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DETECTION OF METASTATIC CANCERS IN BONE: A COMPARISON OF MACROSCOPIC AND RADIOGRAPHIC METHODS

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To the Graduate Council:

I am submitting herewith a thesis written by Sara Fatula entitled "DETECTION OF METASTATIC CANCERS IN BONE: A COMPARISON OF MACROSCOPIC AND RADIOGRAPHIC METHODS." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Anthropology.

Lee Meadows Jantz, Major Professor

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(Original signatures are on file with official student records.)

**DETECTION OF METASTATIC CANCERS IN BONE: A
COMPARISON OF MACROSCOPIC AND
RADIOGRAPHIC METHODS**

A Thesis Presented for the
Master of Arts
Degree
The University of Tennessee, Knoxville

Sara Fatula
May 2020

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ABSTRACT

The bioarcheology and forensic anthropology literature describes the visual detection of cancerous lesions on dry bone, including those that metastasize from soft tissues. While the field of paleopathology has recently brought attention to use of radiographs in detecting cancerous lesions, these developments have not become standard in forensic anthropology. The objective of this study is to determine if the presence of cancer in a sample of contemporary skeletons is detected more frequently when assessed using radiographs compared to simply macroscopic examination of dry bone. Given the mechanisms of cancer metastasis, it is expected that more cancerous lesions within the study sample would begin inside the bone, thus visible only with the aid of medical imaging equipment. Potentially individuating cancerous lesions may not be detected when employing only macroscopic analysis of dry bone and may be excluded from the biological profile, impeding the identification of unknown remains.

The study sample consisted of 30 individuals with reported cancer from the William M. Bass Donated Skeletal Collection. All elements were examined in isolation for the presence of visible lesions following the criteria for differential diagnosis established in the paleopathology literature (Ortner 2003; Roberts and Manchester 2005). Each element was also radiographed at the University of Tennessee Student Health Center by a trained radiology technician. Dry bone and radiographs were examined in isolation with statistical methods comparing the frequencies of lesion detection assessing the strength of agreement between the methods.

Results show that more individuals displayed lesions radiographically compared to macroscopically (63%), with 42% of those individuals exhibiting lesions only visible on the radiographs. Of the elements selected, lesions appeared most frequently on the skull, followed by the os coxae. Agreement between the methods ranged from slight to substantial, depending on

the element under analysis, with substantial agreement occurring in elements when individuals exhibited no lesions. Given these results, the argument can be made that radiographing skeletal material is a necessity for detecting the full range of disease presence in a contemporary forensic context.

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CHAPTER ONE

INTRODUCTION AND GENERAL INFORMATION

Cancer

Cancer is a disease that significantly impacts contemporary populations, with the World Health Organization (2020) attributing one in every six deaths to cancer worldwide. In 2018, cancer was estimated to cause 9.6 million deaths globally (World Health Organization 2020). In addition to the deaths caused by cancer, the diagnosis and treatment of cancer can create social, physical, and economic strains on the patient, their families, and their community. In the United States alone, there were 1,658,716 new cases of cancer and 598,031 deaths due to cancer in 2016, the most recent year for which official federal records are available (U.S. Cancer Statistics Working Group 2019). Of these new cases, the most diagnosed was female breast cancer at a rate of 124.2 per 100,000 people, followed by prostate (101.4) and lung and bronchus cancer (56) (U.S. Cancer Statistics Working Group 2019). In terms of deaths caused by cancer in 2016, the leading cancer was that of the lung and bronchus at a rate of 38.5 per 10,000 people, followed by female breast (20.0) and prostate (19.4). Cancer is the second leading cause of death in the United States, with one in every four deaths being attributed to it. According to the United States Cancer Statistics (2019), the rate of cancer has been steadily decreasing since 1999, however, risk factors for cancer have been increasing, such as exposure to environmental carcinogens and increased lifespans.

Also referred to as neoplasms, cancerous tumors are characterized by the uncontrollable and abnormal spread and growth of tissue cells (American Cancer Society 2018: 1; Roberts and Manchester 2005: 252). These neoplasms have the potential to be either benign or malignant. Of

concern to this study are malignant tumors, those that have the potential to spread beyond the site of origin to other tissues. Soft tissue cancers, especially those of the breast, prostate, thyroid, lung, and kidney, are likely to metastasize to bone. The nature of metastatic lesions on bone can be either osteoblastic, resulting in bone formation, or osteolytic, resulting in bone destruction. There are two forms of lytic lesions: 1) those that destroy the cancellous bone first, then work their way outward to eventually destroy the cortex and produce a macroscopically visible lesion, and 2) those that begin on the surface of the cortex and are visible using macroscopic examination. Presently, studies of cancer in dry bone provide detailed descriptions of the lesion morphology being noted for different types of metastatic cancers on a case-study basis (Biehler-Gomez et al. 2019; Brunetti et al. 2017, Marks and Hamilton 2007).

Detection of Cancerous Lesions on Bone

The bioarcheology and forensic anthropology literature (Aufderheide and Rodríguez-Martin 2006; Ortner 2003; Roberts and Manchester 2005) details the visual detection of cancerous lesions on dry bone, including those that metastasize from soft tissues. However, the use of radiographs to examine skeletal material, until recently, has seldom been mentioned in the literature. In the field of paleopathology, recent attention has been brought to the fact that visual inspection alone is not enough to account for the true scope of a cancerous lesion (Marques et al. 2019; Villa et al. 2019). While the use of radiographs to detect cancerous lesions in skeletal material has received increased attention within the field of paleopathology, the same rigor and momentum has not permeated the forensic anthropology community. This is problematic since one route for cancerous lesions in bone to form is from the inner cortical bone outward, thus invisible without the aid of medical imaging equipment (Marques 2019). Rothschild and

Rothschild (1995) remains the only systematic large-scale analysis of the presence of metastatic cancer using macroscopic and radiographic methods. Their findings indicated that two-thirds of all cancer cases within their sample would have been missed by visual inspection alone.

However, their study was limited to the Hamman-Todd collection, comprising individuals who lived during the late 1800s. The 1920 mortality statistics issued by the Department of Commerce Bureau of the Census stated that cancer caused 72,931 deaths at a rate of 83.4 per 100,000 people that year (Steuart 1922: 40). That number is significantly lower than the 598,031 deaths attributed to cancer in 2016 (U.S. Cancer Statistics Working Group 2019). Therefore, cancer is more prevalent in today's society than when the individuals in the Hamman-Todd Collection were alive. Additionally, their results may not apply to a contemporary population living in a vastly different environment and having access to treatment options, such as chemotherapy and radiation therapy, that would not have been available during that time.

Today, multiple modality treatment that combines chemotherapy, radiation, and surgery is standard practice (DeVita and Chu 2008). Research in the clinical sphere has illustrated that chemotherapy and hormonal therapies used to treat certain soft tissue cancers, such as breast and prostate can have negative impacts on bone. Some of these impacts include: accelerated bone loss, lower bone mineral density, osteopenia, and pathological fractures (Vehmanen et al. 2001; Hadji et al. 2009; Greep et al. 2003; Georgiou et al. 2011; and Holmes et al. 1994). While this research has been conducted in the clinical sphere, no current study explicitly examines how these treatments may alter the way lesions manifest on dry bone in an anthropological context. A study using a contemporary collection is necessary to understand how many instances of cancer may be missed using only visual inspection.

Hypothesis

It is hypothesized that more individuals in this study sample will exhibit visible lesions on the radiographs when compared to only macroscopic analysis. This is because lesions have the potential to take a hematogenous route into the medullary cavity of long bones and the spongy bone of flat bones, forming endocortical lesions. These endocortical lesions would begin inside the bone and initially only be visible with the aid of medical imaging equipment. It is also hypothesized that porous lesions visible on the outside of the bone may exhibit evidence of increased trabecular bone destruction on the radiographs that exceeds the boundaries visible on the surface.

Objective and Implications

The objective of this study is to determine if visible cancerous lesions within a sample of contemporary skeletons are detected more frequently when assessed using radiographs compared to macroscopic examination of dry bone. Detecting cancerous lesion presence in bone is important because skeletal material is sometimes all that remains in forensic and archaeological contexts. According to the United States Cancer Statistics (2019), the rate of cancer has been steadily decreasing since 1999, but the risk factors for cancer have been increasing. If these trends continue, cancer may become a more common occurrence in forensic investigations, providing more potentially individuating lesions that can be incorporated into the biological profile. Individuals that sought treatment for cancer may have antemortem scans on file that can be compared to postmortem scans for positive identification purposes. In paleopathological and archaeological contexts, cancer is often considered rare (Assis and Codhina 2010; Capasso 2005; David and Zimmerman 2010). A study that allows for the full range of metastatic cancer

manifestations to be detected within a contemporary population is necessary to formulate better methods of cancerous lesion detection in dry bone that can be incorporated into the biological profile in forensic contexts.

CHAPTER TWO

LITERATURE REVIEW

Cancer significantly impacts contemporary populations and exhibits a wide range of variation in its manifestation. Generally, cancerous tumors are categorized as benign or malignant. Benign tumors remain confined to the site of origin and exhibit well-differentiated borders from the surrounding tissue, while malignant tumors consist of poorly differentiated borders and may spread from the site of origin (Ortner 2003; Roberts and Manchester 2005). Neoplasms can also be divided into primary or secondary tumors. Primary tumors are associated with the tissue of origin, while secondary tumors originate in one tissue and metastasize to another. Therefore, there is the potential for primary benign, primary malignant, and secondary malignant tumors. The following subsections will discuss cancerous tumors that affect bone.

Primary Cancer

Primary bone cancers, those that are the result of uncontrolled growth and proliferation of cells originating in bone, are generally rare and tend to occur in younger individuals (Ortner 2003). Osteomas are the most common form of benign tumor. There are three common types of malignant primary bone neoplasms that each leave their own unique markers on the skeleton, including: osteosarcoma, Ewing's sarcoma, and chondrosarcoma (Fisher 2013). These tend to be rare and occur in individuals 10-24 years of age (Fisher 2013).

Benign Cancers

The most common form of benign primary cancer is the osteoma, which consists of dense lamellar bone on the outer surface of the bone, usually on the frontal and parietal bones of the

skull (Aufderheide and Rodríguez-Martin 2006; Brothwell 2008; Marques 2019). This form of osteoma is usually referred to as a button osteoma. Osteoid osteomas also may occur, which consist of a dense area of cortical bone with a center of osteoid and bone trabeculae (Aufderheide and Rodríguez-Martin 2006; Brothwell 2008). The most common locations for these tumors include the diaphysis of the femur, tibia, fibula, and humerus, as well as the vertebrae and tarsal bones (Ortner 2003). Osteoid osteomas more often affect females and individuals within the active growing period between the ages of 10-30 (Aufderheide and Rodríguez-Martin 2006). However, it is possible for older individuals to be afflicted, though it is rare. Osteblastomas are another form of benign primary tumors that affect the thoracic and lumbar spine and the diaphysis of long bones (Ortner 2003). However, they tend to affect individuals under the age of 20. Yet another form of primary benign tumor that affect individuals under the age of 20 is the ossifying fibroma, which has the potential to affect the long bones (Aufderheide and Rodríguez-Martin 2006).

Malignant Tumors - Osteosarcoma

Osteosarcoma is the most common primary malignant bone tumor, accounting for 40-60% of all primary malignant tumors (Hansen 2009). It arises from the connective tissue elements of bone and is characterized by the production of osteoid (Hansen 2009). The most commonly affected areas include the active growth sites in the metaphyses of the distal femur, proximal tibia, and proximal humerus (Aufderheide and Rodríguez-Martin 2006; Ortner 2003). This predilection for the metaphyses is due to abundant sinuses, blood supply, and a direct connection between the epithelium and the tumor cells, all of which are necessary for tumor cells to proliferate (Virk and Lieberman 2007). Males are more often affected than females and

adolescents and young adults are more often affected (Ortner 2003; Hansen 2009). Osteosarcoma is thought to arise from a mutation in p53 or retinoblastoma (Rb) genes, which would normally suppress tumor growth. These mutations allow tumor cells to replicate without any sort of restraint, producing growth factors such as Transforming Growth Factor (TGF), Insulin Growth Factor (IGF), and connective tissue growth factor (CTGF), which leads to angiogenesis, the formation of new blood vessels, of the tumor cells and more accelerated cell replication and growth (Broadhead et al. 2011). These growth factors lead to increased osteoblast expression, with osteoblasts being important mediators of bone resorption through their expression of RANKL (Broadhead et al. 2011).

Like cancerous tumors that affect other tissues, osteosarcoma has the potential to metastasize, with the osteosarcoma cell breaking away from the primary tumor, adhering to the extracellular matrix (ECM), migrating via intravasation, and finding a new site via extravasation (Broadhead et al., 2011). When assessing its appearance on dry bone, radiographs are necessary to assess the level of cortical penetration achieved. Initial lytic activity within the metaphysis results in alternating areas of osteopenia and normal density bone resulting a “cloudy” appearance (Aufderheide and Rodríguez-Martin 2006). With increasing time, the lesion moves outward towards the cortex. If cortical penetration is achieved, the result is a “sunburst” appearance whereby spicules of new bone formation occur as surrounding structures are penetrated, which may be visible without the aid of radiographs (Aufdherheide and Rodríguez-Martin 2006; Roberts and Manchester 2005). If the osteosarcoma began outside the bone between the periosteum and cortical bone, rather than within the metaphysis itself, the manifestation is different. The growing tumor can lift the periosteum, forming a Codman’s triangle that demarcates the boundaries of the tumor (Aufderheide and Rodríguez-Martin 2006).

There is also the possibility of these lesions becoming sclerotic following treatment, with sclerotic areas of calcification occurring both within and outside the bone (Aufderheide and Rodríguez-Martin 2006; Wu and Hochman 2012). This can be an indication of slowed tumor growth and attempts by the bone to repair itself.

Malignant Tumors – Ewing’s Sarcoma and Chondrosarcoma

Ewing’s sarcoma arises from undifferentiated mesenchymal stem cells, forming a permeative lesion that is loosely held together by matrix (Ortner 2003). This allows the lesion to spread from the intertrabecular spaces to the outer cortex over a large surface area, eliciting a bony response on the surface of the bone (Ortner 2003). While the most commonly affected areas are the long bone extremities and the pelvic bones, other sites with trabecular marrow can also be affected (Ortner 2003). Ewing’s sarcoma is differentiated from osteosarcoma in that it does not produce osteoid or any other matrix. If reactive bone is present, distinguishing Ewing’s sarcoma from osteosarcoma is accomplished by taking into account the permeative intracortical destruction and the lack of involvement along the diaphysis (Ortner 2003). When assessing its appearance radiologically, the formation of different lamellar layers may produce an “onion-skin” appearance (Aufderheide and Rodríguez-Martin 2006). This condition occurs in children and adolescents (Ortner 2003).

Chondrosarcoma arises in the metaphases of long bones, especially those of the proximal and distal femur and the proximal humerus (Ortner 2003: 526; Roberts and Manchester 2005). It is also important to note that bone metastases frequently originate within the bone and progress outward to the cortical bone, thus producing endosteal scalloping of the cortex only visible with the aid of medical imaging equipment (Ortner 2003; Roberts and Manchester; Marques 2019).

There is no sex predilection and individuals from late-adolescence into adulthood are affected (Ortner 2003).

Secondary Cancer

Cancers that arise in soft tissues and later spread to bone are more common within adult populations. Five soft tissue cancers have a propensity to metastasize to bone include: breast, prostate, thyroid, lung, and kidney (Coleman 1997; Coleman and Rubens 1987; Roberts and Manchester 2005; Taxel and Mirza 2009). Breast and prostate are considered the most prevalent, accounting for more than 65% of all skeletal metastases (Lukaszewski et al. 2017; Mundy 2002). Skeletal lesions have also been attributed to bladder, pancreas, esophagus, and melanoma cancers, though these are considered rare (Assis and Codhina 2010). Once a cancer metastasizes to bone, it can become a major cause of morbidity and mortality for the patient with bone pain being a common ailment (Jimenez-Andrade et al. 2010; Taxel and Mirza 2009; Macedo et al. 2017; Coleman 1997). Other problems associated with cancer metastases include pathological fractures, anemia, impaired mobility, spinal cord compression, and hypercalcemia (Jimenez-Andrade et al. 2010; Macedo et al 2017; Coleman 1997; Taxel and Mirza 2009; Lukazewski et al. 2017).

Mechanisms of Cancer Metastases

The propensity for cancers to metastasize to bone is tied to Stephen Paget's 1889 "seed and soil hypothesis" whereby the "microenvironment of the new organ serves as the fertile soil for the cancer cells" (Gosman 2012). This idea was later challenged by James Ewing in the 1920s when he suggested a circulatory pattern between primary and secondary tumor sites, thus

suggesting a mechanical route for cancer metastases (Chambers et al. 2002). This idea found a resurgence in the 1940s when Batson noted a direct linkage between a group of veins in the prostate and the capillary beds of the lumbar spine (Robinson et al. 2004). Both were correct in their assumptions. Once a primary tumor invades the underlying tissue, the tumor cells can enter the blood or lymph in a process known as intravasation, which allows them to circulate throughout the body (Couzin 2003; Chambers et al. 2002). The anatomical nature of blood vessels and capillaries may assist in the transmission of cancer cells from the primary site to the secondary site. However, according to the “seed and soil” hypothesis, not all microenvironments are suitable for the growth of metastatic cancer, therefore certain cancers can only metastasize to particular sites, such as the bone marrow cavity (Macedo et al., 2017; Mundy 2002). Once an ideal site is found, one of two processes may occur. The cancer cells may adhere to the blood vessels and proliferate within them, or extravasation occurs, and the cancer cells begin to interact with the endothelium of the new host site (Macedo et al., 2017; Robinson et al. 2004). Once in the new site, proliferation and angiogenesis can occur (Couzin, 2003). As the cells constantly divide, they cause inflammation via pro-inflammatory cytokines and growth factors and disrupt various components including: cytotoxic lymphocytes, macrophages, Tregs, and Th1/Th2 cells (Curiel 2007). By causing inflammation, disrupting the immune system, and promoting the development of new blood vessels, an ideal environment is maintained whereby the tumor cells can continue to proliferate (Chambers et al. 2002).

Bone is an extremely “fertile” ground for cancer cells to grow and proliferate due to the rich blood supply in the hematopoietic bone marrow, as well as the scaffold structure necessary to support the growth of cells (Mundy 2009). As such, bones of the axial skeleton tend to be affected more often, including: the vertebrae, proximal femur, ribs and sternum, skull, os coxa,

sacrum, proximal humerus, and shoulder girdle (Aufderheide and Rodríguez-Martin 2006; Ragsdale and Lehmer 2012; Binder et al. 2014; Assis and Codhina 2010; Coleman 2006; Macedo et al. 2017). These areas of the skeleton were confirmed as preferred sites of breast cancer metastases in a contemporary sample from the CAL Milano Cemetery Skeletal Collection (Biehler-Gomez et al. 2019). Some uncommon sites of cancer metastases include the distal elbow and knee due to their distance from the axial skeleton (Leeson et al. 1986). It is also important to note that metastatic lesions on bone almost always tend to be multiple, with solitary lesions being a rarity and associated with slow-growing cancers, such as chondrosarcoma (Macedo et al. 2017; Ortner 2003). The nature of metastatic lesions can be either osteolytic, resulting in bone destruction, osteoblastic, resulting in bone formation, or a mixture of the two.

Cancerous Lesions - Lytic

Some lytic metastases destroy the cancellous bone first, then work their way outward to eventually destroy the cortex and produce a macroscopically visible lesion, while other lytic lesions may begin on the surface of the cortex (Ortner 2003; Roberts and Manchester 2005; Wu and Hochman 2012). Lytic lesions tend to be associated with cancers of the lung, thyroid, and kidney (Ortner 2003; Roberts and Manchester 2005; Wu and Hochman 2012).

Lytic cancerous lesions are primarily the result of unchecked osteoclastogenesis, or the formation of bone destroying osteoclasts, that would normally be balanced by osteoblastogenesis (formation of bone-forming osteoblasts) in growth and remodeling. Therefore, an understanding of the underlying cellular pathways that stimulate bone destruction is necessary. The primary cellular pathway for this process is the OPG/RANK/RANKL pathway. Receptor Activator of the Nuclear Factor Kappa – β Ligand (RANKL) is a member of the Tumor Necrosis Factor (TNF)

superfamily that is secreted by osteoblasts, osteocytes, bone lining cells, and other stromal cells (Florencio-Silva et al. 2015; Iñiguez-Ariza and Clarke 2015). To stimulate osteoclast formation, RANKL must bind to RANK (Receptor Activator of the Nuclear Factor Kappa – β) on osteoclast precursor cells (Florencio-Silva et al. 2015). RANKL must also bind with Macrophage Colony Stimulating Factor (M-CSF), which is secreted by mesenchymal cells and osteoblasts, for proper stimulation of osteoclast expression (Florencio-Silva, 2015). The third piece of the OPG/RANK/RANKL pathway is Osteoprotegerin (OPG), which is produced by bone lining cells, osteoblasts, and stromal cells (Florencio-Silva et al. 2015). OPG acts as a decoy by binding to RANKL instead of allowing RANKL to bind to RANK, which inhibits the cascade necessary for osteoclastogenesis (Sherman 2012). For lytic cancerous lesions, a cycle is initiated, where factors that are secreted by the tumor cell, such as Parathyroid Hormone Related Peptide (PTHrP), Interleukins (IL-1 and IL-6), Prostaglandin E2 (PGE2), and Tumor Necrosis Factor (TNF), promote bone resorption via RANKL (Macedo et al. 2017). As the bone is resorbed, factors in the extracellular matrix (ECM), such as TGF- β , fibroblast growth factor (FGF), insulin-like growth factor (IGF), and Bone Morphogenetic Protein (BMP-2) are released from the bone matrix and promote growth of the tumor cells (Macedo et al. 2017). As tumor cells grow, they secrete more factors that stimulate RANKL, further breaking the bone down, releasing more factors that promote growth of the tumor cell. This creates a feedback loop that highlights the unstoppable nature of soft tissue lytic cancer metastases to bone.

In addition to the OPG/RANK/RANKL pathway, the Wnt/ β -Catenin signaling pathway is of importance since it mediates the OPG/RANK/RANKL pathway by differentiating cells into the osteogenic (bone forming) lineage, allowing for osteoblastogenesis to occur. In instances of cancer metastasis, this pathway is inhibited, thus allowing for the OPG/RANKL/RANK pathway

and osteoclastogenesis to go unchecked. There are many factors that inhibit the Wnt/ β -Catenin signaling pathway, including sclerostin, which is produced by the SOST gene in osteocytes and tends to be overexpressed in breast cancer (Iñiguez-Ariza and Clarke 2015). Sclerostin acts as an antagonist to the Wnt/ β -Catenin signaling pathway by binding to Wnt co-receptors Lipoprotein Receptor-related Protein (LRP) 5/6 in osteoblasts, thus preventing Wnt and LRP 5/6 from binding to the Frizzled co-receptor complex (Iñiguez-Ariza and Clarke 2015). If Wnt cannot bind to Frizzled, osteoblastogenesis is suppressed, leading to the enhancement of osteoclastogenesis through increased osteoblast apoptosis (Bermeo et al. 2014). Dickkopf 1 (Dkk1) is another factor produced by osteocytes that leads to increased osteoclastogenesis through the inhibition of the Wnt signaling pathway in a similar fashion to sclerostin (Iñiguez-Ariza and Clarke 2015; Brunetti et al. 2017).

Cancerous Lesions - Blastic

In contrast to lytic cancerous lesions, the cellular mechanisms surrounding osteoblastic lesions are less understood. Osteoblastic lesions tend to appear as either rough, porous masses, or as fine “spicules,” and tend to be associated with cancers of the prostate, uterus, and ovaries (Ortner 2003; Roberts and Manchester 2005). The cellular mechanisms responsible for osteoblastic lesions are thought to primarily be the result of factors such as endothelin-1, BMPs, TGF- β 2, FGF, RUNX2 and osteocalcin, which increases cancer cell proliferation and osteoblast formation (Macedo et al. 2017; Mundy 2002, Esposito et al. 2018). For example, Endothelin-1 was found in higher levels in individuals with prostate cancer and breast cancer that produced osteoblastic metastases (Mundy 2002). Research suggests that osteoblasts are vital not only for the normal regulation of bone homeostasis, but for maintaining the survival of cancer cells that

metastasize to bone. Similar to the feedback seen in lytic lesions, osteoblasts secrete factors such as PTHrP, VEGF, IL-6, and IL-8 that can be taken up by the cancerous tumor cells, thus promoting a feedback cycle of growth and survival of tumor (Shupp et al. 2018). With time, osteoblastic lesions can give way to lytic lesions as osteoblasts begin to produce more inflammatory cytokines, including RANKL, which are vital for stimulating osteoclastogenesis (Esposito et al. 2018; Shupp et al. 2018).

Cancerous Lesions – Mixed

Breast cancer produces a mixture of osteolytic and osteoblastic lesions (Aufderheide and Rodríguez-Martin 2006). In breast cancer especially, the main mediator of osteoclast activation is parathyroid hormone related peptide (PTHrP), which stimulates the binding of RANKL to RANK and is found in elevated levels in individuals with breast cancer (Coleman 2006; Mundy 2002; McCauley and Schneider 2004). Increased expression of PTHrP also downregulates the production of OPG, further promoting osteoclast formation and bone resorption (Gosman 2012). While the lesions may be primarily osteolytic due to the stimulation of osteoclasts, increased serum-alkaline phosphate levels, PTH, and PTHrP produce a local bone-forming response adjacent to the lytic lesion (Mundy 2002; McCauley and Schneider 2004). Therefore, these lesions can manifest as primarily lytic with blastic margins.

Differential Diagnosis of Cancerous Lesions

Assessment of Dry Bone

Significant portions of the paleopathology literature describe the macroscopic detection of cancer on dry bone because of the propensity of primary cancers and soft tissue metastatic

cancers to leave macroscopically visible lesions (Ortner 2003, Roberts and Manchester 2005; Aufderheide and Rodríguez-Martin 2006). The characteristics of lesions are described, including: whether the lesion is osteolytic, osteoblastic, or mixed; whether it is a solitary lesion multifocal; whether the margins of the lesion show activity at the time of death; and whether their distribution throughout the skeleton predilects the axial or appendicular skeleton or the diaphysis or epiphysis of particular elements (Marques 2019). Specific lesions are commonly described with descriptive terms, such as “sunburst appearance,” “Codman’s triangle,” and “onion-skin” (Aufderheide and Rodríguez-Martin 2006; Ortner 2003; Roberts and Manchester 2005). However, using radiographs to examine skeletal material, until recently, has seldom been mentioned in the paleopathology literature.

Detection Using Radiographs

Roberts and Manchester (2005:253) claim that “ideally radiographic analysis should be undertaken whenever possible to ensure that internal lesions are identified.” They further state that visual inspection alone is not a reliable enough method to account for the true scope of cancerous lesions. This is because metastatic cancers may begin in the marrow inside the bone, not on the outside, and that “mere inspection may fail to reveal these deep truths, which may only be demonstrated radiographically on bone” (Roberts and Manchester 2005: 261). A similar sentiment has been echoed by Marques (2019: 645) who claims that “systematic radiographic evaluation of bones with changes to the external surface is essential for diagnosis, as intramedullary lesions may be discovered, and the differential diagnosis is also more reliable.” Brothwell (2012), Ragsdale et al. (2018), and Villa et al. (2019) also advocate for the combined macroscopic study of bones and radiographs whenever possible.

Marks and Hamilton (2007) have also advocated for the use of combined gross morphological examination and radiographic analysis in the differential diagnosis of metastatic cancer in cases where differential diagnoses may overlap. Their study detailed the lesions present in a 62-year old contemporary white female who did not seek any formal medical treatments for her metastatic breast cancer. The use of radiographs allowed them to rule out multiple myeloma as a potential diagnosis and clarify that metastatic breast cancer was the correct diagnosis. While their case does use a contemporary example, the fact that this individual did not seek any sort of treatment for her condition may not be an accurate representation of how cancerous lesions manifest in contemporary remains.

Radiography

Radiographs offer many advantages, such as their ease of access and relatively low cost when compared to other imaging modalities (Tins et al. 2009; Villa et al. 2019). However, compared to other imaging modalities, bone destruction needs to be significant before it can be visualized radiographically. Tins et al. (2009) estimate destruction needs to reach between 30-75% of the bone mass before it can be noted on a radiograph. Therefore, lesions that manifest as porosity without extensive cortical destruction may not appear on radiographic images. Despite the requirement of substantial bone destruction, radiographs are more likely to be available to forensic anthropologists and archaeologists than other imaging modalities, such as computed tomography (CT) and Magnetic Resonance Imaging (MRI) (Villa et al. 2019).

Radiography – Technical Notes

Radiographic images allow one to visualize the internal structures of the bone and can highlight differences in densities easily. An X-ray machine consists of the X-ray tube, or the source of the X-rays, and an image detector, usually a film, where the X-ray attenuation is recorded and an image is produced (Villa et al. 2019). When reading a radiograph, the image will be darkest where the greatest number of x-rays have permeated the image detector. Therefore, empty spaces will be colored black and areas of lower density will be colored varying shades of grey. The image will be lightest where more X-rays were absorbed by the sample, therefore the most dense parts of bones would appear white (Villa et al. 2019).

There are many systems of classifying skeletal lesions on radiographs on living individuals for risk of malignancy in the literature (Lodwick 1964; Madewell et al. 1981a, 1981b, 1981c; Miller 2008; Costelloe and Madewell 2013; Ragsdale and Lehmar 2012). Ragsdale et al. (2018) has outlined a scoring system meant for radiographing dry bone specimens that classifies cancerous lesions based on the nature of their borders that is a modified version of the Lodwick-Madewell Grading System, which is commonly used in radiology to classify lytic lesions in the living for risk of malignancy. Table 2.1 shows the scores and descriptions for the modified Lodwick-Madewell Grading System present in Ragsdale et al. (2018), while Figure 2.1 illustrates these descriptions.

Uses for Radiographs Within Forensics

Medical imaging equipment has increasingly been employed in forensic pathology and forensic medicine, especially in the growing trend known as the “virtual autopsy” or “virtopsy.” Virtopsy is considered a noninvasive tool that can be used in conjunction with traditional autopsy

or ultimately replace traditional autopsy in cases where religious or cultural taboos prohibit such (Thali et al. 2006; Dirnhofer et al. 2006; Buck et al. 2009; Bollinger et al. 2008) The use of x-ray, CT, and MRI has been employed in forensics as an aid to traditional autopsy by allowing for gas (such as in a pneumothorax), foreign bodies (such as bullets or shrapnel), and bone fractures (such as in motor vehicle accidents) to be visualized prior to a traditional autopsy (Grabher et al. 2007; Bollinger et al. 2008). While Virtopsy has gained increasing acceptance in forensic pathology and medicine, these methods are primarily used on fleshed remains. Therefore, these methods have not received the same traction in an anthropological scenario where skeletonized remains are found.

The use of medical imaging equipment during forensic anthropological analysis has mostly involved the comparison of antemortem and postmortem radiographs for establishing positive identification (Ross et al. 2016, Stephan et al. 2011, Watamaniuk and Rogers 2010), the use of trabecular bone pattern as a means of identification (Kahana et al. 1998, Kahana and Hiss 1994), development of methods for estimating measurements from radiographic images or 3D reconstructions of the skull (Schroeder et al. 1997, Verhoff et al. 2007), and methods of age or

Table 2.1 Scores and descriptions for Modified Lodwick-Madwell Grading System.

IA	Well-defined geographic lytic lesion with a sclerotic rim/margin
IB	Well-defined geographic lytic lesion with a sharp margin without a sclerotic rim. The sclerotic rim fades and looks “punched out”
IC	The lesion extends between the trabeculae, covering a larger area. Therefore, the margin is ill-defined.
II	“Moth-eaten” margins. The cortex has many overlapping lesions.
III	Permeative destruction. Margins not clearly defined.

(Ragsdale et al. 2018: 30).

MARGIN TYPES

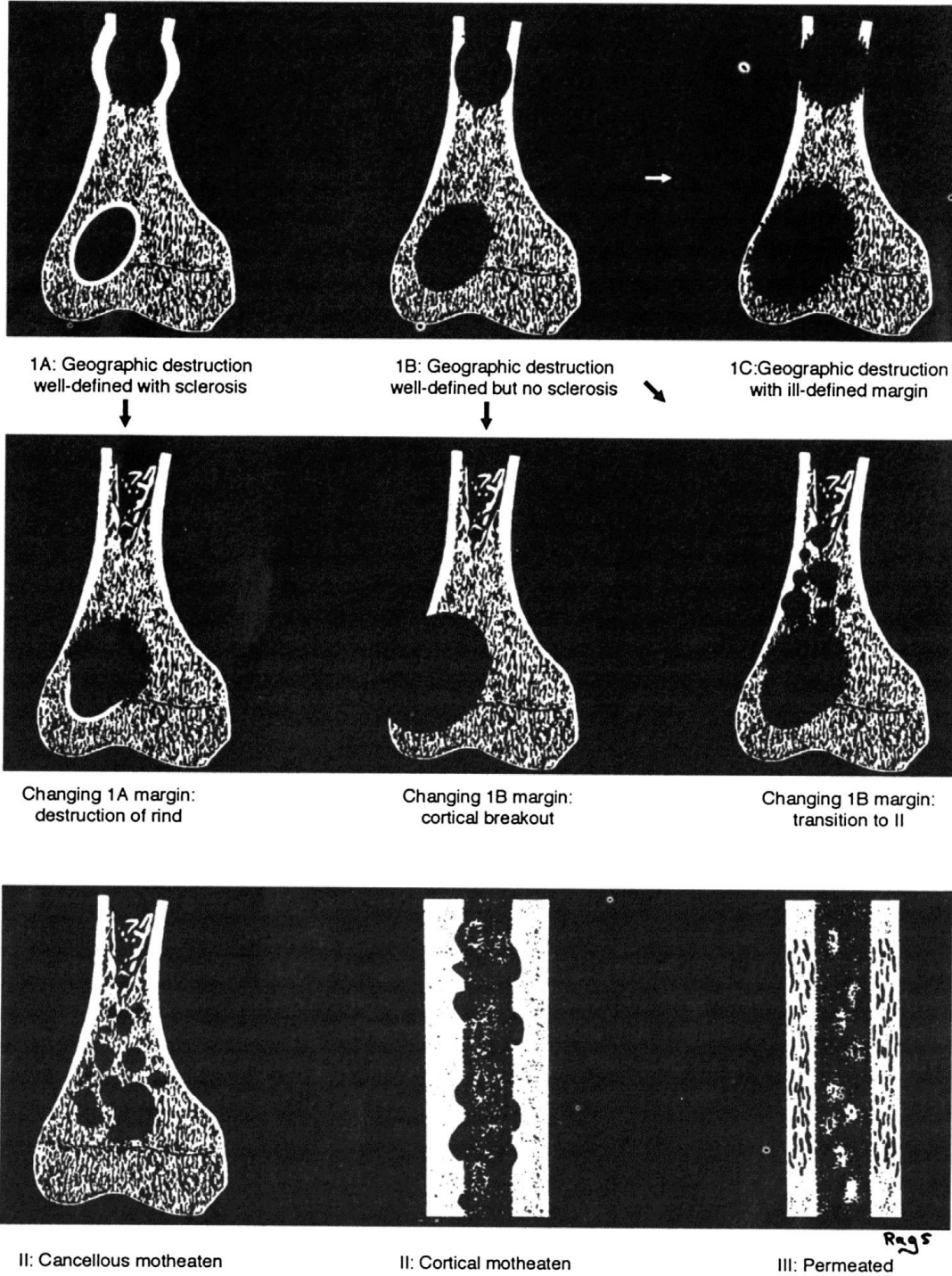


Figure 2.1 schematic illustrating the Modified Lodwick-Madwell Grading System (Ragsdale et al. 2018, p.32, Figure 2. Originally from Ragsdale, 1993, p. 452, Figure 2).

sex estimation based on radiographs (Aly et al. 2016; Garamendi et al. 2011; Wittschieber et al. 2013, Dedouit et al. 2010). However, the contributions of radiographs to the detection of disease processes is not a prominent part of the forensic anthropology literature, despite mention in the paleopathology literature. Lack of radiographing skeletal material could be due to many factors, such as a lack of resources or misunderstandings surrounding the benefits of medical imaging equipment (Biehler-Gomez et al. 2019). Despite these setbacks, the necessity of radiographing of skeletal material has been addressed for cases of dental disease and for porotic hyperostosis (Linn et al. 1987; Macadam 1987). Therefore, the value of radiographs to cancer detection should be recognized.

Radiography and Cancer Detection

The need to radiograph skeletal material for the detection of cancerous lesions has received attention within the paleopathology literature, however, this same attention has not permeated the forensic sphere (Ortner 2003; Roberts and Manchester 2005; Brothwell 2012). The necessity of radiographs was systematically tested by Rothschild and Rothschild (1995). They visually examined the entire skeleton of 129 individuals with documented cancer in the Hamman Todd Collection, documenting the form and distribution of bony lesions. They then subjected the entire skeleton to radiographic analysis in a blind manner whereby previous presence or absence of skeletal lesions visible were unknown. Their analysis indicated that of the 129 individuals examined, only 11 showed signs of cancer macroscopically but 33 individuals exhibited cancer radiologically, mostly in the form of increased or decreased bone density (Rothschild and Rothschild 1995: 358). Only four individuals exhibited lesions both macroscopically and radiologically (Rothschild and Rothschild 1995). Therefore, if visual

inspection alone was used to detect cancer, two-thirds of all cases would have been missed. Another key observation in their study was that there was no notable relationship between the distribution of metastatic diseases and the location of the primary soft tissue tumor, contrary to what is noted macroscopically in the paleopathology literature (Rothschild and Rothschild 1995). This observation justifies the need to radiograph the entire skeleton instead of selecting only a few elements. The authors also found that 91% of radiographic metastases appeared on the ilia, while femoral metastases were also noted in high frequencies (Rothschild and Rothschild 1995). Therefore, if one were to select elements for x-ray due to time or budget constraints, the ilia and femora should be considered. While the use of radiographs to detect cancerous lesions in skeletal material has received increased attention within the field of paleopathology, the same rigor and momentum has not been accepted within the forensic anthropology community, primarily utilizing visual methods of detecting disease frequency.

Cancer in Contemporary Populations

With the exception of Rothschild and Rothschild's (1995) study, no other large-scale studies explicitly compared the presence of cancer lesions both macroscopically and radiologically. However, their use of the Hamman-Todd Collection is not an accurate representation of cancer prevalence in a contemporary population. The 1920 mortality statistics issued by the Department of Commerce Bureau of the Census stated that cancer caused 72,931 deaths at a rate of 83.4 per 100,000 people that year (Steuart 1922: 40). That number is significantly lower than the 598,031 deaths attributed to cancer in 2016 (U.S. Cancer Statistics Working Group 2019). Researchers have addressed the topic of cancer in antiquity, and the general consensus is that there is a lower frequency of malignant conditions in the past than there

are in clinical practice today (Assis and Codhina 2010; Capasso 2005; David and Zimmerman 2010). This lack of cancer presence in antiquity can be due to many factors, including differences in diet, life expectancy, and environment. These differences can also impact the way cancers manifest in bone, therefore, a study utilizing a contemporary collection is of importance,

The modes of cancer treatment commonly employed today have rapidly advanced and would not have been common practice when individuals in the Hamman-Todd Collection were alive. The use of hormonal therapy to treat breast and prostate cancer was first employed in 1939 (DeVita and Chu 2008). Experimental treatments in the early 1940s included the use of nitrogen mustard and folic acid (DeVita and Chu 2008). Chemotherapy, or the “use of chemicals to treat cancer,” was not systematically employed until the mid-1960s with proof that childhood leukemia and adult Hodgkins lymphoma could be cured (DeVita and Chu 2008). Today, multiple modality treatment that combines chemotherapy, radiation, and surgery is standard practice (DeVita and Chu 2008). In certain types of cancer, such as breast and prostate, hormone-based therapies may also be employed, including ovarian ablation and androgen deprivation therapy (Garlow 2007; Fraenkel et al. 2013; VanderWalde and Hurria 2011; Saad et al. 2008).

Clinical data has illustrated that chemotherapy and hormonal therapies used to treat certain soft tissue cancers, such as breast and prostate can have negative impacts on bone. Some of these impacts include accelerated bone loss, lower bone mineral density, osteopenia, and pathological fractures (Vehmanen et al. 2001; Hadji et al. 2009; Greep et al. 2003; Georgiou et al. 2011; and Holmes et al. 1994). This could be due to the chemicals used pushing bone marrow stromal cells to an adipogenic lineage instead of an osteoblastic lineage (Georgiou et al. 2012). While much work has been done in assessing the clinical effects of contemporary cancer treatments on bone in living patients, no current study explicitly examines how these treatments

may alter the way lesions manifest on dry bone in an anthropological context. The advancements in cancer treatments since the 1950s relegates earlier studies on cancer using collections comprised of individuals born before 1920 as not representative of cancer prevalence in contemporary populations. A study using a contemporary collection is necessary to truly understand how instances of metastatic cancer are visualized on bone, and how many cases are missed if visual inspection alone is employed.

CHAPTER THREE

MATERIALS AND METHODS

This study compared recognition of cancer on skeletal material macroscopically and radiologically to assess which method would more frequently detect cancerous lesions. The visualization of cancer radiologically was considered because some cancerous lesions begin on the inside of the bone due to the mechanisms of soft tissue cancer metastases. Therefore, it was expected that if a lesion were to develop at all, it would first be visible radiologically. This chapter will cover the process of selecting the study sample, as well as the methodologies employed for both radiographic and visual assessment. Statistical tests used to compare the frequencies of lesion presence and agreement between the methods will be described.

Sample

The study sample for this project was drawn from the William M. Bass Donated Skeletal Collection housed at the University of Tennessee. A spreadsheet of all donors with reported cancer was generated from the collection database, resulting in a list of 139 individuals with general cancer presence reported, such as “cancer” or “metastatic cancer.” Within this group of 139, a list of 55 individuals with specific cancers reported, such as breast or prostate cancer was also generated. A power analysis utilizing the chi-squared test was conducted in R. The chi-squared test was chosen because it measures goodness of fit and was appropriate for the data being examined. Based on the literature regarding skeletal metastases and identification of lesions using both radiographs and macroscopic examination, the difference in lesion detection between radiographic examination and macroscopic examination would exhibit a difference

between 0-34% of the time. If this holds true for this study sample, would 30 donors be enough to capture that difference? The significance level was set at 0.05. The results of the power analysis determined that a sample of 30 donors would have at least 76.6 power to identify the difference in lesion detection between differing methods. Thus, a sample of 30 individuals was selected from the generated lists at random to include fifteen male and fifteen female individuals over the age of 40 and having reported soft tissue cancers.

Given the nature of the research question, a control group of individuals without reported cancer was not sampled. All donors received an anonymized number (1-30) as an attempt to avoid cognitive bias. Table 3.1 lists the sex, age, and reported cancer within the study sample. Elements selected for analysis from each donor included: the cranium, mandible, all vertebrae, sacrum, clavicles, scapulae, sternum, ribs, humerii, os coxae, and femora. The radii, ulnae, tibiae, fibulae, and bones of the hands and feet were excluded from analysis due to not being common sites of soft tissue cancer metastasis (Aufderheide and Rodriguez-Martin 2006; Coleman 2006; Ortner 2003; Roberts and Manchester 2005).

Assessment of Visible Lesion Presence

Radiological Assessment

Selected elements from each donor were radiographed at the University of Tennessee Student Health Center by a radiology technician. Radiographs were chosen over computed tomography due to both budget constraints and that radiographs are generally more accessible than other medical imaging modalities. Radiograph images taken included: an anterior-posterior (AP) view of the cranium, a lateral view of the left side of the cranium, superior-inferior views of

TABLE 3.1 Sex, age, and reported cancer for study sample

Anonymized Number	Sex	Age	Cancer Reported
1	Male	67	Lung
2	Female	65	Breast with metastases
3	Female	62	Lung with metastases to brain and bone
4	Female	52	Lung
5	Female	68	Breast with metastases
6	Male	57	Prostate with metastases
7	Female	40	Breast
8	Male	82	Prostate with lymph-node
9	Female	65	Lung
10	Male	79	Lung
11	Male	74	Lung
12	Male	68	Metastatic
13	Male	59	Prostate
14	Male	53	Lung
15	Female	70	Breast
16	Male	56	Lung
17	Male	56	Prostate with metastases
18	Female	54	Lung
19	Female	76	Lung with brain metastases
20	Female	67	Breast with metastases
21	Male	67	Lung
22	Male	72	Lung
23	Female	60	Breast with metastases
24	Female	67	Lung
25	Male	59	Cancer, metastatic
26	Male	48	Lung
27	Female	72	Lung
28	Female	55	Lung
29	Male	82	Prostate
30	Female	51	Breast with metastases



Figure 3.1 Illustration of post-cranial elements arranged to avoid element overlap.

the mandible, vertebrae, and ribs, posterior-anterior (PA) views of the sacrum and scapulae, and AP views of the clavicles, sternum, humerii, os coxae, and femora. Cranial images were taken in isolation, while the remaining elements were arranged on the image receptor in a way that simultaneously minimized the number of images necessary while allowing for no elements to overlap. Figure 3.1 provides an example of how post-cranial elements were arranged on the image receptor.

As per the Bass Collection protocol, the cervical, thoracic, and lumbar vertebrae are strung together in anatomical order. These elements were left in this state to allow different vertebrae to be easily sequenced and identified by number. Images were taken by a trained radiology technician at the exposure settings she decided were appropriate on a case by case basis. This allowed for the exposure setting to be adjusted for each round of radiographs to

prevent over or underexposure. The angles at which the images were taken were chosen to allow for a clear view of the inner trabecular bone where the red bone marrow would have been in life. While these angles allowed for significant portions of the elements to be analyzed, there were drawbacks. One such issue was the presence of processes on certain elements overlapping other parts of that element (ie. acromial process of scapula). The chosen angles also precluded visualization of certain portions of elements to distinguish lesions, such as the lamina of the vertebrae or portions of the ischiopubic rami of the os coxae. It is recommended that future studies take multiple angles of skeletal elements if time and budgets allow. Digital images were stored on a CD-rom burned by the radiology technician. Syngo FastView, a standalone viewer for DICOM images, allowed for the contrast and lighting of the images to be altered, certain parts of images to be analyzed more closely, and measurements of lesions to be taken.

The radiographs were visually analyzed by the author to determine the presence and location of any lesions. The nature of the lesions (osteolytic, osteoblastic, or mixed), whether the lesions were solitary or multifocal, and location of the lesions were recorded in an excel file compatible with SAS software. Additional pathology notes for each donor detailed the nature of the margins surrounding each lesion, the location of the lesion in relation to other lesions, the size of the lesion, any other noticeable attributes of the lesion, and the presence of non-cancerous pathological conditions such as arthritis or healed fractures. The nature of the margins surrounding the lesion was assessed using the criteria outlined in Ragsdale et al. (2018: 30). Refer to table 2.1 for a description of Ragsdale et al. (2018) Modified Lodwick-Madwell

Grading system. Radiographic analyses were conducted prior to the macroscopic assessment of visible lesions.

Visual Assessment

Following assessment of the radiographs, skeletal elements were examined macroscopically for the presence of visible lesions. This analysis occurred one week after radiographic analysis as an attempt to avoid cognitive bias. Ideally, the waiting period between each analysis would have been longer, however, time constraints prohibited such. Visual analysis followed the criteria for differential diagnosis established in the paleopathology literature (Ortner 2003, Roberts and Manchester 2005; Aufderheide and Rodríguez-Martin 2006). Such analyses describe the characteristics of the lesion, such as whether it is lytic or blastic, whether it is solitary or multiple, whether there is active bone formation around the margins, and where within the skeleton the lesions are distributed as an attempt to narrow down the pool of potential disease processes that could have produced the lesion in question. Nature of the lesions (osteolytic, osteoblastic, or mixed), whether the lesions were solitary or multiple, and location of the lesions were recorded in an excel spreadsheet compatible with SAS software. Additional pathology notes for each donor detailed the nature of the margins surrounding each lesion, the location of the lesion in relation to other lesions, the size of the lesion, the degree of cortical destruction, and other distinguishable features of the lesions. Other observable pathological conditions, such as arthritis and healed fractures, were also noted. An example of the data collection spreadsheet is in Appendix A.

Simultaneous Assessment

Following observation of both the radiographs and dry bone in isolation, the selected elements were re-analyzed using both methods simultaneously. This analysis occurred one week after visual assessment to avoid cognitive bias. Simultaneous assessment clarified why discrepancies between macroscopic and radiographic assessment occurred. Discrepancies between number of lesions seen macroscopically and radiologically were attributed to either the angle of the radiograph not allowing a particular lesion to be seen, the condition being too small of an area of bone formation or destruction to be able to be visible on radiographs, or the lesion being located in an area of bone already of a lesser density, thus not apparent on the radiograph in isolation. New notes were taken for each donor during this assessment, so that any differences in observer opinion between isolated and simultaneous analysis could be assessed.

Statistical Methods Employed

Statistical analyses were based on the donor sample size of thirty (30) instead of individual lesion counts. All statistical tests were completed in SAS v 9.4 (Cary, NC). Frequency counts and percentages of lesion presence, lesion nature and lesion location were acquired using the Frequency procedure. Agreement between macroscopic and radiographic methods for each element were conducted using the “agree” option within PROC FREQ and the Landis and Koch (1977) scale for Kappa values, outlined in Table 3.2. This analysis was run for all elements, such as comparing the lesion presence of the right clavicle macroscopically and radiographically, or the lesion nature of the left os coxa macroscopically and radiographically. Fisher’s Exact Chi-square tests were conducted to assess the association between lesion detection using macroscopic analysis and radiographic analysis.

Using both statistical methods and detailed pathology notes, this study compared the frequency of recognizing cancer macroscopically and radiographically to determine if more lesions could be visualized using macroscopic examination, radiographic examination, or a combination of both. Agreement between the methods was assessed, as well as reasons for disagreement between the two methods, to provide a framework for detecting cancer presence in skeletal remains within a contemporary context.

TABLE 3.2. Landis and Koch (1977) Kappa Statistic Agreements

Kappa Statistic	Strength of Agreement
<0.00	Poor
0.00-0.20	Slight
0.21-0.40	Fair
0.41-0.60	Moderate
0.61-0.80	Substantial
0.81-1.00	Almost Perfect

CHAPTER FOUR

RESULTS

The results of this study indicate that identifiable lesions varied between the methods of analysis and between the elements studied. Overall, more lesions were identified radiographically compared to macroscopically. Simple Kappa and Fischer's Exact Tests assessed the agreement between radiographic and macroscopic analysis, indicating agreement to range from slight to substantial depending on the element under analysis, with substantial agreement occurring where no lesions were identified macroscopically or radiologically. The high levels of disagreement between the two methods suggest that employing macroscopic examination alone will not capture the full scope of lesion presence. Therefore, radiographing skeletal material, in conjunction with macroscopic analysis of dry bone, within a forensic context is recommended to accurately detect cancerous lesions on bone.

Lesion Frequencies

Within the sample of 30 individuals, lesions were noted either macroscopically or radiographically in 19 individuals (63%), while 11 (37%) individuals exhibited no lesions. The possibility of no lesions being observed is expected due to the nature of reporting soft tissue cancers. Just because a soft tissue cancer is reported does not necessarily indicate that the cancer metastasized to the bone. When isolated macroscopic and radiographic analyses for those individuals that exhibited lesions was compared, more individuals exhibited lesions that could be visualized with radiographs overall (100%). Figure 4.1 illustrates lesion presence frequencies for all elements for those individuals with lesions present (N = 19). More individuals exhibited

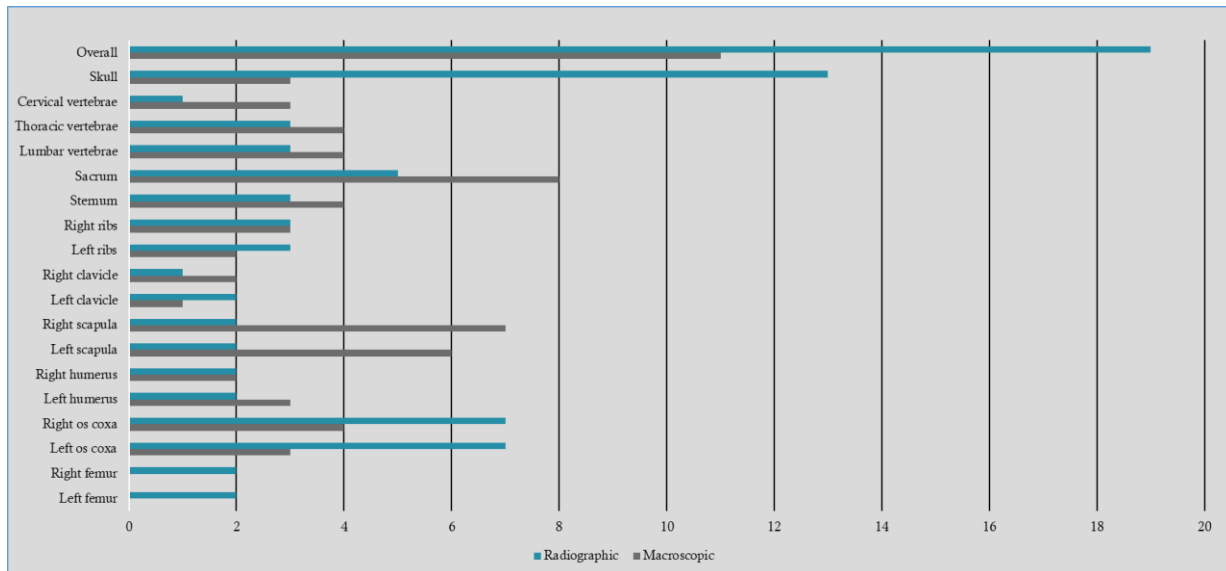


Figure 4.1 Lesion Presence Frequencies Separated by Element (N = 19 Donors).

lesions on radiographs compared to macroscopic analysis on: the skull, left ribs, left clavicle, right and left os coxae, and right and left femora. More individuals were observed as having lesions present macroscopically, compared to radiographically, on the vertebrae, sacrum, sternum, right ribs, right clavicle, right and left scapulae, and the right and left humeri (Figure 4.1). Potential error can be introduced when considering actual lesion counts when assessing radiographic images. Radiographs show overlap of anterior and posterior surfaces in an AP view, which may result in multiple visible lesions on dry bone assuming the appearance of a single lesion on radiographs. Given the potential for these errors, assessing lesion visualization in terms of “presence” and “absence” was used. Therefore, if a humerus exhibited one lesion or three lesions, the notation of “lesion present” would be constant.

Figure 4.2 illustrates an example of a cranium that exhibited visible lesions using macroscopic analysis of the dry bone and the radiographs. However, due to the overlap of the parietals and frontal bones, more lesions would appear visible on the radiograph than on the dry

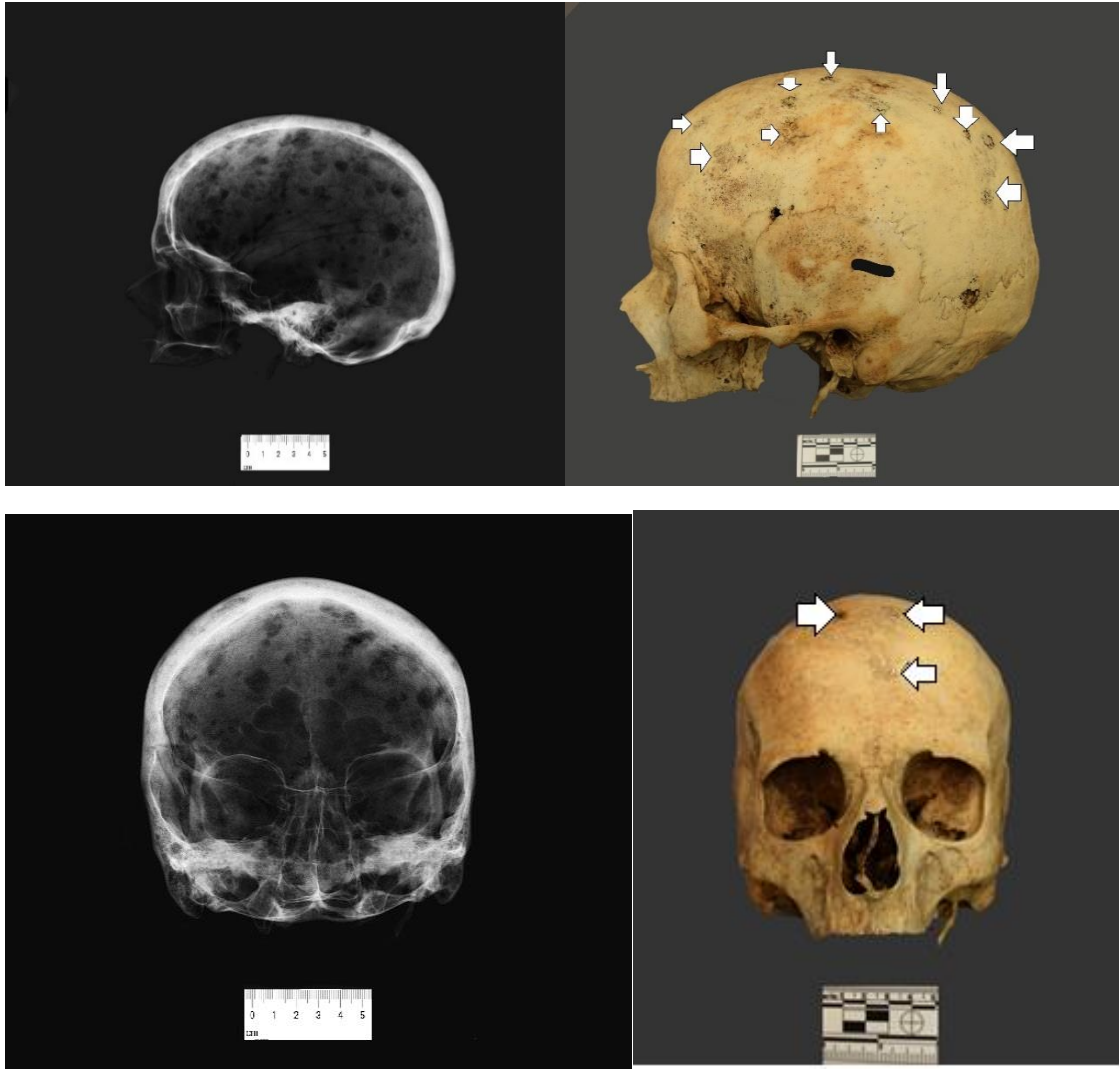


Figure 4.2 Donor 2 lateral cranium radiograph (upper left) and photo (upper right), and anterior-posterior cranium radiograph (lower left) and photograph (lower right) of cranium with white arrows indicating visible lesions.

bone when viewing the cranium from the same angle.

Of the donors that exhibited lesions radiographically, 42% exhibited lesions solely on the radiographs, while 58% exhibited lesions through both macroscopic and radiographic analysis.

Table 4.1 lists lesion presence for all individuals, both macroscopically and radiographically, as well as in which elements there was disagreement. If an individual is noted as “yes” lesion

Table 4.1 Lesion presence for 30 individuals using both methods

Individual	Macroscopic Lesion Presence	Radiographic Lesion Presence	Element(s) where methods disagreed
1	No	Yes	Skull
2	Yes	Yes	Clavicles, humerii
3	Yes	Yes	Skull, left femur
4	No	Yes	Skull
5	No	No	None
6	Yes	Yes	Vertebrae, skull
7	No	No	None
8	No	Yes	Skull, right os coxa
9	No	No	None
10	No	No	None
11	Yes	Yes	Os coxae
12	No	No	None
13	No	No	None
14	No	No	None
15	Yes	Yes	Right scapulae and clavicle
16	No	No	None
17	Yes	Yes	Right humerus, 1 st ribs
18	Yes	Yes	Skull, sacrum
19	Yes	Yes	Skull, sacrum
20	No	Yes	Skull
21	Yes	Yes	Os coxae
22	Yes	Yes	Skull, os coxae, vertebrae
23	No	No	None
24	Yes	Yes	Vertebrae, sternum, scapulae
25	No	No	None
26	No	Yes	Skull
27	No	No	None
28	No	Yes	Skull
29	No	Yes	Os coxae
30	Yes	Yes	Vertebrae, sacrum, sternum, os coxae, skull, femora.

*When there were discrepancies between the methods, the skull was the element in question 12 times (40%), the os coxa six times (23%), the vertebrae four times (13%), the sacrum three times (10%), the humerii, femora, clavicles, scapulae, and sternum two times (6%).

present, that indicates that a lesion was found on any of elements studied. Therefore, an individual with one lesion or 20 lesions would still be marked as “yes.” When there was disagreement between the methods, the elements were more likely the skull (40%) and the os coxae (23%), followed by vertebrae (13%), sacrum (10%), humerii (6%), femora (6%), scapulae (6%), clavicles (6%), sternum (6%), and 1st ribs (3%) (Table 4.1).

Other Lesion Attributes

Of the lesions detected in this study, more individuals exhibited visible lesions that were lytic in nature, when compared to blastic. Of the lytic lesions, more were observed overall using radiographs (60%), particularly on the skull and os coxae. Of the blastic lesions observed, only one lesion of a questionable etiology was observed macroscopically, while two individuals exhibited areas of increased density radiographically. However, the nature one of lesions is uncertain, while the other was deemed cancerous. Locations of lesions also varied between the methods. Unsurprisingly, more individuals exhibited lesions radiographically in areas of better visibility (cranial vault, sternal body, and ilia), while more individuals exhibited visible lesions macroscopically in areas that are difficult to see on radiographs (basicranium, vertebral arches, acromion and coracoid process of scapulae, etc.).

Agreement Between the Methods

A Kappa agreement was conducted to assess the strength of agreement between macroscopic and radiographic methods. Table 4.2 illustrates the results of the Kappa agreement for lesions visually identified between macroscopic and radiographic methods. There were more individuals with agreement between macroscopic and radiographic assessment when lesion

presence was considered “absent,” with as many as 27 individuals having no lesions visible using either method on the left femur. All skeletal elements showed more instances of individuals having disagreement over lesion presence than agreement over the lesion being “present” (Table 4.3). Highest agreement for lesions visualized as present between the methods occurred in the sacrum (4), while the least agreement occurred in the skull (14). Clearly, agreement between the two methods vary by individuals and by skeletal elements.

A Simple Kappa Agreement was conducted to assess the strengths of agreement of lesion observation for all elements. Table 4.3 outlines the results of this test and assesses strength of agreement using Landis and Koch (1977) (refer to table 3.2 for Landis and Koch criteria).

Table 4.2 Kappa agreement for lesion presence between macroscopic and radiographic methods

	agree present	agree absent	disagree
Skull	3	13	14
Cervical vertebrae	1	23	6
Thoracic vertebrae	2	23	5
Lumbar vertebrae	2	23	5
Sacrum	4	21	5
Sternum	2	22	6
Right ribs	2	20	8
Left ribs	1	22	7
Right clavicle	1	26	3
Left clavicle	1	26	3
Right scapula	2	22	6
Left scapula	2	23	5
Right humerus	0	26	4
Left humerus	1	25	4
Right os coxa	4	17	9
Left os coxa	3	18	9
Right femur	0	26	4
Left femur	0	27	3

Table 4.3 Simple Kappa Agreements for lesion presence using macroscopic and radiographic methods

	Estimate	Standard Error	95% Confidence Limits		
			min	max	
overall	0.2077	Fair agreement	0.1022	0.0075	0.4080
skull	0.1498	Slight Agreement	0.1057	-0.0573	0.3569
Cervical vertebrae	-	-	-	-	-
Thoracic Vertebrae	0.4718	Moderate Agreement	0.1602	0.1578	0.7859
Lumbar vertebrae	0.4275	Moderate Agreement	0.1844	0.0661	0.7889
Sacrum	-	-	-	-	-
Sternum	0.3662	Fair Agreement	0.1824	0.0086	0.7238
Right ribs	0.3931	Fair Agreement	0.1760	0.0481	0.7380
Left ribs	-	-	-	-	-
Right clavicle	0.6429	Substantial Agreement	0.2299	0.1923	1.0000
Left clavicle	0.6407	Substantial Agreement	0.2338	0.1825	1.0000
Right scapula	0.4231	Moderate Agreement	0.1456	0.1377	0.7084
Left scapula	-	-	-	-	-
Right humerus	-	-	-	-	-
Left humerus	0.4470	Moderate Agreement	0.1596	0.1342	0.7598
Right os coxa	-	-	-	-	-
Left os coxa	-	-	-	-	-
Right femur	-	-	-	-	-
Left femur	-	-	-	-	-

*Any value with a “-“ denotes a rare event, thus the statistic could not be run

Depending on the element, agreement between the methods varied, ranging from slight to substantial. Most notable was the substantial agreement between the methods for the clavicles and slight agreement for the skull. A closer examination of why these agreement strengths vary is warranted. In six individuals (32% of the sample), lesions were observed radiographically and not macroscopically in the skull, thus producing slight agreement (Table 4.4). However, when assessing the clavicles, agreement is high because 26 individuals (92.86% of the sample),

exhibited no lesions using either method. Therefore, the agreement was because of the high absence of lesions, not agreement over the presence of lesions.

Given this study's small sample size, a Fisher's Exact Chi-Square test was considered appropriate to assess agreement of lesion detection between macroscopic and radiographic assessment. Table 4.4 outlines the results of the Fisher's Exact test for lesion presence between macroscopic and radiographic examination. A P-value of $<.05$ is considered significant under this test. The results of this analysis show that there is a significant difference between detection of lesions using macroscopic and radiographic methods overall, and for all elements except the cervical vertebrae, right humerus, and right femur.

Table 4.4 Fischer's exact test for lesion presence agreement between macroscopic and radiographic lesion presence

	Probability	Pr \leq P
Overall	0.0107	0.2016
Skull	0.0365	0.3342
Cervical vertebrae	0.0828	0.3655
Thoracic vertebrae	0.0001	0.0004
Lumbar vertebrae	0.0026	0.0128
Sacrum	0.0002	0.0003
Sternum	0.0046	0.0377
Right ribs	0.0009	0.0128
Left ribs	0.0004	0.0069
Right clavicle	0.0046	0.0138
Left clavicle	0.0044	0.0202
Right scapula	0.0013	0.0026
Left scapula	0.0002	0.0004
Right humerus	0.0621	0.0690
Left Humerus	0.0010	0.0025
Right os coxa	0.0001	0.0052
Left os coxa	0.0007	0.0191
Right femur	0.9000	1.0000
Left Femur	-	-

*Any value with a “-“ denotes a rare event, thus the statistic could not be run

CHAPTER FIVE

DISCUSSION

The results of this study reflect the complexity in identifying whether a lesion is visible in unbiased viewing of either dry bone or radiograph images. Whether an instance of reported cancer that metastasizes to bone is visible is influenced by a multitude of factors. Some of these issues include: the angle at which the radiograph was taken, the overall complexity of the skeletal element being imaged, the amount of cortex destroyed by a lytic lesion, and the distribution of lesions throughout the element as either localized or diffuse. The results of this study indicate poor agreement between the methods in detecting lesions, with more than half of the study sample exhibiting visible lesions that were missed if macroscopic analysis of dry bone alone is employed. This contemporary study sample exhibited results not entirely different from Rothschild and Rothschild (1995), with similar proportions of lesions being noted on radiographs compared to macroscopically. Overall, the high levels of disagreement between the two methods suggest that employing macroscopic examination alone will not capture the full scope of lesion presence. Therefore, radiographing skeletal material, in conjunction with macroscopic analysis of dry bone, within a forensic context is recommended to accurately detect cancerous lesions on bone.

Lesion Frequencies

The results of this study indicate that visible lesions were noted on 63% of the study sample, which was composed only of individuals with reported cancer. It should be remembered that not all soft tissue cancers metastasize to the bone. Therefore, it was expected that in selecting a sample of individuals with reported cancer, there would be individuals in this study

that exhibited no lesions using either method. While there were individuals in this sample that did not exhibit visible lesions, it should be remembered that roughly 70% of patients with advanced prostate and breast cancer develop skeletal metastases (Coleman 1997, Saad et al. 2008). Therefore, the proportion of individuals within this sample with skeletal lesions is similar to clinical reports and could be representative of a contemporary population.

More individuals in this study sample exhibited visible lesions radiographically for the cranium, left ribs, left clavicle, right and left os coxae, and right and left femora while more individuals exhibited lesions macroscopically for the vertebrae, sacrum, sternum, right ribs, right clavicle, right and left scapulae, and the right and left humeri (Figure 4.1). When the complexity of the bone in question is considered, these results appear clearer. Save for the cranium, elements where more lesions were noted radiographically are relatively simple and were able to be viewed easier on the radiographs. This is because they either lacked excessive processes, laid flat on the image receptor, or lacked areas of dramatic density differences. Other elements that had excessive processes or areas of thin bone were difficult to capture clearly in the radiograph and exhibited more lesions viewable macroscopically. The reasons for the distribution throughout the right and left halves of the body are unknown, as there is no mention in the literature of metastatic cancers predilecting one side of the body over another. Without information pertaining to location of any soft-tissue lesions prior to skeletonization (ie. lung cancer in the right lung), the reasons for this distribution cannot be ascertained.

Of the donors that exhibited lesions on radiographs, 42% exhibited lesions solely on the radiographs not visible macroscopically, while 58% exhibited lesions macroscopically, with additional lesions being noted using the aid of radiographs. Therefore, macroscopic examination alone did not capture the full range of visibly detectable lesions within the sample, validating

statements that radiography is necessary for the detection of internal lesions (Roberts and Manchester 2005, Marques 2019).

Case Studies

Donor three exhibited no visible lesions that would indicate cancer during macroscopic analysis. The only noticeable pathologies included a button osteoma on the occipital and roughened porosity scattered throughout the vertebrae and ribs. Upon radiographic analysis, four noticeable areas of radiolucency were noted on the cranium. Three of these were concentrated on the frontal near midline and ranged from ~2-11mm in diameter. The margins of these defects were gradual and followed a 1C classification according to Ragsdale et al. (2018)'s modified Lodwick-Madewell Grading System. The fourth area of radiolucency was located on the inferior frontal, was ~4mm in diameter, and exhibited gradual 1C margins. Figure 5.1 illustrates both the dry bone and radiographs of the cranium for donor three.

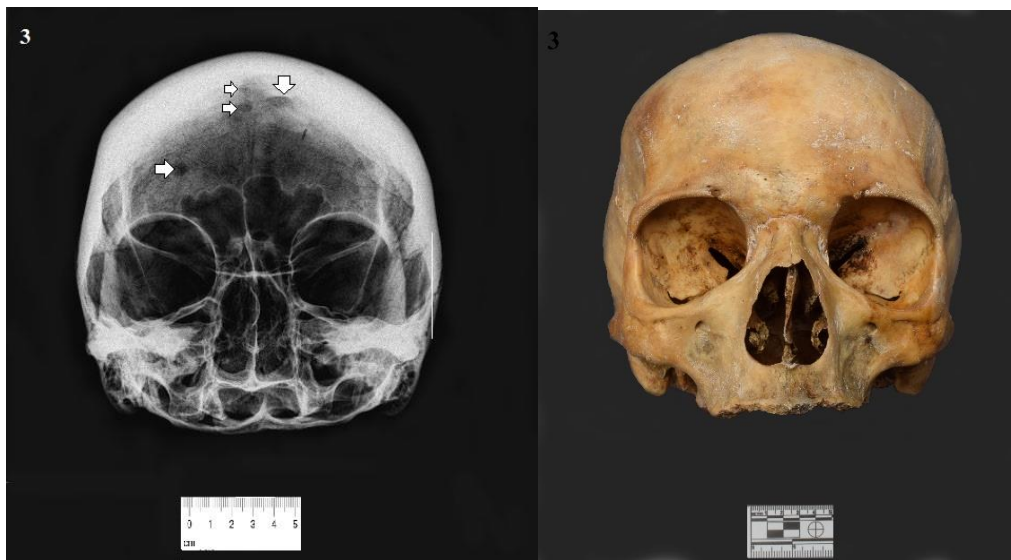


Figure 5.1 Donor 3 cranium radiograph (left) and dry bone (right), with white arrows illustrating the presence of lesions only visible on the radiographs.

Donor two exhibited instances of lytic cancerous lesions throughout the dry bone on the cranium, mandible, clavicles, right and left scapulae, multiple vertebrae, multiple ribs, the sacrum, and the right and left os coxa. Figure 5.2 illustrates some examples of these lytic lesions. Donor two, however, only exhibited signs of diffuse periosteal reaction along the diaphysis of the right humerus when macroscopic analysis was employed. Periosteal reaction does not automatically indicate cancer as there are multiple etiologies that can be linked to periostitis, such as infection, nutritional deficiencies, and trauma. Therefore, in an unbiased viewing of the dry bone the periostitis was noted but not considered a cancerous lesion. Radiographic analysis, on the other hand, exhibited multiple lytic lesions throughout the cortex of the diaphysis on the right humerus. Figure 5.3 illustrates the right humerus dry bone and radiographs, illustrating the presence of additional lytic lesions not seen on the dry bone. This case provides an example of how radiographs can detect additional lesions within the cortex, even in cases where there may be excessive lytic lesions visible on the dry bone of other skeletal elements. The use of radiographs in this case aided in understanding the scope of these cancerous lesions, especially



Figure 5.2. Donor 2 right os coxa (far left), right scapula (middle), and lateral view of the cranium (far right) with white arrows illustrating visible lesions.

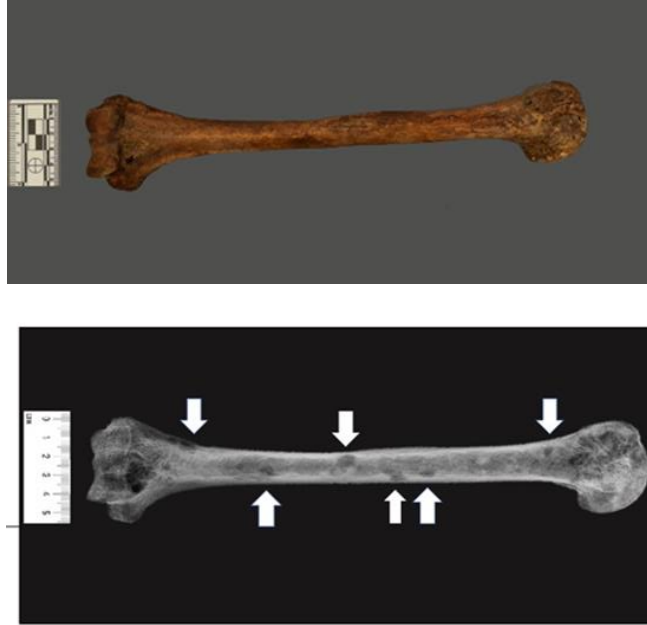


Figure 5.3. Donor 2 right humerus bone (top) and radiograph (bottom) with white arrows illustrating lesions visible on the radiograph throughout the shaft that are not visible on the dry bone.

since the humeral diaphysis is not considered a common site of soft tissue cancer metastases (Ortner 2003).

Donor 21 exhibited a large lytic lesion on the right os coxa near the auricular surface. The lesion spanned ~31mm and exhibited porous and dark-colored margins. The anterior cortical bone and underlying trabecular bone were destroyed, leaving the posterior cortical bone with small pinprick-sized holes of porosity. The right os coxa illustrated grey-colored diffuse porosity around the iliac fossa and along the obturator foramen on the dry bone. Upon radiographic examination, the lytic lesion near the auricular surface spanned ~35mm, indicating that the lesion extended additional millimeters underneath the cortex. An additional lesion was noted on the radiographs near the anterior inferior iliac spine that measures ~17mm and exhibits gradual 1C margins. This case highlights how radiographs can detect additional lesions on the same element



Figure 5.4. Donor 21 right os dry bone (left) and radiograph (right) with arrows indicating lytic lesions present.

where there are already macroscopically visible lesions. Figure 5.4 illustrates the presence of an additional lesion on the right os coxa of donor 21, exhibiting how lesions can still be missed if macroscopic analysis alone was employed. As the above three case studies have shown, the use of radiographs was necessary to visualize the full range of cancerous manifestations in these this study sample. This illustrates that even in cases where there are visible lesions on the bone, the true nature of the cancerous metastasis may be more complicated and more extensive, warranting further analysis.

Other Lesion Attributes

Of the 19 donors that exhibited visible lesions, five were males with reported lung cancer, four were females with reported breast cancer, seven were females with reported lung cancer,

and three were males with reported prostate cancer. These trends are not atypical, given that lung, breast, and prostate are considered among the top five cancers that metastasize to bone (Ortner 2003). More lesions noted were lytic in nature, which corresponds with the attributes of the soft tissue cancers noted in the literature (Ortner 2003, Roberts and Manchester 2005, Wu and Hochman 2012). There was one instance of a blastic lesion noted. The amount of lytic lesions within this study sample should not be surprising given the cellular mechanisms of cancer metastases where osteoblastic and osteoclastic responses may be coupled. Certain growth factors, such as TGF- β 2 and FGF, can initially promote osteoblastic lesions in cases of prostate cancer. Those same growth factors can be taken up by the tumor cell itself, leading to secretion of factors that stimulate RANKL, further breaking the bone down, releasing more factors that promote growth of the tumor cell. This creates a feedback loop that highlights the unstoppable nature of soft tissue lytic cancer metastases to bone. Therefore, even in cases of cancer that are typically reported as being blastic in the literature, such as breast and prostate cancer, lytic lesions may form if the cancer persisted long enough. This should highlight the importance of considering the influence of contemporary medical interventions on the manifestation of cancerous conditions in dry bone. The aid of contemporary cancer treatments prolongs the timeline of cancer manifestations and can provide more time for lytic lesions to develop in conditions that would have previously been considered blastic.

As noted in the results, variation in detection of lesions occurred within the same elements, with more lesions being noted radiographically in areas of better visibility (the cranial vault or the ilia), while clear macroscopic lesions were not visible on radiographs in areas of elements that are difficult to capture in A-P radiographs. These shortcomings should prompt future studies to radiograph material from multiple angles and ensure all portions of the elements



Figure 5.5. Donor 15 basicranial view of dry bone (far left), lateral radiograph (middle) and AP radiograph (far right), with white arrow indicating a visible lytic lesion on the dry bone.

are visible. Figure 5.5 illustrates an example of an instance where multiple angles would be beneficial. There is a sizeable lytic lesion on the base of the occipital, however, this lesion is not captured when only an A-P and lateral view of the skull is taken. One can see in the radiograph image that the dense areas of the skull, such as the petrous portion of the temporals, obscures a clear view of the lesion occurring in the basicranial areas. A superior-inferior radiograph of the skull would be recommended in future studies. Figure 5.6 illustrates a case of a lesion in a location that is difficult to see using either method. The lytic lesion occurs in the posterior body of the first sacral segment. The lesion is of the median sacral crests overlapping the body. This same issue occurs on the radiograph when taking a posterior-anterior view. To accurately see the lesion on the dry bone, the bone itself needed to be angled so one is viewing into the sacral canal. Therefore, an A-P view and an oblique angle should also be taken in addition to the P-A radiograph.



Figure 5.6. Donor 18 sacrum with a close-up view of lytic lesion in the sacral canal indicated by white arrow (top image), radiograph (bottom left) and dry bone (bottom right).

Agreement Between the Methods

Overall, the agreement between macroscopic and radiographic methods of lesion detection was poor. When there was agreement, it was because a lesion was marked as “absent.” When lesions were viewed as present, there were disagreements between the methods. When a Simple Kappa Agreement was conducted to assess the strength of agreement, the results ranged from slight to substantial. As mentioned in the results chapter, the substantial agreement occurred in the clavicle where the agreement was due to lesion being marked “absent.” The slight agreement between the methods for the detection of lesions in the skull was due to more lesions being detected radiographically than macroscopically. The results of the Fisher’s Exact Test for lesion presence showed that there is a significant difference between detection of lesions using macroscopic and radiographic methods overall, and for all elements except the cervical vertebrae, the right humerus, and the right femur. The reasons the agreement for the cervical vertebrae, right humerus, and right femur are once again due to agreement over lesions being “absent” as opposed to present. The propensity for disagreement between the methods reflects that simply employing macroscopic analysis cannot account for the full range of skeletal manifestations of metastatic cancer. If it could, the agreement between macroscopic and radiographic assessment would be more similar. Since they are not in exact agreement, radiographing skeletal material is necessary.

Simultaneous Assessment

Following isolated analysis of the dry bone and radiographic images, all skeletal elements were analyzed simultaneously using both methods. Due to the introduction of this bias, agreement between the methods improved with lesions not previously noted on radiographs

being now visible. Throughout the course of the simultaneous assessment, a few noticeable patterns emerged as to why there was such high disagreement between the two methods. Due to the time and budget constraints of this project, multiple angles of skeletal elements were not taken, which would affect whether lesions are visible. An example would be that more individuals exhibited lesions macroscopically on the vertebrae, especially on the lamina. These lamina lesions are not apparent in an A-P view of the vertebrae. Another example would be that in taking a P-A view of the scapula, the spine and acromion process would obscure lesions on the supraspinous fossa. There were also instances of disagreement within the same element, such as the os coxae, where lesions on the ilium could be seen easily on radiographs while lesions on the ischiopubic ramus or pubis could not. Figure 5.7 illustrates a case where the porous lesion on the ilium could be visualized with radiographs, but the porous lesions near the obturator foramen were better visualized using macroscopic analysis due to A-P views of os coxae not being able to capture the iliopubic and ischiopubic rami.



Figure 5.7. Donor 11 right os coxa dry bone (far left and middle images) and radiograph (far right image) with white arrows illustrating visible lesions.

Another factor that could influence visibility of lesions radiographically is that not enough of the cortex was destroyed to be visible on radiographic images, as with lytic lesions that manifest as porosity without extensive cortical destruction. Very few lesions noted within the study sample destroyed significant portions of the cortex, while more lesions were pockets of increased, dark-colored porosity. This was apparent especially in thin elements, such as the scapulae, where the radiolucency of the image is already high and could further obscure the presence of porous lesions. There were also instances where the porosity was more diffuse and spread throughout the element instead of forming a localized area of porosity. When the porosity was diffuse, it appeared like normal, internal bone on the radiographs.

Figure 5.8 illustrates an example where lesions on the posterior right scapula manifested as slightly grey-colored porosity, especially along the scapular spine, superior angle, medial and lateral borders, and inferior angle. Lesions also manifested as porosity on the anterior surface of

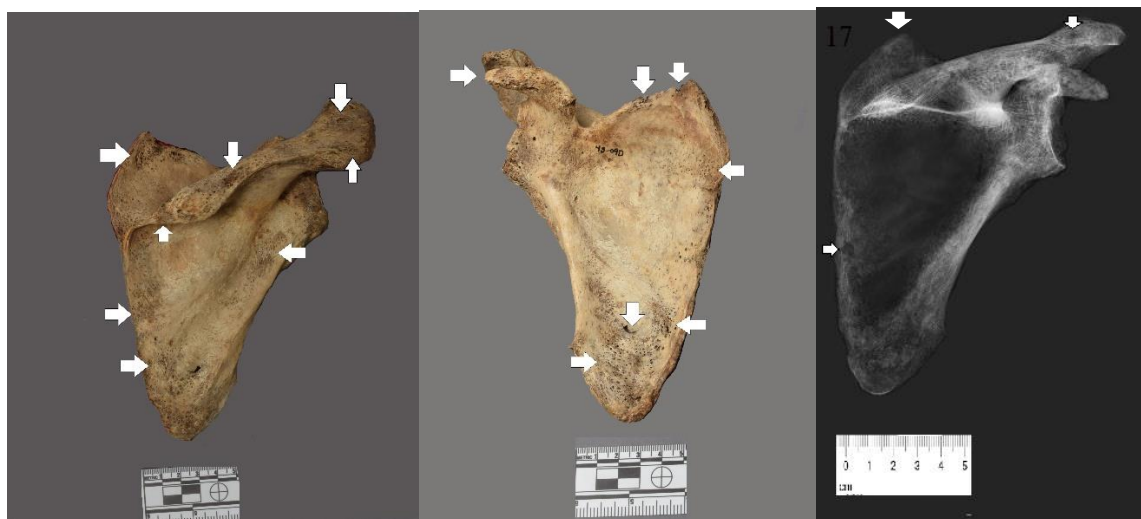


Figure 5.8 Donor 17 right scapula posterior view of dry bone (far left), anterior view of dry bone (middle), and posterior radiograph (far right), with arrows indicating visible porous lesions.

the right scapula as grey-colored porosity, especially at the inferior angle. The posterior-anterior view of the radiograph pictured illustrates how an element with thin portions may not provide the contrast necessary to see porous lesions.

Potential Conflicts

As with any research project, there are inherent limitations to this project. The study sample came from individuals who self-reported the presence of cancer. Without official medical records, one does not know the details of their diagnosis, including whether skeletal metastases were known. As with any population, the potential for comorbidities exists within this sample. The individuals within this study sample are over the age of 40 and are prone to other conditions, such as osteoporosis, that could possibly alter the integrity of the trabecular bone, thus distorting the results of radiographic analysis. While some comorbidities are more apparent, such as arthritis, healed fractures, or Schmorl's nodes, others are not as apparent. There were multiple instances in the pathology notes that questionable lesions were noted. This was especially the case when porosity was diffuse throughout the element or appeared in an area unexpected given the literature on cancer metastases. Some of these comorbidities could include infections or injury that induce inflammation at the site, resulting in an osteoclastic response similar to a cancerous lesion. Figure 5.9 illustrates an example of diffuse porosity on an ilium that is not visible on radiographs. Diffuse porosity is often attributed to a multitude of etiologies, including infection and nutritional deficiencies (Ortner 2003). Since the porosity is not visible on the radiograph, details about the etiology may not be able to be elaborated.

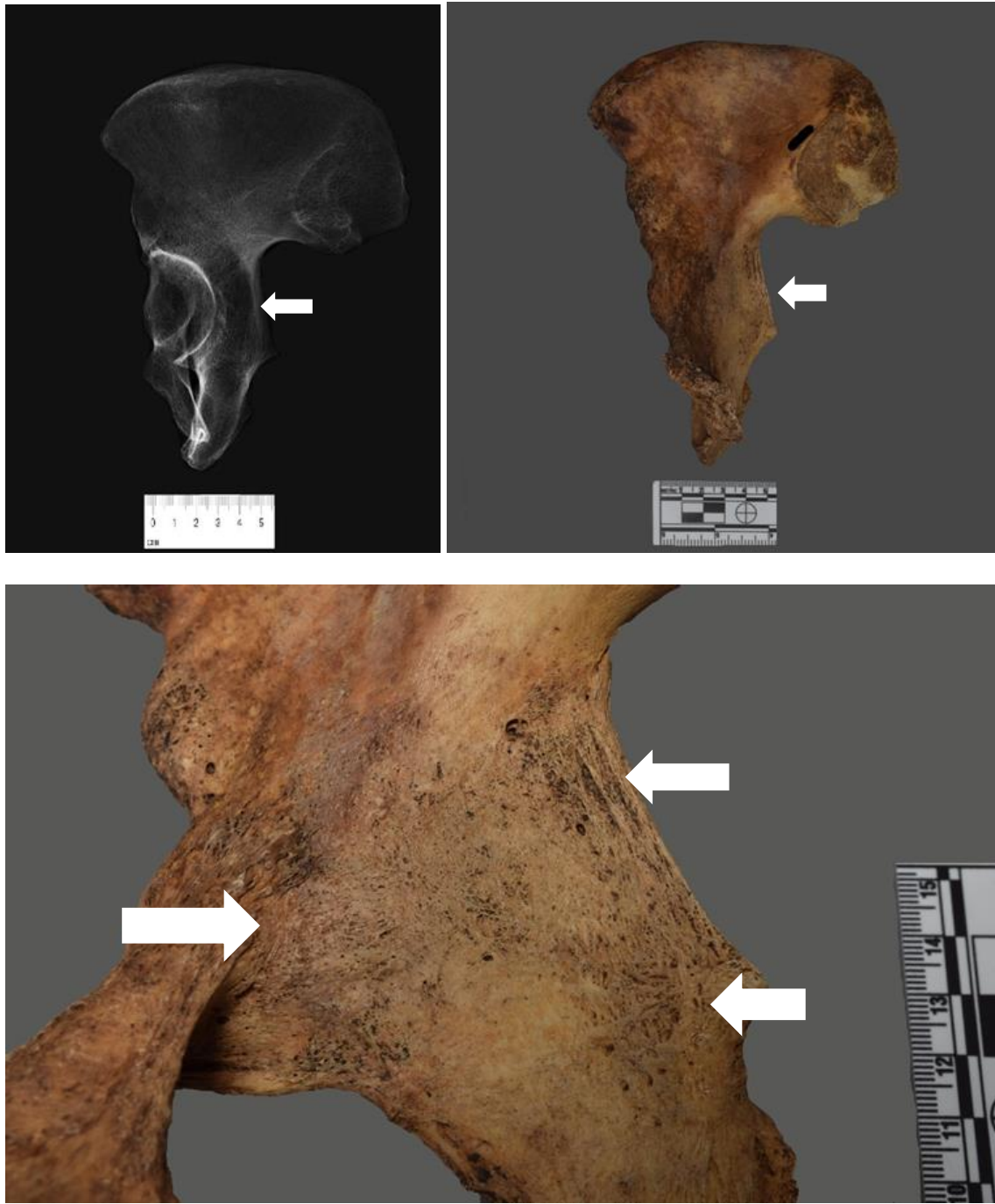


Figure 5.9 Donor 24 right os coxa radiograph (upper left) dry bone (upper right) and closeup of periosteal bone reaction (lower) illustrated by arrows.

Case Study

There were also instances where ambiguous lesions were clarified based on radiographic assessment. Donor 30 exhibited a single lesion during macroscopic analysis, consisting of a thick, roughened coating of porous bone on the shaft of the left first rib. The entire length of the rib was surrounded by this roughened bone formation, with some areas becoming as thick as ~12mm. Figure 5.10 illustrates both the dry bone and radiograph of this rib. The particular etiology of this lesion is unknown as it does not appear typical of a healed fracture. Upon radiographic analysis, the skull, thoracic and lumbar vertebrae, sacrum, right and left ribs, right and left os coxa, and right and left femur exhibited small, circular clusters of increased radiodensity. These lesions are characteristic of internal osteoblastic lesions and were only visible with the aid of medical imaging equipment. Therefore, the use of radiographs clarified that there were cancerous lesions present within this individual, thus providing a potential etiology to the blastic rib lesion. Figure 5.11 provides an illustration of these osteoblastic lesions only visible on radiographs. In this case, radiographs provided additional information to accompany an ambiguous case. While the lesion on the rib may not be able to conclusively be attributed to cancer, the presence of cancerous lesions throughout other portions of the skeleton does make the diagnosis of cancer for the rib lesion more likely.

Sensitivity and Specificity

Instances such as the above highlight the importance of improving lesion detection methods. With the implementation of radiographs, more lesions were being noted and attributed to cancer, which added to the sensitivity, or true positive rate, of the study sample. Sensitivity and specificity are concepts that are commonly employed in epidemiology, however, they are

difficult to employ in paleopathological contexts. Sensitivity refers to the true positive rate, or the “proportion of people with a condition who are correctly identified by a test as having the condition” (Buikstra 2019: 15). When using skeletal samples, sensitivity is limited due to the fact that not every condition having the potential to affect bone actually manifests as a visible lesion (Buikstra 2019). In the case of metastatic cancer, an individual may have breast cancer that remained localized to the breast and did not metastasize to the bone. Specificity refers to the true negative rate, or the “proportion of people without a condition who are correctly identified by the test as not having it” (Buikstra 2019: 15). When using skeletal samples, specificity is limited due to bone being able to respond to multiple diseases in one of three ways: bone resorption, bone formation, and a mixture of the two. Therefore, lesions with different etiologies may have a similar appearance, resulting in false positives (Buikstra 2019). Examples of this encountered within the study sample include elements with diffuse periostitis that could be attributed to other conditions. By implementing radiographs into the analysis of dry bone for the detection of cancerous lesions, a broader range of manifestations can be accounted for and added to the identification criteria for cancerous lesions, thus increasing the sensitivity.

Results of Using a Contemporary Collection

Rothschild and Rothschild (1995) indicated that of the 26% of their sample that exhibited lesions radiographically, only 3% exhibited lesions using both methods. The present study’s sample, though much smaller, exhibited a higher percentage (37%) of individuals with lesions visible using both methods of analysis. In Rothschild and Rothschild (1995), 23% of their sample exhibited lesions solely using radiographs, whereas 42% of the current study’s sample exhibited lesions solely using radiographs. Despite the smaller sample size, the proportions of individuals



Figure 5.10. Donor 30 left first rib radiograph (left) and dry bone (right) exhibiting an ambiguous case of excessive bone formation (indicated by arrows)

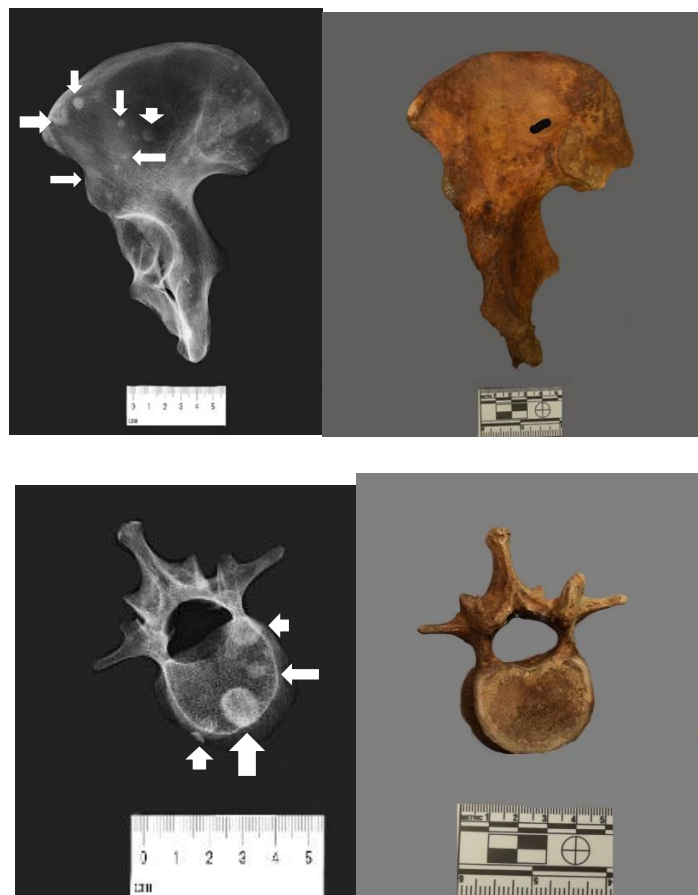


Figure 5.11 Donor 30 right os coxa radiograph (upper left) and dry bone (upper right), lumbar vertebra radiograph (lower left) and dry bone (lower right). The arrows in both images illustrate the presence of osteoblastic cancerous lesions.

displaying lesions on radiographs not encountered macroscopically are similar. However, the present study's sample exhibited more instances where radiographs added to the nuance of visible lesion presence in elements where there were already macroscopically visible lesions. Therefore, it can be argued that radiographing skeletal material for cancer presence is necessary for contemporary populations. Another finding that was similar to Rothschild and Rothschild (1995) was that the current study sample contained more individuals that exhibited lesions radiographically on the ilia and femora, however, the present study sample also contained more individuals that exhibited visible lesions radiographically on the cranium, which was not a finding in Rothschild and Rothschild (1995). The reasons for this difference are unknown.

Given the nature of the donation process for the Bass Collection, the individuals that comprised the study sample may have had access to contemporary cancer treatments, such as chemotherapy, radiation therapy, surgery, or hormonal therapies that would not have been available to individuals in the Hamman-Todd collection. Clinical data has illustrated that chemotherapy and hormonal therapies used to treat certain soft tissue cancers, such as breast and prostate can have negative impacts on bone. Some of these impacts include: accelerated bone loss, lower bone mineral density, osteopenia, and pathological fractures (Vehmanen et al. 2001; Hadji et al. 2009; Greep et al. 2003; Georgiou et al. 2011; and Holmes et al. 1994). Given the influence that cancer treatments have on the proliferation of cancer cells, either prohibiting the spread of metastases or prolonging the process longer for more metastases to form, it is surprising that the proportion of individuals within this study displaying lesions on radiographs is like that of Rothschild and Rothschild (1995). These results support that radiographing skeletal material for instances of disease presence should be a necessary part of any contemporary

forensic investigation, since macroscopic analysis fails to capture the full range of manifestations of cancer presence.

Further Implications

Cancer is the second leading cause of death in the United States, with one in every four deaths being attributed to it. According to the United States Cancer Statistics (2019), the rate of cancer has been steadily increasing since 1999. If these trends continue, cancer will continue to play a pivotal role in contemporary society. This study has illustrated how an understanding of cancer can be beneficial in multiple contexts. This study can have implications for forensic contexts since cancer is a more individuating pathological condition that can be added to the biological profile when compared to other conditions, such as arthritis. Individuals that sought treatment for cancer may have antemortem scans on file that can be compared to postmortem scans for positive identification purposes. This study has also illuminated that the use of medical imaging equipment can also aid in clarifying ambiguous cases of disease presence, with additional noncancerous, potentially individuating conditions also being captured with the aid of radiographs. By increasing the range of manifestations of metastatic cancer, the sensitivity of cancerous lesions within the sample also increased.

Beyond forensics, this study can also have paleopathological implications in that a fuller understanding of how metastatic cancers manifest in contemporary remains may impact the way cancer is understood in past populations as well. Researchers have addressed the topic of cancer in antiquity, and the general consensus is that there is a lower frequency of malignant conditions in the past than there are in clinical practice today (Assis and Codhina 2010; Capasso 2005; David and Zimmerman 2010). This lack of cancer presence in antiquity can be due to many factors, including differences in diet, life expectancy, and environment. However, these

differences may also be due to the intricate process of soft tissue metastasis in bone. Clinical studies have illustrated that contemporary medical interventions may have adverse effects on bone, including: accelerated bone loss, lower bone mineral density, osteopenia, and pathological fractures (Vehmanen et al. 2001; Hadji et al. 2009; Greep et al. 2003; Georgiou et al. 2011; and Holmes et al. 1994). While much work has been done in assessing the clinical effects of contemporary cancer treatments on bone in living patients, no current study explicitly examines how these treatments may alter the way lesions manifest on dry bone in an anthropological context.

This study has examined contemporary skeletal material for instances of cancer metastasis, and has highlighted some interesting observations, including: instances of lesions visible radiographically that were not visible macroscopically, additional lesions visible on radiographs that were not visible macroscopically on elements already exhibiting macroscopically visible lesions, lesions visible radiographically in areas that are not commonly considered sites of soft tissue cancer metastasis, and lytic lesions manifesting in cancer cases where lesions are typically blastic or mixed. This study sample has the benefit of consisting of individuals with reported cancer, which allows for detailed examination of how lesions manifested within specific forms of cancer. Therefore, this study could provide information pertaining to the patterns of soft tissue metastasis specific to contemporary contexts.

CHAPTER SIX

CONCLUSIONS AND RECCOMENDATIONS

Cancer is a disease which significantly impacts contemporary populations, with the World Health Organization (2020) attributing 1 in every 6 deaths to cancer worldwide. In 2018, cancer was estimated to cause 9.6 million deaths globally (World Health Organization 2020). In addition to the deaths caused by cancer, the diagnosis and treatment of cancer can create social, physical, and economic strains on the patient, their families, and their community. According to the United States Cancer Statistics (2019), the rate of cancer has been steadily increasing since 1999. This is due to many factors, such as improvements in screening methods, exposure to different environmental risk factors, and increased lifespans. If these trends continue, cancer will continue to play a prominent role in contemporary society.

In the field of paleopathology, recent attention has shown that visual inspection alone is not enough to account for the true scope of a cancerous lesion (Marques 2019; Ortner 2003; Roberts and Manchester 2005; Brothwell 2012). While the use of radiographs to detect cancerous lesions in skeletal material has received increased attention within the field of paleopathology, the same rigor and momentum has not permeated the forensic anthropology community. This is especially surprising given the rise of virtual autopsy within forensic pathology and forensic medicine. The results of this study indicated that radiographing skeletal material was a necessity to capture the full manifestation of metastatic soft tissue cancer presence in bone. This sample exhibited poor agreement between the methods in assessing lesion presence, with more than half of lesions being missed if macroscopic analysis of dry bone alone is employed. The levels of agreement between the two methods ranged from slight to substantial,

with substantial agreement only occurring in instances where the agreement was showing lesions being absent. Therefore, macroscopic analysis alone cannot accurately capture disease presence. This contemporary study sample exhibited results not entirely different from Rothschild and Rothschild (1995), with similar proportions of lesions being noted on radiographs compared to macroscopically. This indicates that even with contemporary influences, such as advancements in cancer treatment, lesions permeating the inner cortex are being missed without the aid of medical imaging equipment. Radiographing skeletal material for instances of potentially individuating disease presence should be a necessary part of any forensic investigation.

Recommendations and Future Research

Future studies should radiograph skeletal material from multiple angles if time and budgets allow to ensure that all areas of the element are visible. Images should be taken and viewed by a trained radiology techologist when available. This study should also highlight the importance of properly training forensic anthropologists in the imaging and interpretation of radiographs, since a trained radiology technologist may not always be available. If possible, future iterations of this study should employ the use of commuted tomography (CT) to assess lesion presence due to the inherent benefits of this imaging modality. One such benefit is that lesions lacking 30-75% of cortical destruction that would otherwise not be visible on a radiograph might be visible using CT (Tins et al. 2009). Another benefit would be the ability to compare anthropological CT scans and the information obtained from them to those that were obtained in a virtual autopsy or antemortem CT scan context. This may allow for clarification in how many lesions are clearly visible only in skeletonized material. Studies of this nature can

provide a framework for visualizing potentially individuating lesions and contribute to a detailed biological profile that aids in victim identification.

While this study has the benefit of comprising of individuals with reported cancer, a more ideal sample would have complete medical histories that include the timing of the cancer onset, the length of time the disease persisted, and any medical treatments received to provide a more nuanced understanding of any contemporary interventions employed that could influence lesion visibility. Having more complete medical histories would allow for lesion distributions to be mapped across individuals with specific types of cancer. Clinical studies have illustrated that contemporary medical interventions may have adverse effects on bone, including accelerated bone loss, lower bone mineral density, osteopenia, and pathological fractures (Vehmanen et al. 2001; Hadji et al. 2009; Greep et al. 2003; Georgiou et al. 2011; and Holmes et al. 1994). While much work has been done in assessing the clinical effects of contemporary cancer treatments on bone in living patients, no current study explicitly examines how these treatments may alter the way lesions manifest on dry bone in an anthropological context. Future research can expand upon this idea and analyze skeletal collections from both pre and post-chemotherapy periods. A nuanced understanding of the relationship between treatment options and the cellular mechanisms that allow for soft tissue metastases to bone can aid in diagnosing cancer in dry bone in both forensic and paleopathological contexts.

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APPENDIX

ID	Sex	Age	Cancer	MGenLes	MGenNat	MSkullLesion	MSkullNat	MSkullNum	MSkullLoc
1	male	67	lung	No	N/A	No	N/A	N/A	N/A
2	Female	65	breast with metastases	Yes	Lytic	Yes	Lytic	Multiple	occipital (~2), right parietal (~10), left parietal (~14), spheNoid, frontal (~12), mandible (~2).
3	Female	62	lung, metastatic to brain and bone	Maybe	Lytic	No	N/A	N/A	N/A
4	Female	52	Lung	No	N/A	No	N/A	N/A	N/A
5	female	68	breast with metastases	No	N/A	No	N/A	N/A	N/A
6	Male	57	Prostate with metastases	Yes	Mixed	No	N/A	N/A	N/A
7	Female	40	Breast	No	N/A	No	N/A	N/A	N/A
8	male	82	Prostate and LymphNode Cancer	No	N/A	No	N/A	N/A	N/A
9	female	65	lung	No	N/A	No	N/A	N/A	N/A
10	Male	79	Lung	No	N/A	No	N/A	N/A	N/A
11	male	74	lung	Yes	Lytic	No	N/A	N/A	N/A
12	male	68	metastatic	No	N/A	No	N/A	N/A	N/A
13	Male	59	prostate	No	N/A	No	N/A	N/A	N/A
14	Male	53	Lung	Yes	Mixed	No	N/A	N/A	N/A
15	female	70	breast	Yes	Mixed	Yes	Mixed	Multiple	vault, occipital
16	male	56	lung	No	N/A	No	N/A	N/A	N/A
17	male	56	metastatic prostate cancer	Yes	Lytic	Maybe	Lytic	Multiple	spheNoid, frontal, mandible
18	Female	54	Lung	Yes	Lytic	No	N/A	N/A	N/A
19	Female	76	lung cancer with brain metastases	Yes	Lytic	No	N/A	N/A	N/A

Example of the Data Collection Sheet for lesion recording

Vita

Sara Fatula was born and raised in Pennsylvania. She graduated from Mercyhurst University in Erie, Pennsylvania in May of 2017 with a Bachelor of Science in Archaeology and Anthropology. Sara began her Master of Arts Degree in Anthropology at the University of Tennessee under Dr. Lee Meadows Jantz in 2017. While at UTK, she was a graduate teaching assistant for human osteology and was a member of the Forensic Anthropology Center's Facilities Documentation and Maintenance team. In addition to these duties, Sara has been an active participant in the annual short courses offered by the Forensic Anthropology Center, serving as both a lecturer and a team leader for surface recoveries. Sara will complete her Master of Arts Degree in May of 2020, before continuing in the Doctoral Program at the University of Tennessee under Dr. Dawnie Steadman in fall 2020.