# EFFECTS OF CHEMOTHERAPY ON BONE MINERAL DENSITY IN A POST-MORTEM CONTEXT

by

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## LIST OF ABBREVIATIONS

| Abbreviation | Description |
| --- | --- |
| BMD | Bone Mineral Density |

## ABSTRACT

Chemotherapy has been shown to have a significant impact on bone mineral density and bone quality in cancer patients. This research aims to study the impact of chemotherapy on bone mineral density and bone quality as seen in the skeletal remains of cancer patients. The Texas State Donated Skeletal Collection was utilized with a total of 20 individuals in the sample size of which 10 were cancer patients and 10 were demographically matched control individuals. The distal left tibia and first lumbar vertebra were scanned using microCT technology and the images were processed to determine bone mineral density, trabecular thickness, and cortical thickness for each element. There is no statistically significant difference between the trabecular and cortical thickness between donor groups. The average bone mineral density is statistically significantly greater for the chemotherapy group than the control group. The difference between donor groups is likely due to outside factors including in-depth medical information and lifestyle that were unable to be accounted for. The post-menopausal impact on the female donors is also another factor that likely has a significant impact that could not fully be accounted for. Further research should take into account and factor in the medical records and lifestyle of individuals with a larger and more comprehensive sample size as well.

# I. INTRODUCTION

This study aims to perform a comparative analysis of the bone quality in the skeletal remains of individuals who have undergone chemotherapy and those who have not. The purpose of this research is to determine the impact that chemotherapy has on the human skeletal system in a post-mortem context. Both bone mineral density and bone porosity will be evaluated to test for a statistically significant difference between the test groups. This research will also allow for a more in-depth look at bone mineral density and quality that cannot be observed in living patients.

## Mechanisms of Cancer

A cancer diagnosis is a dramatically disrupting event that changes an individual's entire life plan and trajectory. Not only does a diagnosis with as much gravity as cancer bring the burden of health stress, but also a significant mental and emotional toll. For many cancer patients the decision to accept and undergo treatment itself can be a heavy and arduous process (Gassmann *et al.*, 2016).

Cancer is the abnormal and dangerous rapid proliferation of healthy cells caused by mutations and damage in the DNA. Cells become cancerous when they can independently signal cell proliferation beyond healthy capacity, avoid cell death and proliferation inhibitors, promote blood vessel formation, and invade other tissues. In order to reach this cancerous state, the cells' DNA and growth signaling pathways must sustain damage that goes unchecked and uninhibited by the cell. Cells have internal regulatory systems which signal for the cell to grow and replicate and initiate cell death in the case of DNA damage. A significant amount of DNA and cell signaling pathways must be sustained to overcome more of the cells' internal regulations. If a cell is able to

signal to itself to proliferate without cell death, then it is able to overcome these regulatory systems. Once all of these checks which are established to maintain healthy cell proliferation are overcome, mutated cells proliferate at an uncontrolled rate causing damage to nearby tissues and the body (Cree, 2011).

**Mechanisms of Chemotherapy**

Chemotherapy is a common treatment option that works to inhibit cancer growth by targeting pathways of cell proliferation to stop the unimpeded replication of cancer cells. Cytotoxicity of chemotherapy leads to the healthy tissues of the body becoming inadvertent targets of cancer-killing mechanisms, leading to illness and frailty. The skeletal system is strongly impacted by this nonspecific function of chemotherapy, leading to bone loss and destabilization of the skeleton. The impact on the skeleton alone can interrupt daily function, as bone loss leads to complications with mobility and an increased risk of fractures (D'Oronzo *et al.*, 2015).

Chemotherapy works in fighting cancer by targeting the cellular functions which allow cells to rapidly divide. This function is to stop tumors and cancer cells from proliferating, as the cell signals work unintendedly in these cells to enact regulation as healthy human cells would. Unfortunately, the mechanisms to stop rapid cell division and proliferation are not specific enough to cancer cells, as healthy, but proliferating cells and tissue can be targeted. As discussed, cancer cells are derived from healthy cells with DNA mutations which prevent the cell from correctly regulating itself and preventing damage. The same signaling pathways which are mutated in cancer cells exist in healthy cells as well, which leads to the rise of the notorious side effects of chemotherapy from damage to healthy tissues and cells (Gassmann *et al.*, 2016; Gleeson *et al.*, 2002).

Chemotherapy is known to have many painful and even debilitating side effects, including fatigue, pain sensitivity, nausea, and sickness. Patients have reported that the decision to maintain treatment through chemotherapy can become a process of weighing the cost and benefits of chemotherapy, due to the toll it takes on the body to keep them alive. While the experiences with different types of cancers and chemotherapy treatments may not be precisely universal, dealing with cancer and the cytotoxic impact of chemotherapy is an incredibly difficult and disrupting time (Gassmann *et al.*, 2016).

The impact of chemotherapy on the skeletal system has significant ramifications on the human body, as a central and integral structure (Gleeson *et al.*, 2002). Chemotherapy impacts the skeletal system by reducing bone density, which reduces bone integrity and increases the risk for fractures (Winters-Stone *et al.*, 2009). The skeletal system is finely regulated and under constant remodeling. Osteoblasts, which contribute to new bone growth, must be kept in balance with osteoclasts, which resorb old bone material, to maintain a healthy skeleton (D'Oronzo, *et al.*, 2015). Bone density is measured by the concentration of hydroxyapatite per volume (Mei *et al.*, 2017). Hydroxyapatite is a vital component of bones, contributing to the stability and strength of the skeletal system. An imbalance of bone resorption, in which osteoclasts are more active than osteoblasts, leads to hydroxyapatite loss in the increased reabsorption with a lack of new bone. Decreased bone mass and bone density result from increased activity of osteoclasts without sufficient osteoblastic activity to replace the bone that is taken away. Conditions, such as osteoporosis, decrease the amount of hydroxyapatite and bone density, thus leading to increased porosity, fragility of the skeleton, and greater risk for fractures (Liu *et al.*, 2018).

**Indirect Chemotherapy Pathways**

One way that chemotherapy affects the skeletal system is indirectly. The endocrine system and hormone levels are altered by chemotherapy, which in turn affects bone remodeling (Winters-Stone *et al.*, 2009; Howell *et al.*, 2000). Hormones, including testosterone, estrogen, and androgens, allow for cells to grow and reproduce at a healthy rate through cell signaling and regulation. Estrogen regulates bone turnover by signaling for osteoprotegerin, which is secreted by osteoblasts to prevent an overproduction of mature osteoclasts, keeping bone resorption in check. Androgens aid estrogen as well in further inhibiting the overproduction of osteoclasts by blocking the expression of cytokines in the pro-osteoclastogenic pathway. Testosterone is converted to estradiol which signals the production of osteoblastic activity as well as prevents apoptosis (D'Oronzo *et al.*, 2015). When levels of these hormones are significantly disrupted, these cell-signaling pathways are disrupted and healthy tissue damage and bone loss can occur (Howell *et al.*, 2000). Winters-Stone *et al.* (2009) showed how estrogen and progesterone deprivation caused by cytotoxic side-effects of chemotherapy can induce bone loss and lead to an increased risk for fracture. Bone loss is induced as estrogen deprivation leads to an imbalance in osteoblast and osteoclast signaling (D'Oronzo, *et al.*, 2015). Howell *et al.* (2000) explained how chemotherapy can inhibit testicular function through cytotoxicity, leading to reduced testosterone production. Bone mineral density was found to experience a correlated decrease as testosterone aids in maintaining proper bone remodeling (Howell *et al.*, 2000). The chemotherapy agent cisplatin has been known to cause damage to the kidneys, leading to an imbalance in magnesium in the body which impedes bone remodeling and growth (van Leeuwen *et al.*, 2003). The chemotherapy

agent ifosfamide similarly causes indirect bone loss through kidney damage, resulting in the drastic decrease of vital minerals, proteins, and electrolytes (D'Oronzo *et al.*, 2015).

Cell receptors are often the targets of chemotherapy, as blocking those receptors helps to mitigate cancerous growth and inducing cessation of the cell cycle. However, as shown in the study of the IKKbeta receptor, inhibiting those receptors also inhibits intercellular signaling which induces cell growth for other organs and tissues, such as bone. IKKbeta phosphorylates IκB proteins which activate NFκB, a protein complex that aids in DNA transcription (Le Sommer *et al.*, 2015). The overexpression of NFκB has been implicated in breast cancer metastases, which are often radiotherapy and drug-resistant (Marino *et al.*, 2018). Deficiency in minerals, such as calcium, has also been found to be caused by chemotherapy and can also lead to an interruption of cell growth (Orgel, 2016). Understanding the various factors and pathways that can lead to bone loss allows for better insight into the depths of the impact of chemotherapy.

**Direct Chemotherapy Pathways**

The skeletal system itself can be directly impacted by chemotherapy regimens as well. In these cases, the pathways which signal osteoblast and osteoclast activity are disrupted and no longer in equilibrium leading to a decrease in both trabecular and cortical bone (D'Oronzo *et al.*, 2015). The trabecular bone and bone marrow have been shown to express greater rates of bone loss than the cortical bone, being more significantly impacted by cytotoxic side effects (Orgel *et al.*, 2016; Koh *et al.*, 2017). Etoposide is a widely used chemotherapy agent which induces apoptosis by inhibiting topoisomerase which prevents the rebinding of DNA strands, leading to the breaking of DNA strands and the death of the cell. It has been found to be significantly effective to

decrease tumor mass and cancer cell count but has also been found to greatly affect the cells of bone marrow. Etoposide is highly associated with increased fracture risk, as bone cell production and maturation become greatly affected and inhibited, as the bone marrow is the center for these activities (Koh *et al.*, 2017). Cell signal cascades in the bone marrow are interrupted due to deprivation of essential proteins, preventing maturation of osteoblasts, significantly impacting bone growth. Hematopoietic functions may also be inhibited, as some chemotherapy regimens, such as melphalan, target the formation of new blood vessels to the tumor to stop tumor proliferation. This may prevent proper blood supply to the skeletal system as well, only furthering tissue damage (Gencheva *et al.*, 2013). The agent doxorubicin has been shown to impede chondrocyte proliferation, leading to decreased bone mass in the trabeculae and thinning of the skeletal growth plate. Methotrexate has been established as a cause for significant side effects in the skeletal system, including increased fractures, general pain, and osteoporosis (van Leeuwen *et al.*, 2003). Cyclophosphamide also directly impacts the bone remodeling process by impeding the cell division process, as it is an alkylating agent causing cross-linking of DNA and RNA during cell replication and division, leading to cell death due to cross-linking interrupting this process (McCann *et al.*, 1971). Thus, bone mineral density is impacted due to the division of osteocytes and pre-osteoblasts being inhibited leading to an overall decrease in bone activity (D'Oronzo *et al.*, 2015). An individual may experience multiple factors at once, such as mineral deficiency, hormone imbalance, and cell signal interruptions, which induce bone loss during chemotherapy (Howell *et al.*, 2000).

**Variables and Considerations of Chemotherapy**

Although research has supported the loss of bone density in individuals from various backgrounds, the variability of the types of chemotherapy itself must be considered as chemotherapy regimens are adjusted for the type of cancer (Stephens and Aigner, 2016). Different targets of chemotherapy impact how healthy body tissue may be impacted, as the target changes, different healthy tissues are inadvertently affected. Chemotherapy regimens, as seen in doxorubicin and cyclophosphamide, may impact the skeletal system through both direct and indirect pathways (D'Oronzo *et al.*, 2015). Compounding effects of chemotherapy and multiple chemotherapy regimens administered at once potentially lead to further intensified loss of bone density (Orgel *et al.*, 2016). These types of cancer and the types of chemotherapy regimens need to be considered in this research as a variable for differences in bone density among the individuals who have undergone chemotherapy.

Orgel *et al.* (2016) showed how the treatment of acute lymphoblastic leukemia with a chemotherapy regimen, composed of vincristine, a glucocorticoid, an anthracycline, and pegylated L-asparaginase, can induce bone loss as early as 28 days into treatment. This early onset loss of bone density is important to consider in this research as the findings support that chemotherapy patients are significantly impacted by chemotherapy regardless of the length of time chemotherapy is administered (Orgel *et al.*, 2016). As presented in a study on childhood patients by Gleeson *et al.* (2002), the effects of chemotherapy in decreasing bone mineral density last many years after treatment and remission when compared to people who have not undergone chemotherapy treatment. Acknowledging these findings is important to the study at hand, as it supports the

supposition that the chemotherapy patients being studied will exhibit a significant decrease in bone density due to the impact of chemotherapy, while considering the variables of age, time after treatment, and duration of treatment. While it has been shown that the effects of chemotherapy persist long after the initial treatment (Winters-Stone *et al.*, 2009; Gleeson *et al.*, 2002), the length of time may impact an individual's ability to recover from or receive treatment for bone loss.

**Research Questions**

This study will focus on the impact of chemotherapy on bone quality, including bone mineral density, porosity, and cortical thickness in human skeletal remains. It also allows for possible insight into the human remains of various cancer patients found in various contexts. This research will also examine if there are long-term effects on bone quality after chemotherapy has been administered.

The study will test two hypotheses:

H1: Individuals who have undergone chemotherapy will have lower volumetric bone mineral density relative to age and sex matched counterparts.

H2: Individuals who have undergone chemotherapy will have greater cortical bone porosity relative to age and sex matched counterparts.

These hypotheses have been informed by previous research conducted on the impacts of chemotherapy on bone quality. The existing literature has supported that bone integrity and bone mineral quality are negatively impacted by chemotherapy, leading to the formulation of hypotheses that this will be reflected in skeletal remains.

## II. MATERIALS AND METHODS

### Sample Size

This study utilized the Texas State Donated Skeletal Collection for skeletal analysis (Gocha *et al.* 2022). The Texas State Donated Skeletal Collection consists of self and next of kin willed body donations, with medical and demographic information provided by the individual and/or the next of kin. This information includes pertinent information which aided in this research including age at death, ancestry, height, weight, medical diagnoses, as in this case cancer, and treatment. The sample size consists of 20 individuals, half of whom had received chemotherapy and half of whom had not. The sample size was restricted by the availability of donors within the collection who have received only chemotherapy, and no radiation or hormone therapy. These criteria were determined to mitigate variables influencing bone quality and to focus on the effects of chemotherapy. These ten donors who had received chemotherapy in life were then matched with donors who had not received chemotherapy according to the medical data provided. These donors were matched based on sex, ancestry, age at death (± 5 years), weight, and stature to mitigate the differences in bone mineral density and bone quality being attributed to biological factors rather than the effects of chemotherapy. Table 1 shows the list of donors from the chemotherapy and control groups matched with one another.

### Data Collection

The left distal tibia and first lumbar vertebra from each donor were imaged using a North Star Imaging, Inc. X5000 Computed Tomography System at Texas State University. Both of these bony regions provide a sufficient amount of trabecular bone to

be analyzed (American Bone Health, 2019; Koh *et al.*, 2017; Orgel *et al.*, 2016). Previous research has established that these regions of bone are impacted by chemotherapy in terms of bone quality and bone mineral density. These skeletal elements were imaged with a bone densitometry phantom as a calcium hydroxyapatite reference bone mineral density to estimate the bone mineral density values from the skeletal elements during data analysis. The elements were fixtured together in an acrylic tube and secured with low density foam. They were scanned at 64 microns, 75 kV, and 185 mA from 3 views. The radiographs were then reconstructed using NSI efX-CT software® and stacks were saved as 16-bit tiff grayscale images.

**Bone Mineral Density**

After reconstruction, bone volumetric bone mineral density was calculated for each element. Using the Dragonfly® program, the mean grayscale values were acquired from the histogram of the bone phantom by creating regions of interest around the control columns within the bone phantom. These grayscale values and the respective known densities (in grams per cubic centimeter) were used to set up a scatter plot graph in Microsoft Excel®. A line of best fit was plotted on this graph and an equation was derived from this line to later calculate the unknown densities for the tibia and first lumbar vertebra in the same scan. The skeletal elements were then analyzed by capturing the entire bone as a region of interest for both the lumbar vertebra and the distal tibia. The mean grayscale values for each skeletal element were then computed by Dragonfly®. This greyscale value was used to derive the unknown density values from the equation set up earlier with the bone phantom. Using the Bone Analysis widget within the Dragonfly® program, the bone volume fraction was computed from the regions of

interest of each skeletal element. The bone volume fraction of each element was multiplied by the density of its respective calculation to derive the bone mineral density values for each skeletal element.

**Bone Quality/Porosity**

Volumetric measurements were calculated from these skeletal segments to better assess bone volume and greyscale values for the bone utilizing the Bone Analysis tool. Average trabecular and cortical thickness was also calculated and recorded for each element scanned during this process. This was performed in the Bone Analysis Widget in Dragonfly®. Using the algorithm developed by Kohler *et al.* (2007) the trabecular bone was segmented separately from the cortical bone, allowing average thicknesses to be calculated by Dragonfly®. These average thicknesses were then recorded for each element and donor.

If a statistical difference is determined between the bone mineral density values for the chemotherapy and the control group, then a qualitative visual assessment of the skeletal elements was performed. The Dragonfly® program was used to analyze the bone quality as seen in the cortical bone. To better visualize the bone and see density levels in the CT scan, volume rendering tools were used to isolate the bone and provide better visualization. The skeletal elements were then isolated to be analyzed separately by creating a Region of Interest just around the skeletal material. This was performed by isolating the skeletal material from the surrounding air and extraneous material using the greyscale values to differentiate between these regions. Using the Thickness Contour Mesh tool, a 3D isosurface was generated to visualize the skeletal elements and visualize color-coded regions of thinning and porosity in the cortical bone. These regions of

porosity and thinning were recorded for the amount of the surface that they occur on and the regions they occur on. The donors were then placed into categories based on the amount of porosity and thinning exhibited, whether mild, moderate, or significant. The mild category is denoted as porosity occurring in approximately a quarter of the surface with mild thinning. The moderate category is denoted by approximately half of the surface exhibiting thinning and porosity. The significant category is denoted by the majority of the skeletal element exhibiting cortical thinning and over half of the surface exhibiting porosity.

**Data Analysis**

A Shapiro-Wilk test was performed to test whether or not the data is normally distributed. The result of the Shapiro-Wilk test determined the type of test that was used to test for a statistically significant difference between the groups. Normal distribution would warrant a two-tailed t-test of unequal variances while non-normal distribution would indicate a Mann-Whitney U test should be used. A test was performed for the bone mineral density values for the tibia, first lumbar vertebra, and the total bone mineral density values comparing the control and chemotherapy groups. The average trabecular and cortical thickness was also tested for a statistically significant difference between the chemotherapy and control groups. They were tested for normal distribution and the appropriate test was applied accordingly.

## III. RESULTS

Descriptive statistics, including the mean, standard deviation, and range, for bone mineral density, cortical thickness, and trabecular thickness are shown by treatment group in Table 5, Table 6, and Table 7 respectively.

A Shapiro-Wilk test was performed to test if the bone mineral density data were normally distributed. The bone mineral density values were normally distributed, so a t-test of equal variances was performed. This test was performed to compare the bone mineral density values for the tibia, first lumbar vertebra, and the total bone mineral density values between the control and chemotherapy groups. The t-tests were performed at a 95% confidence interval. The p-value calculated for comparing the aggregated bone mineral densities is 0.0105. The p-value calculated for the tibia is 0.0498 and for the first lumbar vertebra is 0.0919. In general, the chemotherapy group had higher bone mineral density, thicker cortical and trabecular bone.

A Shapiro-Wilk test revealed that the data for trabecular and cortical bone thickness is not normally distributed, so a Mann-Whitney U test was chosen to test for a statistical difference between the chemotherapy and the control groups at a 95% confidence interval. The p-values for the total trabecular thickness, the trabecular thickness of the tibia, and the trabecular thickness of the first lumbar vertebra are 0.4328, 0.9450, and 0.2568 respectively. The p-values for total cortical thickness, cortical thickness of the tibia, and cortical thickness of the first lumbar vertebra are 0.2315, 0.1431, and 0.5288 respectively.

There was a statistically significant difference between the total bone mineral density values and the BMD values of the tibia, so a visual and qualitative assessment

was performed in Dragonfly®. There was no statistically significant difference between the chemotherapy patients and the control for the lumbar vertebra, so special attention was given to the tibia in the scans.

The donors from the control group were examined first. The donors were divided into three categories: significant porosity and thinning, moderate porosity and thinning, and mild porosity and thinning. Donors 2015.021, 2014.001, 2016.029, and 2016.011 exhibited dramatic thinning occurring over the majority of the bone and significant porosity, with gaps in the cortical bone near the articular surface. Donors 2008.001, 2016.041, and 2017.003 exhibited moderate porosity and thinning, with thinning and porosity occurring on approximately half of the tibial surface. Donors 2018.063, 2013.008, and 2014.066 exhibited minor thinning and porosity with less than half of the surface exhibiting porosity.

The donors from the chemotherapy group were analyzed and categorized into the same three previously defined categories. Donor 2012.004 exhibited significant thinning and porosity. Donors 2012.014, 2012.040, 2014.016, and 2014.037 exhibited moderate porosity and thinning. Donors 2012.024, 2014.039, 2015.022, 2016.040, and 2016.051 exhibited minor porosity and thinning.

A pattern was detected among all donors across both groups with a correlation between the donors with smaller average cortical and trabecular thickness exhibit more porosity and cortical thinning, especially at the articular surface. The region of bone directly above the articular surface of the tibia was the most porous region in all donors. The articular surface exhibited the most consistent thinning among all groups but exhibited the least amount of porosity. There was no difference in the porosity and

thinning patterns exhibited between the control and chemotherapy groups.

## IV. DISCUSSION

The purpose of this study was to compare bone quality between individuals who had received chemotherapy compared to a demographically matched control group. The volumetric BMD was statistically significantly different between the donor groups ($p = 0.0105$). The volumetric BMD was also statistically significantly different for the tibia, with a p-value of 0.0498, however, it was not statistically significantly different for the first lumbar vertebra comparison with a p-value of 0.0919. The research hypothesis that individuals who have undergone chemotherapy will have lower volumetric bone mineral density relative to age and sex matched counterparts was rejected. The average volumetric BMD for the chemotherapy group is greater than the average BMD value for the control group. As this was not expected according to research on previous literature, other explanations and factors in this study were investigated.

Although the donors were demographically and physically matched as close as possible between the groups, multiple variables including lifestyle and medical history could not be controlled for, as this information was not available. One of the most important variables that could not be accounted for is remission, including if the donors were able to achieve remission and for how long. Such variables could have explanatory power and could offer significant insight into the results observed in this study.

Taking into account the idea of mechanostat, it is possible that the individuals in the chemotherapy group recovered a significant bone mineral density if remission was achieved. Mechanostat, as developed by Frost (1987), describes the way that use, strain, and excessive mechanical load on bones elicit a response to begin remodeling, resulting in bone formation through osteoclast activity, resulting in an increase in bone mineral

density. During chemotherapy, individuals experience a decreased mechanical load on bones due to treatment and the accompanying side effects. However, in the period after chemotherapy has ended, an increased mechanical load will lead to a more significant bone remodeling response than prior to when chemotherapy was initiated. This is due to the bone having been in a state of disuse and a different point at which the bone would respond to mechanical load. This can lead to an overcompensation in the bone remodeling response as the bones are adjusting to the strain that is now more significant to a weaker bone (Frost, 1987).

Diseases, conditions, and events like chemotherapy and cancer that cause a significant impact on bone mineral density have been shown to result in a different setpoint at which the bone will respond to mechanical loads, resulting in this increased remodeling response (Frost, 1987). Increased activity and exercise after chemotherapy has ended can exacerbate this overcompensation further, leading to significant recovery and increase of bone mineral density among these patients. When considering mechanostat in regard to this study, it is possible that the higher level of bone mineral density observed in the chemotherapy group is due to overcompensation of bone remodeling activity after recovering from chemotherapy (Di Masso *et al.,* 1997).

Visualizing the skeletal elements offered a more in-depth view of the bone quality of the sample. The visual assessment did not reveal any difference in patterns between the donor groups. Bone loss occurred in the same regions in both groups, with most porosity and thinning occurring just above the articular surface of the distal tibia and on the medial malleolus. Regions that were consistently thicker and had less porosity were observed in the superior portion of the shaft and the inferior articular surface. This pattern

of bone mass retention and loss follows the expected pattern of bone loss in osteopenic and osteoporotic individuals (Kamer *et al.*, 2016). This further supports the interpretation that too many factors other than chemotherapy were impacting bone mineral density and bone quality. Factors such as age and activity level in life were likely much more impactful on the donors, thus creating similar patterns of bone loss between both groups. While the contribution of chemotherapy to bone loss cannot be fully discounted, other causational routes to bone loss had significantly more impact, and possibly have more impact than chemotherapy at an older age range.

The change in hormone distribution in the body after menopause is a significant factor that leads to bone loss in women. With menopause cessation, estrogen levels decrease which leads to an increase in bone resorption with increased osteoclast activity. However, there is not sufficient osteoblast activity signaled in this stage of life which can often lead to loss of bone quality (Joshi, 2013). As almost every donor in this study is female of the postmenopausal demographic, postmenopausal hormone changes likely had a more significant impact than chemotherapy. Previous research conducted by Cameron *et al.* (2010) has also established that ovarian function dictates bone loss and changes in bone density after chemotherapy has been stopped. Research has also established that menopause induced by chemotherapy leads to a significant decrease in bone quality (Cameron *et al.*, 2010). Due to the results seen in this study, it is likely that the change in bone remodeling regulation facilitated by menopause was more significant than the change in bone regulation caused by chemotherapy.

## Limitations

A significant limitation of this study is the cross-sectional nature of the study. This study did not allow insight into changes before and after chemotherapy for individuals as well as the recovery period if remission was achieved. The starting BMD values for the chemotherapy group are also unknown, which could impact the difference as seen in a post-mortem context. It is also possible that the control donors began losing BMD earlier than the chemotherapy donors with no preventative measures taken, resulting in lower BMD values. As elaborated upon in the discussion, there is also a possibility that bone mineral density could have been greatly recovered and overcompensated for after chemotherapy or if remission was achieved, which could lead to the increased BMD in the chemotherapy group. Insight into the changes in bone mineral density when remission is initially achieved and after a period of recovery would allow significant insight into how chemotherapy affects the trajectory of bone mineral density loss and gain.

Unfortunately, very few donors had extensive medical records available, leaving many gaps in knowledge regarding the types of chemotherapy used in treatment as well as the duration of treatment itself. This does leave a significant gap in knowledge that could aid in interpreting the bone mineral density values as impacted by different types of chemotherapy treatment. The duration of chemotherapy treatment may have a significant impact on the amount of bone mineral density lost (Winters-Stone *et al.*, 2009; Gleeson *et al.*, 2002). It is also unknown if some donors achieved remission and the duration of remission as well. The duration after remission from chemotherapy also has an impact on bone mineral density as established by Seland *et al.* (2017) and Anargyrou *et al.* (2019).

However, research by Yao *et al.* (2008) has established some level of BMD loss up to 12 years after chemotherapy. The dosage administered, which is unknown for the donors being analyzed, is another contributing factor that can lead to significant differences in BMD (Ozdemir *et al.*, 2003). As the average BMD was less for the chemotherapy group than the control group, the amount of chemotherapy administered and the duration may have been a less than significant portion, resulting in the conclusion interpreted from the results seen here.

The lived experiences of donors, including differences in occupations, diet, illnesses, and other health conditions, could also not be fully accounted for as factors that may impact differences in bone mineral density. Behaviors such as sedentary lifestyle and exercise routines have been shown to impact BMD, especially in the elderly (Godinho-Mota *et al.*, 2003). These factors are acknowledged as having a potential impact on bone quality, and while they cannot be explicitly accounted for according to each donor, it is acknowledged that bone remodeling processes are impacted by almost every aspect of life. The unknown factor of lifestyle could be a major contributing factor that would provide further explanation of the difference in BMD between the chemotherapy and control groups. These unknown factors of medical history and lifestyle had a more significant impact than chemotherapy alone. If such factors of lifestyle, occupation, and medical history could be understood and known, then a more substantial and robust explanation for the difference in bone mineral density and bone quality could be offered based on a comparison of these factors between donor groups.

## Future Considerations

This study opens many questions left by unknown documentation that medical records and personal health documentation could answer. Future research could benefit greatly from studying donor groups with well recorded and documented medical histories available. Controlling for the types of chemotherapy or the duration of chemotherapy could offer significant knowledge into the impacts of the duration of chemotherapy or certain types. Controlling for the time of remission would also allow for insight into bone recovery from chemotherapy, with known occupation or activity level to also aid in such knowledge. With medical histories available, more meaningful conclusions could possibly be reached in terms of stress and boney reactions to chemotherapy as seen in postmortem skeletal remains. A comparative study of the bone loss due to menopause compared to chemotherapy could offer insight into both postmenopausal health and cancer treatment in postmenopausal individuals. As this group focuses largely on women in the sample size, looking into the changes in life for men could also reveal insight into bone health and bone maintenance for men in elder years as well as elderly men receiving care for cancer. A wealth of knowledge can still be obtained from looking into the impacts of chemotherapy and cancer care in a postmortem context.

**Table 1.** List of donors divided by group and including demographic information.

| **Chemotherapy** | | | | | | |
|---|---|---|---|---|---|---|
| Donor Matches | Donor Number | Age | Sex | Ancestry | Height | Weight |
| A | 2012.004 | 63 | Female | White | 168cm | 154lb. |
| B | 2012.014 | 85 | Male | Hispanic | 168cm | 135lb. |
| C | 2012.024 | 83 | Female | White | 152cm | 172lb. |
| D | 2012.040 | 67 | Female | Hispanic | 155cm | 120lb. |
| E | 2014.016 | 59 | Female | White | 168cm | 160lb. |
| F | 2014.037 | 73 | Female | White | 165cm | 130lb. |
| G | 2014.039 | 70 | Female | White | 170cm | 190lb. |
| H | 2015.022 | 67 | Female | White | 172cm | 140lb. |
| I | 2016.040 | 69 | Female | White | 170cm | 120lb. |
| J | 2016.051 | 77 | Female | White | 150cm | 105lb. |
| **Control** | | | | | | |
| Donor Matches | Donor Number | Age | Sex | Ancestry | Height | Weight |
| A | 2015.021 | 63 | Female | White | 173cm | 148lb. |
| B | 2008.001 | 81 | Male | Hispanic | 168cm | 140lb. |
| C | 2018.063 | 84 | Female | White | 170cm | 173lb. |
| D | 2014.001 | 72 | Female | Hispanic | 152cm | 124lb. |
| E | 2014.066 | 59 | Female | White | 170cm | 150lb. |
| F | 2016.029 | 73 | Female | White | 173cm | 160lb. |
| G | 2016.011 | 70 | Female | White | 165cm | 165lb. |
| H | 2017.003 | 67 | Female | White | 178cm | 170lb. |
| I | 2013.008 | 68 | Female | White | 165cm | 125lb. |
| J | 2016.041 | 77 | Female | White | 152cm | 110lb. |

**Table 2.** Bone Mineral Density values in grams per cubic centimeter given for each element from each donor.

| Bone Mineral Density (g.cm-3) | | | | |
|---|---|---|---|---|
| Donor Match | Chemotherapy | | Control | |
| **A** | **2012.004** | | **2015.021** | |
| | Tibia | 0.55915 | Tibia | 0.42611 |
| | L1 | 0.40706 | L1 | 0.39232 |
| **B** | **2012.014** | | **2008.001** | |
| | Tibia | 0.45701 | Tibia | 0.44189 |
| | L1 | 0.42958 | L1 | 0.36950 |
| **C** | **2012.024** | | **2018.063** | |
| | Tibia | 0.39970 | Tibia | 0.36979 |
| | L1 | 0.38873 | L1 | 0.47378 |
| **D** | **2012.040** | | **2014.001** | |
| | Tibia | 0.47433 | Tibia | 0.38191 |
| | L1 | 0.42171 | L1 | 0.45826 |
| **E** | **2014.016** | | **2014.066** | |
| | Tibia | 0.34663 | Tibia | 0.57495 |
| | L1 | 0.48981 | L1 | 0.40247 |
| **F** | **2014.037** | | **2016.029** | |
| | Tibia | 0.54010 | Tibia | 0.32986 |
| | L1 | 0.41602 | L1 | 0.29531 |
| **G** | **2014.039** | | **2016.011** | |
| | Tibia | 0.51924 | Tibia | 0.38184 |
| | L1 | 0.48188 | L1 | 0.18724 |
| **H** | **2015.022** | | **2017.003** | |
| | Tibia | 0.51892 | Tibia | 0.46988 |
| | L1 | 0.45863 | L1 | 0.46543 |
| **I** | **2016.040** | | **2013.008** | |
| | Tibia | 0.56127 | Tibia | 0.41982 |
| | L1 | 0.56085 | L1 | 0.46331 |
| **J** | **2016.051** | | **2016.041** | |
| | Tibia | 0.48931 | Tibia | 0.42513 |
| | L1 | 0.39010 | L1 | 0.33719 |

**Table 3.** Average trabecular thickness in micrometers given for each element from each donor.

| Trabecular Thickness (μm) | | | | |
|---|---|---|---|---|
| Donor Match | Chemotherapy | | Control | |
| **A** | **2012.004** | | **2015.021** | |
| | Tibia | 158.6287 | Tibia | 187.1169 |
| | L1 | 188.5083 | L1 | 179.4343 |
| **B** | **2012.014** | | **2008.001** | |
| | Tibia | 157.0329 | Tibia | 154.6371 |
| | L1 | 178.51 | L1 | 168.974 |
| **C** | **2012.024** | | **2018.063** | |
| | Tibia | 153.9964 | Tibia | 170.3897 |
| | L1 | 185.481 | L1 | 209.4242 |
| **D** | **2012.040** | | **2014.001** | |
| | Tibia | 182.502 | Tibia | 207.371 |
| | L1 | 185.1778 | L1 | 180.8861 |
| **E** | **2014.016** | | **2014.066** | |
| | Tibia | 199.4785 | Tibia | 191.1646 |
| | L1 | 219.2635 | L1 | 171.0047 |
| **F** | **2014.037** | | **2016.029** | |
| | Tibia | 207.1677 | Tibia | 162.283 |
| | L1 | 237.9886 | L1 | 179.4133 |
| **G** | **2014.039** | | **2016.011** | |
| | Tibia | 191.3842 | Tibia | 181.8407 |
| | L1 | 227.1538 | L1 | 200.956 |
| **H** | **2015.022** | | **2017.003** | |
| | Tibia | 182.7296 | Tibia | 196.8755 |
| | L1 | 203.4682 | L1 | 203.2761 |
| **I** | **2016.040** | | **2013.008** | |
| | Tibia | 193.0504 | Tibia | 177.2425 |
| | L1 | 194.895 | L1 | 192.1161 |
| **J** | **2016.051** | | **2016.041** | |
| | Tibia | 185.5282 | Tibia | 193.7989 |
| | L1 | 176.2146 | L1 | 184.8695 |

**Table 4.** Average cortical thickness in micrometers given for each element from each donor.

| Cortical Thickness (μm) | | | | |
|---|---|---|---|---|
| Donor Match | Chemotherapy | | Control | |
| **A** | **2012.004** | | **2015.021** | |
| | Tibia | 253.9502 | Tibia | 186.772 |
| | L1 | 369.4444 | L1 | 557.8208 |
| **B** | **2012.014** | | **2008.001** | |
| | Tibia | 230.5812 | Tibia | 167.4522 |
| | L1 | 371.9848 | L1 | 294.0599 |
| **C** | **2012.024** | | **2018.063** | |
| | Tibia | 256.3037 | Tibia | 278.8116 |
| | L1 | 316.6614 | L1 | 323.9006 |
| **D** | **2012.040** | | **2014.001** | |
| | Tibia | 480.3174 | Tibia | 206.8128 |
| | L1 | 425.4499 | L1 | 476.7762 |
| **E** | **2014.016** | | **2014.066** | |
| | Tibia | 303.6805 | Tibia | 262.0388 |
| | L1 | 503.6471 | L1 | 599.3903 |
| **F** | **2014.037** | | **2016.029** | |
| | Tibia | 258.621 | Tibia | 204.1022 |
| | L1 | 546.6805 | L1 | 263.0745 |
| **G** | **2014.039** | | **2016.011** | |
| | Tibia | 310.1307 | Tibia | 217.0269 |
| | L1 | 526.5188 | L1 | 517.2396 |
| **H** | **2015.022** | | **2017.003** | |
| | Tibia | 204.4828 | Tibia | 223.7841 |
| | L1 | 699.5842 | L1 | 522.5667 |
| **I** | **2016.040** | | **2013.008** | |
| | Tibia | 354.8386 | Tibia | 261.5008 |
| | L1 | 631.5414 | L1 | 518.8005 |
| **J** | **2016.051** | | **2016.041** | |
| | Tibia | 388.8403 | Tibia | 304.8985 |
| | L1 | 373.8989 | L1 | 353.4295 |

**Table 5.** Descriptive statistics for bone mineral density.

| | Bone Mineral Density (g.cm-3) | | |
|---|---|---|---|
| | Average | Standard Deviation | Range |
| Chemotherapy Total | 0.4655 | 0.0645 | 0.2146 |
| Control Total | 0.4033 | 0.0806 | 0.3877 |
| Chemotherapy Tibia | 0.4866 | 0.0699 | 0.2146 |
| Control Tibia | 0.4221 | 0.0671 | 0.2451 |
| Chemotherapy L1 | 0.4444 | 0.0540 | 0.1721 |
| Control L1 | 0.3845 | 0.0918 | 0.2865 |

**Table 6.** Descriptive statistics for cortical thickness.

| | Cortical Thickness (μm) | | |
|---|---|---|---|
| | Average | Standard Deviation | Range |
| Chemotherapy Total | 390.36 | 136.62 | 495.10 |
| Control Total | 344.21 | 136.87 | 431.94 |
| Chemotherapy Tibia | 304.17 | 83.69 | 275.83 |
| Control Tibia | 245.17 | 56.54 | 183.27 |
| Chemotherapy L1 | 476.54 | 126.07 | 382.92 |
| Control L1 | 442.71 | 121.63 | 336.32 |

**Table 7.** Descriptive statistics for trabecular thickness.

| | Trabecular Thickness (μm) | | |
|---|---|---|---|
| | Average | Standard Deviation | Range |
| Chemotherapy Total | 190.41 | 21.77 | 83.99 |
| Control Total | 184.65 | 14.95 | 54.79 |
| Chemotherapy Tibia | 181.15 | 18.59 | 53.17 |
| Control Tibia | 182.27 | 16.36 | 52.73 |
| Chemotherapy L1 | 199.67 | 21.55 | 49.48 |
| Control L1 | 187.04 | 13.85 | 40.45 |

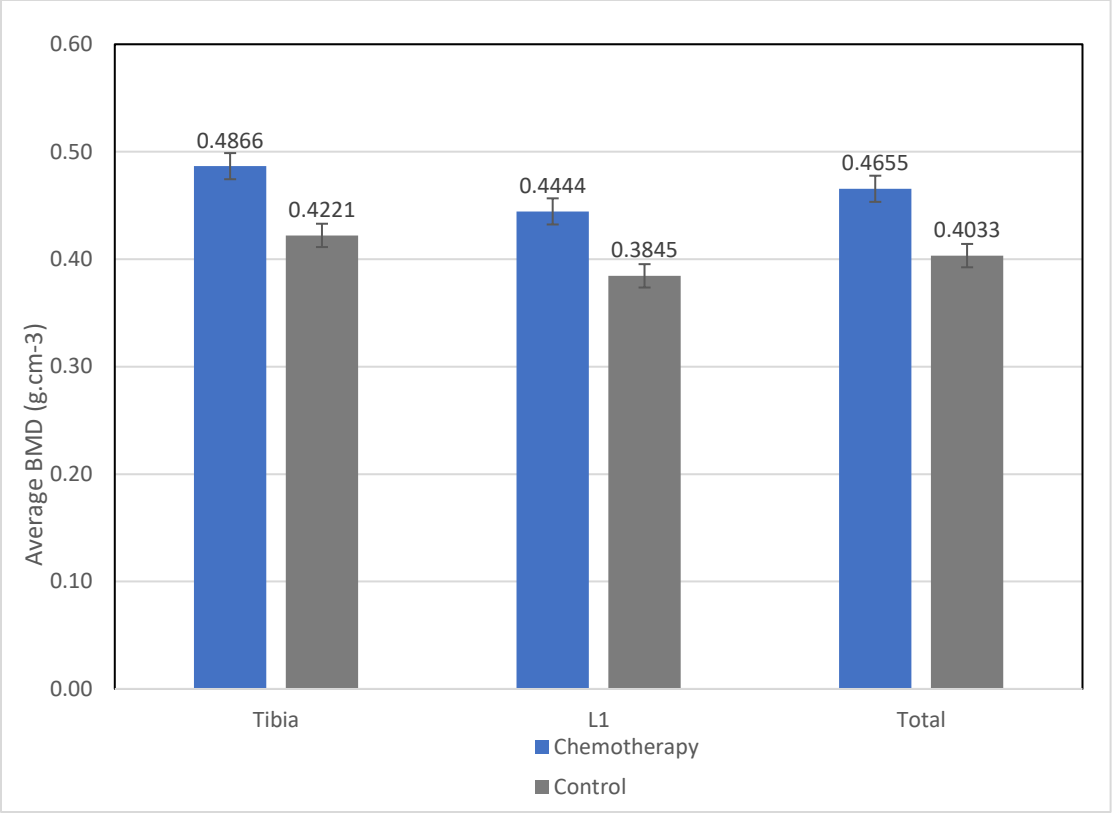


**Figure 1.** Average Bone Mineral Density values in grams per cubic centimeter for each element and respective donor groups.

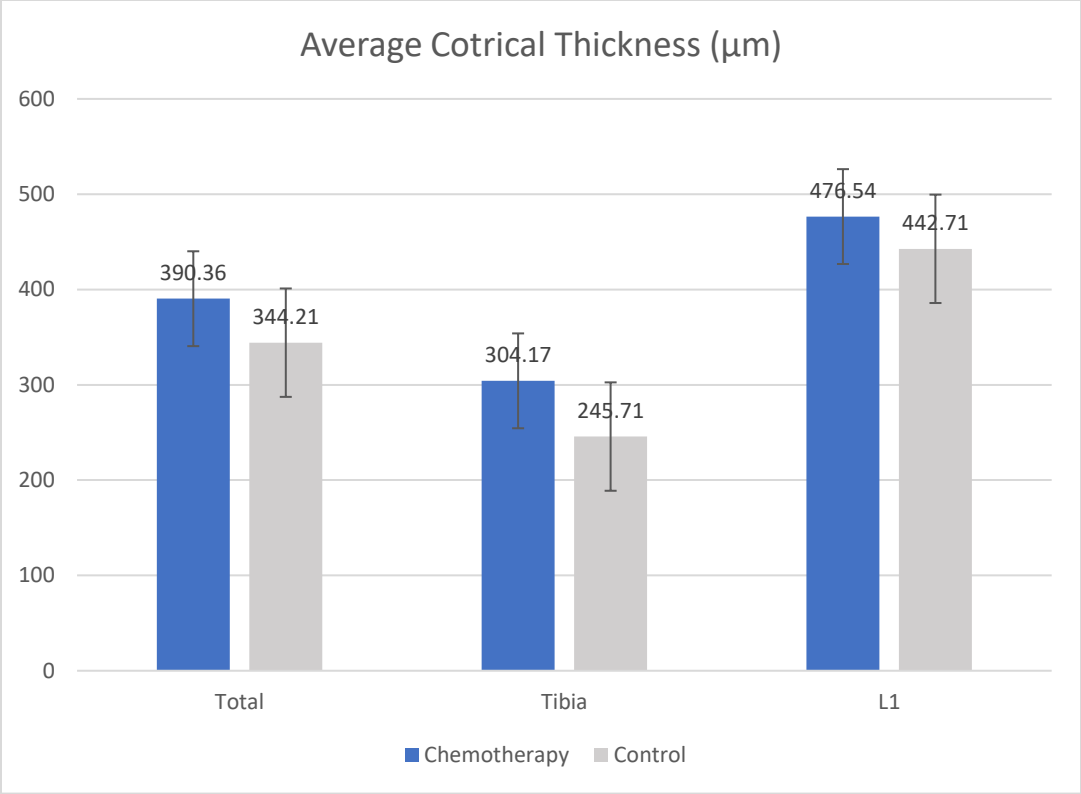


**Figure 2.** Average cortical thickness values in micrometers for each element and respective donor groups.

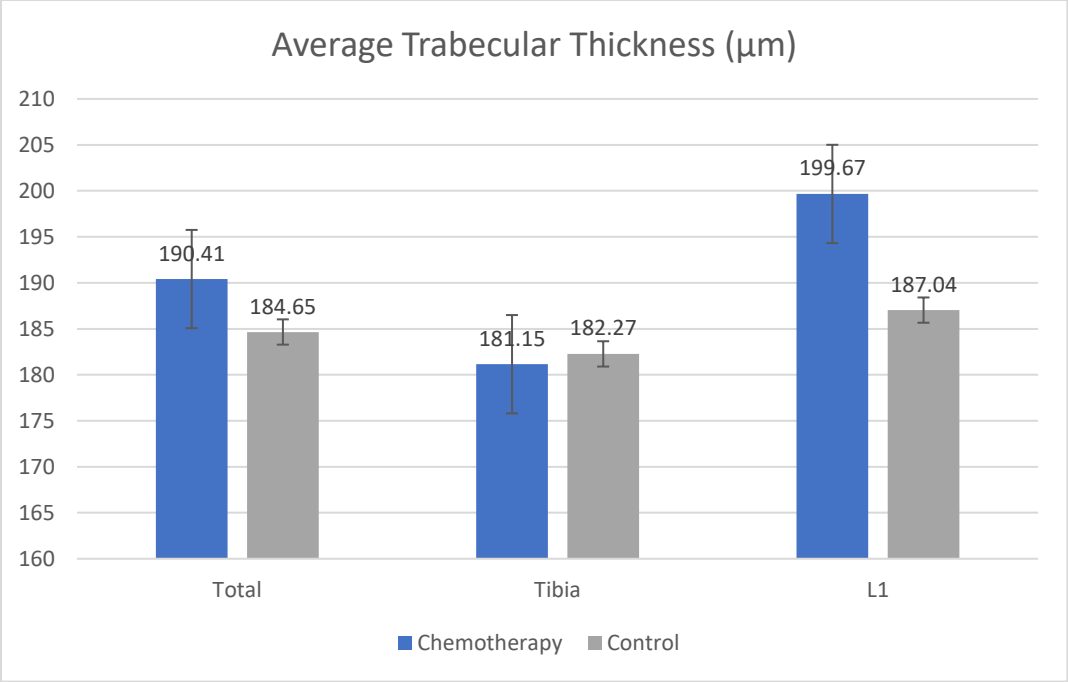


**Figure 3.** Average trabecular thickness values in micrometers for each element and respective donor groups.

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