# UNIVERSITY OF CAPE TOWN FACULTY OF HEALTH SCIENCES




Bone Mineral Density in HIV: A comparison of HIV-positive versus HIV-negative patients with lower limb long bone fractures.

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**MASTERS IN MEDICINE**
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## Declaration: Student

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This study was conducted from under the supervision of Professor Maritz Laubscher, Department of Orthopaedic Surgery, University of Cape Town.

As the candidate's Supervisor, I have approved this dissertation for submission.

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## Ethics statement

The authors declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.

Prior to commencement of the study ethical approval was obtained from the ethical review board: HREC reference number 590/2016

All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.


## Acknowledgements, format and contributions

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The format of this manuscript is in publication-ready format.

This manuscript has not been submitted, already published, or accepted for publication.

I will be publishing this manuscript in the South African Orthopaedic Journal after successful completion of my minor dissertation.

**P. Botha:** literature reviews, analysis and interpretation of relevant data, writing up of this manuscript.

**M. Laubscher:** conception and study design, acquisition of data for the work (data collection), review of manuscript, final approval prior to submission for MMed marking.

**M. Held**: review of manuscript and final approval.

**S. Maqungo:** review of manuscript and final approval.

**N. Ferreira**: review of manuscript and final approval

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**R. Waters:** review of manuscript

**S. Graham:** study design, data collection, acquisition, analysis and interpretation of relevant data, review of manuscript and final approval for submission.

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**ABBREVIATIONS**

| Abbreviation | Definition |
|---|---|
| 3TC | Lamivudine |
| ARV | Antiretrovirals |
| ART | Antiretroviral Therapy |
| CT | Computerised-Tomography |
| DXA | Dual Xray Absorptiometry |
| EFV | Efavirenz |
| GSH | Groote Schuur Hospital |
| HOST | HIV in Orthopaedic Skeletal Trauma |
| IQR | Interquartile Range |
| IM | Intramedullary |
| NHLS | National Health Laboratory System |
| NRTI | Nucleoside Reverse Transcriptase Inhibitors |
| NNTRI | Non-nucleoside Reverse Transcriptase Inhibitors |
| PI | Protease Inhibitors |
| TDF | Tenofovir Disoproxil Fumarate |
| WHO | World Health Organization |

**Abstract**

**Background**: Osteoporosis is a global health issue causing a deterioration in bone microarchitecture, compromising bone strength. In South Africa (SA), the prevalence of osteoporosis is a growing concern for both HIV-positive and HIV-negative individuals. The aim of this study was to assess the impact of HIV infection on bone mineral density (BMD) in HIV-positive individuals and compare this to the BMD in HIV-negative individuals with traumatic long-bone fractures. In addition, we compared the impact of demographics, nutritional (albumin), Vitamin D status and smoking between HIV-positive and negative individuals.

**Patients and Methods:** We retrospectively reviewed data from a prospectively collected database within the HIV in Orthopaedic Skeletal Trauma (HOST) study conducted at tertiary care hospitals. This study included all individuals with a confirmed HIV positive or negative status who had their BMD measurement performed using a calcaneal quantitative ultrasound scan (cQUS) and excluded those individuals who had never had a cQUS performed.

**Results:** Of 400 individuals recruited from the parent study, 172 (43%) had their BMD measured. 27(15.7%) were HIV-positive. Overall, the BMD and T-scores were similar in both the HIV-positive and HIV-negative participants with a median BMD of 0.49 g/cm$^2$ (0.23 – 0.71) within the HIV positive group; 0.49 (0.3-0.71) within the HIV negative group and an overall T-score of -0.8, with no statistical significance found between the two groups (p>0.050). Age, smoking status, Vitamin D, Albumin and BMI had no effect on BMD (p> 0.050).

**Conclusion:** We found a higher proportion of patients with osteoporosis within the HIV-positive cohort, although not statistically significant and no statistically significant difference in

the median BMD between the HIV -positive and HIV-negative subgroups. Neither age, smoking, vitamin D, albumin nor BMI was associated with a lower BMD.

*Keywords: HIV Bone mineral density, calcaneal QUS, long bone fractures*

**Part A: Manuscript in Article format**

**Title**

**Bone mineral density in HIV: a comparison of HIV-positive versus negative patients with lower limb long bone fractures**

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**Abstract**

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**Conclusion:** We found a higher proportion of patients with osteoporosis within the HIV-positive cohort, although not statistically significant and no statistically significant difference in the median BMD between the HIV -positive and HIV-negative subgroups.(Neither age, smoking, vitamin D, albumin nor BMI was associated with a lower BMD.

*Keywords: HIV Bone mineral density, calcaneal QUS, long bone fractures*

## Introduction

Osteoporosis is a global health issue causing a deterioration in bone microarchitecture, thereby compromising bone strength. In South Africa, the prevalence of osteoporosis is a growing concern for both HIV-positive and HIV-negative individuals, particularly those living in low socio-economic settings.[1–3] Poor accessibility to healthcare, general lack of knowledge about the disease process, and an aging population contribute to this problem.[1,4]

Recent studies suggest the incidence of osteoporosis is increasing globally.[1] In South Africa, the National Osteoporosis Foundation (NOF) reports one in three women and one in five men are at risk of developing this condition during their lifetime.[5] This means that potentially between four and six million South Africans are affected by osteoporosis.[6] In a systematic review and meta-analysis, Salari et al[1] estimated the worldwide prevalence of osteoporosis to be 18.3% (95% CI 16.2–20.7). Prevalence is higher in women, with a reported rate of 23.1% (95% CI 19.8–26.9), compared to 11.7% in men (95% CI 9.6–14.1).[4]The highest prevalence of osteoporosis was reported in Africa, with 39.5%. (95% CI 22.3–59.7).[1,2]

The interplay of HIV infection, antiretroviral use, lifestyle factors and nutritional deficiencies is thought to contribute to an increased risk of osteoporosis in the aging HIV population.[7-9] With appropriate treatment, the life expectancy of HIV-positive individuals is now more similar to that of the HIV-negative population.[6] There may be a strong association between HIV and reduced bone mineral density (BMD) which may be directly due to the virus itself, to comorbidities, increasing life expectancy or secondary to the antiviral medication.[9-13]¶This has been documented in several cross-sectional studies, with low BMD being found in both male and female HIV-infected individuals.[9-13] There are multiple factors playing a role in the aetiology of osteopenia and osteoporosis.

Osteoporosis is mostly diagnosed through low-energy traumatic fracture – that is, fragility fractures and BMD measurement – by utilizing the gold standard, Dual X-ray Absorptiometry

(DXA) scan. This valuable scan is often not available to a significant portion of the South African population, due to a lack of access to equipment. According to the World Health Organization (WHO) classification, osteoporosis is defined as a bone mineral density (BMD) that is 2.5 standard deviations (SDs) or more below the average value for young healthy women, indicated by a T-score of less than -2.5 SD. Osteopenia is defined as a T-score between -1 and -2.5 SD.[14]

Point of care measurement of BMD has been developed and is available through cQUS scan, i.e. Calscan DXL which is a quick, cost effective, simple to use peripheral imaging device using lower radiation doses providing information on bone mass and bone micro architecture.[15] The system employs dual X-ray absorptiometry (DXA) with a fan beam configuration and incorporates a laser measurement of heel thickness to enhance accuracy.[16]

The aim of this study was to assess the impact of HIV infection on BMD in HIV-positive individuals and compare this to the BMD in HIV -negative individuals with traumatic long-bone fractures. In addition, secondary parameters that may affect BMD - demographics, nutritional status (albumin), Vitamin D status and smoking, were assessed and compared between both subgroups.

## Patients and methods

We retrospectively reviewed data from a prospectively collected database. This patient cohort was collected as part of the HIV in Orthopaedic Skeletal Trauma (HOST) study (NCT03131947).[17] This database contains all cases (both HIV-negative and -positive) of femoral and tibial intramedullary (IM) nailings involving 400 participants with a total of 442 IM nails performed for fracture fixation in patients older than 18 years old, during the period September 2017 to December 2018.

This study included all individuals with a confirmed HIV positive or negative status (n=172) who had their BMD measurement performed using a, cQUS scan – i.e. Calscan DXL(Demetech AB, Solna, Sweden) – at Groote Schuur Hospital. This Ipsilateral calcaneal scan of the uninvolved limb was performed within 6 weeks of the definitive primary surgery. The study excluded participants who had never had a cQUS performed.[17]

In this study, all included participants, whether previously diagnosed with HIV or newly diagnosed, underwent laboratory measurements of CD4 cell count and viral load after providing formal consent through a standardized hospital protocol.

If the individual's HIV status was known to be positive (confirmed through laboratory system) and taking their antiretroviral therapy, then the antiretroviral treatment regime was recorded at the time of assessment. Demographic data such as age and gender, as well as potential clinical and non-clinical risk factors associated with reduced BMD such as smoking status, Vitamin D, albumin levels and body mass index (BMI), were recorded.

According to the definitions established by the WHO, BMD was recorded for each subgroup and defined as either normal, osteopenic or osteoporotic.(**Table I**)[14] Ethical and hospital institutional approval was obtained for this study (HREC 590/2016).

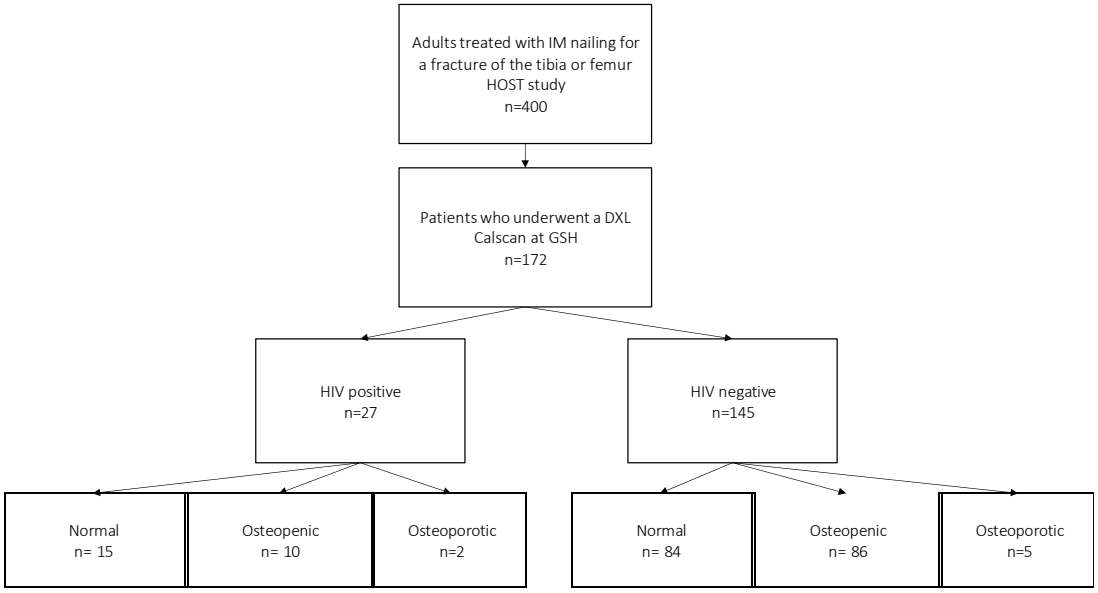


Figure 1. Outlines number within the HOST parent study of HIV positive and negative subgroups

## Statistical analysis

Data analysis was performed using R for statistical computing.[18] Numerical variables were assessed for normality using the Shapiro-Wilk test, with central tendency and dispersion represented as appropriate. Analysis of continuous data was done using the T-test and Mann-Whitney test. Categorical variables were compared using the Chi-squared and Fisher's exact test, where appropriate. A confidence interval of 95% was used, with a two-tail test hypothesis using an alpha value of 0.05 as discriminator for rejection of the null- hypothesis.

## Results

172 of 400 individuals (43%) had their BMD measured. 27(15.7%) were HIV-positive(Figure 1). A greater male predominance in both groups with 117 of 145;80.7% within the HIV-negative group and 21 of 27;77.7% within the HIV-positive group was found (**Table II**).

The median age in the HIV-negative subgroup was 30 years (IQR 30–38 years) years, and 33.50 (IQR 29.25–40.75) years in the HIV-positive group, with no significant difference noted between the two groups (p> 0.050). **Table II** outlines the baseline characteristics, demographic data and laboratory values of albumin and Vitamin D of both groups, with no comparable differences noted (p>0.050). The prevalence of osteoporosis and osteopenia in the HIV-positive group was 7.4 % and 37% respectively, compared to 3.4% and 38.6 % in the HIV-negative subgroup (**Table III**). Seven participants had a BMD T-score that was diagnostic of osteoporosis (T<-2.5). There was a slightly higher proportion of HIV-positive participants who were classified as osteoporotic compared to HIV-negative participants (2/27 [7.4%] vs 5/145 [3.4%]) but no statistical significance was found between these two groups (p>0.050) (**Table III**).

Of the 66 participants classified as osteopenic (T-score between -1.0 and -2.5), the males (n =51) outnumbered the females (n=15). Among these osteopenic participants, 10 were HIV-positive and 56 were HIV-negative (**Table IV**).

Overall, the BMD and T-scores were similar in both the HIV-positive and HIV-negative participants with the median BMD 0.49 g/cm2 (IQR 0.23 – 0.73) and a T-score of -0.8 with no statistical significance found between the two groups (p>0.050) (**Table III**).

Within this study cohort, a higher percentage of females were osteopenic and osteoporotic compared to the male group (p= 0.029) (**Table IV**). Classified with low BMD were 19/34 (55%) females in comparison to 54/138 (39%) males found to have low BMD.

A higher proportion of non-smokers was found in the total cohort: 110/172 (64%). No statistically significant effect of smoking on BMD in the entire cohort study was noted (p= 0.544) (**Table IV**).

Vitamin D levels were insufficient in 44/66 (78.3%) individuals in the osteopenic group and 3/7 (42.9%) in the osteoporotic group. Insufficient levels were also noted in the normal categorized BMD group, with 49/99 (49.5%) participants recorded as being insufficient. Within the HIV-positive subgroup, 11/27 (41%) participants were recorded as being insufficient in comparison to 16 / 27 (59%) having normal Vitamin D levels.

Albumin levels were found to be slightly lower in the osteoporotic group, with 4/7 (57.1%) having low recorded albumin levels, but this was not statistically significant: (p>0.050). Among HIV-positive individuals, 15 out of 27 had low albumin levels, accounting for 55.6% of the group. The remaining 12/ 27 (44%) of individuals had normal albumin levels. No significant influence of albumin on BMD was noted (p=0.057).

We did not find any statistical difference between viral load, ART treatment status, and CD4 count on BMD within the HIV-positive cohort (p> 0.050) (**Table V**).

## Discussion

In this study, we found a higher proportion of patients within HIV-positive cohort although this was not statistically significant. We report a 15.7% HIV-positivity rate. Overall, the median BMD and T-scores were similar in both the HIV-positive and HIV-negative participants (median BMD of 0.49 g/cm$^2$ (0.23 – 0.71) within the HIV positive group; 0.49 (0.3-0.71) within the HIV negative group) and an overall T-score of -0.8, with no statistical significance found between the two groups (p>0.050). Age, smoking status, Vitamin D, Albumin and BMI had no effect on BMD (p> 0.050).

According to the WHO criteria, osteoporosis is defined as a BMD that lies 2.5 standard deviations or more below the average value for young, healthy sex-matched population:(a T-score of < − 2.5 SD) (**Table I**).

Osteoporosis is usually diagnosed in the presence of low energy fractures in combination with imaging, such as the gold standard DEXA scan, allowing for the most accurate assessment of BMD status.[15,16] This study utilized the DXL Calscan, a calcaneal dual X- ray absorptiometry device, which has become most certainly valuable and a widely used screening tool in the setting of resource-limited countries like South Africa where the gold standard DXA scan is not always freely accessible and available. Studies have demonstrated that calcaneal BMD measurements using DXL have predictive ability for osteoporosis-related fractures, similar to gold standard measurements of lumbar spine and hip BMD using a standard DXA machine.[19] DXL measurement at the heel demonstrating a T-score threshold of -2.5, corresponds with the World Health Organization's definition of osteoporosis.[15,19]

Previous literature reports a reduction in BMD with a greater risk of fragility fractures as a complication of HIV infection and associated treatment in both males and females.[1,20] These studies showed that patients living with HIV had lower BMD at the hip (Z-score −0.31,

95% CI: −0.46 to −0.27) and lumbar spine (Z-score −0.36, 95% CI: −0.39 to −0.15) compared to their controls.[21-23]

SA has one of the highest HIV infection rates in the world and as of 2019, the national data base survey revealed that an estimated 7.7 million people were living with HIV in South Africa, which accounted for approximately 20% of the global HIV burden.[24,25]

The prevalence of HIV in this study population is 15.7 % (27/172) individuals. The prevalence in the Western Cape is 5.6%.[26] Therefore, the prevalence of HIV in the study population was significantly higher at 15.7 % (10.1% higher) compared to the Western Cape. This is possibly due to the fact this study cohort consists of young adult males living within low- income areas of the Western Cape, where HIV is considered more prevalent. HIV prevalence has been found to vary across different regions of South Africa.[27]

The prevalence of osteoporosis in HIV-positive patients is significantly higher compared to the general population, with modifiable and non-modifiable risk factors playing a key aetiological role.[9,10] Despite this, our study cohort only identified a slightly higher proportion of HIV-negative individuals who were classified as osteopenic compared to HIV-positive individuals – 10/ 27 (37%) vs 56/145 (38.6%) – and in the osteoporotic group, 2/27 (7.4%) vs 5/145 (3.4%) with no statistical difference found between the two (p=0.050). These findings could most likely be explained by the limited number of study participants within the HIV-positive subgroup, as well as by the subgroup being younger in age. Such findings differ in studies with larger numbers of cases. One must take into consideration that this study was not statistically powered to compare BMD.

These findings showed age to have no significant effect on BMD (p=0.079); however, the age differences between both groups were fairly minor, with median age of 30(IQR (30 - 38) years in the HIV-negative group and 33.50(IQR 29.25-40.75) years in the HIV-positive group.

In an unmatched, younger cohort group one would normally expect to find a normal BMD in comparison to the elderly generation, with expected age-related changes in bone micro-architecture deterioration.

Existing literature highlights the prevalence of osteopenia and osteoporosis among individuals with HIV occurring in the age group over 50 years old.[28] Low bone mineral density is also specifically influenced by the type and duration of ARV treatment.

Despite our study age group being fairly young, the median age within the osteoporotic group was 46 (IQR 30 – 61) and the osteopenic group was 32 (IQR 24 – 40), there was shown to be no statistically significant effect of age on BMD (p>0.050).

Within our study cohort, a higher percentage of females were osteopenic and osteoporotic compared to the male group (p= 0.03) (**Table IV).** Amongst the females, 19/34 (55%) were classified as having low BMD in comparison to 54/138 (39%) males. This finding is in accordance with the literature where females are at a higher risk for osteoporosis than males [29–31] especially among post-menopausal woman who undergo a natural physiological process with subsequent accelerated loss in bone microarchitecture.[1] One meta-analysis (Gourlay et al. 2012)[32] found that women had significantly lower BMD at the hip and at the spine compared to men. While gender plays a role in the development of poor bone mineral density, it is essential to consider other factors such as age, genetics, lifestyle behaviours, and certain medical conditions that can also contribute to low BMD.

Within the subgroup analysis of the HIV cohort (**Table V**), our confounding factors of viral load, treatment status and CD4 count had no effect on BMD (p> 0.050). Of the 27 individuals within this cohort, 12/27 (44.4%) were virally suppressed and 17/27 (63%) of individuals were noted to be taking treatment at the time of assessment. During the study period (09/2017 – 12/2018), the WHO recommended a combination of two nucleoside reverse transcriptase inhibitors (NRTIs), with the non-nucleoside reverse transcriptase inhibitors (NNTRI) which consists of Tenofovir (TDF) + Lamivudine (3TC) (or emtricitabine [FTC] + Efavirenz (EFV) for the management of HIV infection. These first-line therapies and regimens have evolved with

the development of newer – and fewer – medications, which has improved the overall compliance and dosing requirements.

Despite this study finding no effect of treatment on BMD, multiple studies have shown a positive correlation between ART and a reduced BMD status.[9,21,31,33,34] ARV treatment, specifically TDF and protease inhibitors (PI) has been noted to have an association with low BMD, with PI having a greater association of bone loss.[35]

The difference in our findings from the literature could possibly be explained by low numbers within the subgroups, making subgroup analysis less accurate. Limited information was also available with regards to the duration of the ARVs each member was taking and whether there was a transition from one regimen to another during this study period.

In a meta-analysis, Brown *et al* [34] found that HIV-positive patients treated with antiretroviral therapy (ART) were more likely to have osteoporosis compared to ART-naïve patients. In 11 studies with 884 HIV-positive patients, 67% had reduced bone mineral density (BMD), and 15% had osteoporosis, compared to HIV-negative controls.[9,34] ART-treated individuals had 2.5-fold increased odds of presenting with reduced BMD compared to ART- naïve patients. In other studies, ART duration has been associated with lower BMD in some groups.[36]

The relationship between Vitamin D, as measured by serum 25(OH)D, and bone health is biologically plausible and the former plays a well-established role in several known diseases, including those related to skeletal health.[37,38] However, it is important to note that this relationship is complex and factors such as age, genetics and overall lifestyle all play a role in BMD.

Vitamin D deficiency has been considered a potential contributor to bone loss in HIV-infected individuals, described in several HIV populations – both ART-naïve individuals and those associated with ART exposure.[39] Lower levels than normal can be expected within the HIV

positive individuals.[9,40,41] One study within the Netherlands reported a prevalence of Vitamin D deficiency at 29 % amongst HIV-positive individuals.[40]

Our study showed very little comparable difference in Vitamin D levels between the two subgroups (**Table I**) with 11/27 (40.7%) HIV-positive and 85/145(57.9%) HIV-negative being insufficient (< 50) (p=0.218).This slight difference is most likely explained owing to the low numbers in the HIV-positive group, younger age group. This specific study did not review the type and duration of ARVs for the HIV-positive subgroup other than the information provided in Table V. The literature identifies ARVs as having an influential role in Vitamin D levels, with lower levels occurring in NNRTI-treated regimes compared with PI- treated individuals. TDF has specifically been highlighted as the main ART contributing to Vitamin D deficiency in HIV-positive individuals.[42,43]

Within our study group, Vitamin D levels were insufficient in 44/66 (66.7%) individuals within the osteopenic group and in 3/7(42.9%) in the osteoporotic group. Interestingly, insufficient levels were also noted in the normal categorized BMD group, with 49/99 (49.5%) individuals recorded as insufficient.(p> 0.050) (**Table IV**).

Our findings noted no significant effect of Vitamin D levels on BMD (**Table IV**) (p=0.221) which differs from reported literature. De Martinis et al. stated that a deficiency of Vitamin D accelerates bone turnover, bone loss, and increases the risk of osteoporotic fractures.[37] However, a metanalysis by Reid et al. found very little evidence to support the overall benefit of Vitamin D supplementation on BMD.[44]

In 2021, the prevalence of smoking in South Africa was estimated to be around 19% among adults aged 15 years and older.[45] However, importantly, smoking rates can vary among different populations, genders, age groups and across socio-economic status within the country.[45]

In this study group, the prevalence is 62/172 (36%) individuals who smoked, which is almost double that of the general South African population. Despite a high prevalence of smoking in the study populations, smoking was not found to be a factor associated with the development of a low BMD (p=0.544). Between BMD subgroups, a slightly higher proportion of non -smokers within the osteopenic and osteoporotic sub-groups was noted. This finding is contrary to the established literature, which has identified smoking as a risk factor for deterioration in bony infrastructure.[46] Ward *et al* [46] identified smokers as having significantly reduced bone mass compared with non-smokers (never and former smokers) at all skeletal sites.

The prevalence of hypoalbuminemia in this study cohort was 69/172 (40,1%). Our study showed a slightly higher number of individuals within the HIV subgroup – 15/27 (55.6%) – with low albumin levels (< 34) compared to the HIV-negative group – 54/145 (37.2%) – with no statistically significant difference noted between the two. Albumin testing is routinely performed at our institution as a marker as nutritional status despite this use being criticized as an unreliable marker and correlation to nutritional parameters.[47–49] Albumin does not independently indicate differences in nutritional status between HIV-negative and HIV-positive individuals[48],however, it is used in the stratification of HIV-infected individuals. There may be other factors or comprehensive assessments needed to accurately assess nutritional status in these populations.

## Limitations to the study

We identify limitations to this study, the first being an un-matched cohort study between HIV-positive and HIV-negative individuals with a younger generation of both cases and controls. Limited information with regards to duration of ARV treatment for each HIV positive individual was noted. This was a small sample group (n= 27) of HIV-positive individuals who were included within the data collection: a greater sample size would be advised for future research. Statistical analysis includes a univariate analysis. Multi – variate analysis was not possible due to its small population size of study.

## Conclusion

In conclusion, we found a higher proportion of patients with osteoporosis within the HIV-positive cohort, although not statistically significant and no statistically significant difference in the median BMD between the HIV -positive and HIV-negative subgroups. Neither age, smoking, vitamin D, albumin nor BMI was associated with a lower BMD,contrary to previous findings. A higher percentage of females were osteopenic and osteoporotic compared to the male group. Future studies should focus on incorporating more patients enabling us to see significant relationships.

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**Table I**. Value Ranges and classifications for BMD, Vitamin D and Albumin

| | Status | Range |
|---|---|---|
| BMD T-score* (g/cm2) | Normal | >-1 |
| | Osteopenia | -1 to -2.5 |
| | Osteoporosis | At or below -2.5 |
| Vitamin D level (nmol/L) | Deficient | <30 |
| | Insufficient | 31-49 |
| | Normal | >50 |
| Albumin (g/L) | Normal | >35-54 |
| | Low | <34 |

* WHO classification of BMD. European reference values were used since no comparison values for BMD within the South African population group.

**Table II**. baseline characteristics, demographic data and modifiable risk factors of HIV positive and negative subgroups.

| | HIV-Negative n=145 patients ( %) | HIV-Positive n=27 patients(%) | P value |
|---|---|---|---|
| Gender Female: Male | 28 (82.4) : 117 (84.8) | 6 (17.6) : 21 ( 15.2) | 0.822 |
| Age Median (IQR) | 30 (30 - 38) | 33.50 (29.25-40.75) | 0.079 |
| Smoking No:Yes | 91 (82.7) : 54 (87.1) | 19 (17.3) : 8 ( 12.9) | 0.681 |
| Albumin Normal : Low | 89 (88.1) : 54 (78.3) | 12 (11.9) : 15 (21.7) | 0.092 |
| Vitamin D Normal: Insufficient: Deficient | 57 (78.1) : 54( 87.1): 31 (91.2) | 16 (21.9) : 8 (12.9) : 3 (8.8 ) | 0.218 |
| BMI Median (IQR) | 22 (19.6-26) | 21 (20-24) | 0.663 |

**Table III**. Bone mineral density within the study cohort and two subgroups

| **Bone Mineral Density** | **Study Cohort** | **HIV-negative** | **HIV-positive** | **P -value** |
|---|---|---|---|---|
| | **n = 172 ( %)** | **n= 145 ( %)** | **n =27 ( %)** | |
| **T - score** [WHO]* | | | | |
| **Classification** | | | | |
| Normal | 99 (57.6) | 84 (58) | 15 (55.6) | 0.500 |
| Osteopenia | 66 (38.4) | 56 (38.6) | 10 (37) | |
| Osteoporosis | 7 (4) | 5 (3.4) | 2 ( 7.4) | |
| **BMD** (g/cm2: Median, IQR) | 0.49 (0.23 - 0.73) | 0.49 (0.23-0.73) | 0.49 (0.3 - 0.71) | 0.700 |
| **T score** (median, IQR) | -0.8 (-3.8 - -2.4) | -0.8 (-3.8 - 2.4) | -0.8 (-3.2 - 2.1) | 0.790 |

**Table IV**. Modifiable and non- modifiable risk factors influencing BMD

| Factors influencing BMD | Normal n= 99 patients(%) | Osteopenia n=66 patients( %) | Osteoporosis n=7 patients(%) | p- value |
|---|---|---|---|---|
| Age (yrs) Median(IQR) | 30 (25.25-36.75) | 32 (24-40.25) | 46 (30-61) | 0.081 |
| HIV Negative: Positive | 84 (57.9): 15 (55.6) | 56 (38.6) : 10 (37 ) | 5 (3.4) : 2 (7.4) | 0.515 |
| Gender Females: Males | 15 (44.1) : 84 (60.9) | 15 (44.1) : 51 (36.9) | 4 (11.8) : 3 (2.2) | **0.029** |
| Smoking No: Yes | 67 (60.9) : 32 (51.6) | 39 (35.5) : 27 (43.5) | 4 (3.6 ) : 3 (4.8) | 0.544 |
| Vitamin D Normal: Insufficient: Deficient | 48 (65.8) : 30 (48.4) : 19 (55.9) | 21(28.8 ) : 30 (48.8) : 14 (41,2 ) | 4 (5.5) : 2 (3.2) : 1 (2.9) | 0.221 |
| Albumin Normal: Low | 60 (59.4) : 37 (53.6) | 38 (37.6) : 28 (40.6) | 3 (3) : 4 (5.8) | 0.577 |
| BMI MEDIAN (IQR) | 22.20 (20-26.8) | 20.80 (19-24) | 23 (20-24) | 0.145 |

**Table V**. Subgroup analysis of the HIV positive cohort.

| Subgroup analysis of the HIV positive cohort | Normal n=15 patients(%) | Osteopenia n=10 patients(%) | Osteoporosis n=2 patients(%) | p-value |
|---|---|---|---|---|
| Viral Load Suppressed : Not suppressed | 6 (50) : 9 (64.3) | 5 (41.7 ) : 5 (35.7) | 1 (8.3 ) : 0 | 0.552 |
| Treatment status On treatment : Not on treatment | 10 (58.8 ) : 5 (50) | 6 (35.3) : 4 (40) | 1 (5.9) : 1 (10) | 1 |
| CD4 count Median (IQR,cell/mm3) | 263 (202,5 - 460,5) | 406 (354,8 - 536,25) | 282 (282- 282) | 0.300 |

## Appendix 1: HREC approval letter

# UNIVERSITY OF CAPE TOWN

**Department of Surgery**
**Departmental Research Committee**
**A/Prof Maritz Laubscher**
Groote Schuur Hospital
Observatory 7925
South Africa
**Tel** (021) 404 5108
**Email**: **maritz.laubscher@uct.ac.za**

14 Sep 2021

Dr P Botha

Department of Surgery
University of Cape Town

Dear Dr Botha

RE: Project 2021/254

**PROJECT TITLE: Bone Mineral Density In Hiv: A Comparison Of Hiv Positive Versus Negative Patient With Lower Limb Long Bone Fractures Within The Traumatic Setting**

The above protocol has been reviewed by the Department of Surgery Research Committee. I am pleased to inform you that the committee approved the scientific merit of the study, and endorse the protocol for submission to the relevant ethics committee.

Although this letter serves as confirmation that the above protocol has successfully passed through the surgical DRC, respective ethics committees still require DRC chair signature before submission.

Please use the above project number in all future correspondence,

Yours sincerely

A/PROF MARITZ LAUBSCHER
CHAIR SURGICAL DRC

## Appendix 2: Copy of Ethics approval for HOST study

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

### FHS016: Annual Progress Report / Renewal

| HREC office use only (FWA00001637; IRB00001938) | | | |
|---|---|---|---|
| This serves as notification of annual approval, including any documentation described below. | | | |
| ☑ Approved | Annual progress report | Approved until/next renewal date | 30.01.2025 |
| ☐ Not approved | See attached comments | | |
| Signature Chairperson of the HREC/ Designee | | Date Signed | 1/2/2024 |

**Note**: Please email this form and supporting documents (if applicable) in a combined pdf file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

HUMAN RESEARCH ETHICS COMMITTEE
31 JAN 2024
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

| Comments to PI from the HREC |
|---|
| |

**Principal Investigator to complete the following:**

**1. Protocol information**

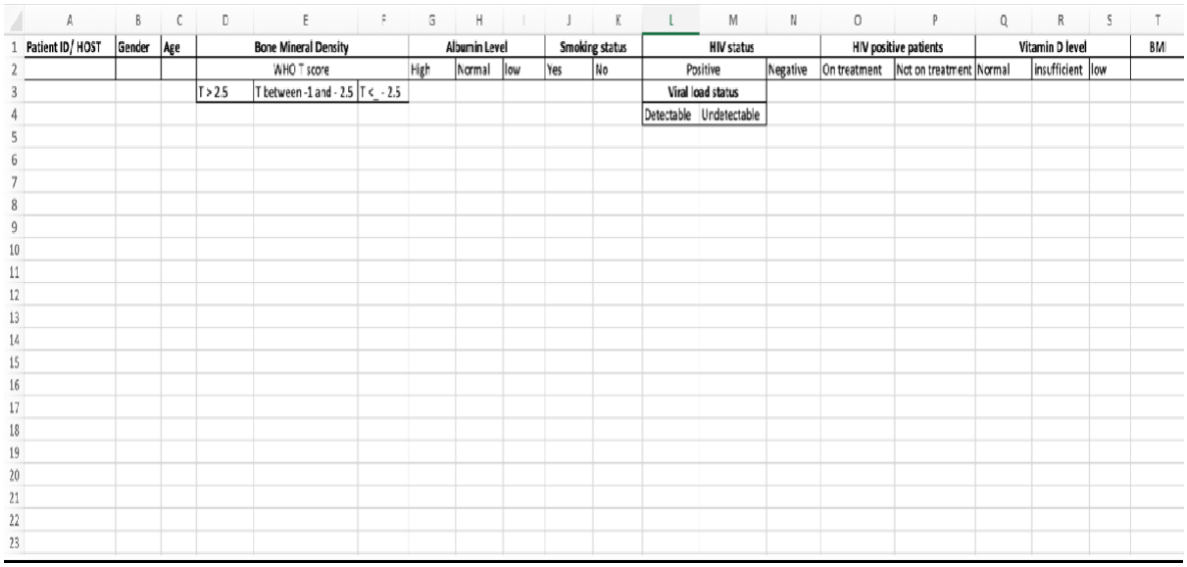
| | | | |
|---|---|---|---|
| Date (when submitting this form) | 29/01/2024 | | |
| HREC REF Number | 590/2016 | Current Ethics Approval was granted until | 30/01/2024 |
| Protocol title | Fracture healing in HIV positive patients – HIV in Orthopaedic Skeletal Trauma Study (HOST study) | | |
| Protocol number (if applicable) | | | |
| Are there any sub-studies linked to this study? | | ☐ Yes | x No |
| If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study. | | | |
| Principal Investigator | Prof Sithombo Maqungo | | |
| Department / Office Internal Mail Address | Division of Orthopaedic Surgery, H49 OMB, Groote Schuur Hospital<br>Marilyn van der Berg, (021) 404 5108, marilyn.vanderberg@uct.ac.za | | |

| 1.1 Does this protocol receive US Federal funding? | ☐ Yes | x No |
|---|---|---|



(Note: **Please complete the Closure form (FHS010) if the study is completed within the approval period)**

**Appendix 3: Data Collection and interpretation spreadsheet**



| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Patient ID/ HOST | Gender | Age | Bone Mineral Density | | | Albumin Level | | | Smoking status | | HIV status | | | HIV positive patients | | Vitamin D level | | | BMI |
| 2 | | | | WHO T score | | | High | Normal | low | Yes | No | Positive | | Negative | On treatment | Not on treatment | Normal | insufficient | low | |
| 3 | | | | T > 2.5 | T between -1 and - 2.5 | T < _ - 2.5 | | | | | | Viral load status | | | | | | | | |
| 4 | | | | | | | | | | | | Detectable | Urdetectable | | | | | | | |
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**Appendix 4: Information for Author Guidelines as per**

**South African Orthopaedic Journal**

**Information for Authors**

**To submit a manuscript click here**

Authors submitting articles for consideration for publication by the journal are required to familiarise themselves with the journal Ethics and Malpractice policy prior to submission. The policy is available on the journal website: https://www.saoj.org.za

**Criteria for publication**

- The article falls within the scope of the journal.
- Methods, statistics, and other analyses are performed to a high technical standard and are described in sufficient detail.
- Results reported have not been published elsewhere.
- Conclusions are presented appropriately fashion and are supported by the data.

- The article is presented in an intelligible fashion and is written in standard English (British usage).
- The research meets all applicable ethical standards.
- The article adheres to guidelines provided in the instructions for authors section.

**Guidelines for authorship**

- Each author should participate and is responsible for the content and design of the study, the preparation of the manuscript and its revisions, and final approval.
- In order to qualify for authorship, authors should satisfy all four the criteria for authorship as specified by the ICMJE:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

- Other ‘contributors’ or ‘collaborators’ can be acknowledged at the end of the manuscript together with their contribution. Those whose contributions do not justify authorship may be acknowledged individually or together as a group under a single heading (e.g., “Clinical Investigators” or “Participating Investigators”), and their contributions should be specified (e.g., “served as scientific advisors,” “critically reviewed the study proposal,” “collected data,” “provided and cared for study patients”, “participated in writing or technical editing of the manuscript”.
- The South African Orthopaedic Journal accepts a maximum of 8 authors per article. If there are more than eight authors, the first eight authors must be listed along with the group name at the end. The remaining authors and their affiliations must then be listed in an appendix.
- On submission of your article, the ORCID (Open Researcher and Contributor ID) identifier of at least the corresponding author will be required. ORCID provides a persistent digital identifier that distinguishes you from every other researcher and supports automated linkages between you and your

professional activities, ensuring that your work is recognised. To register and find more information, please visit: http://orcid.org

**Registration of clinical trials**

- A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Interventions include drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes.
- Clinical trials should be registered in a public trials registry in accordance with International Committee of Medical Journal Editors
- Trials must be registered and approved by the relevant authorities before the onset of patient enrolment.
- The Medicines Control Council (MCC) reference number and the SA National Clinical Trial Register (SANCTR) registration number should be included at the end of the abstract of the article.
- Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) do not require registration.

**Reporting guidelines**

- All articles should be prepared in accordance with the guidelines relevant to the study design, as described in the Equator Network Guidelines (https://www.equator-network.org/reporting-guidelines/)
- Randomised trials should be accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrolment, randomisation, withdrawal and completion, and a detailed description of the randomisation procedure.

**Reporting of statistics**

In terms of the statistical reporting, the Equator Network advises on the use of the SAMPL guideline: https://www.equator-network.org/2013/02/11/sampl-guidelines-for-statistical-reporting/

The SAMPL guidelines provide two guiding principles

1. *"Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results."* When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as *P* values, which fail to convey important information about effect size.
2. *Provide enough detail that the results can be incorporated into other analyses.* This requires reporting the descriptive statistics from which other statistics are derived, such as the numerators and denominators of percentages, especially in risk, odds, and hazards ratios. Likewise, P-values are not sufficient for re-analysis. Needed instead are descriptive statistics for the variables being compared, including sample size of the groups involved, the estimate (or effect size) associated with the P-value, and a measure of precision for the estimate, usually a 95% confidence interval.

Some specific guidelines applicable to the SAOJ:

- Consistency is one of the most important factors in presenting a well-formatted, professional manuscript.
- The nature of the measurements and variables reported on will often dictate the amount of precision required. Report numbers - especially measurements? with an appropriate degree of precision. For ease of comprehension and simplicity, round to a reasonable extent.
- The recommendation is to report the number of decimals that have both clinical and statistical meaning and consistently reporting all other variables in the same manner.
- Note: Generally, for descriptive purposes, percentages are reported as whole numbers except when dealing with really large sample sizes
- At least for the primary outcomes, report a measure of precision (a confidence interval).
- Although not preferred to confidence intervals, if desired, *p* values should be reported as equalities to three decimal places (e.g., $p = 0.031$ and not as inequalities: e.g., $p < 0.05$). Do NOT report NS; give the actual *P-value*. The smallest *P*-value that needs to be reported is $P < 0.001$.

- Report numerators and denominators for all percentages
- Summarize data that are approximately normally distributed with means and standard deviations (SD). Use the format: mean (SD) not mean ?
- Summarize data that are not normally distributed with medians and interpercentile ranges, ranges, or both.
- Do NOT use the standard error of the mean (SE) to indicate the variability of a data set. Use standard deviations, inter-percentile ranges, or ranges instead.

Formatting examples:

- p = 0.028 or p < 0.001
- (43% vs 21%; p = 0.002)
- (odds ratio (OR) 0.38; 95% confidence interval (CI) 0.71 to 1.82; p = 0.822) or after first use (OR 1.62; 95% CI 1.41 to 1.86; p < 0.001)
- *Descriptive stats normal distribution:* mean age 36 years (SD 4 years) or 36 years (SD 4; range 40 to 97 years)
- *Descriptive stats non-normal distribution:* median age 36 years (IQR 44 to 88 years) or 36 years (IQR 44 to 88 years; range 40 to 97 years)
- *Descriptive stats percentage:* (149 of 202; 74%)

**Formatting of submissions**

Text formatting

- Use Helvetica or Arial font, size 11.
- Use double line spacing throughout the document.
- Number the pages of the blinded manuscript consecutively.
- Use italics for emphasis.
- When referring to an article with multiple authors, please use the following format: Rabinowitz et al. published their retrospective review.

- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or Math Type for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

- Use no more than three levels of displayed headings.

Abbreviations

- Define abbreviations and acronyms at first mention and use consistently thereafter.

Units

- Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Figures

- Figures should be numbered consecutively with illustration Arabic numbers 1, 2, 3, etc.
- The figure should be listed in the text as follows: … wound irrigation and splinting (*Figure 1*).
- Figures should be clear and easily understandable with a full descriptive legend stating any areas of interest and explaining any markings, letterings or notations. All figures and figure legends should be understandable as a stand-alone item, without having to read the main body of the text.
- For radiographs, please ensure you state the view used and the time point at which it was taken, as well as the demographic details of the patient if applicable.
- Please submit the original JPEG (300 dpi) or TIFF of all photographs, as well as the figure saved as a Word document. The Word version of the figure should be complete with the legend and any necessary markings such as letters or arrows.
- Figures such as graphs and algorithms should be in Word or PowerPoint in order to be editable.

- Figures should not be imbedded in the text file but should be submitted as separate individual files. Each figure should be a separate file, entitled Figure 1, Figure 2, etc.
- Remove all markings, such as patient identification, from radiographs before photographing. Clinical photos must be adequately anonymised.
- A statement of patient consent for clinical photographs must be provided on the title page.
- In images depicting X-rays of children there should exhibit adequate shielding of radiation.
- All line or original drawings must be done by a professional medical illustrator.
- We accept a maximum of six figures. You may apply to the Editor-in-Chief for permission to include more figures if considered critical to the clarity and completeness of the submission.
- Do not submit any figures, photos, tables, or other works that have been previously copyrighted or contain proprietary data unless you have obtained and can supply written permission from the copyright holder to use that content.

Tables

- Tables should carry uppercase Roman numerals, I, II, III, etc.
- Tables should always be cited in the text in consecutive numerical order.
- The table should be identified in the text as follows: Details of results are listed in *Table I*. Or, alternatively, high-energy trauma that is often associated with these fractures (*Table II*).
- Tables should be used to present information in a clear and concise manner. All tables should be understandable without the main text.
- For each table, please supply a table heading explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table heading.
- Footnotes to tables should be indicated by superscript lower-case letters and included beneath the table body.
- Please submit tables as editable text and not as images. They should be created using the Table tool in Word.

- Do not embed tables in the text file but submit them as separate individual files. Each table should be a separate file, entitled Table I, Table II, etc.
- We accept a maximum of eight tables.
- Do not duplicate information given already in the text.
- Do not submit any figures, photos, tables or other works that have been previously copyrighted or contain proprietary data unless you have obtained and can supply written permission from the copyright holder to use that content.

References

- References should be numbered consecutively in the order that they are first mentioned in the text and listed at the end in numerical order of appearance.
- Identify references in the text by Arabic numerals in superscript after punctuation.
- References should not be a listing of a computerised literature search but should have been read by the authors and have pertinence to the manuscript.
- Accuracy of references is the authors' responsibility, and the author is to verify the references against the original documents.
- Manuscripts in preparation, unpublished data (including articles submitted but not in the press) and personal communications may not be included in the reference listing. They may be listed in the text in parentheses only if absolutely necessary to the contents and meaning of the article.
- The titles of journals should be abbreviated according to the style used in Index Medicus, obtainable through the website http://www.nlm.nih.govshould
- The following format should be used for references:

*Journal article:*

Sidhu GS, Ghag A, Prokuski V, Vaccaro AR, Radcliff KE. Civilian gunshot injuries of the spinal cord: a systematic review of the current literature. *Clin Orthop Relat Res* 2013;**471**:3945-55.

Ideally, the names of all authors should be provided, but the usage of *et al*. in long author lists (more

than six authors) will also be accepted: Fong K, Truong V, Foote CJ, *et al*. Predictors of nonunion and reoperation in patients with fractures of the tibia: an observational study. *BMC Musculoskelet Disord* 2013;**14**:103.

*Online journal article:*

Caetano-Lopes J, Lopes A, Rodrigues A, *et al*. Upregulation of inflammatory genes and downregulation of sclerostin gene expression are key elements in the early phase of fragility fracture healing. *PLoS One* 2011;**6**:e16947.

*Web reference (with authors):*

Cierny G, DiPasquale D. Adult osteomyelitis protocol. http://www.osteomyelitis.com/pdf/treatment_protocol.pdf.

(date last accessed 05 March 2013).

*Web reference (no authors listed):*

No authors listed. International commission on radiological protection. http://www.icrp.org (date last accessed 20 September 2009).

*Chapter in a book:*

Young W. Neurophysiology of spinal cord injury. In: Errico TJ, Bauer RD, Waugh T (eds). *Spinal Trauma*. 3rd ed. Philadelphia: JB Lippincott; 1991: 377-94.

*Dissertation:*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

*Abstract:*

Peterson L. Osteochondritis of the knee treated with autologous chondrocyte transplantation [abstract]. ISAKOS Congress, 2001.

**Structure and content of submission**

- We accept a maximum of 3 500 words, including the abstract and body of the text (excluding references).
- Exceptions to this rule may be made for systematic reviews and meta-analysis at the discretion of the Editor-in-Chief.
- Please follow the following structure when preparing your submission. Each of the following should be submitted as a separate file.
- Title page (title, authors and affiliations, corresponding author and declarations)
- Blinded manuscript (Abstract, keywords, introduction, methods, results, discussion, funding sources, conflict of interest statement, ethics statement, acknowledgements and references)
- Tables (with headings), each table as a separate file.
- Figures (with legends), each figure as a separate file.

Title page

*Title*

- The title should be concise and informative.

*Author names and affiliations*

- Please provide the following information for each author:
  - Full names and surname, as well as title
  - Qualifications
  - Designation
  - Affiliation and address
  - ORCID ID (see Article Submission section)

- Please check that all names are accurately spelled.
- Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate affiliation details.
- Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

*Corresponding author*

- Clearly indicate who will handle correspondence at all stages of refereeing and publication, including post-publication.
- Ensure that the e-mail address and permanent address is given and that contact details are kept up to date by the corresponding author.
- Please note that the corresponding author's contact details will be provided in the final article.
- Provide the following information for the corresponding author:
  - Full names and title
  - Affiliation
  - Physical address
  - Postal address
  - Telephone number
  - E-mail address

*Declarations*

Authors are to insert a section at the end of the title page entitled declarations (please provide the author's name, signature and date). The following statements are required under the declarations section:

*Authorship*

The authors confirm that all authors have made substantial contributions to all of the following:

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
- The drafting of the article or its critical revision for important intellectual content.
- Final approval of the version to be submitted.

*Sound scientific research practice*

The authors further confirm that:

- The manuscript, including related data, figures and tables, has not been previously published and is not under consideration elsewhere.
- No data have been fabricated or manipulated (including images) to support conclusions.
- This submission does not represent part of a single study that has been split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. 'salami-publishing').

*Plagiarism*

The authors confirm that the work submitted is original and does not transgress the plagiarism policy of the journal.

- No data, text or theories by others are presented as if they were the authors' own.
- Proper acknowledgements of others' work have been given (this includes material that is closely copied, summarised and/or paraphrased); quotation marks are used for verbatim copying of material.
- Permissions have been secured for copyrighted material.

*Conflict of interest statement*

A conflicting interest exists when professional judgment concerning a primary interest (such as the patient's welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). It represents a situation in which financial or other personal considerations from authors, reviewers or editors have the potential to compromise or bias professional judgment and objectivity. It may arise for the authors when they have a financial interest that may

influence their interpretation of their results or those of others. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, grants or other funding. All potential conflicts of interest need to be declared. The conflict of interest statement should list each author separately by name, e.g.,

'Author A.B. (*use initials of relevant author, not full name in order for the document to remain blinded*) has received research grants from Company A. Author B.C. has received a speaker honorarium from Company X and owns stock in Company Y. Author C.D. is a member of committee Z.'

If no conflicts of interest exist, state this as follows:

'The authors declare they have no conflicts of interest that are directly or indirectly related to the research.'

*Funding sources*

All sources of funding should be declared. Also, define the involvement of study sponsors in the study design, collection, analysis and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication.

List all funding sources as follows:

'This work was supported by the xxxx (grant numbers xxxx, yyyy).'

When funding is from a block grant or other resources available to a university, college or other research institution, submit the name of the institute or organisation that provided the funding.

If no funding was received, state as follows:

'No funding was received for this study.'

*Compliance with ethical guidelines*

- For all publications:

  ‘The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.’

  Available from: http://publicationethics.org/resources/international-standards-for-editors-and-authors

  Institutional Review Board (IRB) ethical approval must have been given if the study involves human subjects or animals. Please provide the approval number. IRB documentation should be available upon request.

  ‘Prior to the commencement of the study ethical approval was obtained from the following ethical review board: *Provide name and reference number’*

- For studies with human subjects include the following:

  ‘All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.’

  ‘Informed written consent was or was not obtained from all patients for being included in the study.’

  ‘Consent was obtained from patients for the use of clinical photographs and these images were adequately anonymised.'

- For studies with animals, include the following sentence:

  ‘All institutional and national guidelines for the care and use of laboratory animals were followed.’

- For articles that do not contain studies with human or animal subjects:

  ‘This article does not contain any studies with human or animal subjects.’

- If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. If any identifying information about patients is included in the article, the following sentence should also be included: Additional informed consent was obtained from all patients for which identifying information is included in this article. The Helsinki Declaration 2008 can be found at http://www.wma.net/en/30publications/10policies/b3/

  Please provide the names and email addresses of two reviewers.

  *Title Page Example*

  Title of Submission

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**Declarations:**

*Authorship*

The authors confirm that all authors have made substantial contributions to all of the following:

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
- The drafting of the article or its critical revision for important intellectual content.
- Final approval of the version to be submitted.

*Sound scientific research practice*

The authors further confirm that:

- The manuscript, including related data, figures and tables, has not been previously published and is not under consideration elsewhere.
- No data have been fabricated or manipulated (including images) to support conclusions.
- This submission does not represent part of a single study that has been split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. 'salami-publishing').

*Plagiarism*

The authors confirm that the work submitted is original and does not transgress the plagiarism policy of the journal.

- No data, text or theories by others are presented as if they were the authors' own.
- Proper acknowledgements of others' work have been given (this includes material that is closely copied, summarised and/or paraphrased); quotation marks are used for verbatim copying of material.
- Permissions have been secured for copyrighteed material.

*Conflict of interest statement*

John Smith declares that he has no conflict of interest. Paula Taylor has received research grants from Drug Company A.

*Funding sources*

No funding was received for the purposes of performing this study.

*Compliance with ethical guidelines*

The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.

Prior to the commencement of the study ethical approval was obtained from the following ethical review board: *Provide name and reference number.*

All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed written consent was or was not obtained from all patients for being included in the study.

Consent were obtained from patients for the use of clinical photographs/ and these images were adequately anonymised.

| Author Name | Signature | Date |
|---|---|---|
| J Smith | | 15/8/2017 |
| P Taylor | | 16/8/2017 |

Blinded manuscript

To ensure a blinded review, the main body of the manuscript should not contain any identifying information, including author's names, institutions or affiliations. Please do not include the name of the ethics committee, this information should be provided in the title page.

*Abstract*

- A structured abstract (maximum of 350 words) summarising the most important points in the article is required.
- The abstract consists of four paragraphs with the subheadings:
  - Background (must include the aim of the study)
  - Patients and methods
  - Results
  - Conclusion
- References should be avoided. Avoid uncommon abbreviations. If essential, they must be defined at their first mention in the abstract itself.

*Keywords*

- Immediately after the abstract, provide a maximum of six keywords using standard searchable terms. These keywords will be used for indexing purposes.

*Level of evidence*

- Level 1 to 5.

- Please follow the level of evidence guidelines provided by the Oxford Centre for Evidence-Based Medicine (OCEBM); version 2.1.
- Available from: OCEBM Levels of Evidence Working Group. 'The Oxford Levels of Evidence 2'.Oxford Centre for Evidence-Based Medicine. http://www.cebm.net/index.aspx?o=5653

*Introduction*

- The introduction should contextualise the study by providing the background to the research; explain the problem that is to be addressed, and provide the rationale for the study.
- Briefly outline the relevance of the study with respect to the current literature. Avoid a detailed literature survey or a summary of the results.
- The last sentence should outline the research question or hypothesis.

*Patients (or Materials) and methods*

- State the methods, outcome measures, and selection criteria. The following aspects need to be described:
  - The study design and research methodology
  - Whether randomisation (with methods) was applied
  - If case-controlled, how the controls were selected
  - The time period under review
  - Number of patients/subjects under investigation and why this number was chosen
  - Inclusion and exclusion criteria
  - Case and outcome definitions
  - A description of the procedure or intervention, including post-operative protocol

- The outcome measures or scores used
- The minimum follow-up period
- Statistical analysis paragraph. This should be included at the end of this section to detail statistical tests and package used, the reasons why these tests were used, and what p-value was considered statistically significant. A power analysis is recommended for studies comparing two or more groups.

- Provide sufficient detail so that another researcher can replicate the study.
- The reader should understand from this description all potential sources of bias such as referral, diagnosis, exclusion, recall or treatment bias. This includes the manner in which investigators selected the patients. Consecutive inclusion implies all patients with a given diagnosis are included, while selective implies patients with a given diagnosis but selected according to certain explicit criteria (e.g., state of disease, choice of treatment).
- Do not describe standard procedures for common operations. Only include new procedures or adaptations to standard procedures.
- If you name any specific product, it requires the manufacturer's name, city and state/country.
- Present information in the narrative format and use the past tense.
- Where relevant, tables or figures may be included to provide information more clearly.
- Generally, no data should be presented in this section.

*Results*

- Describe the relevant results and analysis thereof.
- Provide details of the number of patients included and excluded, as well as the reason for exclusion.
- It is important to state the follow-up period (mean and range).
- The results can be broken down into separate sections, e.g. Treatment, Functional outcome, Complications, etc.
- Tables may be used but avoid repeating data reported in the text in the tables.
- All appropriate data should be presented as means with ranges, not with standard deviations (SDs). Medians should only be used when the data is skewed, accompanied by an interquartile range (IQR).
- Avoid using percentages in studies involving well under 100 subjects.

- All results must be backed up with p-values or survivorship analysis. All Kaplan-Meier data should be presented with confidence intervals. Always present exact absolute p-values, whether significant or not, unless $p<0.001$.
- However, *P-values* do not always convey the entire picture and where relevant, the confidence interval will also be required (in addition to the power of the study reported in the methods section).

*Discussion*

- The question or hypothesis stated at the end of the introduction should be discussed and either supported or rejected.
- The results must be interpreted clearly, and any deficiencies expressed. All possible confounding factors, sources of bias or weaknesses in the study should be identified.
- Explore the significance of the results of the work rather than repeating the results.
- The discussion must point out the relevance of the work described in the paper and its contribution to current knowledge.
- Explain what can be deduced from the results and how will it affect clinical practice.
- Include a review of the relevant literature, placing the results of the study in the context of previous work in this area.
- Discussion of relevant prior research and references must be concise. Avoid extensive citations and discussion of published literature emphasize previous findings that agree (or disagree) with those of the present study.
- Do not repeat the introduction.
- Present the limitations of the study and suggest how the study could have been improved for a future study.
- Avoid making inferences from non-significant trends unless you believe your study is adequately powered to answer the question; in that case, provide a power analysis.

*Conclusion*

- Provide a summary statement that conveys the conclusions of the findings.

- Do not draw conclusions not supported by the data obtained from the specific study presented.

*Ethics statement*

- For studies involving human subjects, please include an ethics statement as follows: ‘All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.’
- For animal studies, please include the following ethical statement: ‘All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.’
- If the study did not involve human or animal subjects, state that: ‘This article does not contain any studies with human participants or animals performed by any of the authors.’
- Please also include an informed consent statement: ‘Informed consent was obtained from all individual participants included in the study.’
- Alternatively, for retrospective studies, please add the following sentence: ‘For this study formal consent was not required.’
- If identifying information about participants is available in the article, the following statement should be included: ‘Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.’

*Acknowledgements*

- Acknowledgements should be placed at the end of the discussion and before the references.
- In this section, persons who were involved but did not earn authorship can be acknowledged.
- Statements should be brief. A person can be thanked for assistance or comments.
- Do not include contributions by editors or referees.

*Author contributions*

- Please state the contributions of each author

- For example: ‘A.B contributed to the study conceptualisation, design, data analysis and manuscript preparation. C.D. contributed to data collection and manuscript preparation. E.F. contributed to ....’
- The types of contributions are:
  - Conceptualisation and design
  - Data collection or contribution
  - Data analysis
  - Manuscript preparation
  - Other contributions (please specify)

*References*

- Please refer to the section on Formatting of submissions.

Tables and figures

- Tables and figures should not be imbedded in the text file but should be submitted as separate individual files. Each table should be a separate file, entitled Table I, Figure 2, etc.
- Each table and figure should be provided with a heading or legend.
- Please refer to the ‘Formatting of submission’ section for further guidelines.

**Case reports**

In addition to the preceding guidelines the following applies:

- The following headings need to be adhered to in the body of the manuscript:
  - Abstract
  - Keywords
  - Background
  - Case report
  - Discussion
  - Conclusion

- Ethics statement
- References

- Abstract: Minimum 250 words (350 maximum), using the following headings:

- Background
- Case report
- Discussion
- Conclusion
- Statement of informed consent must be included in the ethics statement.

**Current Concepts Review Article (by invitation only)**

General Guidelines:

- A narrative review will suffice (and systematic or scoping review not necessary)
- A thorough literature review needs to be done prior to writing the manuscript to ensure that the author is well acquainted with the current concepts related to the topic (with emphasis on the most recent developments)
- A balanced and unbiased view of the current clinical aspects of the topic.
- Focus on clinical aspects like diagnosis and treatment.
- Discuss controversies and state both sides of the argument.
- Avoid extensive discussion of basic science (anatomy/physiology/pathology) aspects, except for some really novel and clinically relevant new developments in the field.
- The topic may be adapted, but only with the permission of the Editor-in-Chief.

Outline of Article:

- Abstract = One paragraph, no headings, ≤350 words.
- Introduction = Brief introduction to the topic

- Contents = Please use headings (in bold) and sub-headings (in italics) to structure the manuscript in a reader-friendly manner
- South African context = Discuss matters which may be particularly relevant or unique to the South African clinical setting.
- Learning points = Make use of tables to summarize important learning points
- Conclusion = Brief evidence-based conclusion and summary
- Conflict of interest statement
- References = As usual

## DECLARATIONS

### Funding


- The authors did not receive support from any organization for this submitted work.
- No funding was received to assist with the preparation of this manuscript.
- No funding was received for conducting this retrospective study.
- No funds, grants, or other support was received.


### Conflicts of interest/ competing interests

- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no conflicts of interest to declare that are relevant to the content of this article.
- All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed in this article.

### Availability of data material

Data and all appropriate documentation are stored on an excel document and are available for consultation if needed.

### Consent to participate and publication

This is an extension of the HIV in Orthopaedic Skeletal Trauma (HOST) study and all patients of the HOST study group have given written consent related to further studies and publications from the collected data.

**Ethics approval**

Ethical approval was obtained by the Human Research Ethics Committee (HREC) of the University of Cape Town. HREC **590/2016**. This article does not contain any studies with human participants or animals performed by any of the author.