an internal consistency and Cronbach's alpha was in the range between 0.70 and 0.95, for both raters (AA and WAW), that is the recommended range (Tavakol and Dennick, 2011).

The revised scoring table showed internal consistency and relatedness of the items for both raters individually, by removing each item in the scoring table the Cronbach's alpha does not fall out of the range of 0.70 to 0.95 again showing internal reliability for each items (Table 3-14, Table 3-16). However, the scoring between two raters had poor to good Kappa values, and so despite our best efforts, we did not end up with a reliable or user friendly, fracture healing outcome measure, which would reliably state that fracture healing was accelerated or delayed.

4.3.11 Quantification of CT scans using Matlab™ and ImageJ™

Computational resources for image quantification are fast, repeatable and objective measurements. This is a unique feasibility study where fracture healing was followed up with CT scans every two weeks for 12 weeks. Matlab and ImageJ software programmes were used on CT scans of a distal fibular fracture (Weber B ankle fractures) to quantify the healing. Trabecular bone region, callus formation and cortical bone changes were assessed quantitatively using Matlab™ and ImageJ™ software. Each of the following parameters cortical bone, mineralized callus and trabecular bone regions were followed with CT scans and changes were quantified. The changes in the number of voxels in a specified range of thresholds for the cortical bone, callus and trabecular region were quantified along a time-line of every two weeks in a fixed volume of interest. As the fracture heals there are no distinct lines demarcating cortical bone, mineralised callus and trabecular bone. The mineralized callus and trabecular bone regions contain blood, fat and bone it is very difficult to separate them apart. Hounsfield unit is a measurement of intensity in a pixel or voxel. The shift of intensity (which is measured by Hounsfield unit) on the images from cortical bone, callus and the trabecular bone region is a gradual and continuous making range of intensities overlap between the cortical bone, mineralised callus and trabecular bone region. Defining a volume of interest (VOI) and matching the same volume of interest in the subsequent images is very central for measurements of numbers of voxels as a slight rotation between the images can change the number of voxels in the region of interest leading to large changes in the specified range of intensities. The threshold range was selected and the software applied it making it a more robust and objective way of assessing the number of voxels in a particular range of intensity in the volume of interest. Others have used segmentation method by choosing the volume of callus this can be misleading as the it is difficult to draw a line between the mineralised callus and bone cortices because the intensity ranges overlaps. Also, a static image is easy to measure as it gives the same results if repeated and the same parameters are instructed to the software programmes the difficulty is when the images change over time and how to measure these differences for the same objects. The difference has to be larger than the

differences due to noise and margin of error. As callus is formed it starts with density of soft tissue and this density gradually increases until cortical bone is formed. Porter et al used a software programme called OrthoRead, which had less than a 5% error in measuring surrogate callus, and it was insensitive to changes in image resolution, image rotation, and the size of the analyzed region of interest. This 5% error can vary with size of the region of interest chosen for quantification (Porter *et al.*, 2016). Computational reading of medical images needs further exploration as there are various factors influencing the reading of the images.

4.4 Patient reported outcome measures

Patient reported outcome measures are extremely important as they can assess the patient's perspective of treatment, which can be different from the health care provider's perception. In recent years regulatory authorities are mandating to include PROMS in the clinical trials for development of drugs. Also, studies which have the patient perspective measured are more likely to be sponsored and approved by health regulatory authorities. In a survey conducted by Zwiers et al, out of 188 who completed their questionnaire, 72% reported using PROMS for research, 39% for routine patient care, 34% for registration of quality or quality care and 17% did not use PROMS. There was no consensus on which PROMS to use, even the most common PROMS mentioned were only 9.7%, which were FAOS and MOXFQ there was a large variability (Zwiers *et al.*, 2018).

The Olerud Molander ankle function score is a validated and much used ankle function scoring system. It measures nine aspects of ankle function which are pain, stiffness, swelling, stair climbing, running, jumping, squatting, use of support, work and activities of daily living, and combines them into a cumulative score at the end from 0 to 100, were 0 is the worst score and 100 is the best score(Olerud and Molander, 1984). Our patients found it easy to complete and user friendly.

The Minimal Clinically Important Differences (MCID) is defined as "The smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management." (Cook, 2008). This is an important concept used to determine if the effect of intervention used improved the perception of outcome by the patient. MCID can be defined at individual level or group level. At individual level larger changes may be required for the individual to consider as a noteworthy change however at the group level although changes can be small but the effect can be large on the population.

Neilson et al reported that the smallest change that indicates a real change of clinical interest for a single subject is 12 points and 15.5% in OMAS score (Nilsson, Eneroth and Ekdahl, 2013). The mean difference of OMAS score between the Standard care

group and Teriparatide group at 6 weeks that is when the cast was taken off was 11 points keeping in mind that the size of the groups was small (5 participants in each group) and the score was the group mean not an individual score which can have big effect on population however this needs further larger sample size groups to validate. There could be downsides with MCID when patient reported outcome are measured. These are: inability of the participants to understand the context of improvement, the subjects can not recall their previous status to compare the changes that is recall bias, influence of baseline severity of the condition, age of the participants older age groups more likely to have comorbidities than younger age group, socioeconomics status of the participants and education levels. Other problems in calculating the MICD is the wide range of the scores or the outliers, ceiling and floor effects (Cook, 2008).

Two RCTs, many case series and case reports have shown acceleration of fracture healing, but to the best of our knowledge this is the first feasibility study reporting the use of Teriparatide to accelerate fracture healing in ankle Weber B fractures. We recommend using the Olerud and Molander ankle function scoring system in any future statistically powered study.

4.5 EQ-5D-5L Health status

EQ-5D-5L quality of life scoring system is a valuable health status indicator for an individual. The National Institutes for Health Excellence (NICE) guideline recommends all health effects should be reported in quality-adjusted life-years (National Insitute for Excellence in Health Care, 2018). There are two types of Quality of life assessment tools (QOL) 1-generic tools 2-specific tools to the disease. Generic tools cover physical wellbeing, functional and psychological aspects of health. EQ-5D-5L is a generic quality of life assessment tool which assess the quality of life using five dimensions of health: mobility, self care, usual activities, pain/discomfort, and anxiety/depression it is a valid and reliable measure in many conditions. We have reported a value set for the EQ-5D-5L for feasibility study of ankle fractures treated with Teriparatide group and standard care group. Although the groups are small, we could see the mean over all self rated health status is very similar between the standard care group and the Teriparatide treatment group until the cast is removed

which is at TV3, some effect on quality of life could be seen in Teriparatide treatment group at TV4 which is the next visit after removal of cast. However this yet needs to be studied further in the larger groups. We could not compare the five dimensions individually with the two study groups due to small number of participants. In a multinational study the values attributed to the different health states of the EQ-5D-5L questionnaire vary substantially by country. When analyzing a multinational clinical trial several relevant value sets should be used to judge the treatment effect and not only one set as it is commonly done (Gerlinger *et al.*, 2019). Should these value sets be applied according to the country or ethnicity of the participant or to the country of participant residence and how long should the participant reside in the country before applying the value sets this need further exploration.

EQ-5D-5L with five domains and five level responses was developed to reduce the ceiling and flooring effect of the EQ-5D-3L. The EQ-5D-5L describes more distinct health states. The existing 3L and 5L valuation tariffs, cost-effectiveness analyses using different versions of the tool are likely to lead to different results and possibly different decisions on the cost-effectiveness of interventions. Cross walking from EQ-5D-5L to EQ-5D-3L seems to be a minor change but these can have huge impacts if conclusions are reached as they give different tariffs (Round, 2018).

Saper et al., stated difference in reported pain by ethnicity, particularly black finding that black patients have a lower pain threshold than white or latino patients given the same injury (Saper *et al.*, 2015). In our feasibility study the pain reported showed a lower trend in the Teriparatide treatment group and the scores remained in the lower range than those of the standard care group even after removal of the cast/walking boot at TV3. However, all of our participants were ethnically white in both study groups.

4.6 Qualitative study

Qualitative research covers a wide range of aspects in clinical trials, intervention being trialled, trial design, trial process and conduct, outcome of the trial, measures used in the trial, and conditions of trial (O'Cathain *et al.*, 2013). Randomised controlled trials which integrate qualitative parts have the potential to optimise the intervention, intervention delivery and acceptability, making trial recruitment and conduction efficient and acceptable to participants, facilitating interpretation of findings, make trial team more sensitive to need of participants, saving resources by directing them efficiently in future trials (Donovan *et al.*, 2002). Having a participant's views on the trial and how the intervention is implemented, how the different components of the intervention are perceived, and factors that affect the intervention, can have huge impact on improving subsequent larger trials.

Participants in the study were eager to fall in the Teriparatide treatment group, which may reflect how they were recruited and what was written in the participant information sheet. It may imply that they perceived the benefits of Teriparatide self-injection, namely the possible acceleration of fracture healing and bone strengthening effects, as a positive benefit of participating in the study. This is also the perception of the physicians prescribing Teriparatide for acceleration of fracture healing and for non-union or delayed union cases of fractures (Figure 3-6). It is speculated that participants gained their information about Teriparatide from the participant information sheet and additionally made them adhere to the trial visits without any dropouts to gain this benefit of bone strengthening

Example quote from participant:

"That was one of the main reasons that I volunteered to take part in the feasibility study because I was hoping to have the injections".

The other perceived advantage participants were expecting to get is monitoring them closely with regular visits and having checked by CT scans which also is a reason to adhere to the trial visits without dropouts (Table 4-1).

Example quote from a participant,

"I think in the later weeks it's just been monitoring me. In the earlier weeks it was just monitoring the plaster."

In study a conducted by Bevan et al on patients' attitudes to participation in clinical trials, for patients involved in the trial (n=66), 62% wanted to help people, and 39% wanted to improve their own treatment (Table 4-1) (Bevan *et al.*, 1993) .

Table 4-1 Reasons given by patients for participating in therapeutic trials (Bevan *et al.*, 1993)

Reasons for participating in the study	Patients involved in the trials Group A=66	Patient who would or might volunteer for a future hypothetical trial Group B = 83
To help people	41 (62%)	47 (57%)
To improve their own treatment	26 (39%)	35 (42%)
Because asked by the doctor	25 (38%)	1 (1%)
To obtain information about disease	3 (5 %(	1 (1%)
Out of gratitude to hospital	3 (5%)	1(1%)
Persuaded by friend/ family	1 (2%)	0 (0%)
To pass time	1 (2%)	1 (1%)
Curiosity	1 (1%)	2 (2%)
Because against animal experiments	0 (0%)	1 (1%)
Don't know	0 (0%)	4 (5%)

Patient acceptance to participate into the study gives an indication of acceptability of the concept of taking Teriparatide drug to accelerate fracture healing. This could apply to other medications to treat other conditions.

Participant compliance to trial visits is crucial to the success of the trial and outcomes. Unlike standard care visits participants required to attend extra visits at specific times, complete additional forms about in between visit status and record

any possible side effects; all these activities are indirect measure of willingness of participants to adhere to the clinical trial visits.

Participants had concerns over radiation exposure from the CT scans during the trial. The radiation risk from exposure to CT scans was calculated by the medical physics department before the trial and the risk was considered low and this was clearly addressed in the participant information sheet. However beliefs about possible radiation harmful effects are difficult to deal or challenge. It was also noticed traveling to and from hospital was an obstacle for patients to participate in the study (Table 4-2) (Bevan *et al.*, 1993).

Example quote from participants

"It's in the back of your mind that you might have had too much radiation. I think that having them every two weeks was quite reassuring, that things were healing as they should have been".

Example quote from another participant

"I'm not an expert, I don't know how much radiation my body should be taking and things like that. I'm just trusting medical science that the radiation I've taken isn't too much".

Radiation exposure and side effects were among the aspects disliked by the patients in the trial, were also reported by Bevan et al in their study (Bevan *et al.*, 1993).

Table 4-2 Aspect of therapeutic trials disliked by patients in response to leading questions (Bevan *et al.*, 1993).

	Patient involved in the trial Group A N=66	Patient declined involvement in the trial Group B N=12	Other unselected out-patients who would not or might not volunteer for a future hypothetical trial. Group C. N=45
Potential side effects of study treatment	16 (25%)	7 (58%)	39 (87)
Taking a new tablet	11 (17%)	7 (58%)	37 (82%)
No potential benefit to the participant	11 (17%)	6 (50%)	35 (78%)
Stopping current medication	5 (8%)	5 (42%)	33 (75%)
Large time commitment	12 (18%)	5 (42%)	19 (42%)
Overnight hospital stays	2 (3%)	6 (50%)	21 (47%)
Travel to and from hospital	12 (18%)	4 (33%)	13 (29%)
Venepuncture	13 (20%)	0 (0%)	5 (11%)
X-rays	6 (9%)	0 (0%)	7 (16%)
ECGS	3 (5%)	0 (0%)	6 (13%)
Urine tests	2 (3%)	0 (0%)	4 (9%)

Retaining participants in clinical trial can have an impact on the trial's outcome. Although retention rate in the trial was 100%, which was more than satisfactory, we tried to understand more about the trials visit adherence. We believe that the visits were not too close, so the patient did not become fed up or tired, and nor were they too far apart to lose interest in follow up. The patient group was 50 years and older, and so most of them were not working or were on sick leave due 1to the fracture, making it easier time wise to attend the study visits. Personal time commitment to research or trial visits is believed to be limiting factor to enrolment into studies. Hollanda et al reported higher score on personal time of the non-enrolling group 3.7 ± 1.6 than enrolling group 2.0 ±1.2 in successful patient

recruitment in CT clinical trials (Hollada *et al.*, 2014). Time, was mentioned by participants as a factor in their making of decision for enrolment in the study. It was more suitable for a retired patient to enrol into the study than for a patient who has work commitments.

Example quote,

"think two weeks is sufficient because if you did it weekly it would be too much to try and, I think two weeks is sufficient because it gives you time to rest in between each visit".

Quality of care or quality of delivering research to participant is intangible, which most clinical studies do not evaluate that can have direct and indirect impact on research. A direct impact is that the participant may withdraw from the study and therefore increase the cost of the study, and indirect is by spreading the word on how bad the study is thus deterring other potential patients to participate in the study.

Patient decisions are made depending on their perception, values, expectations and background. Persons who come first in contact with patients are crucial for patient satisfaction. When participants attend a trial visit, they expect that everyone is aware of the trial being conducted. The first contact points are usually the receptionists. These expectations of the participants should be addressed in future trials in a study conducted by Wei et al, an uncaring attitude of front desk managers were highest complaints 26%, communication problems were 17.5%, unsatisfactory quality of treatment and competence was 26.5%, process of care 13.0% respectively (Wei *et al.*, 2018).

Example quote from participants who had a negative experience,

"It is sort of a notorious hospital parking and getting here and then finding it and then going to reception and then reception haven't got your notes and they don't know what's happening and they'll send you to the cubicles and then they'll find out that you've been looking for me in the waiting room, so the logistics can be a bit frustrating sometimes."

In hospital quality of care is divided in clinical quality (e.g. medical procedures, results of test, diagnosis) and process quality (e.g. physicians bedside manners, wait time to see the physicians, kindness and aptitude of the physicians and staff) clinical quality is difficult for patient to assess as this is more of technical, which assessed by professionals in their field. But process quality which is the process of medical care delivery to the patients, depends on their perception, values, expectation and background (Marley, Collier and Meyer Goldstein, 2004). From the quotes we can conclude participants were worried about waiting time, which they felt that they did not know what do or expect as they were left alone, physicians being always there had reassured them that they would be seen on time. These are some of the thoughts going on in the minds of patients waiting to be seen in out-patient clinics, which if addressed can improve quality of care and make sure that participants end up with a positive experience going through the trial visits.

Example quotes from participants,

Quote from participant A

"You were there every time and that was our biggest worry, we didn't want to be coming on a regular basis and then sat here waiting and you were always there. It was faultless. Really good"

Quote from participants B

"No, I don't this so, as I said I everybody I've encountered has been very helpful, you know, explained what is happening, no I wouldn't say there's any negatives at all. I mean, you've been very explicit in terms of how you've explained things, what's happening and so on. And so from that point of view I've quite enjoyed being part of the trial."

It is important to know what the experiences of participants going through the period of clinical trial are and address them as soon as possible. However some of the components of quality process can be addressed e.g. timeliness, organization and cleanliness, whereas others are more difficult e.g. kindness, concern and bedside manner which takes time to learn and adopt by the researchers (James, Calderon and Cook, 2017). These elements of quality process also depend on the

perception, values, expectations and background of all the research personals staff who are interacting with the participants in the study.

5 Chapter 5 - Limitations and future work

This thesis described a survey and a feasibility study to investigate the usage of Teriparatide to accelerate union of ankle Weber B fractures in elderly.

Limitations:

- The survey in this study was carried out in UK and GCC countries. Expanding the survey to other countries would be costly and time consuming, thus with the limited funding and time available, carrying out the survey in other countries was not possible.
- The outcome of the survey reported, is limited to a specific time and countries. However, it would be interesting to know how Teriparatide is prescribed as off label in other countries, and how its usage to accelerate fractures healing is perceived.
- Patients' willingness to participate in the study did not seem to be limiting factor in this study. Out of 81 patients screened with ankle Weber B fracture who were 50 years old and above 62 (77%) were willing to participate in the study, but due to exclusion criteria and comorbidities they were excluded. Comorbidities associated with aging were the main limiting factors in recruitment.
- Participants were willing to be randomised, however, they preferred to be in the Teriparatide treatment group. The 50:50 chance of being randomised into receiving Teriparatide injection was the main reason for them agreeing to participate in the study. We do not know whether this would have been the case if there had been a placebo injection group.
- Willingness of clinicians to recruit participants could be an issue in other medical centres especially, if they perceive Teriparatide to be high risk of side effects and obligation to patient safety.
- The number of eligible patients was a limiting factor with recruitment of participants in the time frame of the study.

- Some blood test results were abnormal but clinically not significant. Resolving whether to include such patients into the study or not was time pressured as the protocol stated that this had to be done within 10 days of the fracture.
- The cost of Teriparatide was one of the limiting factors in determining the sample size of this study. It is a major limitation of the study, and the Nottingham Research Design Service, recommend approximately 20-30 participants in each arm of a feasibility or pilot study designed to investigate outcome parameters. Large additional costs were the CT scans, staff costs, and number of visits. From this feasibility study we can conclude that, trial visits can be reduced to three trial visits only, base line visit as 1st visit, second visit at four weeks and 3rd visit at six weeks. Hence, the number of CT scan can be reduced also to three CT scans.
- Plain X-rays in the standard care group and the Teriparatide group were not done routinely at standard care visits as part of standard care.
- As there were no gold standards for the assessment of fracture healing parameters, it was difficult to compare the fracture healing parameters assessed in the study with other studies.
- There were differences in sample size calculation for statistically powered RCT with regards to parameters used and between the two raters. For a future RCT we did calculations of sample size with different scenarios. To detect the minimum effect between the standard care group and Teriparatide treatment group, with a 95% confidence interval (2-sided), power 80% and sample size ratio of 1, Table 3-24 shows the sample size required to detect a minimum difference for each parameter at 6 weeks. We considered the difference at 6 weeks, which was at trial visit 4, because normally the cast or the walking boot would be taken off at this time, and the fracture assessed. The two raters differed in the sample size estimations. This issue can be resolved either by taking the mean sample size of the two raters, or by taking the largest sample size of the two raters. We recommend mineralised callus, total fracture healing score and overall fracture healing parameters to be used for any future statistically power study. Table 3-25 shows the sample size, required to detect

the minimum difference with a statistical power, for the quantitative assessment of the parameter; we recommend mineralised callus and cortical bone as the best parameters to use.

- The reading of X-rays or CT scans by Matlab™ and ImageJ™ software is still in its early stages of development but our methods showed promising results, which warrant further exploration.
- Due to the time taken for evaluating CT scans and the outcomes from this study it Is recommended that mineralised callus, cortical bridging and fracture line only are taken into account for assessing in fracture healing.
- Compliance of the patient with Teriparatide self-injection may be an issue. Our participants documented their daily self-injection of Teriparatide in a paper logbook, and the testing of blood and urine were not practical as metabolite levels raises after 12 weeks, which was beyond our period of the study.
- Due to the cost, we were unable to have a placebo injection group.

Future work

1- A multicentre study would increase the rate of recruitment of patients into a study. The study would have to have a placebo arm, and this would require further feasibility work, before an RCT could be considered.

2- Investigating the reasons for potential patients, who said no to participation into the study, is important.

3- There is a further need to explore the optimum radiographic parameters of fracture healing.

4- The quantitative assessment method of fracture healing needs further development.

5- Investigating improved software that can be used in assessment of fracture healing parameters is recommended.

6- There is a need to explore the effect of a placebo injection on patients' willingness to participate in the study. Additionally, what is the effect of placebo injection on fracture healing?

7- Exploration of Teriparatide usage in acceleration of other types of fractures e.g. stress fractures, scaphoid fractures, spine fractures and non-unions etc.
8- Teriparatide usage for acceleration of fractures in younger age groups.
9- Exploration of other drugs as comparator e.g. bisphosphonates, Bone morphogenic proteins etc.

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7 Appendices

Appendix A **Booklet of survey of usage of Teriparatide among physicians**

The University of Nottingham

Survey of usage of Teriparatide (Forsteo/ Forteo) to accelerate fracture healing

Researchers: Prof Brigitte Scammell, Dr Adel Alshaikh, Prof Angus Wallace, Prof Opinder Sahota, Dr Richard Pearson

We would like to invite you to take part in this research survey. Before you decide take part, we would like you to understand why the research is being done and what would be your involvement in it. One of the team members can go through the questions with you and answer any questions you have (see contact details at end). Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
The purpose of this study is to explore if Teriparatide is being used off label in an attempt to accelerate fracture healing, and if it is, what are the doctors' perceptions regarding it's usage to accelerate fracture healing, and what are the barriers for prescribing it? This information will expand our knowledge and will prepare us for better planning of future studies with Teriparatide.

Why have I been invited?
At this stage in our research we don't know who has the knowledge and who prescribes the drug. We are inviting doctors we think are likely to prescribe Teriparatide 1-34. We are sending you this survey questionnaire because you are a doctor and sharing your knowledge and experience is highly valuable for this research.

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?
Nothing will happen to you. Your answers and identity will be kept confidential at all times.

Expenses and inconvenience allowance
There are no reimbursement plans for participating in this study.

What are the possible disadvantages and risks of taking part?
There are no foreseen risk or disadvantages for participants taking part in this survey

What are the possible benefits of taking part?
There is no personal benefits a part from the information which provided to you with this study.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice were all the information that identifies you will be handled in confidence. If you join the study, some parts of the data collected for the study will be looked at by authorised personal from the University of Nottingham who are organising the research. The data may also be looked at by an authorised personal to check that the study is being carried out correctly. All will have the duty of confidentiality to you as a research participant and we will do our best to meet this duty.
Your personal data (email address, telephone number) if known to us will be kept for 12 months or less after the end of the study so that we are able to contact you about the findings of the study and possible follow up studies (unless you advise us that you don't wish to be contacted). The research data will be kept securely for at least 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

We will handle, process, storage and dispose of your data by meeting the requirement of the data protection Act 1998.

What will happen if I don't want to carry on with the study?
Your participation is voluntary and you are free to withdraw at any time, without giving any reason. However the information collected so far cannot be removed and may still be used in the study. Once you have returned your questionnaire back to us, we will give you a unique participant ID and this will be into the database instead of your name to make sure you are anonymous on our system. This is because once the data has been entered into the secure computer system and made anonymous and backed up by the University of Nottingham, which cannot be interfered with.

What will happen to the results of the research study?
The results of the study will be written as part of an educational qualification that is usually published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the participants involved in the survey will be identified in any report or publication. Should you wish to see the results, or the publication, please ask a member of the research team.

Who is organising and funding the research?
This research is being organised by the University of Nottingham.
The funding for this research is part of the student research fund.

Who has reviewed the study?
All research in the University of Nottingham is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Faculty of Medicine & Health Sciences (FMHS) Research Ethics Committee.

Dr Adel Alshaikh
Clinical Research fellow (PhD student)
Academic Orthopaedics, Trauma and Sports Medicine
Division of Rheumatology, Orthopaedics and Dermatology School of Medicine
University of Nottingham
C Floor, West Block Queen's Medical Centre
Nottingham NG7 2UH
Tel: +44 (0) 115 82 31115 FAX: +44 (0) 115 82 31118
E-mail: msxla@nottingham.ac.uk

Introduction:
Teriparatide (Forsteo/ Forteo) is a bone anabolic drug that has been recently approved for the second line treatment of osteoporosis. Some studies have reported accelerated fracture healing with Teriparatide treatment. Teriparatide is not licensed for use in the treatment of fractures, so such use is 'off label'. We are carrying out a survey of doctors to find out the following:

1-Are doctors involved in fracture treatment prescribing Teriparatide to accelerate fracture healing?
2-What are doctors' perceptions with regard to the use of Teriparatide to accelerate fracture healing?
3-What are the barriers to prescribing Teriparatide to accelerate fracture healing if licensed?

The University of Nottingham

Survey of usage of Teriparatide (Forsteo/ Forteo) to accelerate fracture healing

1-How many years have you been practicing medicine?

- ☐ < 5 years
- ☐ 5 to < 10 years
- ☐ 10 to < 15 years
- ☐ 15 to < 20 years
- ☐ 20 to < 25 years
- ☐ 25 to < 30 years
- ☐ 30 years and above

2-In which country are you practising medicine?

- ☐ United Kingdom
- ☐ Kingdom of Bahrain
- ☐ If other, please specify _____

3-What are your qualifications please? (You can choose more than one option)

- ☐ Bachelor of Medicine and Surgery (MBBS)
- ☐ Master's Degree
- ☐ MRCP
- ☐ MRCGP
- ☐ MRCS/FRCS
- ☐ PhD
- ☐ If other, please specify _____

4-What is your medical specialty?

- ☐ Endocrinology / metabolic bone disease
- ☐ Trauma and Orthopaedics
- ☐ Health Care of the Elderly
- ☐ Sports medicine
- ☐ Rheumatology
- ☐ General practice
- ☐ If other, please specify _____

5-Where do you classify yourself with regard to prescribing Teriparatide (Forsteo/Forteo) in your practice?

- ☐ I do prescribe Teriparatide (Forsteo/Forteo) in my practice
- ☐ I have heard of it but I do not prescribe it (✪ If ticked then go to question 9)
- ☐ I have never heard of Teriparatide (✪ If ticked then go to end of page and thank you)

6-Do you prescribe Teriparatide for any of the following osteoporosis groups? (You can choose more than one answer)

- ☐ Treatment of osteoporosis in postmenopausal women
- ☐ Treatment of osteoporosis in men at increased risk of fracture
- ☐ Treatment of corticosteroid-induced osteoporosis
- ☐ I do not prescribe Teriparatide for osteoporosis
- ☐ If other, please specify _____

7-Where do you classify yourself with regard to Teriparatide (Forsteo/Forteo) usage in the treatment of fractures? (You can choose more than one answer)

- ☐ I prescribe it in my practice because there is high quality evidence for its usage in the treatment of fractures
- ☐ I only prescribe it for the treatment of fractures as part of a research project
- ☐ I only use it for exceptional individual cases
- ☐ I prescribe it for delayed union of fracture
- ☐ I prescribe it for non-union of fracture
- ☐ I prescribe it for acceleration of fracture healing
- ☐ I do not prescribe for treatment of fractures
- ☐ If other, please specify ----------

8-If you prescribe Teriparatide, for treatment of fracture for which group of patients do you prescribe it? (You can answer more than one)

- ☐ Elderly
- ☐ Adults
- ☐ Athletes

9-Do you think Teriparatide accelerates fracture healing?

- ☐ Yes
- ☐ No
- ☐ I am not sure

10-What do you think the barriers are to prescribing

- ☐ Teriparatide to patients for fracture healing?
- ☐ This indication is off label
- ☐ The drug is in an injection form
- ☐ Cost of the drug
- ☐ Possible side effects
- ☐ Training of the patient to self-inject
- ☐ Patients are reluctant to use self-injection
- ☐ I do not know
- ☐ Other reasons _____

End

Please drop your completed survey in the box at the registration desk or post it to the address overleaf

Thank you for completing the survey

Appendix B - Survey ethics approval reference letter No: M0605216 SoMOTSM

The University of Nottingham

Faculty of Medicine and Health Sciences

Research Ethics Committee
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham
NG7 2UH

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

27th May 2016

Dr Adel Alshaikh
Clinical Research Fellow/PhD Student
Academic Orthopaedics, Trauma & Sports
C Floor, West Block
School of Medicine
QMC Campus
Nottingham University Hospitals
NG7 2UH

Dear Dr Alshaikh

Ethics Reference No: M06052016 SoMOTSM – **please always quote**
Study Title: Survey of usage of Teriparatide 1-34 hormone (Forsteo/Forteo) to accelerate fracture healing.
Chief Investigator/Supervisors: Professor Brigitte Scammell, Professor of Orthopaedic Sciences, Professor Angus Wallace Emeritus Professor of Orthopaedic & Accident Surgery, Academic Orthopaedics , Trauma and Sports Medicine, School of Medicine
Lead Investigators/student: Dr Adel Alshaikh, Clinical Research Fellow/PhD Student, Academic Orthopaedics , Trauma and Sports Medicine, School of Medicine.
Other Key Investigators: Professor Opinder Sahota, Honorary Professor of Orthogeriatric Medicine/Consultant Physician Health Care of Older People, Dr Richard Pearson, Senior Research Fellow, Academic Orthopaedics , Trauma and Sports Medicine, School of Medicine.
Type of Study: PhD project, questionnaire
Proposed Start Date: 1/6/2016 **Proposed End Date:** 30/11/2016
No of Subjects: 2000+ **Age:** 18+ yrs

Thank you for submitting the above application which was considered by the Committee at its meeting on 10th May 2016 and the following documents were received:

Use of Teriparatide:

- FMHS Research Ethics Application form version 1.0 date: 01/05/2016
- School of Education – PGR statement of Research Ethics
- Study Protocol version 1.0, Date 01/05/2016
- Invitation letter version 1.0 Date: 01/05/2016
- Participant Information Sheet version 1.0 Date: 01/05/2016
- Information and consent page for an Online Survey/Questionnaire, version 1.0 Date: 01/05/2016
- Revised Information and consent page for an Online Survey/Questionnaire, version 2.0 Date: 25/05/2016
- Survey of usage of Teriparatide 1-34 hormone (Forsteo/Forteo) to accelerate fracture healing, version 1.0 Date: 01/05/2016.
- Revised Survey of usage of Teriparatide hormone (Forsteo/Forteo) to accelerate fracture healing version 2.0, Date 25/05/2016

These have been reviewed and are satisfactory and the study is approved.

The University of Nottingham

Approval is given on the understanding that the conditions set out over the page are followed:

1. A Favourable opinion is given on the understanding that all appropriate ethical and regulatory permissions are respected and followed in accordance with all local laws of the country in which the study is being conducted and those required by the host organisation/s involved.
2. Please can you submit copies of letters/e-mails from the Medical Associations when these are available for our records please.
3. You must follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).
4. You must notify the Chair of any serious or unexpected event.
5. This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.
6. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

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Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix C - Survey ethics amendment approval reference letter No. M06052016

University of Nottingham
UK | CHINA | MALAYSIA

Email: FMHS-ResearchEthics@nottingham.ac.uk

Faculty of Medicine & Health Sciences
Research Ethics Committee
c/o Faculty PVC Office
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH

30 August 2017

Dr Adel Alshaikh
Clinical Research Fellow/PhD Student
c/o Professor Brigitte Scammell
Professor of Orthopaedic Sciences
Academic Orthopaedics, Trauma & Sports
C Floor, West Block
School of Medicine
QMC Campus
Nottingham University Hospitals
NG7 2UH

Dear Dr Alshaikh

Ethics Reference No: M06052016 – **please always quote**
Study Title: Survey of usage of Teriparatide 1-34 hormone (Forsteo/Forteo) to accelerate fracture healing.
Chief Investigator/Supervisors: Professor Brigitte Scammell, Professor of Orthopaedic Sciences, Professor Angus Wallace Emeritus Professor of Orthopaedic & Accident Surgery, Academic Orthopaedics , Trauma and Sports Medicine, School of Medicine
Lead Investigators/student: Dr Adel Alshaikh, Clinical Research Fellow/PhD Student, Academic Orthopaedics , Trauma and Sports Medicine, School of Medicine.
Other Key Investigators: Professor Opinder Sahota, Honorary Professor of Orthogeriatric Medicine/Consultant Physician Health Care of Older People, Dr Richard Pearson, Senior Research Fellow, Academic Orthopaedics , Trauma and Sports Medicine, School of Medicine.
Type of Study: PhD project, questionnaire
Proposed Start Date: 1/6/2016 **Proposed End Date:** 30/05/2018
No of Subjects: 2000+ **Age:** 18+ yrs

Thank you for notifying the Committee of amendment no 3: 23/08/17 as follows:

- Time Extension with a new end date of 31/05/18 in order to complete data collection.

These have been reviewed and are satisfactory and the study amendment no 3: 23/09/2017 has been given a favourable opinion.

A favourable opinion is given on the understanding that the conditions set out below are followed:

1. You should follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).
2. You must notify the Chair of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (please request a form).

Yours sincerely

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix D - Audit registration and approval letter

Nottingham University Hospitals NHS
NHS Trust

Miss Jess Nightingale

Research Coordinator
Trauma and Orthopaedics
C Floor, West Block, QMC
Nottingham University Hospitals
Derby Road
NG7 2UH
0115 924 9924 (ext. 67502)
Jessica.nightingale@nuh.nhs.uk
10th October 2015

Dear Dr Alshaikh

Registration confirmed: An audit of osteoporosis incidence in an adult fracture clinic

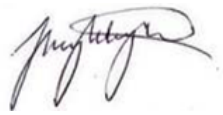
Thank you for submitting your clinical audit registration form.

The form has been reviewed and your project has been registered within the audit service. The project has been approved and this approval gives you permission to complete the project as described in your registration form.

Your project number is: 15-14

If you require further information do not hesitate to contact us.

Yours sincerely



Jess Nightingale
Research Coordinator
Trauma & Orthopaedics

On behalf of
Mr Ben Ollivere
Consultant Orthopaedic Surgeon
Clinical Audit Lead for Orthopaedics

Nottingham University Hospitals NHS
NHS Trust

Study Title: A feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people

Emeritus Prof W Angus Wallace, Dr Daren Forward, Prof Brigitte Scammell, Dr Adel Alshaikh (PhD student), Prof Opinder Sahota, Dr Richard Pearson, Dr Robert Kerslake, Dr Archan Bhattacharya, Sister Lindsey Marshall, Mr Matthew Dunn, Miss Patrice Burke

Ankle fractures are common; they are very disabling and require either a cast or a boot for treatment. The patients initially need to use crutches to maintain their balance. These fractures commonly result in patients needing a temporary period of residential care. Therefore, if the healing time can be reduced, there is a potential for large benefits for the patient and reduced health and social care costs.

Teriparatide hormone is one of the new medications used for treating osteoporosis, and studies in the USA have shown that Teriparatide hormone treatment has accelerated the healing time of pelvic fractures.

The study we are carrying out is a feasibility study in preparation for a larger study with Teriparatide hormone treatment. When used during the healing of ankle fractures in older people these fractures may heal more quickly and may allow a shorter time for using the cast and crutches and possibly less pain. If the study shows positive results, this will mean we can reduce the time for the patient wearing a cast and using crutches thus improving their health as well as their health and social care costs.

Appendix F - Participant information sheet

Nottingham University Hospitals NHS
NHS Trust

Study Title: A feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people

Emeritus Prof W Angus Wallace, Dr Daren Forward, Prof Brigitte Scammell, Dr Adel Alshaikh (PhD student), Prof Opinder Sahota, Dr Richard Pearson, Dr Robert Kerslake, Dr Archan Bhattacharya, Sister Lindsey Marshall, Mr Matthew Dunn, Miss Patrice Burke

PART 1

1 – Invitation - You are invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being carried out and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish.

PART 1 tells you the purpose of this study and what will happen if you take part.

PART 2 gives you more detailed information about how the study will be conducted.

Please ask questions 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.

2 - What is the purpose of the study? – To measure the effect of an injected medication called Teriparatide on the healing rate of ankle fractures. The effect of this treatment on the healing fracture will be monitored using ankle scans. Any improvement to the pain and function of the healing ankle provided by the treatment will be investigated through questionnaires. Participants will be asked a few questions about their experience of taking part in the study. All this information will help us to see how best to perform a proposed follow-on larger study.

3 - Why have I been chosen? - Because you have an ankle fracture (Weber Type B).

4 - Do I have to take part? - No. It is up to you to decide whether or not to take part. If you decide to take part you are still free to leave the study at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect in any way the quality of the routine care you receive.

5 - What will happen to me if I take part? - If you accept the invitation to take part in the study you will be given this participant information sheet to keep. You will need to give your written consent, a copy of which will also be given to you.

You will be asked some screening questions and will give a small amount of blood for screening blood tests. After screening your involvement in the study will be confirmed or declined and you will continue with your standard care.

You will then be allocated randomly into one of two groups to receive the Teriparatide (a medication that strengthens bone and may speed up fracture healing) medication or not. If you

We are here for you

are in the Teriparatide medication group, a nurse will train you on how to self-inject the medication at the Queen's Medical Centre. Teriparatide comes in a pre-filled injection pen, which means you will be self-injecting daily with a very small needle for 3 months (84 days).

CT scans (computer generated 3D x-rays) will be repeated every 2 weeks over 12 weeks (total of 7 CT scans) to identify how the fracture healing is progressing. These scans will be additional to your routine x-rays (usually x-rays are taken at baseline week 1 & 7 post injury)

We will ask you to complete questionnaires during your visits about any ankle pain and once the plaster cast is removed how well your ankle functions. When you come for your final appointment one of the research team will ask you a few questions about your experience of taking part in the research study this will be recorded on audio recording device.

6 - **What do I have to do?** – If you accept the invitation to take part in the study you will need to visit the hospital for a blood test and then attend clinic every 2 weeks for a CT scan a total of 8 visits. You will also be asked to complete some questionnaires and answer a few questions. If you are in the Teriparatide group you will need to take the medication as prescribed by a doctor on the study team.

7 - **What is the medication that is being tested?** - The medication that is being tested is Teriparatide (called Forsteo) an injection in a pre-filled syringe. Teriparatide (medication) makes bone stronger and is similar in function to a naturally occurring hormone in your body.

8 - **What are the alternatives for diagnosis or treatment?** - There are currently no other medications available which are considered to accelerate fracture healing.

9 - **What are the side effects of any treatment received when taking part?** - The medication (Teriparatide hormone) is relatively safe and has been used to treat patients with osteoporosis (condition where bones are weaker than usual) for the last 10 years. These are the possible side effects of the medication – all of which are uncommon:

1) Hypercalcemia, raised calcium levels in your blood, which increases in 4-6 hours after dose, (11% of women and 6% of men).
2) Arthralgia or joint pain (10%)
3) Rhinits or a runny nose (10%)
4) Nausea or sickness (9%)
5) Weakness (9%)
6) Dizziness (8%)
7) Pharyngitis sore throat (6%)
8) Dyspesia or indigestion (5%)
9) Skin Rash (5%)
10) Depression (4%)
11) Dyspnea or shortness of breath, (4%)
12) Pneumonia (4%)
13) Antibodies to teriparatide (3%)

14) Chest pain (3%)

15) Hyperuricemia - raised level of uric acid in the blood (3%)

16) Leg cramps (3%)

17) Syncope or fainting (3%)

18) Vomiting (3%)

19) Tooth disorder (2%).

20) Orthostatic hypotension, drop in your blood pressure when suddenly standing from a sitting position, which usually occurs in the first 4 hours of taking the drug for the first few doses.

Some patients have a small drop in blood pressure when standing up suddenly from a sitting position, which usually occurs in the first 4 hours of taking the drug and only for the first few doses.

Studies carried out on rats have shown, an increased risk of bone tumour (osteosarcoma) when the hormone is used in large doses. However no bone tumour (Osteosarcoma) has ever been observed in humans, in clinical trials or during post treatments follow up study with this medication (Teriparatide hormone).

Studies in rabbits have shown reproductive toxicity The effect of teriparatide on human foetal development has not been studied. The potential risk for the unborn baby is currently unknown,. As a safety precaution, if you are women who is, or who may become pregnant you are not eligible to take part in this study.

As a safety consideration the maximum total duration of this medication (Teriparatide hormone) is limited to 24 months and a further course of the drug should not be repeated over a lifetime. The maximum duration of medication in this study is 84 days (12 weeks i.e. one eighth of the normally prescribed dose over 24 months). All of the above possible side effects will be detailed in a leaflet, which will be provided to you when your medication (Teriparatide) is dispensed. The leaflet refers to when the drug is being used in the treatment of osteoporosis and in this trial the drug is being used off label. Again a member of the research team will explain the leaflet to you on request.

10 - What are other possible disadvantages and risks of taking part? - Clearly having to attend hospital every 2 weeks is an inconvenience and participation in this trial will involve exposure to a small amount of ionising radiation. As part of everyday living everyone is exposed to naturally occurring background radiation and the exposure you will receive, as part of this trial is roughly equivalent to about 12 weeks of background radiation received by an average UK resident.

11 - What are the possible benefits of taking part? - The research literature indicates that healing of the fracture may be accelerated when taking the study medication (Teriparatide). Teriparatide is currently prescribed to increase bone strength to reduce the risk of fracture. If this research is successful then it will lead to a large scale trial which has the potential to change the routine care of fracture patients.

12 - What happens when the research study stops? - You will need to continue your routine follow up with your GP if that is necessary.

13 - What if there is a problem? - If you have a concern about any aspect of this study, you

should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (For more information please contact patient advice & liaison service (PALS) on 0800 1830204). Details can be obtained from the hospital.

In the event that something does go wrong and you come to any harm during the research study there are no special compensation arrangements. If you were to come to harm and this is due to someone's negligence then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

14 - Will my taking part in this study be kept confidential? - Yes. All the information about your participation in this study will be kept confidential. Details are included in Part 2.

15 - Will I receive expenses for taking part in this study? - No. However we will arrange transportation for all your extra visits.

16 - Doctors Contact Detail

Emeritus Prof W Angus Wallace	**Telephone Number:**	**07836699600**
Principle Investigator Dr. Daren Forward	**Telephone Number:**	**07767818463**
Clinical Research fellow **Dr. Adel Alshaikh (PhD student)**	**Telephone Number:**	**07455965952**

This completes Part 1 of the Information Sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making your decision.

23 - What will happen to the results of this clinical trial? - The results of the study will be made available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication. Should you wish to see the results, or the publication, please ask a member of the research team.

24 - Who is organizing and funding this clinical trial? - The Nottingham University Hospitals NHS Trust will act as a sponsor for the research and the study funded by the Nottingham University Hospital charity.

25 - Who has reviewed the study? - All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favorable opinion by the NHS and Research Ethics Committee. The study has also been reviewed and approved by the Research & Innovation department of Nottingham University Hospitals NHS Trust. In addition to that the study has been reviewed by two independent external reviewers who have also given favorable opinions.

26 - Contact for further information
You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your research study nurse or doctor, who will be able to provide you with up to date information about the drug(s)/procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study please contact one of the following people:

Lead clinical investigator - Emeritus Prof W Angus Wallace Tel. Number: 07836699600

Academic Orthopaedics, Trauma and Sports Medicine
Division of Rheumatology, Orthopaedics and Dermatology, School of Medicine,
University of Nottingham
C Floor, West Block Queen's Medical Centre Nottingham, NG7 2UH
Tel: +44 (0) 115 82 31115
Fax: +44 (0) 115 82 31118
Mobile: 07836 699 600
E-mail Angus.Wallace@RCSEd.ac.uk
CV:- www.rcsed.ac.uk/fellows/wawallace
Website:- www.nottingham.ac.uk/orthopaedics/

Clinical research fellow
Dr Adel Alshaikh (PhD student) Tel. Number: 07455965952

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this participant information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.
You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this participant information sheet and to consider this study.

PART 2

17 - What if new information becomes available? - Sometimes during the course of a research study, new information becomes available on the medication in the study. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the study. If you decide not to continue in the study you will be asked to sign an updated consent form. On receiving new information, we might consider it to be in your best interests to withdraw you from the study. If so, we will explain the reasons and arrange for your care to continue.
If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

18 - What will happen if I do not want to carry on with the study? - Your participation in the study is completely voluntary and if you wish to withdraw from the study it is completely up to you. Your continued care will be provided to you with no prejudice.

19 - Will my part in this study be kept confidential? - If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital and the main hospital site managing this research under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms.

Your records will be available to people authorised to work on the trial but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree access to your records for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority and Independent Ethics Committee; this is to ensure that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

If you withdraw your consent for continued involvement in the study, unless you object, your data and samples will remain on file and will be included in the final study analysis.
In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 15 years. Arrangements for confidential destruction will then be made. With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study.

20 - Informing your General Practitioner (GP) - With your consent, we will inform your GP that you are taking part in this study.

21 - What will happen to any samples I give? - Not applicable

22 - Will any Genetic testing be done? - No.

23 - What will happen to the results of this clinical trial? - The results of the study will be made available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication. Should you wish to see the results, or the publication, please ask a member of the research team.

24 - Who is organizing and funding this clinical trial? - The Nottingham University Hospitals NHS Trust will act as a sponsor for the research and the study funded by the Nottingham University Hospital charity.

25 - Who has reviewed the study? - All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favorable opinion by the NHS and Research Ethics Committee. The study has also been reviewed and approved by the Research & Innovation department of Nottingham University Hospitals NHS Trust. In addition to that the study has been reviewed by two independent external reviewers who have also given favorable opinions.

26 - Contact for further information
You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your research study nurse or doctor, who will be able to provide you with up to date information about the drug(s)/procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study please contact one of the following people:

Lead clinical investigator - Emeritus Prof W Angus Wallace Tel. Number: 07836699600

Academic Orthopaedics, Trauma and Sports Medicine
Division of Rheumatology, Orthopaedics and Dermatology, School of Medicine,
University of Nottingham
C Floor, West Block Queen's Medical Centre Nottingham, NG7 2UH
Tel: +44 (0) 115 82 31115
Fax: +44 (0) 115 82 31118
Mobile: 07836 699 600
E-mail Angus.Wallace@RCSEd.ac.uk
CV:- www.rcsed.ac.uk/fellows/wawallace
Website:- www.nottingham.ac.uk/orthopaedics/

Clinical research fellow
Dr Adel Alshaikh (PhD student) Tel. Number: 07455965952

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this participant information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.
You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this participant information sheet and to consider this study.

Appendix G - Participant informed consent

Nottingham University Hospitals NHS
NHS Trust

Study Title: A feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people

Emeritus Prof W Angus Wallace, Dr Daren Forward, Prof Brigitte Scammell, Dr Adel Alshaikh (PhD student), Prof Opinder Sahota, Dr Richard Pearson, Dr Robert Kerslake, Dr Archan Bhattacharya, Sister Lindsey Marshall, Mr Matthew Dunn, Miss Patrice Burke

Patient initial each box

1. I confirm that I have read and understand the participant information sheet dated 03/August/2016 (Version 2.0) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. ☐
3. I understand that my medical records may be, looked at by authorised individuals from the sponsor for the study, the UK Regulatory Authority, the Medicines and Healthcare Products Regulatory Agency (MHRA) or the Independent Ethics Committee in order to check that the study is being carried out correctly. ☐
4. I understand that even if I withdraw from the above study, the data collected from me will be used in analysing the results of the trial, unless I specifically withdraw consent for this. ☐
5. I consent to the storage including electronic, of personal information for the purposes of this study. I understand that any information that could identify me will be kept strictly confidential and that no personal information will be included in the study report or other publication. ☐
6. I understand that my GP, or any other doctor treating me, will be informed of my participation in this study. ☐
7. I agree to do a recorded interview at the end of this study ☐
8. I agree to take part in the study ☐

Name of the patient *(Print)*	Date	Patient's signature
Name of person taking consent *(Print)*	Date	Signature

Original to be retained and filed in the study site file, 1 copy to patient, 1 copy to be filed in patient's notes

Sponsor Ref: 13OR006
IRAS ID: 143755

We are here for you

Appendix H pre-screening consent form

Nottingham University Hospitals NHS
NHS Trust

Study Title: A feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people

Emeritus Prof W Angus Wallace, Dr Daren Forward, Prof Brigitte Scammell, Dr Adel Alshaikh (PhD student), Prof Opinder Sahota, Dr Richard Pearson, Dr Robert Kerslake, Dr Archan Bhattacharya, Sister Lindsey Marshall, Mr Matthew Dunn, Miss Patrice Burke

Patient initial each box

1. I hereby consent to be called on this telephone number ……. by a member of the above research team. ☐
2. I agree to have one specimen of blood taken for screening purposes for this trial. ☐
3. I understand that by signing this form I am not providing consent to participate in the full trial at this stage but simply to find out if I am suitable for inclusion in the study. ☐

_____	_____	_____
Name of the patient *(Print)*	Date	Patient's signature
_____	_____	_____
Name of person taking consent *(Print)*	Date	Signature

Original to be retained and filed in the study site file, 1 copy to patient, 1 copy to be filed in patient's notes

Appendix I - Interview questions guid

- What did you think of the quality of care you received whilst taking part in this research study?
- How was your experience with self-injection (specific for treatment group)?
- How did you feel about visiting hospital every 2 weeks?
- What did you think about having computerized scans (CT scans) every 2 weeks?
- How important do you think this study is?
- Would you consider taking part in any future research and if so why?
- Do you have any further comments or suggestions that might improve this study?

Appendix J - Approval letter from East Midland Nottingham-2 Research Ethics committee

NHS
Health Research Authority

East Midlands - Nottingham 2 Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

03 August 2016

Prof Angus Wallace
Academic Orthopaedics, Trauma and Sports Medicine
Division of Rheumatology Orthopaedics and Dermatology/School of Medicine/University of Nottingham
C Floor, West Block, Queen's Medical Centre
NG27UH

Dear Professor Wallace

Study title:	**A feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people**
REC reference:	**16/EM/0299**
Protocol number:	**13OR006**
EudraCT number:	**2015-005423-32**
IRAS project ID:	**143755**

The Research Ethics Committee reviewed the above application at the meeting held on 25 July 2016. Thank you for attending along with Dr Adel Alshaikh to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Ms Carolyn Halliwell, NRESCommittee.EastMidlands-Nottingham2@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

Conditions of the favourable opinion

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

1. The Consent Form should be amended as follows:

   Point 6 needs to be changed to state 'I understand that my GP, or any other doctor treating me, will be informed of my participation in this study' as notification of a patient's GP is mandatory in this category of study.

   An additional point should be added to facilitate agreement for a participant's interviews to be recorded.

2. The Participant Information Sheet should be amended as follows:

   The bracketed information within the final paragraph on page 1 should be moved so that the sentence reads as follows: 'You will then be allocated randomly into one of two groups to receive the Teriparatide (a medication that strengthens bone and may speed up fracture healing) medication or not.

   The final sentence within part 10 (page 3 of 6) 'The risk is considered to be very low.' should be deleted.

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

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

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

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

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

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

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

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

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

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

Ethical review of research sites

NHS Sites

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

Non NHS sites

The Committee has not yet completed any site-specific assessment(s) (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Summary of discussion at the meeting

- **Social or scientific value; scientific design and conduct of the study**

  The Committee asked, given that Teriparatide is normally prescribed in osteoporosis for a minimum of 2 years and in this study it will be prescribed for just 3 months, what effect the applicants think is likely from the Teriparatide in

this timeframe. *The applicants responded due to the way the parathyroid hormone works, it was initially expected this drug would speed up recovery and some randomised studies have shown just that. There have been 2 studies of non-union and delayed union which have shown useful results. One of these, a study on acute pelvic fractures showed the healing rate improved by about 40%. These are complicated fractures, so if healing time can be reduced that is a benefit. A further study showed an improvement of between one and a half and two weeks in healing time.* The Committee asked if the applicants do expect to see an improvement within 3 months.

The Committee acknowledged the study as being a pilot with limited funding, but queried whether the study will be able to provide any useful data in the event of participant withdrawals, due to the very small sample size. *The applicants responded they are primarily interested in sequential CT scans rather than clinical effect. A small number is needed to find out if any difference can be seen those participants on the parathyroid treatment and those who aren't. The hope is to be able to cut the number of CT scans used in standard care and look at establishing that cut down point. More scans will be undertaken in this study than would be required in a definitive study.*

The Committee commented that due to such a small sample, dropouts could adversely affect the study results. The Committee asked how this will be managed. *The applicants responded as long as they already have the participant's CT scan then they will be able to use that data. They are expecting some degree of dropout and whilst they will not exceed the numbers specified in their application, depending on the dropout rate they may replace those participants.* The Committee queried what usual care is for the patient cohort to be recruited to this study. *The applicants responded patients fall into two groups; surgical and non-surgical. For non-surgical patients there are associated costs of follow-up and sequential x-rays at the time of injury and sometimes at 4 and 8 weeks, but this group do not normally require CT scans.*

- **Care and protection of research participants; respect for potential and enrolled participants' welfare and dignity**

The Committee asked how the participant's study ID will be constructed. *The applicants confirmed participant data will be identified by a randomised generated code which will not contain a participant's date of birth, initials or any other identifiable information.*

The Committee commented upon the study application indicating that participant interviews will be recorded. The Committee sought further information on how this will be done. *The applicants confirmed the interviews will be recorded on a digital device and then transcribed. The recordings are usually transferred onto the university storage system and kept securely within the participant's file for a specified period of time.*

- **Suitability of supporting information**

The Committee queried the need for the inclusion of a lay summary of the study in participant documentation. *The applicants responded they have consulted a PPI (patient and public involvement) group in regard to this and their belief was the summary is needed.* The Committee voiced concern that the issue with inclusion of a lay summary in a study is that there is a danger it can lead to participants reading only the summary rather than the full information sheet. The Committee stressed that it must be ensured that

participants do read all the information so that they are fully informed. *The applicants acknowledged the Committee's concerns and agreed they will ensure participants will read all appropriate information.*

- **Other general comments**

  The Committee brought the applicants attention to the Injection Guide described within the IRAS application form and commented upon not being aware of this document having been submitted for review. *The applicants responded they believe this was submitted and shared a copy of the guide with the Committee.*

  The Committee queried if the applicants had any questions for the Committee themselves. *The applicants advised the MHRA have made an objection to the patient information because they think there is a possibility that some female participants could become pregnant, and that the information within the application on the prevention of pregnancy was insufficient. The applicants indicated they are preparing an amendment and asked how they will need to proceed with this from the Committee's perspective.* The Committee advised the amendment will require submission to the REC when ready.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper [HRA covering letter]		
GP/consultant information sheets or letters [GP letter]	1.0	01 May 2016
Investigator's brochure / IMP Dossier [Package leaflet]		01 January 2016
IRAS Application Form [IRAS_Form_01072016]		01 July 2016
Letters of invitation to participant [Lay abstract]	1.0	01 May 2015
Non-validated questionnaire [Interview questions]	1.0	01 May 2016
Other [Prof Opinder CV]	N/A	01 August 2015
Other [Dr Richard CV]	N/A	07 June 2015
Participant consent form [Participant consent form]	1.0	01 May 2015
Participant information sheet (PIS) [Participant information sheet]	1.0	01 May 2016
Referee's report or other scientific critique report [R&D peer review Richardson]	N/A	09 March 2015
Research protocol or project proposal [Study protocol]	1.0	01 May 2015
Sample diary card/patient card [Contact information card]	1.0	01 May 2016
Summary CV for Chief Investigator (CI)	N/A	31 July 2015
Summary CV for student [Curriculum vita]		05 June 2015
Summary CV for supervisor (student research) [Curriculum vita]		13 May 2016
Summary of product characteristics (SmPC) [Summary of product characteristics]	N/A	01 January 2016
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Appendix Participant clinical pathway in the study]	N/A	01 May 2016
Validated questionnaire [EQ-5D-5L health questionnaire]	N/A	09 April 2014

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

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

After ethical review

Reporting requirements

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

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

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

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/EM/0299	**Please quote this number on all correspondence**

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

East Midlands - Nottingham 2 Research Ethics Committee

Attendance at Committee meeting on 25 July 2016

Committee Members:

Name	*Profession*	*Present*	*Notes*
Miss Shamim Byrne	Gynaecologist/Obstetrician	No	
Mr Simon Deery	Junior Doctor	No	
Professor Frances Game (Chair)	Consultant Physician	Yes	
Mrs Jane Hennebury	Lawyer	Yes	
Dr Asam Latif	Research Pharmacist	Yes	
Mr Jon Merrills	Barrister/Pharmacist	Yes	
Mrs Bernadette Roberts	Retired Finance Manager	No	
Dr Ian Ross	Retired Consultant Physician	Yes	
Dr John Shaw	Retired Patent Licensing Manager	Yes	
Miss Catherine Shenton	Lay Member	Yes	
Ms Margret Vince	Translator	No	

Also in attendance:

Name	*Position (or reason for attending)*
Ms Carolyn Halliwell	REC Manager

Yours sincerely

pp C Halliwell

Professor Frances Game
Chair

E-mail: NRESCommittee.EastMidlands-Nottingham2@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

"After ethical review – guidance for researchers"

Copy to: *Dr Maria Koufali, Nottingham University Hospitals NHS Trust*
Dr Alison Steel, Nottingham University hospital

Appendix K - MHRA approval letter

Appendix L 0 HRA approval letter

NHS
Health Research Authority

Prof Angus Wallace
Academic Orthopaedics, Trauma and Sports Medicine
Division of Rheumatology Orthopaedics and
Dermatology/School of Medicine/University of Nottingham
C Floor, West Block, Queen's Medical Centre
NG27UH

Email: hra.approval@nhs.net

6 October 2016

Dear Professor Wallace

Letter of HRA Approval

Study title:	**A feasibility study to explore the difference in healing time between Teriparatide treatment and standard care on Weber type B ankle fractures in older people**
IRAS project ID:	**143755**
EudraCT number:	**2015-005423-32**
Protocol number:	**13OR006**
REC reference:	**16/EM/0299**
Sponsor	**Nottingham University Hospitals NHS Trust**

Thank you for your request to bring the above referenced study under HRA Approval.

I am pleased to confirm that the study has been given **HRA Approval.** This is a study involving a single participating NHS organisation in England and that NHS organisation (or their partner academic organisation) is sponsoring the study.

It is not expected that any other NHS organisations in England will participate in this study. If subsequent NHS organisations in England are added, an amendment should be submitted to the HRA, providing a Statement of Activities and Schedule of Events, upon which a full HRA assessment will be undertaken.

After HRA Approval

In addition to the document, *"After Ethical Review – guidance for sponsors and investigators"*, issued with your REC Favourable Opinion, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.

- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/.

If you have any queries about the issue of this letter please, in the first instance, see the further information provided in the question and answer document on the HRA website.

Your IRAS project ID is 143755. Please quote this on all correspondence.

Yours sincerely

Isobel Lyle
Senior Assessor
Tel: 0207 972 2496

Email: hra.approval@nhs.net

IRAS project ID	143755

Copy

Dr Maria Koufali, Nottingham University Hospitals NHS Trust maria.koufali@nuh.nhs.uk
Dr Alison Steel, Nottingham University hospital alison.steel@nuh.nhs.uk
Dr Adel Alshaikh Nottingham University, msxla@nottingham.ac.uk
Rachelle Ward, R&D Project Manager, Nottingham University Hospitals NHS Trust
rachelle.ward@nuh.nhs.uk

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below

Document	*Version*	*Date*
Confirmation of Clinical Trial Authorisation from MHRA and relevant correspondence [MHRA authorisation]		18 August 2016
Covering letter on headed paper [HRA covering letter]		
GP/consultant information sheets or letters [GP letter]	1.0	01 May 2016
Investigator's brochure / IMP Dossier [Package leaflet]		01 January 2016
IRAS Application Form [IRAS_Form_01072016]		01 July 2016
IRAS Checklist XML [Checklist_05102016]		05 October 2016
Letters of invitation to participant [Lay abstract]	1.0	01 May 2015
Non-validated questionnaire [Interview questions]	1.0	01 May 2016
Other [Prof Opinder CV]	N/A	01 August 2015
Other [Dr Richard CV]	N/A	07 June 2015
Other [Participant information sheet]	2.0	03 August 2016
Other [Participant informed consent]	3.0	05 October 2016
Referee's report or other scientific critique report [R&D peer review Richardson]	N/A	09 March 2015
Research protocol or project proposal [Study protocol]	1.0	01 May 2015
Response to Additional Conditions Met		03 August 2016
Sample diary card/patient card [Contact information card]	1.0	01 May 2016
Summary CV for Chief Investigator (CI)	N/A	31 July 2015
Summary CV for student [Curriculum vita]		05 June 2015
Summary CV for supervisor (student research) [Curriculum vita]		13 May 2016
Summary of product characteristics (SmPC) [Summary of product characteristics]	N/A	01 January 2016
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Appendix Participant clinical pathway in the study]	N/A	01 May 2016
Validated questionnaire [EQ-5D-5L health questionnaire]	N/A	09 April 2014
2016.08.03 Rec Ref 16-0299 IRAS ID 143755 FO with conditions		03 August 2016
2016.10.05 REC Ref 16-0299 IRAS ID 143755 FO Conditions Met		05 October 2016

IRAS project ID	143755

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, *participating NHS organisations, capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	No agreement is expected as the participating NHS organisation is also the study sponsor.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	No funding will be provided to site.
5.1	Compliance with the Data Protection Act and data	Yes	No comments

Section	HRA Assessment Criteria	Compliant with Standards	Comments
	security issues assessed		
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Yes	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Yes	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There is one site in this study which is also the study sponsor. All study activities as detailed in the study documents will take place at site. If this study is subsequently extended to other NHS organisation(s) in England, an amendment should be submitted to the HRA, with a Statement of Activities and Schedule of Events for the newly participating NHS organisation(s) in England. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at

hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.

The HRA has determined that participating NHS organisations in England **are not expected to formally confirm their capacity and capability to host this research,** because the site is also the study sponsor.

- The HRA has informed the relevant research management offices that you intend to undertake the research at their organisation. However, you should still support and liaise with these organisations as necessary.
- Following issue of the Letter of HRA Approval the sponsor may commence the study at these organisations when it is ready to do so.
- The document "Collaborative working between sponsors and NHS organisations in England for HRA Approval studies, where no formal confirmation of capacity and capability is expected" provides further information for the sponsor and NHS organisations on working with NHS organisations in England where no formal confirmation of capacity and capability is expected, and the processes involved in adding new organisations. Further study specific details are provided the *Participating NHS Organisations* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections of this Appendix.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).

A PI is expected.

GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken

Local staff who have a contractual relationship with the organisation will undertake the expected activities. Therefore no honorary research contracts or letters of access are expected for this study.

Other Information to Aid Study Set-up

IRAS project ID	143755

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.
• The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

Appendix M - Sponsors green light to commence recruitment

Nottingham University Hospitals NHS
NHS Trust

research & innovation

Research & Innovation
Nottingham Health Science Partners
C Floor, South Block
Queen's Medical Centre Campus
Derby Road,
Nottingham
NG7 2UH

www.nuhrise.org
Email: ResearchSponsor@nuh.nhs.uk
Tel: 0115 9249924 ext 70258

11th October 2016

Prof Angus Wallace & Mr. Daren Forward
Department of Trauma and Orthopaedics
Queen's Medical Centre
Nottingham University Hospitals

Dear Both,

Study Title:	Ankle Fractures Treated with Teriparatide
Chief Investigator:	Prof Angus Wallace
Sponsor Reference Number:	13OR006
Site Address:	QMC, Nottingham University Hospitals

Following on from the site initiation visit on 6th October 2016, I can hereby confirm that Nottingham University Hospitals NHS Trust, Department of Trauma and Orthopaedics, has been given the **Green Light to commence recruitment**.

You have been allocated a Research Project Manager (RPM) from our Regulatory Team who will be your point of contact for any sponsor related queries.

The department of NUH R&I will request patient recruitment information from you on a monthly basis and a timely response is required. Your site is expected to have performed 'first patient first visit' no later than 15th December 2016.

We look forward to working alongside you and your team please do not hesitate to contact me if you have any queries.

Yours sincerely,

Rachelle Ward
Research & Innovation

Appendix N visual analogue pain scale