The association between skeletal lesions and tuberculosis in a South African sample

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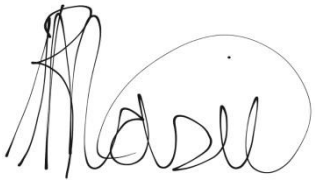
Declaration

I Rethabile Masiu declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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Abstract

Tuberculosis is an infectious disease. Skeletal TB is the extra-pulmonary manifestation of *Mycobacterium tuberculosis* infection and occurs in about 1-5% of all TB cases. The aim of this study was to assess the sensitivity and specificity of skeletal lesions to accurately diagnose TB in a pre-antibiotic South African skeletal sample. For this purpose, 23 skeletal lesions were assessed on 436 skeletons from the Raymond A. Dart Skeletal Collection. These skeletons were divided into three groups: individuals where TB was recorded as cause of death (n=177), individuals where other pulmonary diseases were recorded as cause of death (n=109) and individuals where a variety of diseases, excluding TB and other pulmonary diseases, were recorded as causes of death (n=150). Chi-squared and Fisher's Exact tests were used to assess differences between groups. The skeletal lesions' respective sensitivities and specificities were also calculated and compared to a similar study by Dangvard Pedersen *et al*. (2019a). Lesions on the ventral surface of thoracic and lumbar vertebral bodies were observed significantly more in TB cases than in controls. This lesion type was also found to be the most valuable indicator with a high sensitivity and 55% probability of a true TB diagnosis if observed. An association between skeletal lesions and TB could only be found for rib and vertebral lesions. Distinct differences between this study and the study by Dangvard Pedersen *et al*. (2019a) indicated that TB-related changes were likely to be observed in a South African skeletal sample even when individuals were not documented to have died of the disease.

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Chapter 1: Introduction

Infectious diseases are amongst the most significant selective factors affecting the evolution of human populations (Fumagalli *et al*., 2011; Hajdu *et al*., 2012; Karlsson *et al*., 2014). As a result of either death or reduced health, infections propel selection on genetic variants that affect disease opposition. Pathogens with long-standing evolutionary relationships with humans display the best evidence of selection (Karlsson *et al*., 2014). Tuberculosis, malaria, smallpox and leprosy have affiliations to humans that span between 12 000 to over 100 000 years and variation of the genome as it relates to these diseases has been attributed to the timing, strength and direction of the selection (Anderson and May, 1982; Karlsson *et al*., 2014). The knowledge of host-pathogen connections is essential in the advancement of new therapies in the combating of continued pathogen evolution as well as to manage immune-mediated diseases (Segal and Hill, 2003).

Tuberculosis (TB) is an infectious disease that is spread through the successful passage of the TB bacillus from one person to the next (Aufderheide and Rodriguez-Martin, 1998; Roberts and Buikstra, 2003; Schaaf, 2009). It is a chronic granulomatous disease caused by *Mycobacterium tuberculosis*. The route of infection is usually through the respiratory tract, resulting in the primary focus being situated within the lungs (Ortner, 2003; Goldman and Schafer, 2011; Jameson, 2018). TB is still highly relevant in the world today as it is a global health crisis that continues to affect around 10 million people annually worldwide (World Health Organization, 2017). Despite the fact that it is treatable by antibiotics, it remains one of the main causes of death in South Africa (Statistics South Africa, 2015). In the last three decades, the Human Immunodeficiency Virus (HIV) epidemic has been the

greatest contributory factor to the increase in TB cases in sub-Saharan Africa. HIV has been identified as a leading risk factor that increases the chance of progression from TB infection to active disease. Those who are infected with HIV are more likely to die from TB than any other cause (Markle *et al*., 2014; Ralston *et al*., 2018; Usatine *et al*., 2018).

When the body's defences fail to destroy the inhaled TB bacilli, they multiply logarithmically and accumulate at a primary focus. From the focus they can be transported through lymphatics to regional lymph nodes from which they can reach the bloodstream, with subsequent spread (Marx *et al*., 2013). It is at this point where there may be initiation of bone involvement.

Skeletal TB is an extra-pulmonary manifestation of a mycobacterial infection. Although it is not common, skeletal TB occurs in about 1 to 5% of all TB cases (Kastert and Uehlinger, 1964 cited by Ortner, 2003; Davidson and Horowitz 1970; Vigorita, 2008). However, there have also been other reports that indicate more frequent involvement (Jaff, 1972 as cited by Roberts and Buikstra, 2003; Kastert and Uehlinger, 1964 as cited by Ortner, 2003; Holloway *et al*., 2013). Due to the pattern of lymphatic and haematogenous spread of TB from pleural disease, one-third of skeletal TB cases involve the spine (Bennett *et al*., 2014). The spine often shows focal or generalized bone loss and in more severe cases the vertebral body's trabecular structure is demolished leading to kyphosis. As a result of TB infiltration of the intervertebral spaces, there can be destruction of articular surfaces resulting in deformity or deficit of the bones (Roberts and Buikstra, 2003). It is these signs of skeletal TB that have been used to trace the development and spread of this disease in past populations. Other skeletal manifestations of TB include the ribs, knee, hip

and elbow joints although these may be less specific (Ortner, 2003; Roberts and Buikstra 2003; Vigorita, 2008; Tuli, 2010).

Palaeopathology is the study of prehistoric or early historic disease (Grauer, 2011; Buikstra and Beck, 2017). Palaeopathologists explore singular and multifactorial causes for any consistent anatomical alterations and their ramifications in their attempt to understand human disease (Grauer, 2011). Palaeopathology is the centre of a multi-disciplinary juncture where fields such as medicine, dentistry, anthropology as well as palaeontology meet (Buikstra and Beck, 2017). The most direct approach is through the examination of the physical evidence of skeletal remains, which is possibly the one advantage that a palaeopathologist possesses (Waldron, 2009). The field thus almost entirely hinges on studying of skeletal remains, some of them with pathology, because they are commonly found at archaeological sites (Grauer, 2007; Waldron, 2009; Fernández, 2012). Supplementary forms of evidence include old documents and art (Roberts, 2017). More recently, modern advances have resulted in the introduction of new methods such as DNA and molecular analyses that allow palaeopathologists to detect ancient DNA of pathogenic microorganisms (Pinhasi and Mays, 2008).

It is an intrinsic feature in palaeopathology to use archaeological skeletal series to calculate statistics such as skeletal lesion frequencies and mean age at death and presume a direct relationship between these statistics and health status of that particular population (Wood *et al*., 1992; Wright and Yonder, 2003; Mudry and Pirsig, 2007). Wood *et al*. (1992), however, laid out three vital conceptual problems that confound the interpretation of such statistics. The concept of (1) demographic nonstationarity highlights the reality of past populations' migratory nature which has great effects on age-at-death distribution; making calculations on life expectancy or

mean age at death inherently flawed (Wood *et al*., 1992). Another problem that is of particular significance to the current study is (2) selective mortality that states that samples such as those from skeletal assemblages never have examples of all the individuals who were at risk of disease or death at a given age but only of those who did in reality die at that age (Wood *et al*., 1992). Finally, Wood *et al.* (1992) explained the problem of (3) hidden heterogeneity that denotes that the population from which a skeletal series is sourced comprises an unknown amalgamation of individuals who varied in their susceptibility to disease and death. Due to this, the interpretation of aggregate-level age-specific mortality rates in terms of individual risks of death becomes virtually impossible (Wood *et al*., 1992).

Bony lesions are very difficult to identify, and bone can only respond to injury and disease in a limited number of ways. Miller *et al.* (1996) conducted a study that tested the diagnostic accuracy in dry bone diagnoses during annual meetings of palaeopathology over a period of three years. The study found that there was a significantly higher level of accuracy for general category diagnoses such as whether remains exhibit an anomaly, trauma, inflammatory, circulatory, metabolic, neoplastic or neuro-mechanical alterations, in contrast to the lower level of accuracy when observers were asked to diagnose a specific disease such as TB. This emphasises the importance of obtaining a comparable diagnosis by different observers when assessing a specific lesion. It was suggested that including diagnostic criteria that incorporate subtle osteological changes that are often seen on dry bone but can also be seen on radiographs in a clinical setting can increase diagnostic accuracy. The study made it clear that careful attention to osteological changes and a thorough understanding of conditions that can affect bone may permit reasonable diagnostic inferences.

Wilbur *et al.* (2009) raised concerns regarding research and reports where insufficient care is taken in distinguishing between pathognomonic lesions and those that are 'consistent' with a diagnosis of TB. Much emphasis has been put on the importance of developing differential diagnoses when pathognomonic lesions of a specific disease are absent (Matos and Santos, 2006; Ortner, 2007; Wilbur *et al*., 2008; Barros *et al*., 2015). The specificity of lesions as indicating TB (or any other disease for that matter) remains problematic, due to the limited number of ways in which bone can react, there will be an overlap in the presentation of diseases in the skeleton (Ortner, 2003; Roberts and Buikstra, 2003). Tools have been designed to assist specialists with diagnosis of diseases in skeletal assemblages (Waldron, 2009). These tools include 'operational definitions' which aim to safeguard that all those who study skeletal remains will use the same criteria when diagnosing disease.

Recently, Dangvard Pedersen *et al.* (2019a) conducted a study to assess the probability of a skeletal lesion to be associated with TB, using a 20th century skeletal sample from the United States of America. Using a suite of 18 skeletal lesions, they followed a probabilistic approach to test the association of these lesions with TB, relative to that of a control sample of skeletons. The study found that the most diagnostic lesion, found on the ventral part of thoracic and lumbar vertebrae, provided a probability of only 53.3% of a true tuberculosis lesion.

The ability to correctly diagnose TB from skeletal remains is important in bioarchaeology and in modern skeletal cases that most often are of a forensic nature. Dangvard Pedersen *et al*. (2019a) adopted a model that used TB related lesions together with their diagnostic probability estimates and applied that to an archaeological sample. This process allowed for the generation of estimates of the

prevalence of TB among the people represented in that mortality sample. Such studies allow for palaeopathologists to inch closer towards approximating disease prevalence in once-living populations (Wood *et al*., 1992; Dangvard Pedersen *et al*., 2019b). Additionally, they assist in tracing trends in the skeletal involvement of TB in the pre- and post-antibiotic periods as demonstrated by two South African studies (Steyn *et al*., 2013; Steyn and Buskes, 2016). These studies suggested that contrary to expectations, skeletal lesions in cases of TB are not only increasing but have also changed in pattern in the last 100 years. TB is the most common natural cause of death in South Africa (Lomborg, 2015) and as such it is imperative that we understand its manifestations.

1.1 Purpose of the study

The aim of this study is to assess the sensitivity (the probability of having a particular skeletal lesion, given that the individual actually had the disease) and specificity (the probability of not having the skeletal lesion in people who did not have the disease) (Dangvard Pedersen *et al*., 2019a) of skeletal lesions to accurately diagnose TB in a pre-antibiotic South African skeletal sample. In order to achieve this, the objectives are:

1. To describe and identify lesions that are potentially indicative of TB. These will include lesions identified by Dangvard Pedersen *et al.* (2019a), lesions described by Hershkovitz *et al*. (2002), as well as other TB-associated lesions frequently noted in South African skeletons.

2. To assess the identified lesions in a large skeletal sample of known TB cases and score them as present or absent.

3. To assess these lesions in a skeletal sample of individuals who died of other pulmonary causes and score them as present or absent.

4. To assess these lesions in a large skeletal sample of individuals who died of non-pulmonary causes and score them as present of absent.

5. To use the data from these assessments to determine the sensitivity as well as the specificity of the identified lesions in the correct diagnosis of TB.

6. To assess the frequency of the candidate lesions across the three sample groups and to determine if their occurrence is statistically significant.

7. To compare the results of this study to those of Dangvard Pedersen *et al.* (2019a), in order to observe and quantify possible regional variations.

Chapter 2: Literature Review

2.1 Palaeopathology and the identification of diseases

The study of palaeopathology was necessitated by the desire of physicians and medically trained anthropologists to describe diseases observed in ancient remains of humans and animals (Grauer, 2018). As a discipline, palaeopathology provides basic evidence for the nature of health of ancestors and it does this by amalgamating biological and cultural information (Roberts and Manchester, 2007). In the process of understanding past disease, it is fundamental to also understand the manner in which disease affects the skeleton in the modern clinical sense because that knowledge can then be applied to an archaeological context (Roberts and Manchester, 2007). Rothschild and Martin (1993) suggest that palaeopathology should be anchored upon the assumption that osseous alterations have a reproducible record. This means that it is essential that researchers understand that bone changes caused by a given pathological process will be consistent with each case.

The interest of palaeopathologists is the evidence of disease and so a clear contrast between a typical health and a diseased state had to be outlined (Grauer, 2018). This enables palaeopathologists to distinguish between absence and presence of disease. The models of disease have evolved over time. Initially disease definitions were simplistic and singular causative agents were thought to be involved. However, with time disease descriptions became more nuanced and an understanding developed that there can be multiple forces that play a role, even beyond the causative pathogens themselves (Audy, 1967 cited by Grauer, 2018).

‘The osteological paradox’ as described by Wood *et al*. (1992) can never be understated. Selective mortality, particularly, is a bias that cannot be overcome by merely attaining a greater, more representative skeletal sample. A skeletal sample is an ensemble of individuals who died from diseases such as TB. This means that as a consequence of the disease, individuals’ risk of death is affected resulting in the frequency of the disease being larger among those who died than among the living (Wood *et al*., 1992). This bias remains regardless of sample size. It is incessantly important for palaeopathologists to keep this in mind when attempting to identify diseases particularly in a skeletal sample.

The application of clinical knowledge to ancient remains present challenges (Roberts and Manchester, 2007; Waldron, 2009) as it is an unsubstantiated assumption that bone alterations remain the same even after the diseases evolve over time. Bone changes that are found in ancient remains and associated with a disease may not have modern clinical descriptions (Ortner &Aufderheide 1991; Roberts and Manchester, 2007). Mummies are studied as part of palaeopathology and their soft tissue provides a wealth of information (Waldron, 2009; Grauer, 2011). It is, however, an unavoidable challenge that skeletal analyses cannot be utilised to assess soft tissue thereby limiting the assessment of an individual’s health status when taking into account the reality that skeletal remains are the most commonly discovered evidence (Rühli *et al.,* 2016). There is also very limited information that can be obtained from macroscopic assessments of remains and diagnosis depends almost entirely on the morphology and distribution of the changes found in the skeleton as a result of direct examination (Waldron, 2009). Differential diagnosis is essential in palaeopathology, however, many pathological conditions may cause the same bone reactions making it difficult to assess the true causation behind the

alterations (Goodman and Martin, 2002; Ortner, 2003; Khosla *et al*., 2007; Rühli *et al*., 2016). Palaeopathologists also grapple with pseudopathology caused by taphonomic processes that may be mistaken for true pathological changes (Piepenbrink, 1986; de Souza Barbosa *et al*., 2020). As a final point, the methods used in the field are mostly subjective and rely heavily on the experience of the observer (Bruzek, 2002; Witter-Backonfen *et al.,* 2008; Rühli *et al*., 2016). Interobserver repeatability is not something frequently considered in palaeopathology, and deserves more attention. Careful documentation through photography of lesions in publications may assist in this regard.

One of the most vexing difficulties that palaeopathologists continue to grapple with is the accurate identification of pathological conditions in ancient osteological remains (Byers and Roberts, 2003). When dealing with bioarchaeological remains observed, bony changes can be the result of any number of pathological processes or taphonomic alterations and it is the task of the palaeopathologist to use his or her best judgement to correctly identify the causative agent (Byers and Roberts, 2003). Palaeopathological skeletons can possess many 'diseased' features which have no direct correlation in clinical practice. It is therefore essential for those conducting research in skeletal palaeopathology to be cognisant of the diagnostic boundaries of clinical orthopaedic disease (Ortner and Aufderheide, 1991). Waldron (2009) also outlined ways in which to ensure conformity in order to enable for consistent diagnoses to be made in palaeopathology. He laid out operational definitions of skeletal disease that palaeopathologists could adopt in practice in the hopes to start building consistency and conformity in the field.

Despite its shortcomings, palaeopathology remains a field that is able to explore conditions over vast periods of time and much information can be obtained about the

health of past populations. It allows for the exploration of major alterations in disease patterns that could have been influenced by factors such as the environment and climate or significant changes in occupation or economic status and not by drug therapy (Roberts and Manchester, 2007). The field is ever-evolving due to the conditions of remains that palaeopathologists need to extrapolate information from (Fernández, 2012).

There are key changes in bone and teeth that are associated with pathology and these are used by palaeopathologists as tools that assist them in the identification of disease. Bone formation and bone resorption or a combination of the two are the key changes observed in bone (Roberts, 2017). In the teeth, disease can be characterised by loss or addition of dental tissue (Roberts, 2017). Scholars will then further determine whether the changes that are being observed are active or have healed and follow standard methods that have been outlined in order to make an informed diagnosis and interpretation (Ortner, 2003).

A good study in palaeopathology is usually one that addresses issues such as the feasibility and validity of particular diagnostic techniques. This line of research, like most branches of palaeopathology, is accompanied by some methodological difficulties. The lack of comparability between reports on abnormal specimens and inconsistent terminology are also major problems. This can be a result of varying observer knowledge regarding skeletal diseases and the ambiguity of consistent protocols for the nature of data contained within various reports (Ortner and Aufderheide, 1991). It is, therefore, of the utmost importance for scholars to improve comparability for both content as well as quality of their observations.

2.1.1 The real world implications of Palaeopathology

Fernández (2012) described palaeopathology as a crossroad nurtured by history, archaeology, anthropology as well as medicine that is uniquely placed to provide an integrated visualisation of disease and lifestyle habits. The exploration of past history of the appearance as well as distribution of infectious disease can be of significant theoretical importance and can result in new insights regarding the nature and transmission of diseases that were previously ambiguous (Rothschild and Martin, 1993). Palaeopathology derives its evidence from primary sources such as skeletons and mummified remains - this kind of evidence is the sole reliable indication that a once-living person suffered from a health problem (Roberts and Manchester, 2007; Buzic and Giuffra, 2020). While TB may not be directly indicated as the primary source for skeletal changes, the meticulous documentation of abnormal osseous features has the potential to identify patterns attributable to it (Roberts and Buikstra, 2003).

2.2 History and development of tuberculosis

According to Pezella (2019), the history of *Mycobacterium tuberculosis* (M. tuberculosis) can be traced back 3.3 million years. This means that the disease predates modern humans and has plagued humankind throughout known history (Daniel, 2006). There is evidence of TB living in symbiosis with mankind all over the world since time immemorial. It was a previously generally accepted hypothesis that *M. tuberculosis* was derived from the cattle pathogen, *M. bovis,* due to transmission of the pathogen from cattle to humans following animal husbandry. There has, however, been evidence in numerous regions of the world that suggests that human TB predates animal husbandry (El-Najjar *et al*., 1996; Baker *et al*., 2015). Although it has been suggested that the domestication of animals did perpetuate the

transmission of the disease (Aufderheide and Rodriguez-Martin, 1998; Köhler *et al*., 2014), there is detailed palaeopathological and biomolecular research that supports the existence of TB to before this period (McEvoy *et a*l., 2007; Russell, 2007).

The earliest physical evidence of TB in both humans and animals is provided by bone finds, mainly fragments of vertebrae, showing the gibbus typical of tuberculous Pott's disease (Herzorg, 1998). The earliest known case of TB was not osteological in nature but was found in a 17,000-year-old long-horned extinct bison found in a cave in the United States of America (USA) using mDNA and mycolic lipids (Rothschild *et al*., 2001). Human osteological cases have been found in Great Britain as early as 400 BC, in Hungary 5000 BC and Japan 300 BC (Mays and Taylor, 2003; Suzuki and Inoue, 2007; Tuli, 2010; Köhler *et al*., 2014). However, the oldest such example is a case of spinal TB which was found in human fossil bones that date back to 8000 BC (Ayvazian, 1993 as cited by Herzog, 1998 and Buzic and Giuffra, 2020). TB in Egypt can also be documented to more than 5000 years ago (Daniel, 2006). In recent years, palaeopathological evidence of TB has also been corroborated using biomolecular technologies (Donoghue *et al*., 1998; Haas *et al*., 2000; Spigelman *et a*l., 2002).

Scientific advancements have led the search for the origins of TB to Africa. Modern techniques of molecular genetics and the sequencing of the genome of several strains of *M. tuberculosis* allow a more precise estimation of the time of origin of mycobacteria. These techniques have been used to demonstrate that all the strains currently circulating can be traced back to six lineages which are all present in East Africa (Daniel, 2006; Pezella, 2019). Pezella (2019) wrote that TB diversity increased as a result of the rapid escalation of human populations as well as their migration out of East Africa (Gutierrez *et al*., 2005). Earlier, Wirth *et al*. (2008) used mycobacterial

tandem repeat sequences as genetic markers and successfully demonstrated that the age of the *M. tuberculosis* complex coincided with the expansion of human populations out of Africa 40,000 years ago. They also found that the two independent clades of the *M. tuberculosis* complex were likely both derived from pathogenic lineage, supporting the hypothesis of an original human host. This research built on the foundation previously laid down by Gutierrez *et al*. (2005) and later reiterated by the research conducted by Gagneux (2012).

There is also much evidence in North America of TB. Daniel's work (2000) describes the evidence of early TB in the Americas. This forms part of extensive collection of literature documenting evidence of TB on the various continents. There are almost 400 diagnosed cases of TB that have been found throughout North America, South America as well as Mesoamerica that were dated from 200 AD to 1599 AD (Roberts and Buikstra, 2003). A 1994 study by Salo *et al*. successfully amplified *M. tuberculosis* DNA found in South American remains of a woman who lived approximately 1000 years ago, whereas in Peru, the discovery of bony tuberculous lesions and Pott's disease in Peruvian mummies led to the DNA confirmation of *M. tuberculosis* (Daniel, 2006).

Much osteological evidence of TB also exists in Europe. Palaeopathological studies have a lengthy history in especially Britain, with substantial amounts of evidence of TB from an entire assemblage of cases coming from the agriculturally based rural and urban communities throughout the country's history (Roberts and Buikstra, 2003). Remains from 4000 BC found in the British Isles, for example, displayed new bone growth on ribs that was attributed to TB (Wacher and McWhirr, 1982). Evidence from Hungary indicates that the disease has been present there for at least

1300 years (Hutás, 1999Ba), whereas remains from around 8,300 BC with documented lesions were found in Denmark (Bennike, 1985).

Evidence for the existence of TB has similarly been unearthed in many other parts of the world. For example, there are written texts from 3300 and 2300 years ago that describe TB in India and China respectively (Daniel, 2006; Herzog, 1998). Roberts and Buikstra (2003), in their pursuit of early evidence of TB in the Old World, discuss Egyptian medical papyruses that date back to 1550 BC and describe cases of TB. Similar documents from Mesopotamia date as far back as 675 BC. Writings of Hippocrates, Aristotle as well as Pliny dating between 460 BC and 79 AD (Roberts and Buikstra, 2003) also refer to TB. In Greece around 460-370 BC, TB was known and the famous physician Hippocrates also described cases of TB and coined the term ‘consumption’ (Barbier and Wirth, 2017; Barberis *et al*., 2017). As a physician for the Roman Emperor, Clarissimus Glen documented the symptoms that were common in TB patients and suggested therapies that would alleviate them (Pease, 1940; Barberis *et al*., 2017). TB continued to spread far and wide in Europe following the fall of the Roman Empire (Roberts and Buikstra, 2003).

From the Middle Ages and throughout the Renaissance, more extensive pathological and anatomical descriptions of the disease including those by Francis Sylvius in 1679 emerged (Barberis *et al*., 2017). Skeletons with bony indicators of TB from this time period are also well represented but this may be as a result of larger bioarchaeological samples being available from this time period (Barberis *et al*., 2017). In 1720, English physician Benjamin Marten, displayed a great deal of epidemiological insight in his publication titled “A new theory of Consumption” (Doetsch, 1978). Medical practitioners, however, continued to struggle understanding TB and its aetiology. In parts of Northern Europe it was believed to be

a hereditary disease, whilst in other parts people recognized its infectious nature (Daniel, 2006). It was not until 1843 when French military surgeon Phillip Klencke demonstrated the transmissibility of the disease experimentally using rabbits (Daniel, 2006; Herzog, 1998) that great strides were made with regard to understanding the origin of the disease.

During the early industrialisation period, consumption was associated with harsh living conditions such as hard labour and poor socioeconomic surroundings. Such conditions eventually promoted the spread of the TB pathogen. (Barbier and Wirth, 2017). It is during these modern times that it became apparent that upsurges of the disease have always followed the development of new urban structures which drew increased numbers of people into confined dwellings (Herzog, 1998; Barbier and Wirth, 2017). During the height of the industrial revolution (1830s), challenging social conditions, destitute work environments, shoddy ventilated and overcrowded residential housing and primitive sanitation contributed to the spread of the disease resulting in TB becoming an immense general health issue (Barbier and Wirth, 2017; Barberis *et al*., 2017).

TB ran rampant in Europe and North America so much that by the end of the 19th century, 70 – 90% of the population had been infected (Pezella, 2019) which is why Koch's discovery of the TB pathogen in 1882 was such a monumental feat (Krause, 1984). Koch used sequential procedures in order to cultivate and identify the causative agents concerned using an animal experiment facilitated study (Gradmann, 2006). This advancement quickly gave rise to the race for techniques for diagnosis and treatment. The invention of the X-ray by Conrad Röntgen in 1895 was vital in the diagnosis of TB (Herzog, 1998). The thoracic radiograph quickly became a common diagnostic instrument and often exposed the tubercular lesions before

clinical symptoms had developed (Pezella, 2019). In 1908 a tuberculin sensitivity screening skin test was developed by Mendel and Mantoux (Pezella, 2019).

Effective therapy came with the development of the antibiotic streptomycin in 1944 in Britain (Daniel, 2006; Tuli, 2010; Pezella, 2019). This was a revolutionary antibiotic due to its combined ability to inflict maximum inhibition of the tubercle bacillus with relatively low toxicity. For the first time, the TB bacillus' vulnerabilities were exposed and this paved a way for a rapid succession of more anti-TB drugs (Herzog, 1998). Subsequent years proved, however, that it would take much more than medical interventions to defeat TB. Worldwide, a correlation has been seen between economic conditions and the number of new recorded cases (Herzog, 1998). This has been evident when looking at high TB numbers and low gross national products that Asian, African and Latin American countries report (Herzog, 1998). These challenges coupled with the rise in Human Immunodeficiency Virus (HIV) and the increase in multidrug resistance around the 1980s, have made TB increasingly challenging to overcome (Herzog, 1998; Pezzella, 2019).

Although TB is similar to other infectious diseases in that it has shown a pattern of surging in great epidemics and then receding later, the time scale during which TB displays this pattern challenged accepted explanations for epidemic cycles (Daniel, 2006). As a result of confounding factors such as poverty, resistance to drugs and HIV in the 1990s, TB has shown a massive resurgence and thus remains an enormous issue in global health (Roberts and Buikstra, 2003). It remains as relevant today as it did when Robert Koch first established *M. tuberculosis* as the aetiology and cause of the clinical disease in 1882 (Herzog, 1998; Daniel, 2006; Pezella, 2019). TB may have killed more people than any other microbial pathogen and continues to kill more than 5000 people per day (Roberts and Buikstra, 2003; Daniel,

2006). It has, therefore, remained important to understand TB's epidemiologic transition and thus the evolution of the disease.

2.3 Pathogenesis of TB

The disease progression of TB, or for that matter any disease, is largely dependent on factors such as the size of the inoculum (the size of the infective dose), the virulence of the organism, as well as the resistance of the host (Ortner, 2003). The infective dose includes the number of bacilli per infectious particle and the number of particles inhaled at any one period of exposure (Balasubramanian *et al*., 1994). The clinical manifestations of tuberculosis represent a complex interaction between the causative organism, *M. tuberculosis*, and the human host's immune response (Schluger, 2005). It is widely accepted that TB develops almost exclusively by one of two pathogenic pathways (Schlunger, 2005; Rajaram *et al.,* 2014; Wilson *et al.*, 2015). The first pathogenic pathway suggests that TB arises primarily via the endogenous reactivation pathway which means that the latent primary focus is reactivated. The latent primary focus forms as a result of an initial infection in the lung that healed and covered with scar tissue but with a necrotic area remaining where tubercle bacilli survive (Roberts and Buikstra, 2003; Schlunger, 2005; Tuli, 2010). The second pathogenic pathway is exogenous and happens when the infectious dose inhaled is large or there is prolonged exposure such that a lung infection is initiated and develops into clinical disease (Aufderheide and Rodriguez-Martin, 1998; Roberts and Buikstra, 2003; Schlunger, 2005).

Transmission occurs when droplet nuclei *M. tuberculosis* expectorated via coughing from someone with active TB, are inhaled. Upon entry into the airways, signals that trigger pro- and anti-inflammatory responses are initiated (Flynn *et al*., 2011). Mycobacteria enter alveolar macrophages and can result in the initiation of one of

three processes. The mycobacteria can be (1) eliminated completely, (2) contained in a granuloma for a prolonged period (forever, or with a change in immune status can result in breakdown of the granuloma and reactivation of a latent infection), or (3) immediately progress to active disease with clinical illness (Schluger, 2005).

The granuloma and its formation is considered the principal feature of the preliminary host immune response to tuberculosis. Following the engulfment of mycobacteria, macrophages give off signals to other cells to attract them to the site of infection, leading to the formation of a granuloma (Keane *et al*., 1997; Schluger, 2005; Flynn *et al*., 2011). A granuloma is an organised assemblage of immune cells that forms as a result of chronic antigenic stimulation and is the classic pathological feature of tuberculosis. It functions as both a niche in which the bacilli can grow and an immunologic microenvironment in which the cells with anti-mycobacterial functions interact to control and prevent dissemination of the infection (Flynn *et al*., 2011).

If the primary complex fails to heal, the lung lesion progresses so that tubercle bacilli are disseminated through the bloodstream to other organs and tissues, including the skeleton (Ortner, 2003). A secondary complex is then initiated, often with a large area of necrosis with an accompanying granulomatous inflammatory reaction. A cascade of regional spread ensues, causing progressive pulmonary infection involving bordering structures (Aufderheide and Rodriguez-Martin, 1998). Other immune cells such as the dendritic cells in the lung are also infected with the bacteria, and as a result of their migratory nature, they end up in peripheral locations such as the thoracic lymph nodes which initiates the involvement of the lymphatic system in the progression of the disease (Wolf *et al*., 2008; Flynn *et al*., 2011).

During the exudative phase of the disease fluid filters out of the circulatory system into locations where there is inflammation. The proliferative phase is characterised

by an increase in the quantity of tissue cells (Cardona, 2015). It is during the lymphohematogenous dissemination that the bacilli show preferential spread to kidneys, meningeal areas, the epiphyses of long bones as well as vertebral bodies (Marx *et al*., 2013).

2.4 Skeletal manifestations of TB

Mycobacterial skeletal infections can occur through various different mechanisms, especially given a susceptible host (Hogan *et al*., 2017). The skeletal system is a prime target for the TB bacilli and they can be transported via the bloodstream as well as the lymphatic system and become lodged in any bone of the body (Roberts and Buikstra, 2003). Once the tubercle bacilli begin circulating in the bloodstream, they locate in areas of high circulation and metabolic rate such as areas where there is haematopoetic bone marrow (Roberts and Buikstra, 2003; Hogan *et al*., 2017). Other routes of infection include neighbouring and lymphatic spread (Hogan *et al*., 2017). Once the infection infiltrates the bone, the tissue reacts leaving physical, macroscopic injury. The distribution and appearance of tuberculous lesions reflect the anatomical features of vascularization as well as joint structures related to a bone which allows for possible recognition of this disease (Aufderheide and Rodriguez-Martin, 1998).

According to Kastert and Uehlinger's 1923 study (cited in Ortner 2003), skeletal tuberculosis comprises only 3% of the total and 30% of extra pulmonary tuberculosis. Out of 560 cases of haematogenous TB autopsied over a 10-year period, 115 (21%) had involvement of the skeleton. A more recent study (Holloway *et al*., 2013) showed a much higher percentage of 42% of individuals with skeletal involvement whilst others state percentages as low as 2% (Waldron, 2009). It is important here to distinguish between clinical/living patients with TB, and those who

have died of the disease as they may not have the same frequency of bone involvement.

It is important to point out that the morphology of tuberculous lesions on dry bone is not unambiguous and its manifestations in most cases can overlap with signs of other bone infections (Ortner 2003; Roberts and Buikstra, 2003). There are, however, some general characteristics that may be of diagnostic value. Studies where diagnostic value of lesions can be better understood, considering the distribution and specificity of the lesions on the skeleton, can be of value (Ortner 2003; Dangvard Pedersen *et al*., 2019a). This can be achieved by, amongst other things, assessing the lesions in terms of how they present during the development of the disease. Skeletal changes that occur during the exudative phase differ from those that occur during the proliferative phase (Ortner, 2003).

According to Roberts and Buikstra (2003), TB can be characterized by both bone destruction and new bone formation. As many prior generations have been exposed to TB, the immune response and survival of more recent individuals are stronger and thus the visibility of bone reactions may increase (Roberts and Buikstra, 2003). This is in essence also what was found by Steyn *et al.* (2013) and Steyn and Buskes (2016), who suggested that bone involvement may be increasing in post-antibiotic South Africa as people may live longer with their disease and thus there is more time to develop skeletal lesions.

2.4.1 Spinal manifestation of TB

Different types and regions of bones react in a variety of ways to the intrusion of TB bacilli. The spine has been acknowledged as a chief location for TB lesion involvement which may be due to the large amount of trabecular bone it possesses. High oxygen tension as well as the intricate arterial blood supply of vertebrae makes

for a favourable environment for tubercle bacilli to thrive (Aufderheide and Rodriguez-Martin, 2008; Leonard and Blumberg, 2017). It is widely stated that the characteristic changes to the spine, as a result of TB, usually initiate from the intervertebral space as a result of narrowing and perforation (Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003; Vigorita, 2008). Spreading of the infection up and down the spine can also originate from the primary tubercle via the anterior longitudinal ligament (Waldron, 2009). Cherry *et al.* (2013) state that lesions in the spine can also be initiated by two other mechanisms - the first being direct extension through the lymphatics from caseous paravertebral lymph nodes and the second being direct local haematogeneous or lymphatic extension from a neighbouring bone. The caseous (cheesy-like) appearance is as a result of the antigenic proteins from the TB bacilli triggering the host's immune system cells causing death of the macrophages as well as necrosis of the tissues in the affected area (Aufdeheide and Rodriguez-Martin, 2008). TB bacilli in arterial blood are deposited just above the cartilaginous plate facing the intervertebral space and disseminated along the plane of spongy and trabecular bone (Aufderheide and Rodriguez-Martin, 1998). It is these processes that can result in pressure necrosis and the formation of cold abscesses which will progressively destroy bone. The infiltration of bacilli and liquid necrotic tissue initiates the infectious process which leads to the narrowing of the intervertebral space. Due to this, it is not uncommon to find cold abscesses on adjacent vertebral bodies along the spine, a characteristic discovery in a potential TB case. This is a marked exudative reaction that, unlike normal abscesses, is not the result of an acute pyogenic infection (Tuli, 2010).

A cold abscess forms due to liquefied tissue and bone that is destroyed via caseous necrosis that accumulates around a site of infection (Hogan *et al*., 2019). Trabecular

bone of the infected vertebral body may be damaged due to expansion of the original abscess within the vertebral body, the formation of multiple abscesses in the area or the lack of blood supply to the bone (Aufderheide and Rodriguez-Martin, 1998). Insufficient vertebral trabecular bone due to progressive damage on the body can result in its collapse, bending anteriorly along the spine (kyphosis). With kyphosis, in addition to the dominion of the disc space, vertebral bodies adjacent to the disc space show areas of destruction and wedging (Tuli, 2010). Sclerotic bone destruction in this area can often be continuous with the para-spinal abscesses on the vertebrae and extend into the spinal canal. Calcification of these abscesses is often the result, a conditioned known as TB spondylitis or Pott's disease (Vigorita, 2008). Ortner (2003) also described cavities (cloacae) on the ventral surfaces of thoracic and lumbar vertebral bodies that are usually circular in shape and have smooth edges. These cloacae are characteristic of chronic infection such as in a case of TB. Reactive bone formation may also be seen in that area as a possible result of a paravertebral abscess (Ortner, 2003).

Kotze and Erasmus (2006) conducted a study looking at Magnetic Resonance Imaging (MRI) findings in proven TB spondylitis. Their study identified all patients who had vertebral biopsies for suspected TB spondylitis with positive histological confirmation, as well as a MRI of the affected vertebrae at a medical facility in Bloemfontein between July 2002 and November 2005. They found that thoracic vertebrae were the epicentre of spinal involvement and that 69% of the patients included in the study had at least one vertebral destruction. Typically, more than one vertebra is affected. A recent study by Mann *et al.* (2018) found that there was a substantial burden of spinal TB cases in recent years in the Western Cape with a high proportion of severe presentation, particularly among children.

2.4.2 The manifestations of TB on long bones

In long bones, the bacilli gravitate to red marrow especially at the metaphyses and epiphyses in adults and children (Roberts and Buikstra, 2003). The lesions usually start as an area of endarteritis in the metaphysis, where there is an abundance of trabecular bone with good blood supply. This is thus usually the initial site of infection (Cherry *et al*., 2013; Leonard and Blumberg, 2017). The head and neck of the femur derive their blood supply from several vascular locations namely the lateral epiphyseal, metaphyseal as well as ligamentum teres vessels. As a result, the ample anastomoses in the area provide the perfect environment for multiple infection sites in the area (Aufderheide and Rodriguez-Martin, 1998). In the event of a metaphyseal cancellous bone abscess, the joint will eventually be involved because the hip joint capsule spreads out to the metaphyseal region (Aufderheide and Rodriguez-Martin, 1998).

During the more exudative phase of the tuberculous disease process, once the bacilli have permeated the marrow, there is devitalization of cancellous bone. This results in the formation of centrally located sequestrate of cancellous bone which are also known as caries (Ortner 2003). As a result, there is localized destruction as well as cavitation in the bone which leads to perifocal or broad osteoporosis. In extreme cases, most or the entire head of a femur can be damaged which can cause dislocation (Ortner, 2003). The distal femur and proximal tibia can also show bone changes following an infection initiating from the knee (Ortner, 2003). Linear cortical and undermining damage of the local portion the articular surface may result, often leaving pitting on the articular surfaces of these long bones.

Although uncommon and almost entirely observed in children, the manifestation of TB on the shaft of long bones has been documented (Aufderheide and Rodriguez-

Martin, 1998; Ortner, 2003). There are documented cases where the involvement of the epicondyles of the humerus were observed (Ortner, 2003). Other long bones that have been observed to show TB involvement that does not stem from the joints include the femur, tibia, fibula and the radius (Tuli, 2010).

2.4.3 The effect of TB on joints

Several studies have suggested that joints are the second most affected sites of disease in skeletal TB (Ortner, 2003; Hogan *et al*., 2019) and 90% of tuberculous skeletal lesions involve a joint (Aufderheide and Rodriguez-Martin, 1998). The destruction of articular surfaces of joints is maximized when the tuberculous process starts from within the bone, resulting in the formation of cancellous sequestra and cavitation. After crossing the epiphysis, bacilli can drain into the joint space, resulting in tuberculous arthritis, or form a sinus tract after being released from the destroyed bone (Leonard and Blumberg, 2017). If the infection progresses without effective treatment, as was the case in the pre-antibiotic era, abscesses surrounding the joint or bone may develop and rupture leaving a sinus tract that has long been associated with musculoskeletal TB (Leonard and Blumberg, 2017).

Disintegration of the femur neck secondary to tuberculous damage of the cancellous bone can injure neck vessels, compounding bone necrosis to the changes experienced by the femoral head. The penetration of the joint capsule by the tuberculous infection inside the joint results in cold abscesses. The process is so damaging that joint structures may display complete, fibrous obliteration at the joint (Aufderheide and Rodriguez-Martin, 1998). When the infection begins from within the bone, the articular surface and epiphysis can be destroyed, frequently resulting in the involvement of opposing joint surfaces (Roberts and Buikstra, 2003). The same principles apply to most joints. As a result of an infection in the acetabulum, drainage

channels or additional bony proliferation from extensions of a psoas abscess may lead to the pathological involvement of the lateral body of the ilium (Ortner, 2003).

The knee joint is also a possible area of TB involvement and is believed to be the result of an infection originating in the synovial joint where the capsule attaches to the femur and tibia (Ortner, 2003). Ortner cites an incidence of 15%. The elbow has been found to be affected in about 2% of all cases of skeletal TB. Tuberculous disease of the elbow can be initiated from the olecranon, the distal humerus, the synovia or even the proximal radius (Tuli, 2010). According to Ortner (2003), involvement of the hip and the sacroiliac joint is common.

Davidson and Horowitz (1970) proposed that in skeletal TB, the joints that are predominantly involved are the ones most prone to trauma. They stated that the spine accounted for 50% of patient presentation, the hip for 15% and the knee for 15% (Davidson and Horowitz, 1970). Vigorita (2008) attributed the involvement of these joints to TB targeting the joint space and causing erosion of the bone.

2.4.4 The effect of TB on flat bones

Even though they are flat bones, the ribs and the sternum also have haematopoetic marrow (Ortner, 2003) making them possible sites for haematogeneous bacilli infiltration. Bone formation on ribs has also been attributed to pulmonary TB infection that has disseminated directly from the lung tissue through the pleura to the visceral surfaces of the ribs (Roberts and Buikstra, 2003; Matos and Santos, 2006). Matos and Santos (2006) also found that bilateral proliferative lesions were found frequently in cases of pulmonary TB and that the lesions were likely to be found along the shaft on the visceral surface of ribs.

A study by Eyler *et al.* (1996) proposed that the enlargement of ribs due to bone formation is more likely to happen as a result of TB than any other pulmonary disease. This study reiterated features that Roberts *et al.* (1994) already described earlier, when they found that new periosteal bone formation was found in 61% of individuals who were recorded as having died from TB. The lowest percentage of rib involvement found in individuals who had TB was 60%, while other studies reported that almost all of the individuals who had TB presented with rib lesions (Kelley and Micozzi, 1984; Roberts *et al*., 1994; Santos, 2000). A South African study found that 23.8% of all individuals included in their study had rib lesions (Steyn and Buskes, 2016).

Although there has been a general consensus that rib lesions form as a result of an inflammatory response, the root cause has long been the subject of much debate (Roberts *et al*.,1994). Regardless of the results of a 1974 study (cited by Roberts *et al*.,1994) that proved that the tubercle bacillus can pass from the original focus in the lungs through the pleura to the ribs, the aetiology is still not fully understood.

Cranial involvement in TB is not widely reported and is said to be a rare occurrence (Ortner, 2003). However, Hershkovitz *et al.* (2002) described a phenomenon in the endocranial plate which they termed “serpens endocrania symmetrica”, and demonstrated how it has value as a diagnostic tool for the recognition of intrathoracic disease such as TB. Steyn and Buskes (2016) also found a relatively high incidence of cranial lesions (7%) whilst studying skeletal manifestations of TB in South African samples. These lesions were mostly endocranial, in the cranial fossae, and were thought to be associated with TB meningitis.

2.4.5 Palaeopathological methodology

It is important, when conducting a scholarly investigation, to adopt techniques that improve on error prone approaches (Dangvard Pedersen *et al*., 2019a). A study by Pálfi *et al.* (2012) has laid down the ground work for looking at bone alterations that are less 'classical' to identify possible TB cases on skeletal remains. The study looked at three cases that represented extraordinary manifestations that were uncommon in palaeopathological literature but could be attributed to osteo-articular TB. The cases provided solid evidence that lesions like endocranial symptoms, vertebral hypervasculirization, rib periostitis and periostitis of long bones do have diagnostic value in the identification of TB (Pálfi *et al*., 2012). Posa *et al.* (2013) conducted a study on a Hungarian skeletal series and found that atypical/early stage skeletal TB lesions, such as those found in the Pálfi *et al.* (2012) study, occur significantly more frequently than the 'classical alterations' such as Pott's disease. This is further confirmation that there are less common lesions that warrant further exploration into their diagnostic value as this can result in a more quantifiable assessment of disease prevalence in a sample. It is customary practice in palaeopathology to focus on the typical manifestations of diseases, however, these studies make a case for some adjustments to be made to the methodology employed when dealing with bone alterations than may be considered less conventional of a specific disease.

2.4.6 Sensitivity and specificity of skeletal lesions

Sensitivity is the probability of having a particular lesion given that the individual actually had a specific disease, whilst specificity of a lesion is the probability of not having the skeletal lesion in people who did not have the disease (Dangvard Pedersen *et al.,* 2019a). In the pursuit to estimate disease prevalence in skeletal

samples or trying to establish what happened in once-living populations, it is important to use different approaches that involve a wide array of skeletal lesions, each with an estimated sensitivity and specificity (Boldsen, 2005; Waldron, 2009; Dangvard Pedersen *et al.*, 2019a).

Palaeopathological diagnoses are inherently probabilistic in nature and the estimations derived from studies that look at these specificities of lesions are essential to the field (Waldron, 2009). Dangvard Pedersen *et al.* (2019a) sought to build on previous studies that conducted quantifiable probabilistic assessments and thus they compiled a suite of lesions that would result in sensitivity and specificity estimates of TB-associated lesions. These were identified on the basis of previously published clinical studies of radiographically-obtained images and autopsies, archaeological skeletons, palaeopathology reference works, and their team's research experience examining modern as well archaeological specimens from various eras (Dangvard Pedersen *et al*., 2019a; Dangvard Pedersen *et al*., 2019b). Dangvard Pedersen *et al*. (2019a) investigated a total of 18 lesions that could possibly be associated with TB. These included lesions on the ribs, vertebrae and various joints. They identified two different types of rib lesions, of which low nodules along the costal groove provided highest frequencies but both in cases and controls. Most indicative of TB were bony proliferative lesions or bean-shaped lytic lesions. If present there is a probability of a 33.7% of a true TB diagnosis, if absent there is a1.3% probability of TB.

As expected, the vertebrae were the most diagnostic. They considered three different manifestations of TB on vertebrae including cavitation on the cranial and caudal surfaces of lumbar and thoracic vertebrae. Finding these bony changes on

the vertebrae meant that there was an 8.8% probability of a true TB diagnosis in their absence there was a 5% probability of TB.

Feature “VEN1” is located on the ventral portion of vertebral bodies, and if large holes with a roughly circular shape and rounded edges that pierce the cortical bone are visible, that was considered to be a positive reaction. The manifestation of these cavities on vertebrae suggested a 20.8% chance of a true TB diagnosis and their absence meant that there was 3.8% probability of TB.

“VEN2” is also located on the ventral part of lumbar and thoracic vertebrae but is characterised by bone proliferation. Vertebrae displaying these bony changes mean that there was a 53.3% likelihood of a true TB diagnosis (the highest of the whole suite) and their absence meant there was a 4.1% likelihood of TB.

The “BOD” candidate indicator is located on the lateral body of the ilium between the lower gluteal line and upper acetabular margin. The presence of large pits in this location was considered a positive indication of TB, and if present meant that there was a 33.2% chance of a true TB diagnosis; the absence thereof meant a 5.1% chance of TB.

Lesions found on the articular surface of the acetabulum were given the designation “ACE1” and were characterised by areas of pitting and cavities. The existence of these features meant that there was a 5.5% probability of a true TB diagnosis and lack thereof meant that there was a 0.4% probability of TB.

The “ACE2” indicator was located in the acetabular fossa and was identified as a possible site for osteolytic lesions that are usually surrounded by coarse trabeculae with minimal reactive bone formation. Positive identification of these bone changes meant that there was a 42% chance of a true TB diagnosis and a negative outcome meant that there was a 6.5% chance of TB.

Indicator “PF1” was located at the head of the femur and was characterised by pitting and cavities. The presence of these changes meant there was a 3.4% chance of a positive TB diagnosis and their absence meant that there was a 1.3% chance of TB.

The greater trochanter of the femur was the identified location for indicator “PF2”. Pitting in this location meant that there was 1.3% probability of a true TB diagnosis and the lack of pitting meant that there was a 3.1% probability of TB.

The “ILI” site was identified as a common site of irregular perforations exposing underlying trabecular bone on the iliac articular surface. The presence of these changes was evidence of a 29.6% likelihood of a positive TB diagnosis and their absence meant a 5.8% likelihood of TB.

“DF” was located on the distal femur and was characterised by pitting and cavities on the articular surface. The presence of this characteristic signifies that there is a 2.1% probability of a true TB diagnosis and its absence means that there is 0.9% probability of TB.

“PT1” is the indicator observed on the articular surface of the proximal tibia. Here, when cavities and pits were seen, it meant there was a 0.4% chance of a positive TB diagnosis and when they were not seen this meant that there was a 0.1% chance of TB.

Indicator “PT2” is located on the intercondylar space of the proximal tibia. Pitting and cavities here meant that there was a 2.1% probability of a positive TB diagnosis and their absence suggested that there was a 0.1% probability of TB.

“DH1” was scored at the condylar area of the distal humerus. Moderate to extensive pitting in this area marked a positive TB reaction which meant that there was 3.4 %

likelihood of a True TB diagnosis. However, the lack of a reaction meant that there was a 0.1% probability of TB.

The articular surface of the distal humerus was given the designation “DH2”. Pitting and deep cavities are a characteristic response to TB in this area (Ortner, 2003) and this reaction meant that there was a 2.1% chance of a positive TB diagnosis when no reaction means that there was a 0.1% chance of TB.

“PR” is located at the radial tuberosity of the proximal radius. This area is a frequent location of TB bone involvement (Ortner, 2003). The presence of pitting and cavities means that there was a 2.1% chance of a positive TB diagnosis and the lack of these changes meant that there was a 1.3% chance of TB.

Finally, “PU” was the indicator located on the olecranon process of the proximal ulna. This area is not a common location of TB involvement but has been known to react in some cases (Ortner, 2003). Pitting in this area surrounded by smooth borders was scored as a positive reaction and meant that there was a 9.2% probability of a true TB diagnosis. The absence of the pitting indicated that there was only a 1.7% probability of TB.

The study found that of their 18-lesion-suite, a total of only 6 lesions (RIB2, VEN2, BOD, ACE2, ILI, PU) were significantly associated with a TB diagnosis. In order for these to meet the criteria, they each had to show a significant association with recorded TB diagnoses; a significant relation to 9 or more other indicators as well as a significant sensitivity (>0.05) (Dangvard Pedersen *et al*., 2019a). This study was able to provide quantitative estimates of the association between specific pathological indicators on dry bone and TB, with the most pathognomonic lesion being VEN2. This demonstrates that none of lesions can definitively indicate TB, and

that a differential diagnosis will be needed even when these bony changes are evident.

The Dangvard Pedersen *et al.* (2019a) study selected a research sample and a control sample from two completely different skeletal collections. The TB sample was selected from a collection that was largely comprised of individuals from low socio-economic background and have a high prevalence of people who had died of TB. The control sample was selected from a collection with a very low likelihood of people who had TB, especially during the period of their deaths in the United States when TB was not common (Centers for Disease Control and Prevention, 2008). In a South African sample this approach would be challenging to replicate given the state of TB in the country. South Africa is one of the three countries in the world with the highest burden of TB, TB/HIV and Multi-Drug-Resistant TB according to the latest global tuberculosis report (World Health Organization, 2019).

Chapter 3: Materials and Methods

3.1 Materials

The Raymond A Dart Collection of Human Skeletons, School of Anatomical Sciences, University of the Witwatersrand, houses at least 300 skeletons of individuals who have died of TB before 1950 (Steyn, 2013; Steyn and Buskes, 2016) which is about the time period when streptomycin became commonly available (Pfuetze *et al*., 1955). This is one of the largest collections of pre-antibiotic TB-associated skeletons in the world and is unique in the sense that it is derived mostly from mining contexts around the Witwatersrand (Dayal *et al*., 2009; Steyn *et al.,* 2013). Due to the acquisition procedures, the collection comprises of mostly unclaimed bodies from hospitals around the Gauteng province. It can therefore be surmised that the individuals would have a low socio-economic background. The individuals are known and information regarding sex, age, and cause of death is available (Dayal *et al.,* 2009). All skeletons used in this study were drawn from the Dart Collection.

A total of 177 known cases of individuals who died from TB before 1950 were selected as the research sample. For the purpose of this study sex, ancestry and age were not considered to be mitigating factors and were therefore not taken into account. In order for a skeleton to be included in this study, it had to possess most of the skeletal elements that have been identified as locations of potential TB indicators. A systematic assessment of the skeletons was done to determine which would be included in the study. The skeletons which did not meet these criteria were excluded.

Another 109 cases of individuals who were not recorded as having died from TB, but from other pulmonary diseases (e.g., pneumonia, emphysema, silicosis), were also included in order to be able to better assess the specificity of the lesions (Fig. 3.1).

A control sample of 150 individuals were also selected from the Dart Collection. These were carefully selected to include a variety of causes of death that do not include TB and only included non-pulmonary diseases, non-infectious diseases and non-cancers (Fig. 3.2). The most common causes of death for these individuals were cardiac failure, nephritis, myocarditis, aortic incompetence, cerebral thrombosis, uraemia and ectopic pregnancy.

Many of the skeletons that were initially selected could not be included in the study as a result of missing skeletal elements. Due to the fact that this was a blind study, excluding skeletons meant there was no indication as to which sample groups the excluded skeletons belonged to. As a result, the list of identified skeletons for all of the sample groups was continuously updated. The large number of skeletons that could not be included resulted in limited sample sizes across most of the groups (TB, pulmonary and control).

Permission to study the remains was obtained from the Collections Committee of the School of Anatomical Sciences. An ethics waiver (W-CJ-140604-1) for studies on the remains in the collections was granted (see Appendix 1).

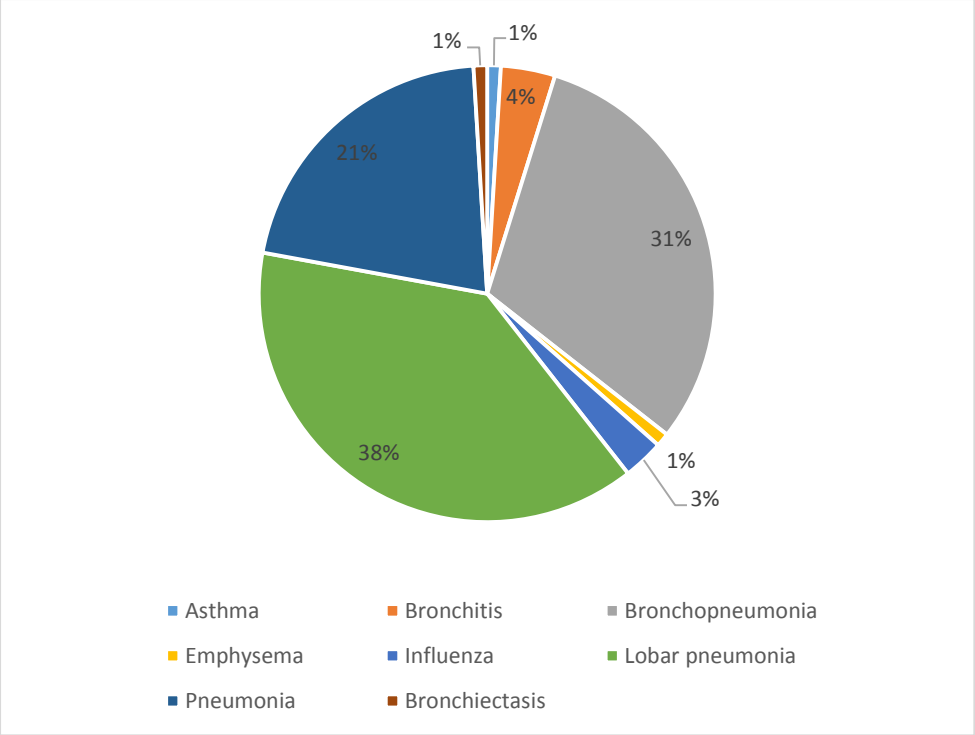


Figure 3.1: Causes of death for pulmonary disease group

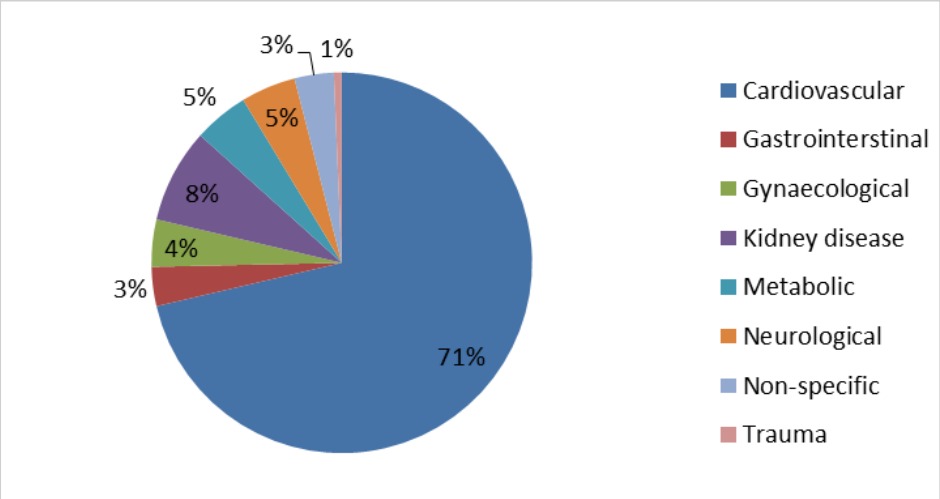


Figure 3.2: Causes of death in the control group

3.2 Methods

Each skeleton was macroscopically assessed for signs of TB using a set of skeletal lesions that are associated with TB (Table 3.1). The primary investigator did not

know from which of the three groups (test sample, pulmonary sample or control sample) a skeleton originated. The majority of the skeletal indicators came from the study by Dangvard Pederson *et a*l. (2018). However, lesions of the crania were added as they were seen in previous South African (Steyn *et al*., 2013; Steyn and Buskes, 2016) and international studies (Hershkovitz *et al*., 2002).

The Dangvard Pedersen *et al.* (2019a) study included a list 18 potential TB indicators. For this study five more potential indicators were identified. All of these additional indicators were located in the endocranial space. The first three were situated in the anterior, middle and posterior cranial fossae respectively, and comprised of lytic lesions seen in the respective fossa (Steyn and Buskes 2016). A study by Hershkovitz *et al.* (2002) is the basis for the inclusion of the last two lesions in, respectively, the inferior and superior elements of the endocranium. These authors found characteristic changes on the superficial surfaces of the endocranium of skeletons from the Hamann-Todd collection. They observed discoloured bone areas exhibiting disruptions of the endocranial surface, giving an appearance of a maze-like appearance with marginal pitting (Hershkovitz *et al.,* 2002). Table 3.1 provides detailed descriptions relating to the location as well as the morphological characteristics of each candidate indicator in the 23-lesion-suite.

A catalogue made available to the researcher by the curator of the Dart collection was used to select skeletons that met the criteria of the current study. The catalogue was used to identify individuals who had died from TB, other pulmonary diseases and those who had died of other causes (controls). Every skeleton is assigned a unique number (Accession number) and this number was randomly added to a list. The list contained an amalgamation of all three sample groups. The list was then used to retrieve skeletons that were going to be scored. As only the number was

used, the researcher had no indication of the sample group each skeleton represented which allowed for the current study to be completed as a blind study.

Each of these potential TB indicators was scored as absent or present. Cut-off points were defined for each of the indicators such that mild progression of disease could be recorded as defined in Table 3.1. Each skeleton was laid out and placed in the anatomical position and each indicator was located and scored on a data collection sheet (See Appendix 2).

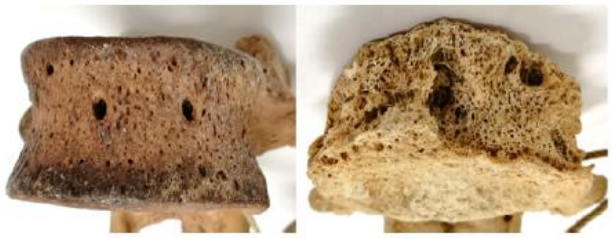
In order to assess how consistently features can be scored, a random sample of six TB cases, five from the control sample and six of the cases of other pulmonary disease-related skeletons were re-scored by the main and another observer (the main supervisor).

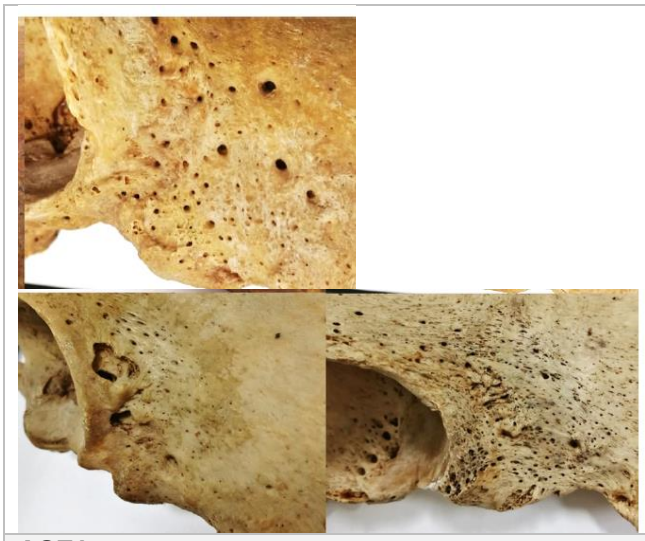
3.2.1 Bony indicators

Table 3.1 provides detailed definitions indicating how each indicator should be scored as well images showing changes frequently associated with TB. For the cranial lesions, images and skeletal changes described in other studies were used (Hershkovitz *et al*., 2002; Steyn and Buskes, 2016), while the remainder were taken from Dangvard Pederson et al., 2019a. This table also provides descriptions of the cut-off parameters.

Table 3.1: Scoring criteria for all 23 indicators

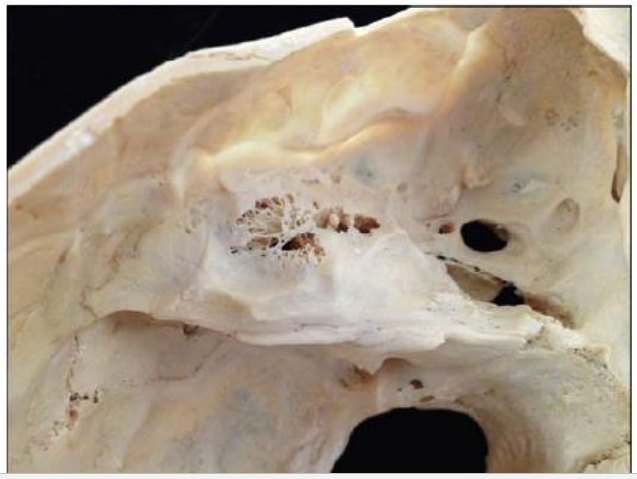
Indicator	Scoring	Description
RIB1 	Location:	Visceral surface of ribs
	0:	No bone change
	1:	Low nodules and rough texture >5cm
RIB2	Location:	Visceral surface of all ribs examined.

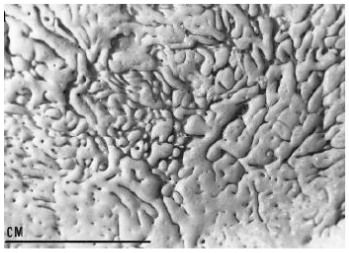
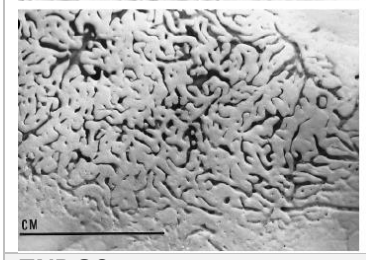
	0:	No bone changes present
	1:	Two or more pieces of ribs larger than 5cm have: • Superficial bone proliferation covering more than 5cm • One or more bean-shaped lytic lesions measuring more than 5mm in diameter
VER 	Location:	Cranial and caudal surfaces of all thoracic and lumbar vertebral bodies
	0:	No bone changes
	1:	Clustered pits or deep cavities in one area measuring more than 10mm
VEN1 	Location:	Ventral part of all thoracic and lumbar vertebral bodies
	0:	No bone changes
	1:	Three or more large pits with a roughly circular shape and rounded edges each measuring at least 3mm in diameter
VEN2 	Location:	Ventral part of all thoracic and lumbar vertebral bodies
	0:	No bone changes
	1:	Two or more thoracic or lumbar vertebrae have a proliferation of bone with a woven appearance that covers 50% of the bone's surface
BOD	Location:	Lateral body of ilium between the lower gluteal line and upper acetabular margin

	0:	No bone changes
	1:	• Three or more large pits with a roughly circular shape and rounded edges each measuring more than 3mm • proliferation of bone with a woven structure is present on at least 50% of the bone's surface
ACE1 	Location:	Articular surface of the acetabulum.
	0:	No bone changes
	1:	Clustered pitting or large cavities occur on the articular surface in two or more areas, each measuring more than 3 mm, or in one area measuring more than 10 mm
ACE2 	Location:	Acetabular fossa of acetabulum
	0:	No bone changes
	1:	Two or more deep cavities in the acetabular fossa, each measuring more than 3 mm or covering more than 50% of the area, with borders having the appearance of a woven structure with dense trabeculae
PF1 	Location:	Head of the femur
	0:	No bone changes
	1:	Clustered pits or cavities occur on the femoral head in two or more areas, each measuring more than 3 mm, or in one area measuring more than 10 mm
PF2	Location:	Greater trochanter of the proximal femur

	0:	No bone changes
	1:	Clustered pits or large cavities on the greater trochanter occur in two or more areas, each measuring more than 3 mm, or in one area measuring more than 10 mm
ILI 	Location:	Iliac auricular surfaces
	0:	No bone changes
	1:	Clustered pits or large cavities in two or more areas, each measuring more than 3 mm, or in one area measuring more than 10 mm
DF 	Location:	Articular surface of the distal femur
	0:	No bone changes
	1:	Clustered pits or cavities are present in two or more areas, each measuring more than 3 mm, or in one area measuring more than 10 mm
PT1 	Location:	Articular surface of the proximal tibia
	0:	No bone changes
	1:	Clustered pits or large cavities occur on the articular surface in two or more areas, each measuring more than 3mm, or one area measuring more

		than 10mm
PT2 	Location:	Intercondylar area of the proximal tibia
	0:	No bone changes
	1:	Three or more large pits are present in the intercondylar area, each measuring more than 3 mm or there is a proliferation of bone with a woven appearance that has spread across at least 50 % of intercondylar area. Bone destruction and proliferation can occur in the same specimen
DH1 	Location:	Condylar area of the distal humerus.
	0:	No bone changes
	1:	Clustered pits covering more than 1 cm^2 are present on the posterior condylar portion of the humerus on the lateral, medial, or both sides
DH2 	Location:	Articular surface of the distal humerus.
	0:	No bone changes
	1:	Clustered pits or large cavities occur in two or more areas on the articular surface, each measuring more than 3 mm, or in one area measuring more than 10mm
PR 	Location:	Radial tuberosity of the proximal radius
	0:	No bone changes
	1:	Clustered pits or large cavities are present in two or more areas, each measuring more than 3

		mm, or in one area exceeding 10mm
PU 	Location:	Olecranon process of proximal ulna.
	0:	No bone changes
	1:	Clustered pits or large cavities are present on the olecranon process in two or more areas, each measuring more than 3 mm, or in one area measuring more than 10 mm
CRA1	Location:	Anterior cranial fossa
	0:	No bone changes
	1:	Clustered pits or large cavities are present in the anterior fossa, similar to what is shown under CRA2
CRA2 	Location:	Middle cranial fossa
	0:	No bone changes
	1:	Clustered pits or large cavities are present in the middle fossa
CRA3	Location:	Posterior cranial fossa
	0:	No bone changes
	1:	Clustered pits or large cavities are present in the posterior fossa, similar to what is shown under CRA2
ENDO1	Location:	Inferior endocranium
	0:	No bone changes
	1:	Discoloured bone with variant serpentine branching excavations measuring more than 1

 		cm
ENDO2	Location:	Superior endocranium
	0:	No bone changes
	1:	Discoloured bone with variant serpentine branching excavations, similar to what is shown under ENDO1

For all the indicators that had to be measured before they could be scored, a sliding calliper was used. For most of the indicators that were scored positive, photos were taken using a Canon camera model EOS 750D.

Figures 3.3 to 3.20 further elucidate the bony lesions associated with each of the scored features. These images are all from skeletons scored in the current study and were taken by the primary investigator.

Figure 3.3: **RIB1** Low nodules on visceral surface of ribs

Figure 3.4: **RIB2** Thin proliferation on visceral surface of ribs (left); loosely adherent proliferation (right)

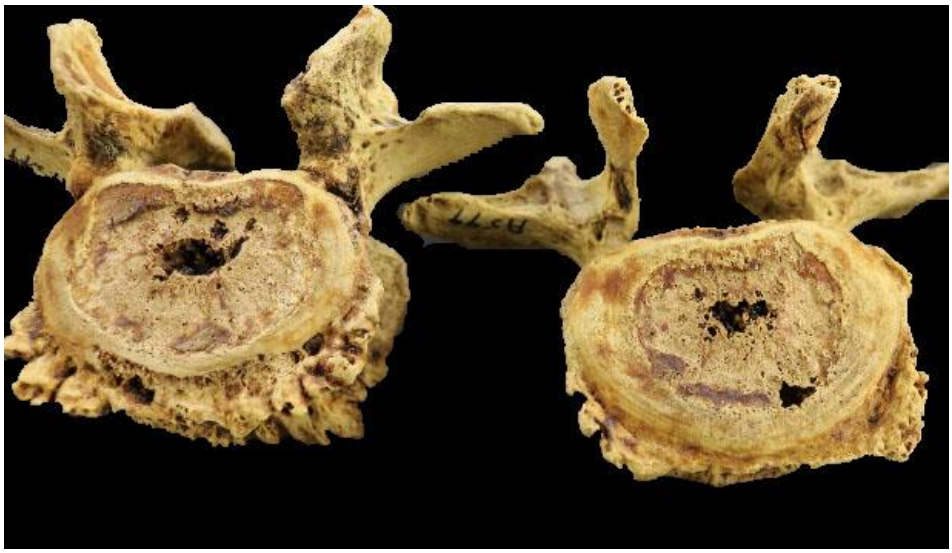


Figure 3.5: **VER** Deep cavities on lumbar vertebrae

Figure 3.6: **VEN1** Large pits on multiple vertebrae (left); medium rounded pits (right)

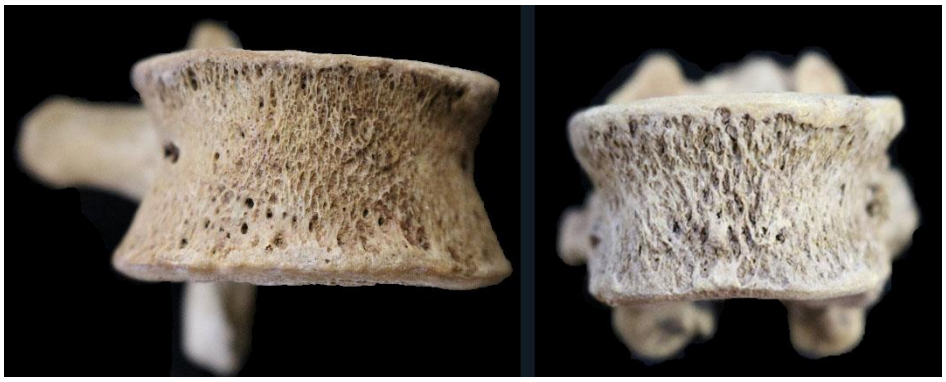


Figure 3.7: **VEN2** Slight bone proliferation (left); moderate proliferation (right)

Figure 3.8: **BOD** Bone proliferation (left); large rounded pits (right)

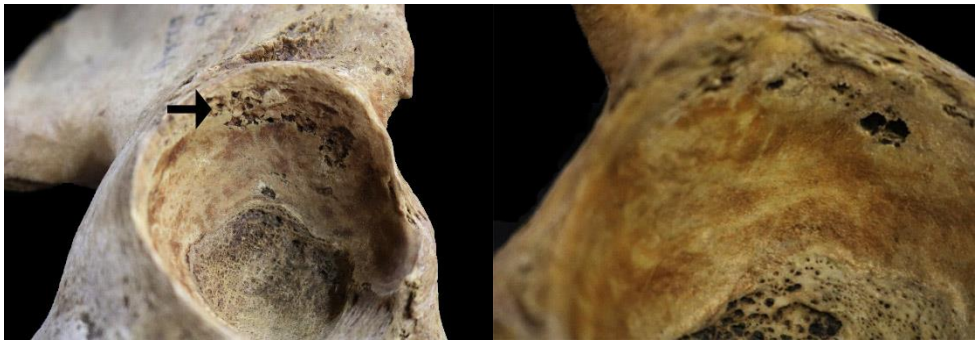


Figure 3.9: **ACE1** Small clustered pits (left); large deep cavities (right)

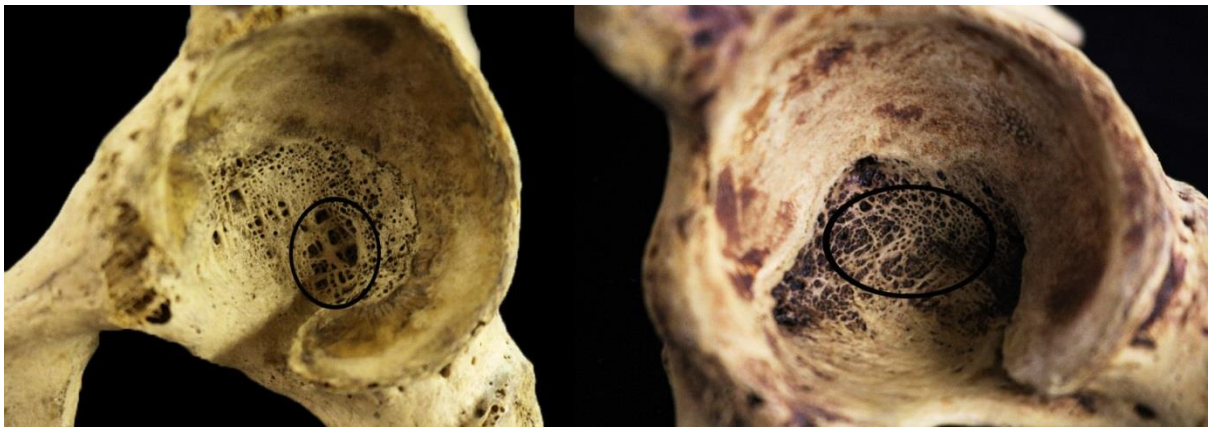


Figure 3.10: ACE2 Deep cavities with borders having woven appearance (left); cavities covering more than 50% of the acetabular fossa

Figure 3.11: **PF2** cavities on the greater trochanter

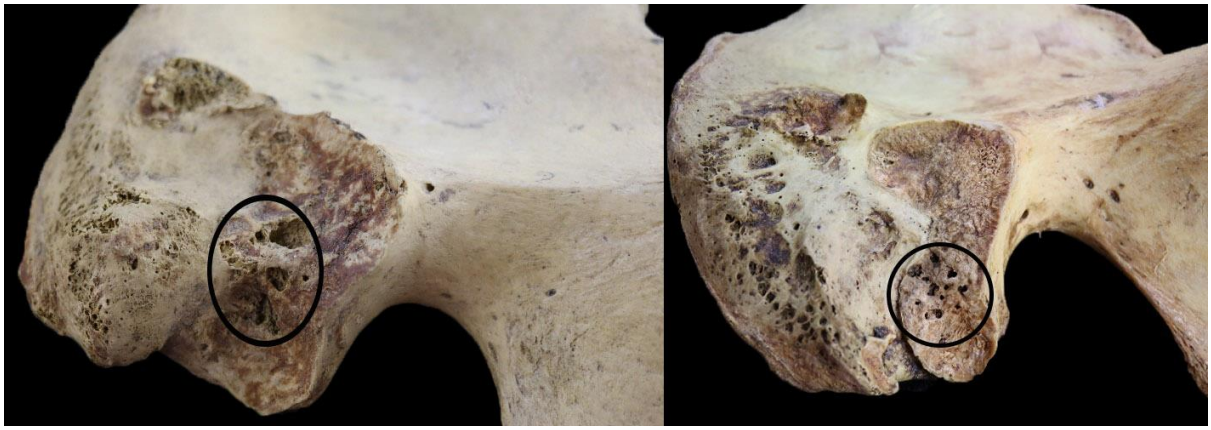


Figure 3.12: **ILI** large cavities (left); clustered pits (right)

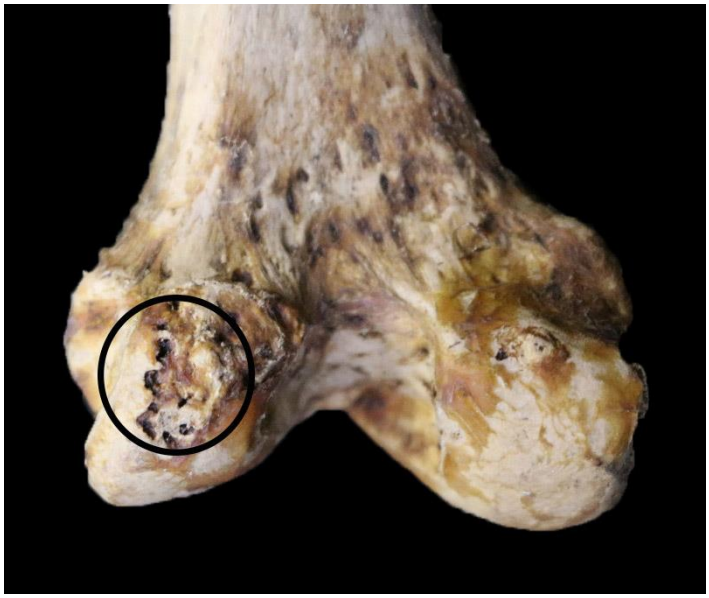


Figure 3.13: **DF** cavities on the posterior surface of the distal femur (encircled)

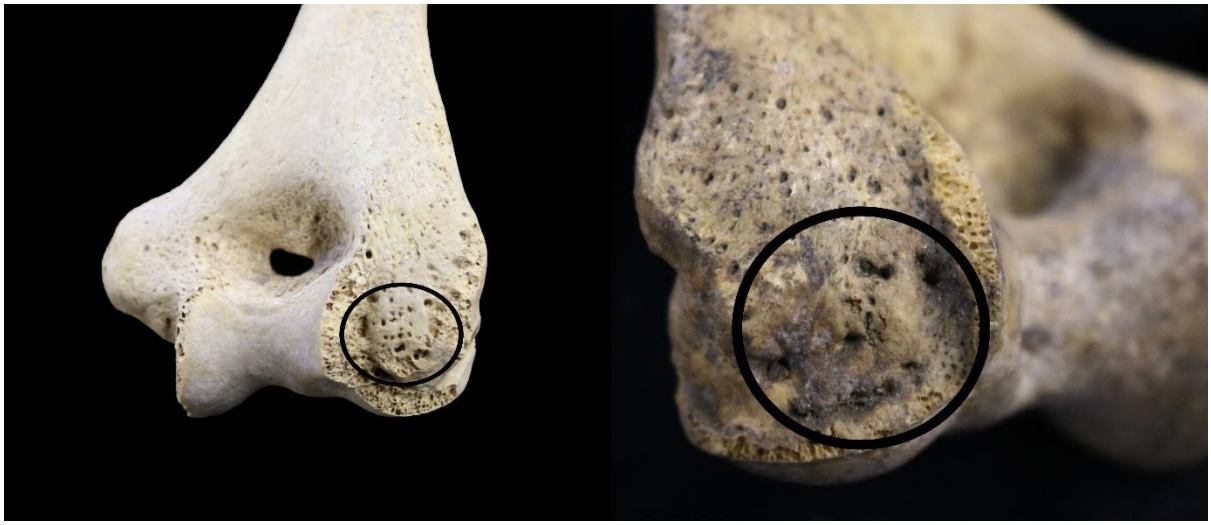


Figure 3.14: **DH1** Small clustered pits (left); large pits (right)

Figure 3.15: **DH2** medium clustered pits (left); small clustered pits (right)

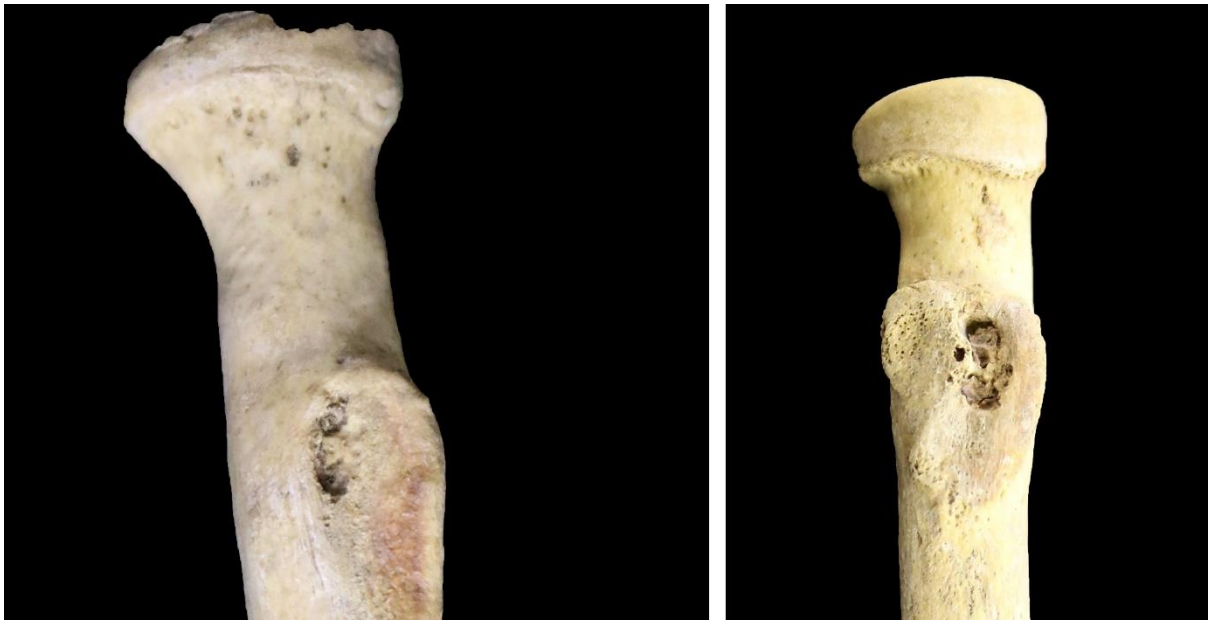


Figure 3.16: **PR** medium shallow cavities (left); large deep cavities (right)

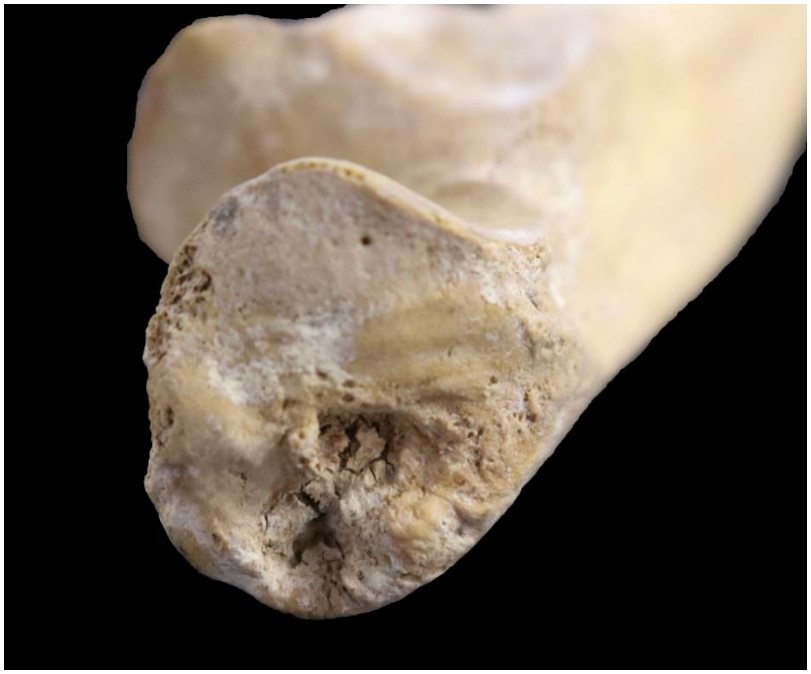


Figure 3.17: **PU** large shallow cavities

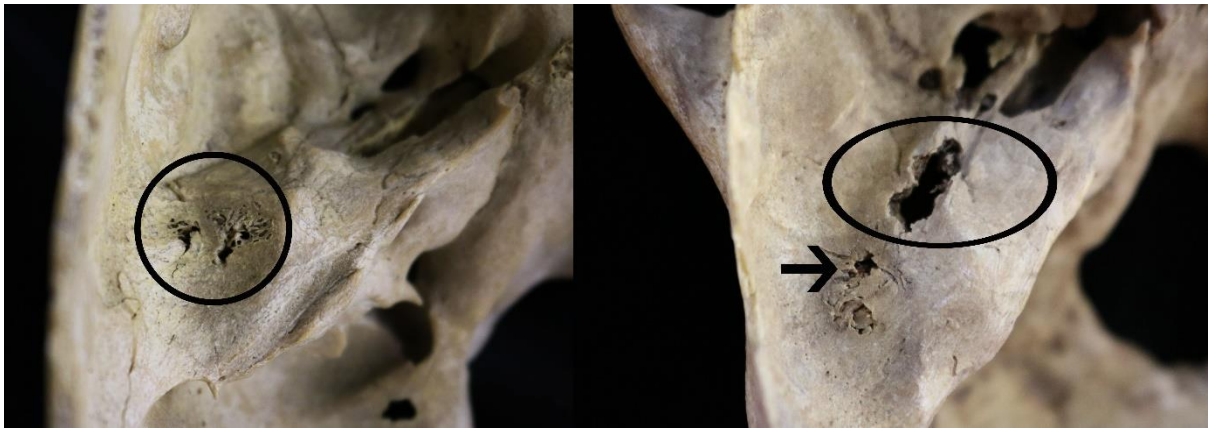


Figure 3.18: **CRA1, CRA2 & CRA3** are all represented by the above images, as they have a similar appearance and only vary in location. Small and medium pits (left) in the middle cranial fossa; large deep cavity (encircled right), arrow pointing at smaller pits

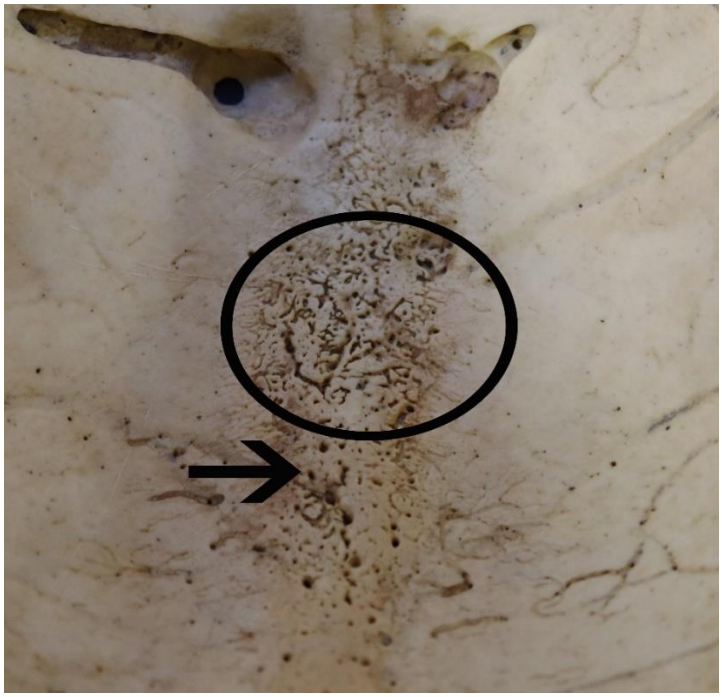


Figure 3.19: **ENDO1** branching excavations (encircled) on the surface of the endocranium; arrow pointing at marginal pitting

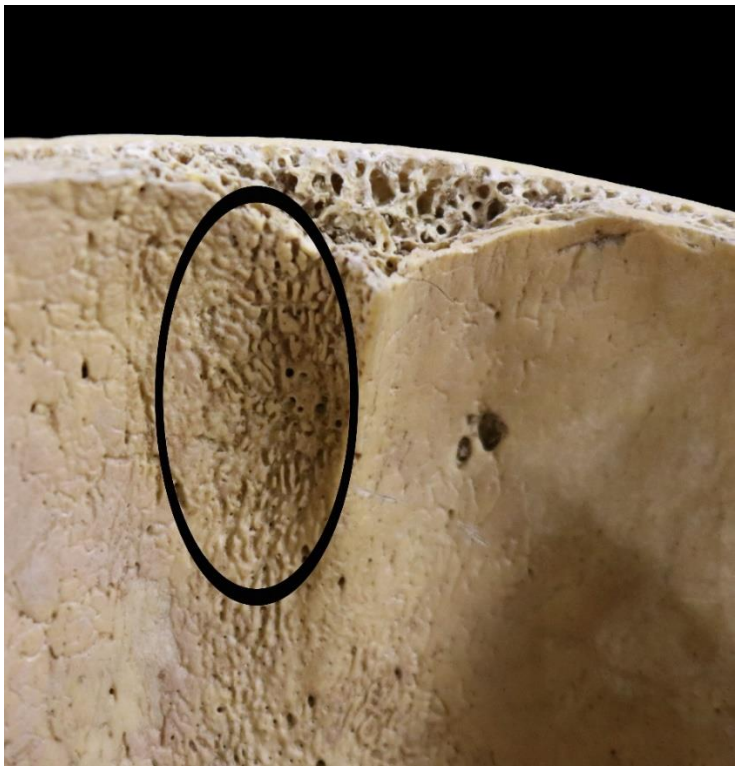


Figure 3.20: **ENDO2** Superficial branching excavations on the endocranium (encircled)

3.3 Statistical analysis

All data management as well as statistical analyses were completed using SAS Enterprise Guide 6.1 and Microsoft Excel 2013. Firstly, analyses of candidate indicators and their relationship to TB was conducted. Fisher's Exact and Chi-squared tests were used to evaluate the differences in frequencies of lesions in the three different samples (TB sample, control sample, pulmonary disease sample). The frequencies were then similarly compared to those found in the Dangvard Pedersen *et al.* (2019a) study. For the current study p-levels were considered to be significant at $p<0.05$.

True positives and true negatives, as well as false positives and false negatives, were then determined, from which the sensitivity and specificity of each lesion were calculated (Table 3.2). Individuals who have skeletal lesions of interest and also have a confirmed TB diagnosis, according to the records, were considered to be true positives (TP). The individuals that lack the lesions and are recorded as being free of TB, were considered to be true negatives (TN). Individuals who have skeletal lesions of interest but have no confirmed TB diagnosis were considered to be false positives (FP). The individuals that lack the lesions but are recorded to have a confirmed TB diagnosis were considered to be false negatives (FN). Indicator specificities as well as sensitivities were calculated according to Table 3.2.

Table 3.2: Sensitivity and specificity calculations (Dangvard Pedersen *et al*., 2019a)

Diagnosis		
Lesion	Affected	Unaffected
Present	True positive (TP)	False positive (FP)
Absent	False negative	True negative (TN)

	(FN)	
Sensitivity =TP/TP+FN **Specificity = TN/TN+FP**		

The sensitivity + specificity column will show a measure of ‘gain in certainty’ from each of the indicators. It is a measure that quantifies the change in the clinical estimate of an individual’s chances of having a disease that occurs as a result of diagnostic testing. When the value exceeds one, the gain in certainty is maximised (Koepsell and Connell, 1985).

Based on these calculations, an assessment could be made as to which lesions are helpful in diagnosing TB, and how much they could be relied upon. The results of this study were then compared to those of Dangvard Pedersen *et al.* (2019a) to make general assumptions on regional differences.

Inter- and intra-rater reliability were assessed using a Cohen’s Kappa test. For this purpose, the following formula was used:

k=(Pr(a) - Pr€)/(1 - Pr€)

Kappa (k) counts for the probability of agreement based on chance. Pr(a) = relative observed agreement, Pr€ = probability of agreement based on chance. Cohen suggested that Kappa results be interpreted as follows (McHugh, 2012):

Values ≤ 0 indicate no agreement

Values between 0.01 - 0.20 indicate none to slight agreement

Values between 0.21 – 0.40 indicate fair agreement

Values between 0.41 – 0.60 indicate moderate agreement

Values between 0.61 – 0.80 indicate substantial agreement

Values between 0.80 – 1.00 indicate almost perfect agreement

Concerns have been raised over the use of Cohen's Kappa particularly in health research (McHugh, 2012). For this reason percentage agreement was also calculated by dividing the sum of the observed agreement by the sum of the row marginal. The sum of the number of times observers agreed there were no lesions present and the number of times they disagreed on whether a lesion was present or not makes up the first row. The second row sums up the number of disagreements in the presence and absence of lesions with the number of times both observers identified the presence of lesions.

Chapter 4: Results

4.1 Introduction

In this study, the remains of 436 individuals were studied. They were divided into three groups: those who died of TB (n=177), those who died of other pulmonary diseases (n= 150) and those who died of diseases not related to lung disease (n=109). Most of the skeletons were complete, however, occasionally there were areas of the skeletons that were missing or damaged and could thus not be scored. Only 25 skeletons had at least one skeletal element missing. In this section, the frequency of lesions in each of the three groups will be reported for each candidate indicator according to its location.

4.2 Occurrence of selected indicators in the three groups

In this section, the number of skeletal elements showing the selected osteological features are indicated for each of the three groups. Figures 4.1 to 4.8 display how all the 23 lesions are distributed across all the sample groups. For purposes of these graphs, the skeletal indicators were grouped according to the bony element on which they occur. For example, both rib indicators are shown in Fig. 4.1, the three vertebral indicators in Fig. 4.2, etc. These graphs display the frequencies of lesions using counts and not percentages.

Figure 4.1 shows the frequencies of the two different kinds of rib lesions in all three samples. As can be seen, RIB1, the nodules, were rare and occurred about equally in the TB and control groups. RIB2, the additional bone formation on the visceral surfaces of the ribs, was more common than RIB1 across all sample groups. RIB 2 also occurred more in the TB sample where 12.1 % were affected compared to the control and pulmonary groups where 5.5% and 4.6% were affected.

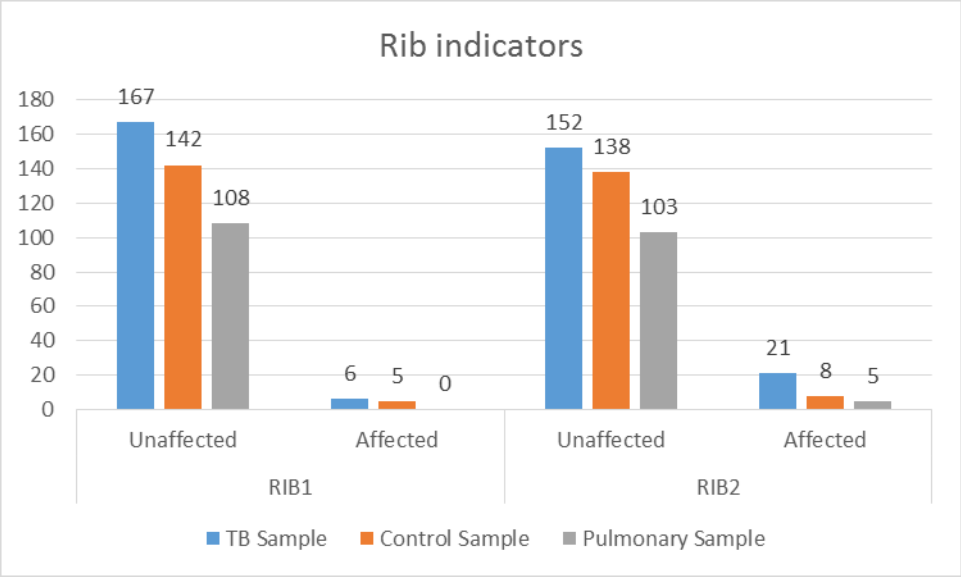


Figure 4.1: The number (N) of affected and unaffected ribs

In Figure 4.2, the distribution of vertebral lesions across the three samples is shown. Vertebral lesions were the most common of all the indicators. Across all the three vertebral indicators, the TB sample was most often affected. VER, clustered pits on the superior and inferior surfaces, had an almost equal count in all three groups, however its percentage was highest in the pulmonary group (9.5%). The most commonly occurring indicator was VEN2 (bone proliferation on the ventral surfaces of the vertebral bodies). This indicator occurred most frequently in the pulmonary group (58.1%) although it was also found in large numbers in the TB (55.0%) and control (32.1%) groups.

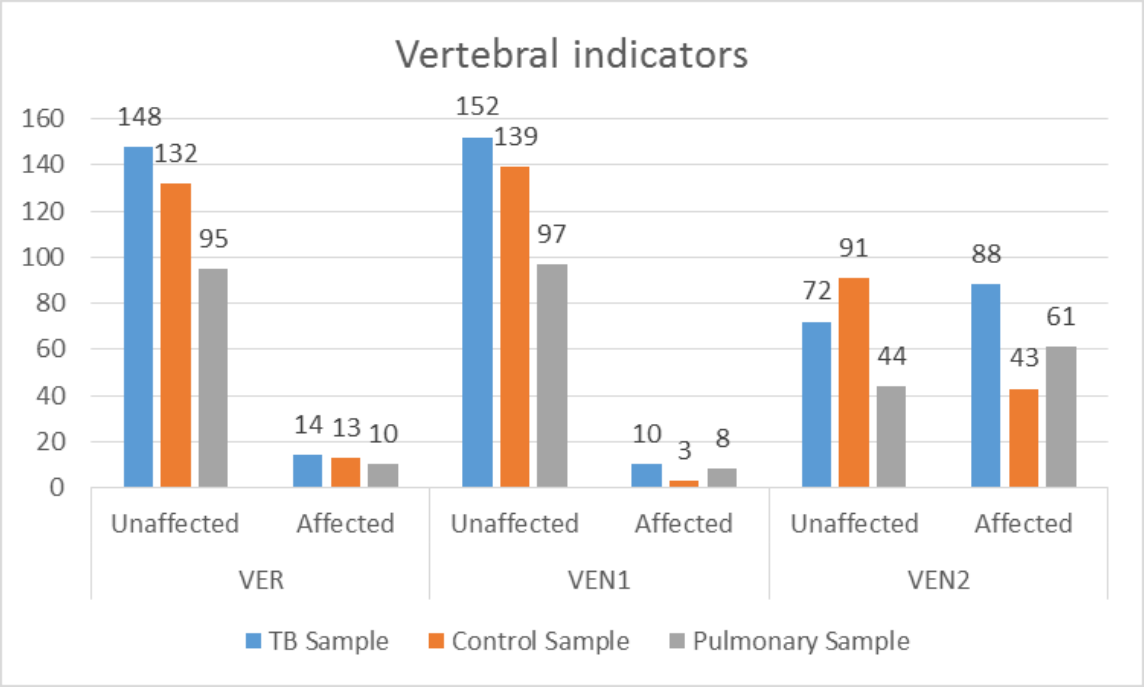


Figure 4.2: Occurrence of vertebral indicators

Fig. 4.3 shows all changes occurring on the os coxa. Acetabular changes were similarly seen in all three groups, with ACE2 (cavities in the acetabular fossa) being the most frequent. It was also by far the most commonly seen in the TB group (27.9%), followed by the pulmonary group (23.7%) and then finally the control group (20.3%). Indicator BOD, was most frequent in the pulmonary group, ACE1 was most frequent in the TB group and ILI was more frequent in the control group.

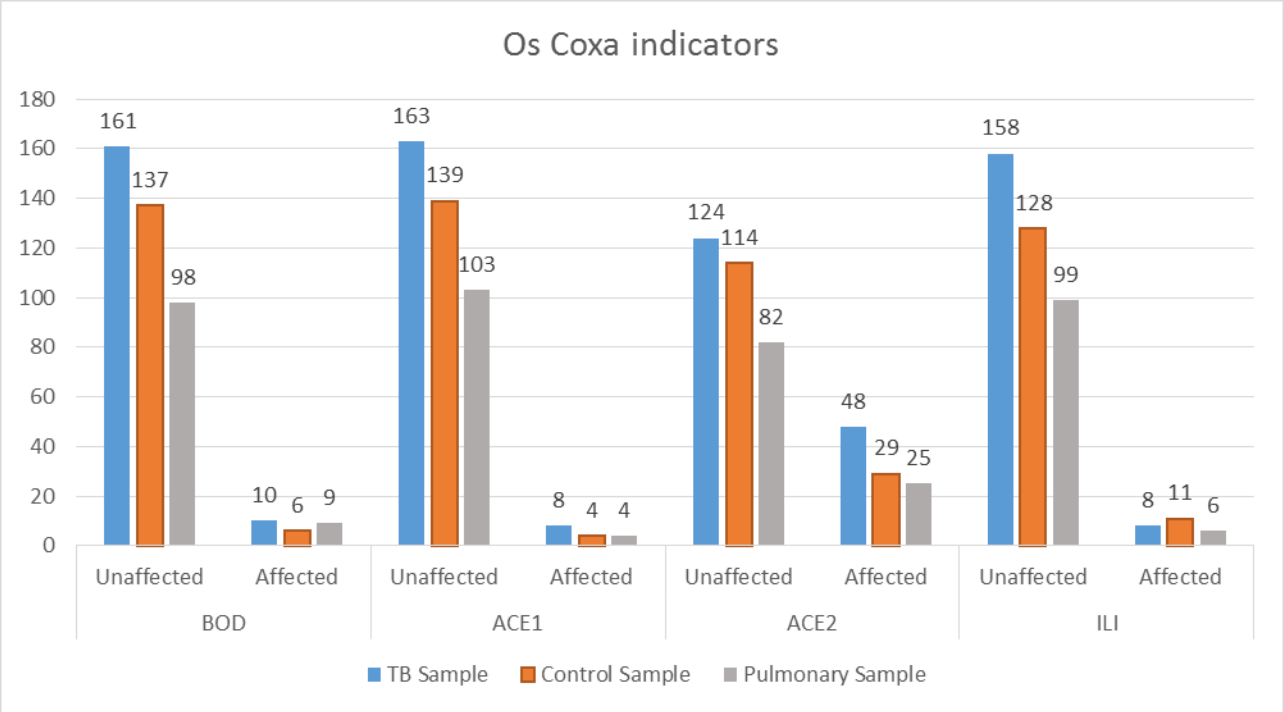


Figure 4.3: Affected and unaffected os coxa (N) indicators

Changes occurring in the femur are shown in Fig. 4.4. As can be seen, bone changes in this region were rare and a total of only eight lesions were found across all three sample groups (Figure 4.4). When they occur, they seem to be more likely in the TB group, but their overall presence is low. PF1 (clustered pits and cavities on the femoral head) were only observed once across all groups and was found in the TB sample. Clustered pits and large cavities on the greater trochanter (PF2) were mostly found in the TB assemblage. The distal femur having clustered pits and large cavities were observed only once and this was in the TB group.

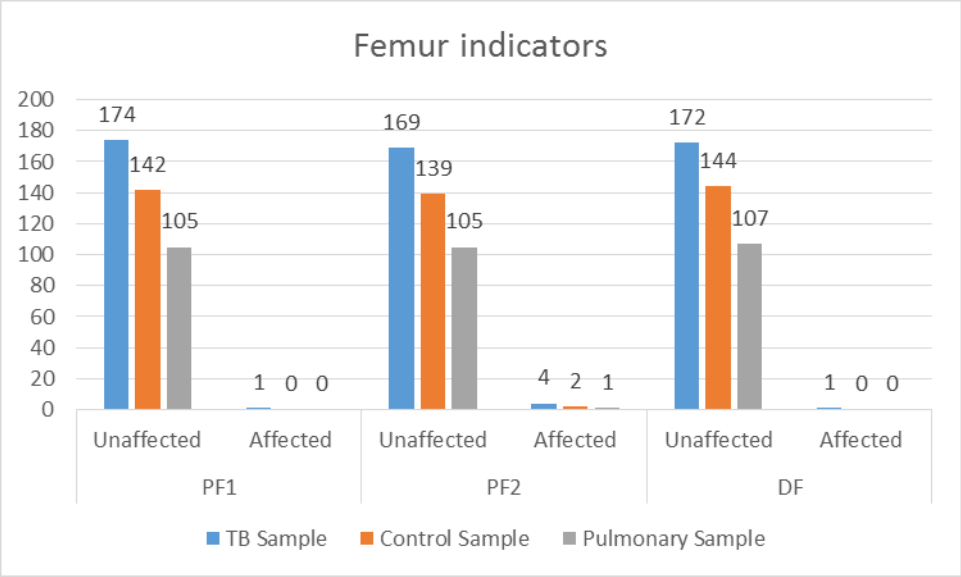


Figure 4.4: The number (N) of affected and unaffected locations on the femur

There were only 4 tibiae with lesions across all samples (Figure 4.5).

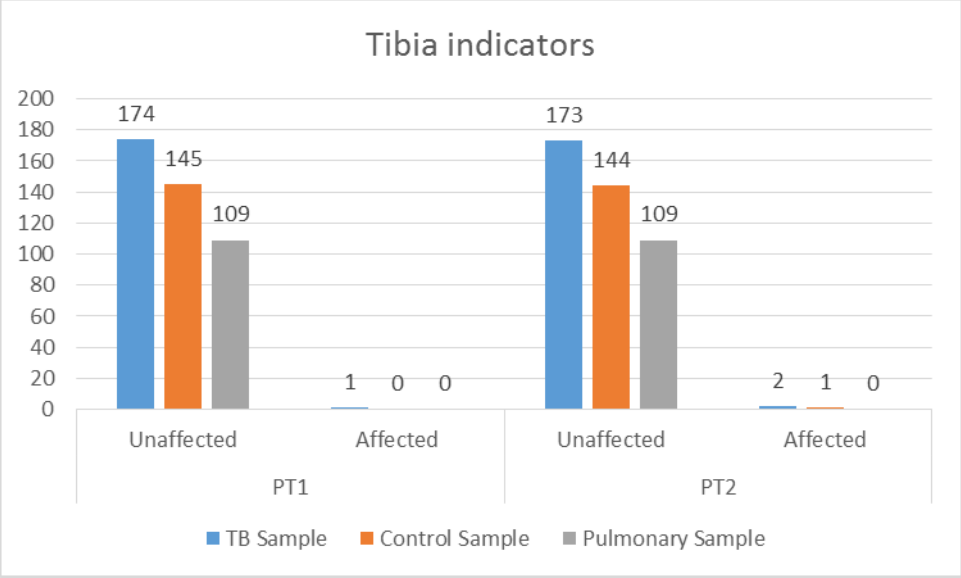


Figure 4.5: The number (N) of unaffected and affected locations on the tibia

PT1 (clustered pits and cavities on the articular surface of the proximal tibia) was observed once in the study - this was found in the TB sample. Of the three individuals found to have clustered pits and cavities on the intercondylar eminence of the proximal tibia, two were found in the TB group and the other one was found in the control.

Of the two locations on the humerus, the most common was the indicator designated DH1 – the clustered pits on the posterior condylar part of the humerus. This occurred in 16 individuals with TB (9.3%), but also in 8 individuals in the control sample (5.6%) and 11 in the pulmonary sample (10.4%) (Fig 4.6). DH2, clustered pits and deep cavities on the articular distal humerus, were observed three times, two of which were from the control group with the other being from the TB sample.

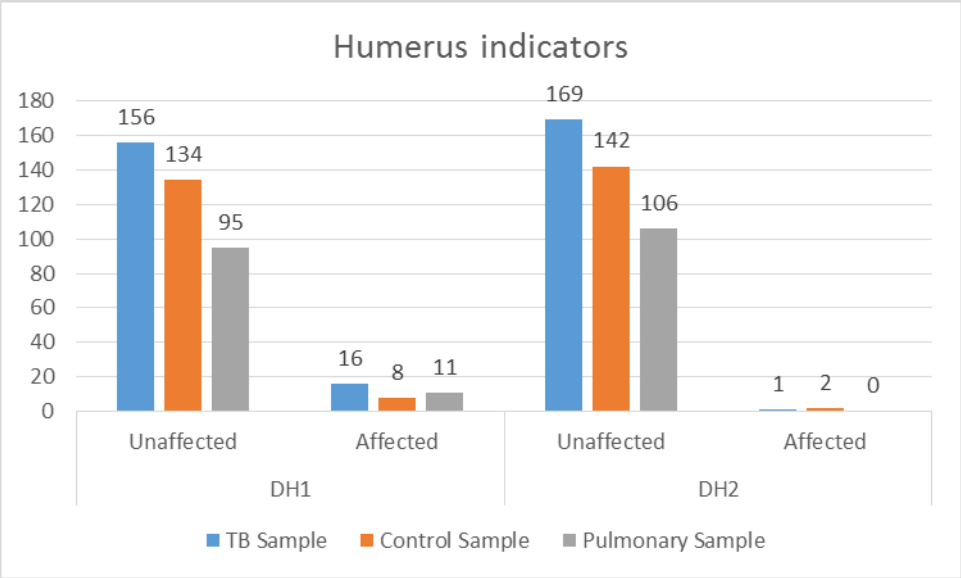


Figure 4.6: The number (N) of affected and unaffected locations on the humerus

Radial and ulnar lesions were rare, although the radius was a somewhat more common location for lesions than the ulna (Figure 4.7). Lesions on the radius (clustered pits and large cavities on the radial tuberosity) occurred more often in individuals with TB (3.5%) than in others (0.7% (control group), 1.0% (pulmonary group)), whereas the only two ulnar lesions (clustered pits and large cavities on the olecranon process) were found in the control group.

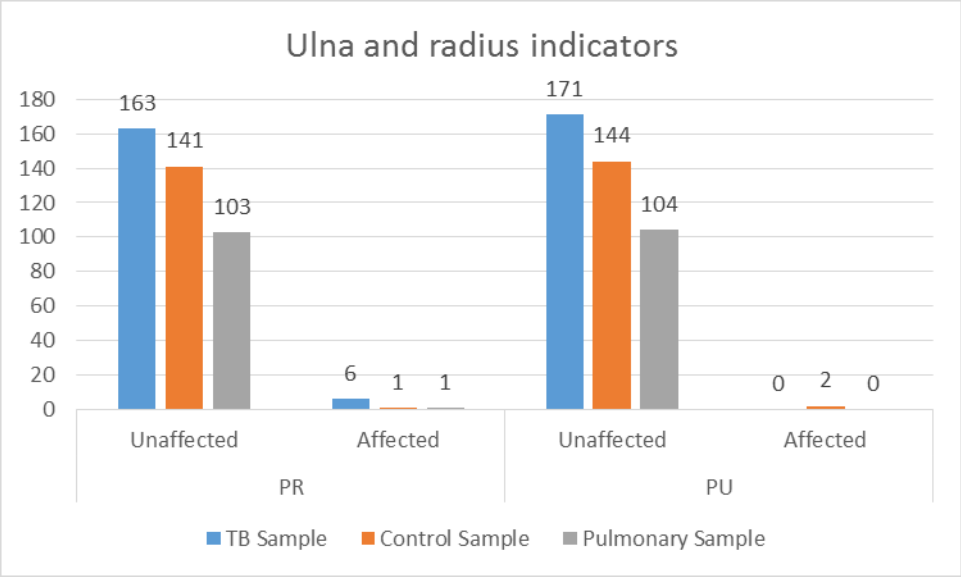


Figure 4.7: Numbers (N) of affected and unaffected locations on radii and ulnae.

The most common cranial lesion was CRA2 (middle cranial fossa), clustered and large cavities on the cranial fossa, while the least common was CRA3 (posterior cranial fossa) with only one individual being observed with the lesions in the control group. CRA1 (anterior cranial fossa) was observed three times, all in the TB sample (Figure 4.8). ENDO2 (superior endocranium), the serpentine branches in the endocranium, was also fairly frequently observed, more so in TB (12.9%) than other conditions (4.6% and 5.9% respectively). ENDO1 (inferior endocranium) were observed five times, four of which were all in the TB assemblage. In general, cranial lesions were not often observed.

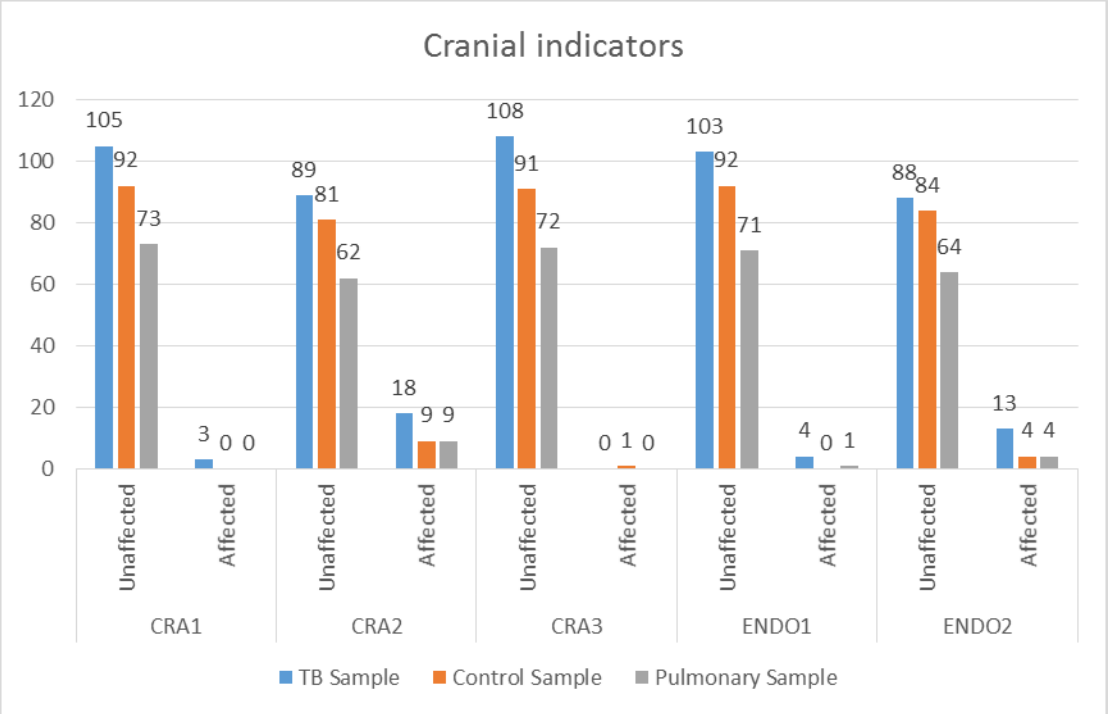


Figure 4.8: Number (N) of present and absent lesions on cranial locations

4.3 Frequencies of skeletal indicators

Table 4.1 shows the frequency of each of the lesions in each of the three sample groups. In all cases, the number of individuals as well as the percentage of affected individuals is given. The differences between the TB, pulmonary diseases and control sample groups were assessed through Fischer's Exact analysis and were deemed statistically significant at $p < 0.05$. Of the 23 skeletal indicators, only 2 were statistically significantly different between the different groups. These were 'VEN2' and 'RIB2'. Most of the lesions occurred in large numbers across the three group that only two indicators *(RIB2 and VEN2) occurred statistically significantly higher in the TB group at $p = 0.030$ and $p<0.001$ respectively.

Table 4.1: Frequency of skeletal indicators in the TB, control and other pulmonary disease sample groups. *indicates statistical significance at p<0.05

Lesion		TB Sample		Control Sample		Pulmonary Sample		p-values
		N	%	N	%	N	%	
RIB1	Unaffected	167	96.5	142	96.6	108	100.0	
	Affected	6	3.5	5	3.4	0	0.0	0.115
RIB2	Unaffected	152	87.9	138	94.5	103	95.4	
	Affected	21	12.1	8	5.5	5	4.6	0.030*
VER	Unaffected	148	91.4	132	91.0	95	90.5	
	Affected	14	8.6	13	9.0	10	9.5	0.975
VEN1	Unaffected	152	93.8	139	97.9	97	92.4	
	Affected	10	6.2	3	2.1	8	7.6	0.085
VEN2	Unaffected	72	45.0	91	67.9	44	41.9	
	Affected	88	55.0	43	32.1	61	58.1	<0.001*
BOD	Unaffected	161	94.2	137	95.8	98	91.6	
	Affected	10	5.8	6	4.2	9	8.4	0.380
ACE1	Unaffected	163	95.3	139	97.2	103	96.3	
	Affected	8	4.7	4	2.8	4	3.7	0.729
ACE2	Unaffected	124	72.1	114	79.7	82	76.6	
	Affected	48	27.9	29	20.3	25	23.7	0.293
PF1	Unaffected	174	99.4	142	100.0	105	100.0	
	Affected	1	0.6	0	0.0	0	0.0	1.000
PF2	Unaffected	169	97.7	139	98.6	105	99.1	
	Affected	4	2.3	2	1.4	1	0.9	0.711
ILI	Unaffected	158	95.2	128	92.1	99	94.3	
	Affected	8	4.8	11	7.9	6	5.7	0.567
DF	Unaffected	172	99.4	144	100.0	107	100.0	
	Affected	1	0.6	0	0.0	0	0.0	1.000
PT1	Unaffected	174	99.4	145	100.0	109	100.0	
	Affected	1	0.6	0	0.0	0	0.0	1.000
PT2	Unaffected	173	98.9	144	99.3	109	100.0	
	Affected	2	1.1	1	0.7	0	0.0	0.788
DH1	Unaffected	156	90.7	134	94.4	95	89.6	
	Affected	16	9.3	8	5.6	11	10.4	0.338
DH2	Unaffected	169	99.4	142	98.6	106	100.0	
	Affected	1	0.6	2	1.4	0	0.0	0.619
PR	Unaffected	163	96.5	141	99.3	103	99.0	
	Affected	6	3.5	1	0.7	1	1.0	0.180
PU	Unaffected	171	100.0	144	98.6	104	100.0	
	Affected	0	0.0	2	1.4	0	0.0	0.180
CRA1	Unaffected	105	97.2	92	100.0	73	100.0	

	Affected	3	2.8	0	0.0	0	0.0	0.116
CRA2	Unaffected	89	83.2	81	90.0	62	87.3	
	Affected	18	16.8	9	10.0	9	12.7	0.391
CRA3	Unaffected	108	100.0	91	98.9	72	100.0	
	Affected	0	0.0	1	1.1	0	0.0	0.602
ENDO1	Unaffected	103	96.3	92	100.0	71	98.6	
	Affected	4	3.7	0	0.0	1	1.4	0.183
ENDO2	Unaffected	88	87.1	84	95.5	64	94.1	
	Affected	13	12.9	4	4.6	4	5.9	0.102

The most common skeletal lesion in the TB sample was bony proliferation located on the ventral surface of vertebral bodies (VEN2), which was evident in 55% of the individuals (Table 4.1). Clustered pits and cavities on the olecranon process (PU) and in the posterior cranial fossa (CRA3) were the least common skeletal lesions in this sample. Similarly, in the control sample, 'VEN2' was again the most common skeletal lesion. The indicators designated 'DF', 'PF1', 'PT1', 'PU', 'CRA1' and 'ENDO1' were the least common as none of these lesions were present in this sample. VEN2 was also the most common skeletal lesion in the pulmonary disease sample, occurring in 58.1%, which is higher than its frequency in the TB group. 'RIB1', 'PF1', 'DF', 'PT1', 'PT2', 'DH2', 'PU', 'CRA1' and 'CRA3' all had no representation in the pulmonary sample.

Table 4.1 shows the frequencies of the two different kinds of rib lesions in all three samples. 'RIB2' was more common than 'RIB1' across all sample groups, and the difference between the three samples, for RIB2, was statistically significantly different ($p<0.030$) indicating that it occurred significantly more frequently in the TB sample. 'RIB1' seemed to have no diagnostic value related to TB.

To better understand these results, Fisher's Exact tests were run separately between the TB and control sample (Table 4.2), control and pulmonary groups (Table 4.3) and TB and pulmonary groups. From Table 4.2, it can be seen that only the same

two indicators (RIB2 and VEN2) were found to be statistically significant at 0.049 and <0.001 respectively.

Table 4.2: Occurrence of skeletal indicators in the TB and control sample groups. *p<0.05.

Lesion		TB Sample		Control Sample		p values
		N	%	N	%	
RIB1	Unaffected	167	96.5	142	96.6	
	Affected	6	3.5	5	3.4	1.000
RIB2	Unaffected	152	87.9	138	94.5	
	Affected	21	12.1	8	5.5	0.049*
VER	Unaffected	148	91.4	132	91.0	
	Affected	14	8.6	13	9.0	1.000
VEN1	Unaffected	152	93.8	139	97.9	
	Affected	10	6.2	3	2.1	0.095
VEN2	Unaffected	72	45.0	91	67.9	
	Affected	88	55.0	43	32.1	<0.001*
BOD	Unaffected	161	94.2	137	95.8	
	Affected	10	5.8	6	4.2	0.611
ACE1	Unaffected	163	95.3	139	97.2	
	Affected	8	4.7	4	2.8	0.557
ACE2	Unaffected	124	72.1	114	79.7	
	Affected	48	27.9	29	20.3	0.147
PF1	Unaffected	174	99.4	142	100.0	
	Affected	1	0.6	0	0.0	1.000
PF2	Unaffected	169	97.7	139	98.6	
	Affected	4	2.3	2	1.4	0.694
ILI	Unaffected	158	95.2	128	92.1	
	Affected	8	4.8	11	7.9	0.342
DF	Unaffected	172	99.4	144	100.0	
	Affected	1	0.6	0	0.0	1.000
PT1	Unaffected	174	99.4	145	100.0	
	Affected	1	0.6	0	0.0	1.000
PT2	Unaffected	173	98.9	144	99.3	
	Affected	2	1.1	1	0.7	1.000
DH1	Unaffected	156	86.7	134	94.4	
	Affected	16	9.3	8	5.6	0.287
DH2	Unaffected	169	99.4	142	98.6	
	Affected	1	0.6	2	1.4	0.595
PR	Unaffected	163	96.4	141	99.3	
	Affected	6	3.6	1	0.7	0.130

PU	Unaffected	171	100.0	144	98.6	
	Affected	0	0.0	2	1.4	0.211
CRA1	Unaffected	105	97.2	92	100.0	
	Affected	3	2.8	0	0.0	0.251
CRA2	Unaffected	89	83.2	81	90	
	Affected	18	16.8	9	10.0	0.213
CRA3	Unaffected	108	100.0	91	98.9	
	Affected	0	0.0	1	1.1	0.460
ENDO1	Unaffected	103	96.3	92	100.0	
	Affected	4	3.7	0	0.0	0.126
ENDO2	Unaffected	88	87.1	84	95.5	
	Affected	13	12.9	4	4.5	0.072

Table 4.3 displays the results of the Fisher's Exact test using the pulmonary and control sample groups, and here it can be seen that only VEN2 now reaches statistical significance, being more prevalent in the pulmonary group.

Table 4.3: Occurrences of skeletal indicators in the pulmonary and control sample. *p<0.05.

Lesion		**Control Sample**		**Pulmonary Sample**		**p values**
		N	**%**	**N**	**%**	
RIB1	Unaffected	142	96.6	108	100.0	
	Affected	5	3.4	0	0.0	0.075
RIB2	Unaffected	138	94.5	103	95.4	
	Affected	8	5.5	5	4.6	1.000
VER	Unaffected	132	91.0	95	90.5	
	Affected	13	9.0	10	9.5	1.000
VEN1	Unaffected	139	97.9	97	92.4	
	Affected	3	2.1	8	7.6	0.058
VEN2	Unaffected	91	67.9	44	41.9	
	Affected	43	32.1	61	58.1	<0.001*
BOD	Unaffected	137	95.8	98	91.6	
	Affected	6	4.2	9	8.4	0.186
ACE1	Unaffected	139	97.2	103	96.3	
	Affected	4	2.1	4	3.7	0.727
ACE2	Unaffected	114	79.7	82	76.6	
	Affected	29	20.3	25	23.4	0.642
PF1	Unaffected	142	100.0	105	100.0	

	Affected	0	0.0	0	0.0	
PF2	Unaffected	139	98.6	105	99.1	
	Affected	2	1.4	1	0.9	1.000
ILI	Unaffected	128	92.1	99	94.3	
	Affected	11	7.9	6	5.7	0.615
DF	Unaffected	144	100.0	107	100.0	
	Affected	0	0.0	0	0.0	-
PT1	Unaffected	145	100.0	109	100.0	
	Affected	0	0.0	0	0.0	-
PT2	Unaffected	144	99.3	109	100.0	
	Affected	1	0.7	0	0.0	1.000
DH1	Unaffected	134	94.4	95	89.6	
	Affected	8	5.6	11	10.4	0.227
DH2	Unaffected	142	98.6	106	100.0	
	Affected	2	1.4	0	0.0	0.510
PR	Unaffected	141	99.3	103	99.0	
	Affected	1	0.7	1	1.0	1.000
PU	Unaffected	144	98.6	104	100.0	
	Affected	2	1.4	0	0.0	0.512
CRA1	Unaffected	92	100.0	73	100.0	
	Affected	0	0.0	0	0.0	-
CRA2	Unaffected	81	90.0	62	87.3	
	Affected	9	10.0	9	12.7	0.622
CRA3	Unaffected	91	98.9	72	100.0	
	Affected	1	1.1	0	0.0	1.000
ENDO1	Unaffected	92	100.0	71	98.6	
	Affected	0	0.0	1	1.4	0.439
ENDO2	Unaffected	84	95.5	64	94.1	
	Affected	4	4.5	4	5.9	0.729

The pulmonary versus TB groups' Fisher's Exact tests are displayed in Table 4.4. Here only RIB2 reaches statistical significance (p=0.0335), being more common in the TB group.

Table 4.4: Occurrences of skeletal indicators in the TB and pulmonary sample groups. *p<0.05.

Lesion		TB Sample		Pulmonary Sample		p values
		N	%	N	%	
RIB1	Unaffected	167	96.5	108	100.0	
	Affected	6	3.5	0	0.0	0.085
RIB2	Unaffected	152	87.9	103	95.4	
	Affected	21	12.1	5	4.6	0.035*
VER	Unaffected	148	91.4	95	90.5	
	Affected	14	8.6	10	9.5	0.829
VEN1	Unaffected	152	93.8	97	92.4	
	Affected	10	6.2	8	7.6	0.629
VEN2	Unaffected	72	45.0	44	41.9	
	Affected	88	55.0	61	58.1	0.704
BOD	Unaffected	161	94.2	98	91.6	
	Affected	10	5.8	9	8.4	0.467
ACE1	Unaffected	163	95.3	103	96.3	
	Affected	8	4.7	4	3.7	0.772
ACE2	Unaffected	124	72.1	82	76.6	
	Affected	48	27.9	25	23.4	0.484
PF1	Unaffected	174	99.4	105	100.0	
	Affected	1	0.6	0	0.0	1.000
PF2	Unaffected	169	97.7	105	99.1	
	Affected	4	2.3	1	0.9	0.652
ILI	Unaffected	158	95.2	99	94.3	
	Affected	8	4.8	6	5.7	0.783
DF	Unaffected	172	99.4	107	100.0	
	Affected	1	0.6	0	0.0	1.000
PT1	Unaffected	174	99.4	109	100.0	
	Affected	1	0.6	0	0.0	1.000
PT2	Unaffected	173	98.9	109	100.0	
	Affected	2	1.1	0	0.0	0.523
DH1	Unaffected	156	90.7	95	89.6	
	Affected	16	9.3	11	10.4	0.836
DH2	Unaffected	169	99.4	106	100.0	
	Affected	1	0.6	0	0.0	1.000

PR	Unaffected	163	96.4	103	99.0	
	Affected	6	3.6	1	1.0	0.258
PU	Unaffected	171	100.0	104	100.0	
	Affected	0	0.0	0	0.0	-
CRA1	Unaffected	105	97.2	73	100.0	
	Affected	3	2.8	0	0.0	0.274
CRA2	Unaffected	89	83.2	62	87.3	
	Affected	18	16.8	9	12.7	0.526
CRA3	Unaffected	108	100.0	72	100.0	
	Affected	0	0.0	0	0.0	-
ENDO1	Unaffected	103	96.3	71	98.6	
	Affected	4	3.7	1	1.4	0.650
ENDO2	Unaffected	88	87.1	64	94.1	
	Affected	13	12.9	4	5.9	0.193

4.4 Sensitivity and specificity of lesions to indicate TB

Table 4.5 shows the sensitivity and specificity calculated using the TB and control samples. It also contains the measure of sensitivity + specificity, indicating which lesions meet the sensitivity + specificity >1 criterion.

Table 4.5: Sensitivity and specificity measures for all 23 indicators in the TB and control samples. *Sensitivity estimates >0.05. The indicators where the sensitivity + specificity > 1 criterion was met are in bold.

Lesions	**Sensitivity**			**Specificity**			**Sens+Spec**
	Estimate	95%CI		Estimate	95%CI		
RIB1	0.035	0.014	0.077	0.966	0.918	0.987	**1.313**
RIB2	0.121*	0.078	0.182	0.945	0.891	0.974	**1.066**
VER	0.086*	0.049	0.143	0.910	0.849	0.950	0.996
VEN1	0.062*	0.032	0.445	0.979	0.935	0.995	**1.041**
VEN2	0.550*	0.469	0.628	0.679	0.592	0.756	**1.229**
BOD	0.058*	0.029	0.107	0.958	0.907	0.982	**1.016**
ACE1	0.047	0.021	0.093	0.972	0.926	0.991	**1.019**

ACE2	0.279*	0.214	0.353	0.797	0.720	0.858	**1.076**
PF1	0.006	0.000	0.036	1.000	0.967	1.000	**1.006**
PF2	0.023	0.007	0.061	0.986	0.945	0.998	**1.009**
ILI	0.048	0.022	0.096	0.921	0.859	0.958	0.969
DF	0.006	0.000	0.037	1.000	0.968	1.000	**1.006**
PT1	0.006	0.000	0.456	1.000	0.968	1.000	**1.006**
PT2	0.011	0.002	0.045	0.993	0.971	0.998	**1.004**
DH1	0.093*	0.056	0.149	0.944	0.888	0.974	**1.037**
DH2	0.006	0.000	0.037	0.986	0.946	0.998	0.992
PR	0.036	0.015	0.079	0.993	0.956	0.99	**1.029**
PU	0.000	0	0.027	0.986	0.975	0.999	0.986
CRA1	0.028	0.338	0.457	1.000	0.953	1.000	**1.028**
CRA2	0.168*	0.105	0.256	0.900	0.814	0.95	**1.068**
CRA3	0.000	0.000	0.043	0.989	0.932	0.999	0.989
ENDO1	0.037	0.012	0.099	1.000	0.95	1.000	**1.037**
ENDO2	0.129*	0.073	0.214	0.955	0.881	0.985	**1.084**

The most sensitive lesion of the suite was the lesion designated ‘VEN2’, which had an estimate of 0.550. It had a specificity estimate of 0.679. This indicates that an osteological reaction on the ventral surface of vertebral bodies means there is a 55% probability of a true TB diagnosis. In the absence of the indicator ‘VEN2’, there is still a 32.1% probability of TB (1 – specificity). Absence of this lesion therefore does not mean that the individual does not have TB. There were eight other indicators with a sensitivity that was higher than 0.05 or 5% (‘RIB2’, ‘VER’, ‘VEN1’, ‘BOD’, ‘ACE2’, ‘DH1’, ‘CRA2’ and ‘ENDO2’).

‘ACE2’ was the second most sensitive indicator in the suite and had a sensitivity estimate of 0.279. This indicates that the occurrence of deep cavities in the acetabular fossa means that there is 27.9% chance of a true TB diagnosis. When these skeletal alterations are absent, there is still a 20.3% chance of a true TB diagnosis. ‘CRA2’ also had a high sensitivity to TB with a sensitivity estimate of 0.168. The presence of clustered pits and large cavities of the middle cranial fossa means that there is a 16.8% probability of a true TB diagnosis. In the absence of these changes there is only a 10.0% chance of the presence of TB in that individual.

On the other end of the spectrum, ILI for example, only provides a probability of 4.8% for a TB diagnosis. When absent, there is a 7.9% probability of the individual having TB. There is thus a greater possibility of having TB among individuals without the specified changes at location ILI than there is for those showing these changes. This is also true for indicators VER and DH2. Two indicators had specificities of zero, namely, the indicator on the proximal ulna (PU) as well as the one located on the posterior cranial fossa (CRA3).

For all but five indicators, the sensitivity + specificity > 1 criterion was met. This indicates that for these indicators the ‘gain in certainty’ was maximized and thus provided information that could not be ascertained without the scrutiny of said indicators. The same could not be concluded when considering indicators VER, ILI, DH2, PU and CRA3.

Table 4.6 shows the sensitivity and specificity estimates calculated using the TB, pulmonary and control samples. A Sensitivity + Specificity column is also present in the table. For the purpose of the determination of the sensitivity and specificity of all three samples, the control and pulmonary sample groups were merged as these

groups were considered not to have had TB as a cause of death. Using all three sample groups only resulted in changes in specificity as the pulmonary group was not considered to have individuals with TB

Table 4.6: Sensitivity and specificity measures for all 23 indicators. *The sensitivity estimates that were >0.05. The indicators where the sensitivity + specificity > 1 criterion was met are in bold.

Lesions	**Sensitivity**			**Specificity**			**Sens+Spec**
	Estimate	95%CI		Estimate	95%CI		
RIB1	0.035	0.014	0.077	0.980	0.952	0.993	**1.327**
RIB2	0.121*	0.078	0.182	0.949	0.912	0.971	**1.070**
VER	0.086*	0.049	0.143	0.908	0.863	0.939	0.994
VEN1	0.062*	0.0316	0.445	0.948	0.955	0.976	**1.010**
VEN2	0.550*	0.469	0.628	0.565	0.499	0.628	**1.115**
BOD	0.058*	0.029	0.107	0.706	0.900	0.964	0.764
ACE1	0.047	0.021	0.093	0.968	0.935	0.985	**1.015**
ACE2	0.279*	0.214	0.353	0.784	0.726	0.832	**1.063**
PF1	0.006	0.000	0.036	1.000	0.98	1.000	**1.006**
PF2	0.023	0.007	0.061	0.988	0.961	0.996	**1.011**
ILI	0.048	0.022	0.096	0.930	0.889	0.958	0.978
DF	0.006	0.000	0.037	1.000	0.981	1.000	**1.006**
PT1	0.006	0.000	0.456	1.000	0.981	1.000	**1.006**
PT2	0.011	0.002	0.045	0.996	0.974	0.9997	**1.007**
DH1	0.093*	0.056	0.149	0.923	0.881	0.952	**1.016**
DH2	0.006	0.000	0.037	0.992	0.968	0.999	**1.006**
PR	0.036	0.015	0.079	0.992	0.968	0.999	**1.028**

PU	0.000	0.000	0.027	0.992	0.968	0.999	0.992
CRA1	0.028	0.338	0.457	1.000	0.972	1.000	**1.028**
CRA2	0.168*	0.105	0.256	0.888	0.827	0.931	**1.056**
CRA3	0.000	0.000	0.043	0.994	0.961	0.9996	0.994
ENDO1	0.037	0.012	0.099	0.994	0.956	0.993	**1.031**
ENDO2	0.129*	0.073	0.214	0.949	0.898	0.976	**1.078**

When all three groups are incorporated, the specificity changes and the absence of VEN2 now means there is 50.1% probability of TB. Using all the groups has decreased the specificity of this lesion. The specificity of indicator ILI only decreased slightly with a 7% chance of a TB diagnosis in its absence.

4.5 Relationship between indicators

The relationships between all the skeletal indicators evaluated through Chi-squared tests are listed in Table 4.7. The null hypothesis states that there is no relationship between indicators, whereas a p-value of ≤0.05 was considered to show close association between indicators wherein the null hypothesis would be rejected.

Table 4.7: Chi-squared test results of the 23 indicators showing the association between indicators

	RIB2	VER	VEN1	VEN2	BOD	ACE1	ACE2	PF1	PF2	ILI	DF	PT1	PT2	DH1	DH2	PR	PU	CRA1	CRA2	CRA3	ENDO1	ENDO2
RIB1	<0.001	<0.001	0.053	<0.001	0.015	0.307	<0.001	0.004	0.362	0.012	0.004	0.004	0.031	<0.001	<0.034	0.530	0.013	0.175	<0.001	0.029	0.532	<0.001
RIB2		0.596	0.099	<0.001	0.247	0.010	<0.001	<0.001	<0.001	0.292	<0.001	<0.001	<0.001	0.844	<0.001	<0.001	<0.001	<0.001	0.020	<0.001	<0.001	0.927
VER			0.032	<0.001	0.094	0.002	<0.001	<0.001	<0.001	0.118	<0.001	<0.001	<0.001	0.740	<0.001	<0.001	<0.001	<0.001	0.067	<0.001	<0.001	0.718
VEN1				<0.001	0.613	0.352	<0.001	<0.001	0.006	0.550	<0.001	<0.001	<0.001	0.067	<0.001	0.013	<0.001	0.005	<0.001	<0.001	0.029	0.117
VEN2					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BOD						0.150	<0.001	<0.001	<0.001	0.923	<0.001	<0.001	<0.001	0.178	<0.001	0.003	<0.001	0.002	<0.001	<0.001	0.010	0.262
ACE1							<0.001	<0.001	0.058	0.126	<0.001	<0.001	0.002	0.006	0.002	0.069	<0.001	0.033	<0.001	0.004	0.143	0.015
ACE2								<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PF1									0.033	<0.001	1.000	0.991	0.324	<0.001	0.314	0.018	0.562	0.142	<0.001	0.754	0.026	<0.001
PF2										<0.001	0.032	0.031	0.192	<0.001	0.203	0.776	0.093	0.540	<0.001	0.118	0.861	<0.001
ILI											<0.001	<0.001	<0.001	0.214	<0.001	0.002	<0.001	0.001	0.001	<0.001	0.008	0.304
DF												0.993	0.322	<0.001	0.312	0.017	0.559	0.141	<0.001	0.751	0.025	<0.001
PT1													0.316	<0.001	0.306	0.017	0.552	0.137	<0.001	0.745	0.024	<0.001
PT2														<0.001	0.979	0.116	0.669	0.575	<0.001	0.570	0.165	<0.001
DH1															<0.001	<0.001	<0.001	<0.001	0.032	<0.001	<0.001	0.941
DH2																0.124	0.652	0.593	0.075	0.557	0.175	<0.001
PR																	0.053	0.396	<0.001	0.079	0.939	<0.001
PU																		0.343	<0.001	0.833	0.079	<0.001
CRA1																			<0.001	<0.001	<0.001	0.053
CRA2																				<0.001	<0.001	<0.001
CRA3																					0.010	<0.001
ENDO1																						<0.001

All but one of the indicators were found to have a close association with more than half (≥12) of the other indicators. Only indicator 'DH1' had significant relationships with less than 12 indicators (10).

4.6 Efficacy of skeletal markers to indicate TB

The results displayed in Tables 4.2, 4.5 and 4.7 were used to compile three criteria used to assess the association of 23 skeletal indicators and TB: (1) Indicator frequency, as per the Fisher's Exact test, where p≤0.001 (the minimum p-value was changed to 0.001 to allow for consistent comparability with the Dangvard Pedersen *et al*. (2019a) study), (2) Indicator sensitivity is >0.05 and (3) Indicator has significant relationship with ≥12 other indicators. Table 4.8 shows where each of the indicators

met the criteria and where the criteria were not met. Criteria not met are indicated as "-" and criteria met is marked with "+".

Table 4.8: Criteria illustrating the efficacy of 23 skeletal indicators in diagnosing TB.

	P≤0.001 (Table 4.2)	Sensitivity >0.05 (Table 4.5)	Significant relation with ≥12 other indicators (Table 4.7)
RIB1	-	-	+
RIB2	-	+	+
VER	-	+	+
VEN1	-	+	+
VEN2	+	+	+
BOD	-	+	+
ACE1	-	-	+
ACE2	-	+	+
PF1	-	-	+
PF2	-	-	+
ILI	-	-	+
DF	-	-	+
PT1	-	-	+
PT2	-	-	+
DH1	-	+	+
DH2	-	-	-
PR	-	-	+
PU	-	-	+
CRA1	-	-	+
CRA2	-	+	+
CRA3	-	-	+
ENDO1	-	-	+
ENDO2	-	+	+

A single indicator out of the 23 met the three criteria (VEN2). However, a total of nine other indicators met at least two of the criteria ('RIB2', 'VER', 'VEN', 'BOD', 'ACE2', 'DH1', 'CRA2' and 'ENDO2').

4.7 Inter-observer and intra-observer reliability

Inter-observer reliability was determined using the values found in a two-by-two assessment (Table 4.9). The numbers indicate the number of times lesions were scored as either present or absent for each of the indicators.

Table 4.9: Inter-observer results

		Observer 1		**Row Marginals (totals)**	
		Absent	Present		
Observer 2	Absent	318	22	340	91.6%
	Present	13	18	31	8.4%
Column Marginals (totals)		331	40	371	
		89%	11%		

Both observers were in agreement pertaining to the absence of lesions in 318 occasions, and both consistently detected possible TB indicators 18 times. However, there was a relatively high level of disagreement between observers pertaining to when lesions were absent or present. There was more disagreement when lesions were present than when lesions were absent. The Cohen's Kappa value was 0.5. This is considered to be a moderate level of agreement for inter-observer reliability. The percent agreement can be calculated as follows (where 371 represent the total number of observations, 318 the agreement that the feature was absent and 18 the agreement that the feature was present):

Percent agreement = (318+18)/371

=0.91

This means that 9.1% of data misrepresents the research data.

Intra-observer reliability was determined using the values found in a two-by-two assessment (Table 4.10). The numbers indicate the number of times lesions were scored as either present or absent for each of the indicators.

Table 4.10: Intra-observer results

		Researcher assessment 1	**Row Marginals (totals)**	
		Absent	Present	
Researcher assessment 2	Absent	326	2	328	95%
	Present	4	15	19	5%
Column Marginals (totals)		330	17	347	
		95%	5%		

The researchers agreed with the initial assessment pertaining to the absence of lesions on 326 occasions, and consistently detected TB indicators 15 times on both occasions. The level of disagreement was low between the first and second assessments pertaining to when lesions were absent or present. This only occurred on 6 occasions. The Cohen's Kappa value was 0.8. This is considered to be a strong level of agreement for reliability.

Percentage agreement = (326+15)/347

= 0.98

This means that 9.8% of the data misrepresents the research data.

4.8 Comparison to results of Dangvard Pedersen *et al*. (2019)

The aim of the Dangvard Pedersen *et al*. (2019a) study was to estimate the sensitivity and specificity of 18 bony lesions that had the likelihood of being related to TB. In this section a comparison is made between the results of the current study and those that were found in the Dangvard Pedersen *et al*. (2019a) study for the 18 features that were used in both studies. For this purpose, the results from the TB sample versus the control sample (which included no pulmonary diseases) were used.

4.8.1 Frequency

The frequency results for the two studies are displayed in Table 4.11, which also shows the p-values of the Fisher's Exact tests.

Table 4.11: Occurrence of skeletal indicators in TB, control samples in the Dangvard Pedersen *et al*. (2019a) study compared to the current study.

Lesion		**Current Study**			**Dangvard Pedersen *et al.* (2019a)**		
		TB Sample %	**Control Sample %**	**p values**	**TB Sample %**	**Control Sample %**	**p values**
RIB1	Unaffected	96.5	96.6		60.0	41.6	
	Affected	3.5	3.4	1.000	40.0	58.4	<0.001*
RIB2	Unaffected	87.9	94.5		66.3	99.7	
	Affected	12.1	5.5	0.049*	33.7	1.3	<0.001*
VER	Unaffected	91.4	91.0		91.3	95.0	
	Affected	8.6	9.0	1.000	8.7	5.0	0.148
VEN1	Unaffected	93.8	97.9		79.2	97.9	
	Affected	6.2	2.1	0.095	20.8	2.1	<0.001
VEN2	Unaffected	45.0	67.9		46.7	95.4	
	Affected	55.0	32.1	<0.001*	53.3	4.6	<0.001*
BOD	Unaffected	94.2	95.8		66.8	97.8	

	Affected	5.8	4.2	0.611	33.2	2.2	<0.001*
ACE1	Unaffected	95.3	97.2		94.5	99.6	
	Affected	4.7	2.8	0.557	5.5	0.4	0.002*
ACE2	Unaffected	72.1	79.7		58.0	93.5	
	Affected	27.9	20.3	0.147	42.0	6.5	<0.001*
PF1	Unaffected	99.4	100.0		96.6	98.7	
	Affected	0.6	0.0	1.000	3.4	1.3	0.221
PF2	Unaffected	97.7	98.6		98.7	96.9	
	Affected	2.3	1.4	0.694	1.3	3.1	0.214
ILI	Unaffected	95.2	92.1		70.4	94.2	
	Affected	4.8	7.9	0.342	29.6	5.8	<0.001*
DF	Unaffected	99.4	100.0		97.9	99.1	
	Affected	0.6	0.0	1.000	2.1	0.9	0.450
PT1	Unaffected	99.4	100.0		99.6	100.0	
	Affected	0.6	0.0	1.000	0.4	0.0	1.000
PT2	Unaffected	98.9	99.3		97.9	100.0	
	Affected	1.1	0.7	1.000	2.1	0.0	0.061
DH1	Unaffected	86.7	94.4		96.6	100.0	
	Affected	13.3	5.6	0.287	3.4	0.0	0.007*
DH2	Unaffected	99.4	98.6		97.9	100.0	
	Affected	0.6	1.4	0.595	2.1	0.0	0.061
PR	Unaffected	96.4	99.3		97.9	98.7	
	Affected	3.6	0.7	0.130	2.1	1.3	0.724
PU	Unaffected	100.0	98.6		90.8	98.3	
	Affected	0.0	1.4	0.211	9.2	1.7	<0.001*

Numerous indicators (RIB1, RIB2, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1 and PU) showed differences between the Dangvard Pedersen and current study, many at the p<0.001 level. The study by Dangvard Pedersen found eight more indicators,

than the current study, that appeared more frequently in the TB group than in the control group. The most frequently observed skeletal lesion in both studies was VEN2 (bony proliferation located on the ventral surface of vertebral bodies). The least common lesions for the current study were DF, PF1, PT1 and PU. In the Dangvard Pedersen *et al*. (2019a) study, the lesion that was least observed was PT1, clustered pits and large cavities on the articular surface of the proximal tibia. Both studies found that indicators 'RIB2' and 'VEN2' were significantly present more frequently in the TB groups than they were in controls.

For the current study, two indicators VER (clustered pits and deep cavities on the caudal and cranial surfaces of vertebral bodies) and ILI (clustered pits and large cavities on the iliac auricular surface) were found more frequently in controls than in TB cases, although these frequencies were not statistically significant. This was not consistent with the Dangvard Pedersen *et al.* (2019a) study which found only one indicator more commonly in the controls, RIB1 (low nodules and rough texture on the visceral surface of ribs) which was statistically significant ($p<0.001$).

4.8.2 Sensitivity and specificity

Table 4.12 shows the sensitivity and specificity calculated using a TB and control sample from both the current study and the Dangvard Pedersen *et al*. (2019a) study. The sensitivity + specificity calculation is also shown.

Table 4.12: Sensitivity and specificity measures for 18 Dangvard Pedersen *et al.* indicators as well as those found in the current study. *Sensitivity estimates at p >0.05. The indicators where the sensitivity + specificity > 1 criterion was met are in bold.

Lesions	**Current Study**			**Dangvard Pedersen *et al*. (2019a) study**		
	Sensitivity Estimate	**Specificity Estimate**	**Sens + Spec**	**Sensitivity Estimate**	**Specificity Estimate**	**Sens+ Spec**

RIB1	0.035	0.966	1.313	0.400*	0.416	0.816
RIB2	0.121*	0.945	1.066	0.337*	0.987	1.324
VER	0.086*	0.910	0.996	0.088*	0.950	1.038
VEN1	0.062*	0.979	1.041	0.208*	0.962	1.170
VEN2	0.550*	0.679	1.229	0.533*	0.959	1.492
BOD	0.058*	0.958	1.016	0.332*	0.949	1.281
ACE1	0.047	0.972	1.019	0.055*	0.996	1.051
ACE2	0.279*	0.797	1.076	0.420*	0.935	1.355
PF1	0.006	1.000	1.006	0.034	0.987	1.021
PF2	0.023	0.986	1.009	0.013	0.969	0.982
ILI	0.048	0.921	0.969	0.296*	0.942	1.238
DF	0.006	1.000	1.006	0.021*	0.991	1.012
PT1	0.006	1.000	1.006	0.004	0.999	1.003
PT2	0.011	0.993	1.004	0.021	0.999	1.020
DH1	0.093*	0.944	1.037	0.034	0.999	1.033
DH2	0.006	0.986	0.992	0.021	0.999	1.020
PR	0.036	0.993	1.029	0.021	0.987	1.008
PU	0.000	0.986	0.986	0.092*	0.983	1.075

The most sensitive lesion of the suite was the indicator designated ‘VEN2’, which had a sensitivity estimate of 0.533. This indicator had a specificity estimate of 0.959. This indicated that an osteological reaction on the ventral surface bodies’ means there is a 53.3% probability of a true TB diagnosis. In the absence of the indicator ‘VEN2’, there is only a 4.1% probability of TB (1 – specificity). This indicator was also found to be the most sensitive in the current study. There were eight indicators with a sensitivity estimate that was higher than 0.05 for both the current and the Dangvard

Pedersen *et al.* (2019a) study (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE2, and DH1). Additionally, Dangvard Pedersen *et al*. found ACE1, ILI, DF, and PU to possess sensitivity estimates that were higher than 0.05.

4.8.3 Relationship between indicators

Chi-squared results of the inter-relationship of the 18 indicators in the Dangvard Pedersen *et al*. (2019a) and the current study are in Table 4.13.

Table 4.13 A-B: x2 -test results of the relationship of the 23 indicators from the current study (B) and the 18 indicators from the Dangvard Pedersen et al. (2019a) study (A).

	RIB2	VER	VEN1	VEN2	BOD	ACE1	ACE2	PF1	PF2	ILI	DF	PT1	PT2	DH1	DH2	PR	PU
RIB1	0.943	0.936	0.393	0.040	0.046	0.644	0.917	0.035	0.505	0.428	0.720	0.313	0.639	0.159	0.182	0.450	1.000
RIB2		0.709	<0.001	<0.001	<0.001	0.279	<0.001	0.404	0.135	<0.001	0.084	0.033	0.198	0.017	0.013	0.693	0.009
VER			0.056	<0.001	0.032	0.933	0.006	0.764	0.088	0.159	0.022	0.786	0.542	0.441	0.238	0.442	0.409
VEN1				<0.001	<0.001	0.236	<0.001	0.225	0.246	0.002	0.164	0.715	0.565	0.237	0.001	0.301	0.106
VEN2					<0.001	0.004	<0.001	0.240	0.986	<0.001	0.426	0.517	0.132	<0.001	<0.001	0.037	0.002
BOD						<0.001	<0.001	0.424	0.504	<0.001	0.473	0.637	0.916	<0.001	0.025	0.112	1.000
ACE1							<0.001	0.003	0.586	0.606	0.637	0.860	0.691	<0.001	0.025	0.112	1.000
ACE2								0.373	0.706	<0.001	0.048	0.083	0.069	0.013	<0.001	0.095	0.001
PF1									0.675	0.016	<0.001	0.876	0.009	<0.001	0.009	<0.001	1.000
PF2										0.854	0.728	0.882	0.738	0.671	0.738	0.043	1.000
ILI											0.465	0.033	0.014	0.001	0.090	0.001	0.003
DF												<0.001	0.001	0.010	<0.001	0.010	0.054
PT1													<0.001	0.895	0.917	0.895	1.000
PT2														0.002	0.815	0.766	0.027
DH1															<0.001	<0.001	<0.001
DH2																0.001	<0.001
PR																	0.369

A

	RIB2	VER	VEN1	VEN2	BOD	ACE1	ACE2	PF1	PF2	ILI	DF	PT1	PT2	DH1	DH2	PR	PU	CRA1	CRA2	CRA3	ENDO1	ENDO2
RIB1	<0.001	<0.001	0.053	<0.001	0.015	0.307	<0.001	0.004	0.362	0.012	0.004	0.004	0.031	<0.001	<0.034	0.530	0.013	0.175	<0.001	0.029	0.532	<0.001
RIB2		0.596	0.099	<0.001	0.247	0.010	<0.001	<0.001	<0.001	0.292	<0.001	<0.001	<0.001	0.844	<0.001	<0.001	<0.001	<0.001	0.020	<0.001	<0.001	0.927
VER			0.032	<0.001	0.094	0.002	<0.001	<0.001	<0.001	0.118	<0.001	<0.001	<0.001	0.740	<0.001	<0.001	<0.001	<0.001	0.067	<0.001	<0.001	0.718
VEN1				<0.001	0.613	0.352	<0.001	<0.001	0.006	0.550	<0.001	<0.001	<0.001	0.067	<0.001	0.013	<0.001	0.005	<0.001	<0.001	0.029	0.117
VEN2					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BOD						0.150	<0.001	<0.001	<0.001	0.923	<0.001	<0.001	<0.001	0.178	<0.001	0.003	<0.001	0.002	<0.001	<0.001	0.010	0.262
ACE1							<0.001	<0.001	0.058	0.126	<0.001	<0.001	0.002	0.006	0.002	0.069	<0.001	0.033	<0.001	0.004	0.143	0.015
ACE2								<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PF1									0.033	<0.001	1.000	0.991	0.324	<0.001	0.314	0.018	0.562	0.142	<0.001	0.754	0.026	<0.001
PF2										<0.001	0.032	0.031	0.192	<0.001	0.203	0.776	0.093	0.540	<0.001	0.118	0.861	<0.001
ILI											<0.001	<0.001	<0.001	0.214	<0.001	0.002	<0.001	0.001	0.001	<0.001	0.008	0.304
DF												0.993	0.322	<0.001	0.312	0.017	0.559	0.141	<0.001	0.751	0.025	<0.001
PT1													0.316	<0.001	0.306	0.017	0.552	0.137	<0.001	0.745	0.024	<0.001
PT2														<0.001	0.979	0.116	0.669	0.575	<0.001	0.570	0.165	<0.001
DH1															<0.001	<0.001	<0.001	<0.001	0.032	<0.001	<0.001	0.941
DH2																0.124	0.652	0.593	0.075	0.557	0.175	<0.001
PR																	0.053	0.396	<0.001	0.079	0.939	<0.001
PU																		0.343	<0.001	0.833	0.079	<0.001
CRA1																			<0.001	<0.001	<0.001	0.053
CRA2																				<0.001	<0.001	<0.001
CRA3																					0.010	<0.001
ENDO1																						<0.001

B

Only half of the indicators in the Dangvard Pedersen *et al*. (2019a) were found to have a close association with more than half (≥9) of the other indicators (‘RIB2’, ‘VEN2’, ‘BOD’, ‘ACE2’, ‘ILI’, ‘DF’, ‘DH1’, ‘DH2’ and ‘PU’). The rest all had a close association with less than half (<9) of the other indicators. For the current study, all

but the indicator designated DH1 (clustered pits on the condylar area of the distal humerus), were found to have a close association with more than half of the other indicators in the suite. A close association between indicators means that the presence of one indicator increases the likelihood of the others with which it has a significant association. There were more such associations in the current study than were found in the study by Dangvard Pedersen *et al*. (2019a).

4.8.4 Good TB indicators

Three criteria (1) indicator frequency, as per the Fisher's Exact test, where p≤0.001, (2) indicator sensitivity is >0.05 and (3) indicator relationships with other indicators describing the association of the 18 bony indicators and TB are tabulated below (Table 4.14). Indicator VEN2 met the three criteria in both studies.

Table 4.14: Criteria results for the current study and the Dangvard Pedersen et al. (2019a) study.

	p≤0.001 Fisher's Exact test		**Sensitivity >0.05**		**Significant relation with other indicators**	
	Current	**Dangvard Pedersen**	**Current**	**Dangvard Pedersen**	**Current**	**Dangvard Pedersen**
RIB1	-	+	-	+	+	-
RIB2	-	+	+	+	+	+
VER	-	-	+	+	+	-
VEN1	-	+	+	+	+	-
VEN2	+	+	+	+	+	+
BOD	-	+	+	+	+	+
ACE1	-	-	-	+	+	-
ACE2	-	+	+	+	+	-
PF1	-	-	-	-	+	-
PF2	-	-	-	-	+	-
ILI	-	+	-	+	+	+
DF	-	-	-	-	+	+
PT1	-	-	-	-	+	-
PT2	-	-	-	-	+	-
DH1	-	-	+	-	+	+
DH2	-	-	-	-	-	+
PR	-	-	-	-	+	-
PU	-	+	-	+	+	+

Six out of the 18 indicators met the three criteria in the Dangvard Pedersen *et al.* (2019a) ('RIB2', 'VEN2', 'BOD', 'ACE2', 'ILI' and 'PU'), but only VEN2 did so in the current study. For the current study 'RIB2', 'VER', 'VEN1', 'BOD', 'ACE2', 'DH1', 'CRA2' and 'ENDO2' met two of the three criteria.

4.9 Summary of results

In order to put each skeletal indicator's efficacy in identifying TB into perspective, it is important for the observations that were made to be discussed in more detail. For an indicator to be a good marker of a disease it would have to show characteristics that can be associated with the disease. For this study three-component criteria were used. It is an expectation that an indicator that is a good marker would be found in significantly large numbers in a sample of people who are known to have died of TB compared to samples of people who are not documented to have died of TB. This was why it was important that the frequency of the indicators be considered when determining their efficacy as a diagnostic tool. Skeletal alterations that resulted as a reaction to the presence of TB would be good markers of the disease. This is why a high sensitivity estimate was considered a good characteristic for candidate indicators. Each indicator that forms part of this suite was selected due to its potential association with TB and as such, a good TB marker would have a significant relationship with other indicators that are associated with TB.

Calculating the lesion frequencies for this study allowed for the assessment of whether the skeletal indicators could be associated with a positive TB diagnosis. Two indicators were found significantly more in the TB cases compared to the controls - superficial bone proliferation and lytic lesions on the visceral surface of ribs (RIB2) and bony proliferation on the ventral vertebral bodies (VEN2). The

frequencies of affected individuals in the TB cohort surpassed those of the pulmonary and the control cohorts. The remaining indicators which were not found to be significant warrant caution with regard to their use when working with composition and sample sizes similar to those of the current study.

The sensitivity and specificity measures were generated from these results because they provide data that can be used to quantify probabilistic assessment of disease (Dangvard Pedersen *et al*., 2019a). Sensitivity, in particular, provides information on the probability of an individual presenting with the skeletal lesions that have been described in this study given that that individual did have TB. Specificity estimates, on the other hand, supply data about the probability of individuals not having these lesions given that they did not have TB. A high sensitivity value, therefore, means that the presence of a lesion is a good indicator of the presence of TB whilst a high specificity means that there are few false positive results. Each indicator was assessed individually, and the discussion below elucidates the meaning of the estimated values in Table 4.6.

When an indicator has a sensitivity estimate of 0.05 that means that there is a 5% probability of a true TB diagnosis. For the purpose of the current study, an indicator with this level of sensitivity was considered to be sensitive enough to TB (criterion 2). The suite has 23 indicators and an indicator that has a close association with half of the other indicators (criterion 3) would be a useful tool in diagnosing TB. For the purpose of this study, an indicator was considered to have met this criterion when it had a close association with at least 12 other indicators. This means that those indicators have such a relationship with more than half of the indicators in the suite of 23. One indicator (VEN2), proliferation of bone on the ventral surface of lumbar and thoracic vertebral bodies, met the three criteria. This means that these bony

alterations were observed significantly more in the TB group; they are significantly associated to at least one half of the other bony indicators; and the sensitivity measure surpassed 0.05. This indicator was associated with a TB diagnosis made when the individuals were alive. Indicator RIB2 was observed significantly more in the TB group than in the control groups with a p-value of 0.049 which did not meet the first criterion.

Seven other indicators, VER, VEN1, BOD, ACE2, DH1, CRA2 and ENDO2 met two of the three criteria. All of these indicators were found in large numbers in the TB as well as the other sample groups. As a result of their statistically insignificant frequency differences across the three sample groups, these indicators did not meet the first criterion.

Chi-squared tests were run in order to determine the relationship between the skeletal indicators. For an indicator to have a significant association with another indicator, it means that a relationship exists between the two indicators. The presence of one indicator can therefore increase the likelihood of the presence of the other indicator with which it has a significant association.

In order to consistently compare the current study to that of Dangvard Pedersen *et al.* (2019a) only the results including the TB and control samples were used. Table 4.2 was used to compare the frequencies of the current study to those found in the Dangvard Pedersen *et al*. (2019a) study. Table 4.5 was used to compare the results pertaining to sensitivity and specificity which only consisted of the TB and control samples.

4.9.1 Spinal indicators

Vertebral lesions were the most common of all the indicators. Each of the three indicators located in the spine was represented differently across the sample groups. In total, there were 250 lesions present across all the groups.

Bone proliferation on the ventral part of vertebral bodies (VEN2) was the most frequent lesion. These changes were observed a total of 192 times in this study, accounting for 76.8% of all the vertebral lesions and 32.5% of lesions in the entire study. The bone alterations were found in 55.0% of the individuals in the TB sample, 58.1% of the individual in the pulmonary group and 32.1% of the individuals in the control group. So, while it is very common and there is a statistically significant difference in occurrence between the groups ($p<0.001$), it is unfortunately not very specific to TB as there were more individuals with this indicator in the pulmonary group than in the TB group. This was also reflected in the measures of sensitivity and specificity.

VEN2 was also the most sensitive indicator. The indicator has a sensitivity estimate of 0.550. This means that when there is proliferation on the ventral part of lumbar and thoracic vertebral bodies, there is a 55.0% probability of a true TB diagnosis. However, the low specificity estimate is indicative of this lesion's lack of specificity for TB in particular. With a specificity estimate of 0.565, when these changes are absent there is still a 43.5% probability that that individual had TB. This indicator met the sensitivity + specificity >1 criterion and can thus be considered to provide substantive information regarding a TB diagnosis.

Indicator VEN2 has a significant relationship with all 22 other indicators. The presence of the bone changes denoted by VEN2 can therefore assist in the prediction of the presence or absence of the other indicators with which it has a

significant relationship. This indicator met the criteria to qualify it as useful in making an association between a TB diagnosis made in life and the bone alterations represented by VEN2.

For VEN2, the findings of the current study are similar to those of Dangvard Pedersen *et al*. (2019a) with an important distinction. The frequency of the indicator was statistically significant in both studies at $p<0.001$ with the indicator being found more often in the TB cohorts over the controls for the current study (55.0%) and the Dangvard Pedersen *et al*. (2019a) study (53.3%). The sensitivity estimates were also similar with the current study's being 0.550 and the Dangvard Pedersen *et al.'s* (2019a) being 0.533. For both of the studies, the presence of this indicator meant a more than 50% possibility of a true TB diagnosis. For the specificity estimates, however, Dangvard Pedersen *et al*. (2019a) found this indicator to be much more specific with only a 4.1% probability of a TB diagnosis when the lesion is absent. For the current study, the specificity estimate when looking at only the TB and control sample means there is a 32.1% chance of a TB diagnosis even in the absence of the skeletal changes represented by VEN2. Both studies found VEN2 having significant relationships with more than half of the other indicators. This means that for both studies there was an association between VEN2 and a TB diagnosis.

Indicator VER is characterized by the observation of clustered pits or deep cavities on either the caudal or cranial surface of a thoracic or lumbar vertebral body. VER was the second most common vertebral lesion. These changes were detected 37 times in this study and accounted for 14.8% of all vertebral lesions and 6.3% of all the lesions observed in the study. VER skeletal alterations were found in 8.6% of individuals in the TB sample, 9.0% of the individuals in the control group and 9.5% of

the individual in the pulmonary cohort. The difference in the indicator's frequency across the three groups was not statistically significant at p=0.975.

VER has a sensitivity estimate of 0.086. This means that there is an 8.6% chance of a true TB diagnosis when clustered pits or deep cavities are observed on the cranial or caudal surface of vertebral bodies. The specificity estimate is 0.908, therefore when these bony changes are absent, there is a 9.2% probability of the individual having TB. This indicator appeared more in individuals in the pulmonary and control groups that it did in the TB cohort. VER skeletal alterations are thus not specific to TB. This indicator did not meet the sensitivity + sensitivity >1 criterion and this means that the changes it represents do not provide useful information with regards to the sensitivity and specificity to TB.

Indicator VER has a significant relationship with 15 other indicators in the suite. The presence of the bone changes seen in VER can, as a result, assist in the prediction of the presence and absence of the indicators RIB1, VEN1, VEN2, ACE1, ACE2, PF1, PF2, DF, PT1, PT2, PR, PU, CRA1, CRA3 and ENDO1 with which it has a significant relationship. This indicator was rejected as having an association with a TB diagnosis.

In the Dangvard Pedersen *et al*. (2019a) study, VER was found in 8.7% of individuals in the TB group and in 5.0% in the control sample. This is not consistent with what was found in the current study, where there was a higher frequency of VER skeletal changes in the control than the TB group. The frequency of this indicator across the TB group and control group was not statistically significant at p=1.000 for the current study and p=0.148 for the Dangvard Pedersen *et al*. (2019a) study. Both studies found sensitivity estimates that were almost identical for this

indicator. For both studies when the indicator is VER is observed, there is a ≥8.6% probability of a true TB diagnosis. VER was more specific to TB in the Dangvard Pedersen *et al*. (2019a) study with 5.0% likelihood for TB in the absence of VER skeletal changes; there was 9.0% likelihood for the current study. The two studies found this indicator to have significant relationships with more than half of the indicators in their respective suites. For both studies this indicator was not deemed to show an association between the skeletal changes it represents and a TB diagnosis.

Large pits on the ventral part of thoracic or lumbar vertebral bodies (VEN1) were the least common vertebral lesions. It was detected 21 times in the study, accounting for 8.4% of all vertebral lesions and 3.6% of all lesions observed in the study. These bone alterations were found ins 6.2% individuals in the TB sample, 2.1% individuals in the control sample and 7.6% individuals in the pulmonary cohort. The frequency of this indicator across the three groups, however, was found not to be statistically significant at p=0.085.

VEN1 has a sensitivity estimate of 0.062 which means that there is a 6.2% probability of a true TB diagnosis. As a result of a specificity estimate of 0.948, the absence of large pits in this area, means that there is a 5.2% probability of TB being present in that individual. This indicator met the sensitivity + specificity >1 criterion and can therefore be accepted as providing valuable information regarding sensitivity and specificity of the bone alterations it represents.

Indicator VEN1 has a significant relationship with 15 other indicators in the suite. The presence of this bone alteration can be used in predicting the presence or absence of the indicators VER, VEN2, ACE2, PF1, PF2, DF, PT1, PT2, DH2, PR, PU, CRA1,

CRA2, CRA3 and ENDO1, with which it has a significant relationship. This indicator only met 2 of the three criteria that makes it a good indicator that shows an association between its presence and a TB diagnosis. It was not found in significantly more TB skeletal remains.

For VEN1, the findings of the current study differ substantially with the findings of the Dangvard Pedersen *et al*. (2019a) study. In the latter study, they found this indicator in 20.8% of their TB remains; however, for both studies the control groups' cases possessed these skeletal alterations only 2.1% of the time. The frequency of VEN1 between the two groups was statistically significant in the Dangvard Pedersen *et al*. (2019a) study ($p<0.001$). This indicator was much more sensitive in the Dangvard Pedersen *et al*. (2019a) study with a 20.8% chance of a true TB diagnosis when VEN1 skeletal changes are identified. Their specificity was only slightly lower with a 3.8% likelihood of a TB diagnosis when the skeletal changes are absent. For the current study, the percentage was 2.1 for specificity. In the Dangvard Pedersen et al. (2019a) study, VEN1 did not have significant relationships with more than half the indicators in their suite. In both studies, there was no association between VEN1 and a TB diagnosis.

4.9.2 Rib indicators

Indicator RIB1 represented the observation of low nodules or rough texture on the visceral surface of ribs. These changes were detected a total of 11 times in this study and accounted for 24.4% of all rib lesions and 1.9% of lesions detected in the entire study. The lesions were found in 3.5% of the individuals in the TB sample, 3.4% of the individuals in the control sample and none were present in the pulmonary sample. The differences in frequencies across the three groups were not statistically significant at $p=0.115$.

RIB1 was the most sensitive of the two rib lesions. The indicator has a sensitivity estimate of 0.035. This means that there is a 3.5% probability of a true TB diagnosis when RIB1 skeletal changes are present. A specificity estimate of 0.980 means that when these bony changes are absent, there is a 2% probability of TB. The indicator met the sensitivity + specificity >1 criterion and can thus be considered as a useful tool for the assessment of the changes it represents regarding sensitivity and specificity.

This indicator has a significant relationship with 16 other indicators in the suite. This means that the presence low nodules and rough texture on the visceral surface of ribs increases the likelihood of RIB2, VER, VEN2, BOD, ACE2, PF1, ILI, DF, PT1, PT2, DH1, DH2, PU, CRA2, CRA3 and ENDO2 with which RIB1 has a significant relationship. RIB1 did not meet the criteria to qualify it as a useful indicator in the association of its specific skeletal changes and TB diagnosis.

The differences between this study and that of Dangvard Pedersen *et al*. (2019a) are significant. This indicator was very common in their study (40.0% in the TB sample and 58.4% in the control sample). What was interesting in their study was that RIB1 was more common in controls than it was in the TB cases. They found the difference in the presence of this indicator in the two samples to be statistically significant at $p<0.001$. For the current study, the difference in frequency across the two groups was not significant ($p=1.000$). This indicator was also not common in this study. For the Dangvard Pedersen *et al*. (2019a) study, the bony alterations described by RIB1 were not specific to TB. The absence of this indicator means that there was still a 58.4% likelihood of a TB diagnosis. The likelihood was only 3.4% in the current study. The Dangvard Pedersen *et al*. (2019a) study found that RIB1 did not have a significant relationship with more than half other indicators in their suite, like was

found in the current study. Both studies could not find an association between RIB1 and TB diagnosis.

Superficial bone proliferation and bean shaped lesions on the ribs (RIB2) were common in this study. These bony alterations were observed, in this study, a total of 34 times and accounted for 75.6% of lesions located on the ribs and 5.8% of all lesions observed in this study. RIB2 lesions were detected in 12.1% of the individuals in the TB sample, 5.5% of the individuals in the control sample and 4.6% in the individuals in the pulmonary sample. This indicator was found frequently in this study and the differences in frequency across the three groups was statistically significant at p=0.030. RIB2 bony changes were found most commonly in the TB sample, and least commonly in the pulmonary group.

RIB2 was the third most sensitive indicator in the suite of 23 lesions. It has a sensitivity estimate of 0.121. The presence of superficial proliferation or lytic lesions on the visceral surface of a rib means that there is a 12.1% probability of a true TB diagnosis. This candidate indicator can be considered as the best in the suite as it is highly sensitive whilst also being very specific to TB. With a specificity estimate of 0.949, this means that in the absence of RIB2 skeletal changes, there is an 8.8% chance of TB being present. Due to this indicator meeting the specificity + sensitivity >1 criterion, the information it provides is considered as beneficial.

Indicator RIB2 had a significant relationship with 16 other indicators (VER, VEN2, ACE1, ACE2, PF1, PF2, DF, PT1, PT2, DH2, PR, PU, CRA1, CRA2, CRA3 and ENDO1). The detection of bony alterations represented by RIB2 therefore means that there is a likelihood of the presence of other indicators with which it has a significant relationship. This indicator was found to be significantly more common in

the TB sample that in controls, however, the p-value was not <0.001. This indicator, therefore, met all but one criterion to qualify it as being useful in showing an association between its skeletal alterations and TB diagnosis.

RIB2 also fared well as a candidate indicator in the Dangvard Pedersen *et al.* (2019a) study. It was found almost three times more frequently in their TB sample (33.7%) and was far less in their control sample (1.3%). These differences were found to be highly statistically significant (p<0.001), a much higher result than what was found in the current study. This indicator was found to be more sensitive (33.7%) and specific (1.3%) to TB than was found in the current study. The Dangvard Pedersen *et al.* (2019a) study's differences in the detection of this indicator across the two groups allowed for RIB2 to meet all the criteria necessary to be accepted as showing an association between its bony changes and TB diagnosis. The differences were, however, not high enough for the current study.

4.9.3 Os coxa indicators

Clustered pits and large cavities found on the articular surface of the acetabulum (ACE1) were the least common lesions located on the os coxa. ACE1 was detected 16 times in this study which accounted for 9.0% of all the os coxa lesions and 2.7% of all the lesions observed in the entire study. These bony changes were seen in 4.7% of the individuals in the TB sample, 2.8% of the individuals in the control sample and 3.7% of the individuals in the pulmonary sample. The study found that this was not a common lesion and its occurrence between groups was not statistically significant (p=0.729).

ACE1 has a sensitivity estimate of 0.047. Clustered pits and large cavities found on the articular surface of the acetabulum of an individual mean that there is a 4.7% probability of that individual having TB. The specificity estimate is 0.968 and in the

absence of these bony changes, there is a probability of 3.2% of that individual having TB. Although this indicator has low sensitivity it is specific enough to be beneficial in the identification of TB in skeletal remains. It does provide useful information pertaining to the sensitivity and specificity of these bony changes as a result of having met the sensitivity + specificity >1 criterion.

Indicator ACE1 has a significant relationship with 13 other indicators (RIB2, VER, VEN2, ACE2, PF1, DF, PT1, PT2, DH1, DH2, PU, CRA1, CRA2, CRA3 and ENDO2) in the suite. The presence of the bony changes represented by this indicator can thus be seen as a good indication of the possible presence of the other indicators with which it has a significant relationship. This indicator was not sensitive enough to TB and was not found significantly more in the TB sample and, as a result, was not deemed to show a useful association between the bony changes it represents and TB diagnosis.

The indicator ACE1 was not common in both the current study and the study by Dangvard Pedersen *et al.* (2019a). That study, however, found that ACE1 was significantly less common in the TB sample than it was in their control sample ($p=0.002$). They also found that ACE1 was relatively sensitive (5.5%) and highly specific (0.4%) to TB which was a stark contrast from the current study (4.7% and 2.8%, respectively). Dangvard Pedersen *et al*. (2019a) did not find ACE1 to have a significant relationship with more than half of the indicators in their suite. As a result, in both studies, ACE1 was rejected as showing an association between the skeletal alterations it represents and TB diagnosis.

ACE2 was represented by deep cavities or the appearance of a woven structure in the acetabular fossa and were the second most common lesions in the study. These

bony alterations were observed a total of 102 times throughout this study and accounted for 60.7% of all os coxa lesions and 17.3% of lesions in the entire study. This indicator was found in 27.9% of the individuals in the TB sample, 20.3% of the individuals in the control sample and 23.7% of the individuals in the pulmonary group. This means that one in every five cases had these skeletal changes across the board, this was reflected in the Fisher's Exact test. The difference in the occurrence of this indicator amongst the three groups was not statistically significant at p=0.293.

ACE2 was the second most sensitive indicator in this study. It has a sensitivity estimate of 0.279. This means that in the presence of deep cavities or the appearance of a woven structure in the acetabular fossa is indicative of a 27.9% probability of a true TB diagnosis. Having a specificity estimate of 0.784 means that when there are no such bone alterations, there is a 21.6% probability of TB being present. This makes ACE2 not very specific to TB, however, due to the fact that it met the sensitivity + specificity >1 criterion it does provide useful information about the said bony changes it represents.

Indicator ACE2 was one of only two indicators which has a significant relationship with all the other indicators in the study. The presence of deep cavities in the acetabular fossa can, as a result, indicate the increased likelihood of the presence of all the indicators in the study. The high occurrence of this indicator across all three groups resulted in it not being a suitable candidate lesion that can be used to show an association between the bone alterations and TB.

ACE2 was one of the candidate indicators that had the best results in the Dangvard Pedersen *et al*. (2019a) study. As with the current study, ACE2 skeletal alterations

were very common in their TB sample (42.0%), the difference here was that there was a very low occurrence of this indicator in their control sample (6.5%). This meant that ACE2 showed statistically significant frequency between the TB and control samples ($p<0.001$). This indicator was also highly sensitive (42.0%) and specific (6.5%) in that study compared to the high sensitivity, but low specificity (20.6%) found in the current study. Both studies determined that ACE2 had a significant relationship with at least half of the indicators in the respective suites. All of this considered, the Dangvard Pedersen *et al*. (2019a) could determine that ACE2 was an indicator that showed an association between the skeletal changes it represented and TB diagnosis whereas the same could not be concluded for the current study.

BOD was seen as proliferation or large pits identified on the lateral body of the ilium. BOD bony alterations were detected a total of 25 times in this study and accounted for 14.9% of all the lesions located on the os coxa and 4.2% of the lesions detected in the entire study. These bony changes were found in 5.8% of the individuals in the TB group, 4.2% individuals in the control and 8.4% in the pulmonary group. Although the differences in the occurrence of BOD across the three groups was statistically insignificant ($p=0.380$), these changes occurred most commonly in the pulmonary group.

The sensitivity estimate for indicator BOD is 0.058. This estimate means that there is a 5.8% chance that a person had TB when proliferation or large pits were present of the lateral body of the ilium. However, with a specificity estimate of 0.706 there is a 29.4% chance of a person having TB when these bony changes are absent in an individual. There is a far greater chance of an individual having TB when they do not possess these changes than there is when the changes are present. Indicator BOD did not meet the sensitivity + specificity >1 criterion and provides no new information

regarding the sensitivity and specificity of these bony alterations. This indicator, therefore, is of no benefit when in the quest of identifying TB in skeletal remains.

Thirteen indicators (RIB1, VEN2, ACE2, PF1, PF2, DF, PT1, PT2, DH1, PR, PU, CRA1, CRA2, CRA3 and ENDO1) had a significant relationship with indicator BOD. This means that the presence of proliferation or large pits identified on the lateral body of the ilium increases the chances of the presence of the other indicators with which it has a significant relationship. This indicator did not meet the criteria to qualify as being useful in showing an association between its skeletal alterations and TB diagnosis.

Indicator BOD tested well in the Dangvard Pedersen *et al*. (2019a) study. The indicator was found significantly more in their TB sample than in controls ($p<0.001$), it was significantly related to at least more than half of the other candidate indicators in their study and had a sensitivity estimate that exceeded 0.05. For their study, indicator BOD showed an association between the bony alterations it represents and TB diagnosis during life. The indicator BOD was not very commonly seen in this current study (5.8%), however, it was relatively common in the Dangvard Pedersen *et al*. (2019a) study as it was present in 33.2% of individuals in the TB sample. For the current study, the difference between the occurrence of BOD in the three samples was very small and the difference was not statistically significant ($p=0.611$) and thus did not meet the criteria.

Indicator ILI represented clustered pits or large cavities observed on the iliac auricular surface. These changes were observed a total of 25 times in the entire study and accounted for 14.9% of lesions located on the os coxa and 4.2% of lesions in the whole study. These bone alterations were observed in 4.8% of the individuals

in the TB sample, 7.9% of the individuals in the control group and 5.7% of the individual in the pulmonary sample. Although the differences in frequency amongst the three groups was statistically insignificant (p=0.567), the TB group had the smallest occurrences of indicator ILI.

The sensitivity estimate for indicator ILI is 0.048. This means that if clustered pits or large cavities are observed on the iliac auricular surface, there is a 4.8% probability of that individual having TB. With a specificity estimate of 0.930 the probability becomes 7.0% when the changes are absent in an individual. This lesion's ability to correctly identify TB is low. Indicator ILI also did not meet the sensitivity + specificity >1 criterion and can therefore not be considered as being reliable as a source of information relating to TB.

Indicator ILI has a significant relationship with 15 other indicators (RIB1, VEN2, ACE2, PF1, PF2, DF, PT1, PT2, DH2, PR, PU, CRA1, CRA2, CRA3 and ENDO1) in the current study. The detection of clustered pits or large cavities are observed on the iliac auricular surface increases the probability of the indicators with which ILI has a significant relationship. This indicator did not meet the criteria necessary to qualify it as showing an association with skeletal TB.

In the Dangvard Pedersen *et al*. (2019a) study, ILI was a commonly observed indicator in their TB group (29.6%). The occurrence was low in the current study (5.8%). For the control group, indicator ILI was more frequent in the current study (7.9%) than it was in the comparative study (5.8%). The results for the Dangvard Pedersen *et al*. (2019a) study were good and found to be statistically significant ($p<0.001$). Contrary to the sensitivity of ILI in this study, it had a sensitivity of 29.6% in the Dangvard Pedersen *et al*. (2019a) study and higher specificity to TB (5.8%).

The indicator had a significant relationship with more than half of the indicators in the respective studies. It met the criteria for showing an association between the skeletal changes it represents and a TB diagnosis in the Dangvard Pedersen *et al*. (2019a) study.

4.9.4 Femur indicators

The presence of clustered pits or cavities on the head of the femur is represented by PF1. These skeletal changes were detected only once in this study and accounted for 11.1% of the femoral cases and 0.2% of lesions found in the entire study. The bone alterations were found in 0.6% of the individuals in the TB sample, and none were found in any of the other sample groups. Femoral lesions were a rare occurrence in this study.

Indicator PF1 has a sensitivity estimate of 0.006. This means that when clustered pits or cavities are present on the head of a femur, there is a 0.6% chance of a true TB diagnosis. The specificity estimate is 1.000, this means that there are no false positives for this skeletal lesion and PF1 is very specific to TB. Changes of this nature are thus only found in individuals that are infected with TB. This result should be treated with caution due to the small number of positives, but this should be further investigated in future.

Indicator PF1 had a significant relationship with 15 other indicators in the study (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, PF2, ILI, DH1, PR, CRA2, ENDO1 and ENDO2). The presence of clustered pits or deep cavities on the head of the femur can be used to determine the presence of absence of the indicators with which PF1 has a significant relationship. This indicator failed to meet the criteria necessary to show that there is an association between the bony reactions seen in

PF1 and a TB diagnosis that was made during life due to its low frequency and sensitivity. It is worth future consideration, however, because its presence in a skeleton is highly characteristic.

Indicator PF1 was also rare in the Dangvard Pedersen *et al*. (2019a) study and the differences found between the two groups were not statistically significant (p=0.221). In both that and the current study, this indicator was not found to be sensitive enough to TB, however, Dangvard Pedersen *et al*. (2019a) found this indicator to be relatively specific to TB (1.3%), as was also found in the current study. PF1 was rejected also by Dangvard Pedersen *et al*. (2019a) as having any association with a TB diagnosis that was made during life due to the reasons already mentioned above.

The presence of clustered pits or large cavities on the greater trochanter of the proximal femur (PF2) was the most common lesions located on the femur. These skeletal changes were observed seven times in this study and accounted for 77.8% of the lesions that were located on the femur and 1.2% of all the lesions found in this study. Indicator PF2 was detected in 2.3% of the individuals in the TB sample, 1.4% of the individuals in the control sample and 0.9% of the individuals in the pulmonary sample. The differences in occurrence across the three groups were found to not be statistically significant (p=0.711), however, more of these bony changes were found in the TB group.

The sensitivity estimate for PF2 is 0.023. This means that there is a probability of 2.3% of a true TB diagnosis if there are clustered pits or large cavities are found on the greater trochanter of the proximal femur. Additionally, in the absence of these alterations, there is a 1.4% chance for the presence of TB because its specificity estimate is 0.986. The sensitivity + specificity criterion was met and thus these

results are considered to provide useable information regarding sensitivity and specificity of the bony alteration in question.

Indicator PF2 has a significant relationship with 13 other indicators in the suite (RIB2, VER, VEN1, VEN2, BOD, ACE2, PF1, ILI, DF, DH1, PR, CRA2 and ENDO2. The presence of clustered pits and deep cavities on the head of the femur increase the likelihood of the indicators with which the indicator has a significant relationship. This indicator did not meet the all three criteria necessary to show and association with the skeletal changes it denotes and a TB diagnosis.

The indicator PF2 was more common in the control than the TB group of the Dangvard Pedersen *et al*. (2019a) study. In both studies the differences between the test and control groups were not statistically significant. This indicator was not sensitive to TB, but the specificity was high in both studies. PF2 did not meet the criteria necessary to illustrate its association to TB diagnoses made in life in both studies.

Indicator DF was identified when clustered pits and large cavities were observed on the articular surface of the distal femur. These skeletal changes were detected only once in the entire study and accounted for 11.1% of lesions located on the femur and 0.7% of lesions detected in the whole study. This indicator was only found in the TB sample (0.6%). These skeletal changes were rare in this study.

The sensitivity estimate for indicator DF is 0.006. This means that there is a 0.6% chance of a true TB diagnosis if clustered pits and large cavities are observed on the articular surface of the distal femur. The specificity estimate is 1.000 and one minus specificity for DF is 0% which means that this indicator has no false positives and only found in individuals with TB. Similar to what was the case for PF1, the lesion

seems to be very specific but due to its low incidence this result needs to be treated with caution. However, it does warrant further investigation.

Even though only one case showed the skeletal changes represented by indicator DF, it had a significant relationship with 15 other indicators (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, PF2, ILI, DH1, PR, CRA2, ENDO1 and ENDO2) in the suite. The presence clustered pits and large cavities observed on the articular surface of the distal femur increases the likelihood of the presence of the bony changes represented by the indicators with which DF has a significant relationship. The indicator did not meet all the criteria that qualifies as having an association with a TB diagnosis made during life, but its high specificity found in the current study may hold promise which should be further investigated.

4.9.5 Tibia indicators

The presence of clustered pits or large cavities observed on the articular surface of the proximal tibia were represented by PT1. These skeletal alterations were only detected once in throughout the study and accounted for 25.0% of the lesions detected on the tibia and 0.2% of lesions detected in the entire study. The lesion was found in the TB sample (0.6%), although it was rare.

The sensitivity estimate for indicator PT1 is 0.006. When clustered pits or large cavities are observed on the articular surface of the proximal tibia, this means that there is a 0.6% chance of that individual having TB. The specificity estimate here is 1.000 and one minus specificity for PT1 is 0% which means that this indicator has no false positives, and it is only found in individuals with TB. As with the femoral lesions, this may not be a useful lesion to use in the identification of TB in skeletal remains as it has a very low frequency. However, its high specificity holds promise.

Indicator PT1 has a significant relationship with 15 other indicators (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, PF2, ILI, DH1, PR, CRA2, ENDO1 and ENDO2) in the suite. The presence of clustered pits or large cavities observed on the articular surface of the proximal tibia increase the probability of the presence of the bony alterations represented by the indicators with which PT1 has a significant relationship. This indicator did not meet all the criteria that qualify these bone changes as having an association with a TB diagnosis, although the high specificity shows that it holds promise.

Indicator PT1 was rare in both the current and the Dangards Pedersen *et al*. (2019) study. The p-value was 1.000 in both studies which means that the differences between TB and control samples are not statistically significant. This indicator was not sensitive enough to detect the presence of TB in both studies.

The presence of large pits or proliferation of bone in the intercondylar area of the proximal tibia was represented by indicator PT2. These bone alterations were observed a total of three times throughout the study and accounted for 75.0% of the lesions identified on the tibia and 0.5% of the lesions found in the entire study. Large pits or proliferation of bone in the intercondylar area of the proximal tibia were observed in 1.1% of the individuals in the TB sample, 0.7% of the individuals in the control group and none were observed in the pulmonary sample group. These skeletal changes were rare in this study.

The sensitivity estimate for indicator PT2 is 0.011. The observation of large pits or proliferation of bone in the intercondylar area of an individual means that there is a 1.1% chance of that individual having TB. Due to a specificity estimate of 0.996, the probability of a true TB diagnosis is 0.4% when these bony changes are absent in an

individual. This lesion is not sensitive or specific enough to aid in the identification of TB in skeletal remains.

Indicator PT2 has a significant relationship with 13 other indicators (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1, CRA2, ENDO1 and ENDO2) in the suite. The detection of PT2 intensifies the likelihood of the presence of the skeletal changes associated with the indicators with which it has a significant relationship. This indicator did not meet the criteria meant to qualify it as having an association with the diagnosis of TB made during life.

Both the current study and that of Dangvard Pedersen *et al*. (2019a) found indicator PT2 to be a rare occurrence and the difference between the TB and control groups not to be statistically significant (p=1.000). This indicator was not sensitive to the presence of TB in both these studies although its specificities were high in that study as well as the current one (0.1 and 0.7%, respectively). Both studies rejected this indicator as not having an association with TB diagnosis.

4.9.6 Humerus indicators

Clustered pits present on the posterior portion of the humerus on either the lateral or medial side (DH1) were relatively common. These bony alterations were observed a total of 35 times in this study and accounted for 92.1% of lesions located on the humerus and 5.9% of lesions found in the entire study. Indicator DH1 was detected in 9.3% of the individuals in the TB sample, 5.6% of the individuals in the control sample and 10.4% of the individuals in the pulmonary sample. Although the difference in occurrences amongst the groups were not found to be statistically significant, DH1 was most common in the pulmonary group and this already provides insight into the indicator's sensitivity to TB.

The sensitivity estimate for indicator DH1 is 0.093. This means that there is a 9.3% probability of an individual having TB if clustered pits are present on the posterior portion of that individual's humerus on either the lateral or medial side. A specificity estimate of 0.923 means that in the absence of these bony alterations, there is a 7.7% probability of that individual having TB. Regardless of indicator DH1 being most commonly present in the pulmonary sample, it was still relatively sensitive for TB.

Indicator DH1 has a significant relationship with 16 other indicators in the suite of 23 lesions (RIB1, VEN2, ACE1, ACE2, PF1, PF2, DF, PT1, PT2, DH2, PR, PU, CRA1, CRA2, CRA3 and ENDO1). The presence of clustered pits on the condylar area of the distal humerus increases the possibility of the presence of the indicators with which DH1 has a significant relationship. This indicator did not meet the criteria necessary to qualify it as having an association to TB diagnosis due to the difference in its occurrence amongst the three sample groups not being statistically significant.

The results pertaining to skeletal alterations of DH1 for the current study are similar to those found by Dangvard Pedersen *et al.* (2019a). They also found that indicator DH1 was more common in the TB sample than in controls and in their study this difference was statistically significant ($p=0.007$). Although this indicator was not sensitive (3.4%) to TB in their study it was highly specific (0.1%). Although this indicator had a significant relation with more than half of the other indicators in the suites in the respective studies, it was not associated with a TB diagnosis because the difference between TB and control samples did not meet the $p<0.001$ criterion.

Clustered pits or large cavities on the articular surface of the humerus (DH2) were rarely detected in this study. These bony alterations were detected a total of 3 times

in this study and these accounted for 7.9% of all lesions that were detected on the humerus and 0.5% of lesions detected in the entire study. DH2 skeletal alterations were found in, 0.6% of the individuals in the TB sample, 1.4% of the individuals in the control sample and none were recorded in the pulmonary sample. Although the difference between the three sample groups was not statistically significant (p=0.619), this indicator was found more commonly in the control sample and this was reflected in the sensitivity estimate.

The sensitivity estimate for indicator DH2 is 0.006. This means that there is a 0.6% chance that an individual with clustered pits or large cavities on the articular surface of the humerus has TB. Indicator DH2 had a specificity estimate of 0.986. The absence of these skeletal alterations means that there is a 1.4% chance of a true TB diagnosis. The sensitivity + specificity being >1 indicate that these estimates provide valuable information about these skeletal changes albeit not beneficial to the detection of TB.

DH2 had significant relationships with only 10 other indicators (RIB1, RIB2, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1 and ENDO2) in the suite, this is less than half of the indicators in the suite. This means that clustered pits or large cavities on the articular surface of the humerus increases the chances of the presence of the indicators with which DH2 has a significant relationship. This indicator, however, did not meet all the criteria meant to qualify it as showing an association between the said bone changes and a TB diagnosis made during life.

A notable difference between the current study and that of Dangvard Pedersen *et al*. (2019a) pertaining to the frequency of indicator DH2 is how in the latter study, this indicator was found more commonly in the TB (2.1%) than the control sample

(0.0%). The difference between the two groups were, however, not statistically significant in both studies. When only comparing the TB and control samples to ascertain the sensitivity and specificity for indicator DH2, the results are not reliable enough to provide useful information (sensitivity + specificity <1) when only using the control and TB samples (Table 4.5). This, however, was not the case in the Dangvard Pedersen *et al*. (2019a) study, where the indicator DH1 was not sensitive enough (2.1%) but was found to be specific to TB (0.1%). For both studies this indicator was determined to not have an association to a TB diagnosis.

4.9.7 Radius and ulna indicators

Clustered pits or large cavities on an individual's radial tuberosity (PR) were rare in this study. It was detected a total of 8 times in the entire study and accounted for 80.0% of all the lesions found in the forearm and 1.4% of the lesions found in the entire study. These bone changes were detected in, 3.5% of the individuals in the TB sample, 0.7% of the individuals in the control sample and 1.0% of the individuals in the pulmonary sample. The difference amongst the three groups was not statistically significant, however, more of these skeletal changes were observed in the TB sample.

The sensitivity estimate for indicator PR is 0.036. The presence of clustered pits or large cavities on an individual's radial tuberosity would mean that there is a 3.6% chance of that individual having TB. A specificity estimate of 0.992 means that there is a 0.8% chance of a true TB diagnosis for that individual. This lesion is therefore not sensitive but it is specific enough to be useful in the identification of TB in a skeletal sample. The estimates determined for indicator PR were considered reliable (sensitivity + specificity >1) although they were not beneficial to TB in particular.

Indicator PR has a significant relationship with 13 other indicators (RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, PF2, ILI, DF, PT1, DH1, PU, CRA2 and ENDO2) in the suite. The presence of clustered pits or large cavities on the radial tuberosity increases the chances of the presence of the bony changes associated with the other indicators with which indicator PR has a significant relationship. The indicator did not meet the criteria necessary to qualify its features as having an association with TB diagnosis as a result of not being sensitive to TB and its frequency not being significantly different amongst the three sample groups.

In both the current and the Dangvard Pedersen *et al.* (2019a) study the skeletal alterations of indicator PR were found more frequently in the TB group than they were in the control group, however, these findings were not statistically significant. In both studies indicator PR was not sensitive to TB but was found to be highly specific to it and both studies provided reliable information in this regard (sensitivity + specificity >1). Both studies rejected indicator PR as not having an association with TB diagnoses made during life due to its insignificant frequency across TB and control samples as well as its low sensitivity to TB and thus only meeting one of the three criterion.

Clustered pits or large cavities on the olecranon process of the proximal ulna (PU) were rare in this study. These bony changes were found a total of 2 times in this study and accounted for 20.0% of the lesions detected on the forearm and 0.3% of lesions detected in the entire study. Both the lesions that were detected for indicator PU were found in the control sample (1.4%); none were detected in the TB or the pulmonary groups.

The sensitivity estimate for indicator PU is 0.000. This means that this indicator is not sensitive to TB. Using this lesion will result in a lot of false positives from the sample that is being tested. The specificity estimate is 0.992 which means that there is a 0.8% probability of a true TB diagnosis if there are no clustered pits or large cavities on the olecranon process of the proximal ulna. This lesion is not useful in the identification of TB in a skeletal sample.

Although rare, indicator PU has a significant relationship with 13 other indicators (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1, PR, CRA2, ENDO1 and ENDO2) in the suite. This means that the presence of clustered pits or large cavities on the olecranon process of the proximal ulna increases the probability of the presence of the bony changes observed for the indicators that indicator PU has a significant relationship with. This indicator failed to meet two of the three criteria necessary to qualify its skeletal changes as having an association with TB diagnoses made during life.

In contrast to the current study, Dangvard Pedersen *et al.* (2019a) observed indicator PU frequently. The difference between the indicator's frequency in the TB (9.2%) and control (1.7%) samples was statistically significant ($p<0.001$) in that study. They also found that this indicator was not only sensitive (9.2%) but also highly specific (1.7%) to TB. The only similarity between the two studies was that it had a significant relationship with more than half the indicators in the respective suites. Indicator PU was, as a result, accepted as having an association to TB diagnoses in the Dangvard Pedersen *et al.* (2019a) study.

4.9.8 Cranial indicators

Clustered pits or large cavities on the anterior cranial fossa (CRA1) were rare in this study. These bony alterations were observed a total of three times and accounted for 4.5% of the lesions located on the cranium and 0.5% of all lesions observed in this study. Indicator CRA1 was detected in, 2.8% of the individuals in the TB sample group. In the pulmonary and control sample groups no such skeletal alterations were observed, however, the difference in frequency across the three groups was not statistically significant (p=0.116).

The sensitivity estimate for indicator CRA1 is 0.028. This means that in the presence of clustered pits or large cavities on the anterior cranial fossa, there is a 2.8% chance of a true TB diagnosis. The specificity estimate is 1.000 which means that indicator CRA1 has no false positives and is only found in individuals with TB. Due to its low frequency it is probably not a very good general indicator of TB, but if present, may assist in providing a diagnosis of TB. More research is needed.

Indicator CRA1 did not have a significant relationship with more than half the indicators in the suite; it only had such a relationship with 12 other indicators (RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1, CRA2, CRA3 and ENDO1). This means that clustered pits or large cavities on the anterior cranial fossa increases the likelihood of the presence of the skeletal alterations observed for the indicators with which these bony changes have a significant relationship. It is notable, though, that this association included the vertebrae, which are common sites for TB infection as was demonstrated in this study. If, for example, both CRA1 and VEN2 are present, the possibility of a TB diagnosis should be considered. This indicator did not meet two of the criteria necessary to qualify it as having an association to TB diagnoses made during life.

Clustered pits and large cavities observed in the middle cranial fossa (CRA2) were not uncommon in this study. These skeletal changes were observed a total of 36 times throughout the study and accounted for 54.5% of the lesions detected on the cranium and 6.1% of the lesions detected in the entire study. Indicator CRA2 was observed in, 16.8% of the individuals in the TB sample, 10.0% of the individuals in the control sample and 12.7% of the individuals in the pulmonary sample. The difference in frequency across all three groups was not statistically significant (p=0.602), however, indicator CRA2 occurred more frequently in the TB group and the least in the control group.

The sensitivity and specificity estimates provided valuable information regarding CRA2 bony changes. The sensitivity estimate for indicator CRA2 is 0.168. This means that there is 16.8% chance of an individual having had TB if clustered pits and large cavities are observed on that individual's middle cranial fossa. The specificity estimate is 0.888 and this means that there is an 11.2% chance of an individual having had TB given that the bony changes were absent. This indicator is thus not highly specific to TB.

Indicator CRA2 had a significant relationship with all the indicators (21) in this suite except for one (DH2). This means that clustered pits and large cavities are observed on that individual's middle cranial fossa increases the probability for the presence of the changes associated with the indicators with which indicator CRA2 has a significant relationship. This indicator missed one requirement necessary to qualify it as having an association with TB diagnoses made during life. Once again this is an indicator that holds promise, as it is associated with other lesions considered to be due to TB, and occurred relatively frequently in TB and pulmonary cases. More research on this is warranted.

Clustered pits or large cavities in the posterior cranial fossa (CRA3) were very rare in this study. These bony alterations were only observed once in this study and accounted for 1.5% of the lesions detected on the cranium and 0.2% of lesions observed in the entire study. The lone identification on indicator CRA3 was done in the control sample; both the pulmonary and TB sample groups had no such bony alterations. The difference in frequency across the three groups were not statistically significant. This lesion will therefore not assist at all to diagnose TB.

The sensitivity estimate for indicator CRA3 is 0.000. This means that this indicator is not sensitive to TB. Using this lesion will result in a lot of false positives from the sample that is being tested. The specificity estimate for indicator CRA3 is 0.994 which means that there is a 0.6% chance of a true TB diagnosis when there are no clustered pits or large cavities in the posterior cranial fossa. These bony changes are reliable in for the identification of TB in a skeletal sample (sensitivity + specificity <1).

Fourteen other indicators (RIB1, RIB2, VER, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1, CRA1, CRA2, ENDO1 and ENDO2) had a significant relationship with indicator CRA3 in the suite. This means that the presence of clustered pits or large cavities in the posterior cranial fossa increases the probability of the presence of the indicators with which indicator CRA3 has a significant relationship. This indicator was shown not have an association to TB diagnoses made during life due to not meeting two of the three criteria.

The discolouration and branching excavations on the inferior endocranium (ENDO1) were uncommon in this study. These bony changes were observed a total of 5 times and accounted for 7.6% of the lesions detected on the cranium and 0.8% of lesions observed in the entire study. Indicator ENDO1 was observed in, 3.7% of the

individuals in the TB group, 1.4% of the individuals in the pulmonary group and no such changes were observed in the control group. The difference in frequency across the three groups were not statistically significant (p=0.183).

The sensitivity estimate for indicator ENDO1 is 0.037. When discolouration and branching excavations are present on the inferior endocranium, there is thus a 3.7% probability of a true TB diagnosis. The specificity estimate is 0.994, this means that there is a 0.6% chance of a true TB diagnosis when these bone alterations are absent.

Indicator ENDO1 has a significant relationship with 15 other indicators (RIB2, VER, VEN1, VEN2, BOD, ACE2, PF1, ILI, DF, PT1, DH1, CRA1, CRA2, CRA3 and ENDO2) in the suite. This means that discolouration and branching excavations are present on the inferior endocranium increases the probability of the presence of the bony alterations observed for the indicators with which ENDO1 has a significant relationship. This indicator did not meet the criteria necessary to qualify its bony alterations as having an association with TB diagnosis made during life.

The presence of discolouration and branching excavations on the superior endocranium (ENDO2) was not uncommon in this study. These skeletal alterations were detected a total of 21 times throughout the study and accounted for 31.9% of the lesions detected on the cranium and 3.6% of lesions detected in the entire study. The indicator ENDO2 was observed in 12.9% of the individuals in the TB sample, 4.6% of the individuals in the control sample and 5.9% of the individuals in the pulmonary sample. The difference in the frequency across all three groups was not statistically significant (p=0.102).

The sensitivity estimate for indicator ENDO2 is 0.129. This means that there is 12.9% probability of an individual presenting with discolouration and branching excavations on the superior endocranium to have had TB. The specificity estimate is 0.949 which means that there is a 5.1% chance of a true TB diagnosis when these bony alterations are absent in an individual. This indicator met the sensitivity + specificity >1 criterion.

Indicator ENDO2 has a significant relationship with 15 other indicators (RIB1, VEN2, ACE1, ACE2, PF1, PF2, DF, PT1, PT2, DH2, PR, PU, CRA2, CRA3 and ENDO1) in the suite. This means that the presence discolouration and branching excavations on the superior endocranium increases the chances of the presence of the indicators with which indicator ENDO2 has a significant relationship. Due to this indicator not being found significantly more in the TB sample than in the pulmonary and control samples, it did not meet the criteria necessary to qualify it as having an association with TB diagnoses made during life. However, it may hold some promise that needs further investigation.

Chapter 5: Discussion

All skeletons used for the current study were sourced from the Raymond A Dart Collection of Human Skeletons housed in the School of Anatomical Sciences at the University of the Witwatersrand. Prior to 1946, the collection comprised entirely of unclaimed bodies which were obtained from the Gauteng Provincial Hospitals (Kramer and Hutchinson, 2015). During that period Gauteng was still experiencing the glory days of 'the gold rush'. Since the discovery of vast gold deposits in the area and its surroundings in 1886, Johannesburg became the epicentre of rapid industrialisation (Callinicos, 1985). The thriving mining industry, industrial development and dreadful living as well as working conditions were favourable conditions for the rapid spread of TB in the region (van Rensburg *et al*., 2005). In assessing the results of the current study, it was essential to keep in mind that in the control and pulmonary diseases sample groups there was a high probability of the individuals in these groups having been infected with TB even if it was not what had ultimately caused their deaths. Comorbidities and undiagnosed disease thus remain major concerns that should be kept in mind when interpreting the results of this study. Some diseases included in the pulmonary group, such as bronchiectasis, may also have been caused by TB.

5.1 Skeletal indicators of TB

5.1.1. Spinal indicators

The spine has been documented as being the most common and most characteristic area of skeletal lesions in cases of TB infection (Ortner, 2003; Roberts and Buikstra, 2003; Vigorita, 2008; Turgut *et al*., 2017). Roberts and Buikstra (2003) outlined the mechanisms that can lead to the infiltration by the TB bacteria into the three different areas of the vertebrae leaving behind the kinds of lesions described in this research.

The findings of the current study relating to indicators VEN2 and VEN1 are consistent with what has been seen in other studies. Vigorita (2008) described how this presents in a clinical setting and how it will look on a radiograph. A study by Resnick and Niwayama (1995) as cited by Ortner (2003) highlighted that the involvement of the spine is almost always isolated to the vertebral body and the anterior portion in particular. As described above, the pulmonary cohort had a higher percentage of individuals who presented with bone proliferation on the ventral part of vertebral bodies than the TB cohort.

The fact that the vertebral lesions were found so often in the pulmonary group as well, could suggest that either (a) pulmonary diseases other than TB also frequently affect the vertebral bodies, or (b) that many people in the pulmonary cohort had, in fact, suffered from TB during some stage of their lives. Some studies would suggest that the latter is more likely. The spinal column is prone to a plethora of diseases; however, the most common chronic infection involvement that affects it is TB (Tuli, 2010). A study by van Schaik *et al.* (2014) which looked into the disease burden of the past using a modern comorbidity index of disease severity found that 15.4% of people who were recorded as having died of pneumonia had concomitant TB. Given the fact that TB is so common in South Africa, this possibility of undiagnosed / unstated TB should be strongly considered. It is thus quite possible that many individuals in the pulmonary group had suffered from TB at some stage during their lifetime. This would explain why, for all the spinal lesions, the pulmonary cohort had the highest percentage of involvement.

Spinal alterations continue to be the most important identifiers of the presence of TB in skeletal remains. The spinal lesions in this study provided very important information that can be used in the identification of TB in this setting. The study has

provided evidence that these lesions are indeed sensitive to TB. Their low specificity to TB is only indicative of the inherent flaw in the methodology utilised to compile the control groups in this study. Adjustments to the problematic elements can help to further demonstrate the extent to which these skeletal changes can be beneficial in TB identification in palaeopathological studies.

Skeletal changes observed on the vertebral column are often characteristic of tuberculous disease (Tuli, 2010). It is, however, important to differentiate between spinal TB from pyogenic spondylitis, brucellar spondylitis and other central nervous system infections (Garg and Somvanshi, 2016). Changes due to brucellar spondylitis are commonly found in the lumbar region and not the thoracic region as is often the case in TB (Tuli, 2010; Garg and Somvanshi, 2016). Although pyogenic spondylitis can result in the destruction of the intervertebral disc, this is not often accompanied by vertebral damage (Garg and Somvanshi, 2016).

5.1.2 Rib indicators

Rib lesions were initially not given much attention because they were considered to be an uncommon manifestation of TB, however, in 1980 Brown studied seven cases with possible TB which showed a higher incidence of rib destruction. Kelley and Micozzi (1984) hypothesised that lesions located on ribs are specifically associated with tubercular infections. To test this, they used a control sample which included remains of people who had died of non-tuberculous pulmonary conditions such as pneumonia and they found their hypothesis to hold true. In 2019 (a), Dangvard Pedersen *et al*. also found more bone proliferation on ribs in their TB sample than in the control. There have been several studies that have highlighted the significance of bone proliferation and lytic lesions in the identification of pulmonary TB (e.g., Matos and Santos, 2006; Raff *et al.*, 2006; Davies-Barrett *et al*., 2019). Bone proliferation or

lytic lesions on the visceral surface of ribs, RIB2, has produced ideal results in the demonstration of these alterations' association to TB. The frequency of these changes was always statistically significant when the other two groups were being compared to TB. This particular indicator can be considered as a bony feature that is a good indication of TB and it can thus be concluded that these skeletal changes occur more in TB cases that they do in other diseases that were in the pulmonary and TB groups. A study by Santos (2000) found that 90% of the individuals who were documented as having died of pulmonary TB had new bone formation on the visceral surface of their ribs. There were almost as many low nodules, RIB1, in the control sample (3.4%) as there were in TB sample (3.5%) and none were observed in the pulmonary sample. In the Dangvard Pedersen *et al*. (2019a) they found that the indicator RIB1 was more common in the control group (58.4%) than it was in the TB group.

The involvement of the ribs due to TB infection varies across scientific studies. Clinically, the involvement of the thoracic cage has been reported to be a rarity (Khan *et al*., 2007; Tuli, 2010). When the changes are observed radiographically, this usually happens long after clinical symptoms (Tuli, 2010). Studies that observed changes in dry bone find higher incidences of rib involvement and Ortner stated that in areas such as South Africa, where TB is endemic, it will indisputably be the most likely cause of rib lesions (Ortner, 2003). For the Dangvard Pedersen *et al*. (2019a) study, however, it may be prudent attempt to differentiate these changes from osteomyelitis, other infectious infections affecting the pleura, fibrous dysplasia etc.

5.1.3 Os coxa indicators

The hip is said to be the second most affected element of the skeleton in skeletal TB (Ortner, 2003; Roberts and Buikstra, 2003; Vigorita, 2008). The possible mechanism

responsible for this sequence of events was eloquently described by Roberts and Buikstra (2003). They indicated that subsequent to vertebral involvement, it is possible for the infection to extend to the psoas muscle which originates from the lumbar vertebrae and inserts at the lesser trochanter. This allows for ease of access to the joint space and secondary infection to the area (Ortner, 2003). Tuli (2010) also indicated that the initial focus of the tuberculous lesions is often the acetabulum, the location of indicators ACE1 and ACE2. It has also been reported that the hip joint accounts for 20% of skeletal TB (Aufderheide and Rodriguez-Martin, 1998). The Dangvard Pedersen study corroborates these findings as can be seen from their results. For the current study hip involvement as described by ACE2, which is reported to be the second most common location of TB involvement, was very common across all the sample groups which corroborates the assertion that there may have been individuals who had suffered from TB that unwittingly ended up in the control and pulmonary samples.

According to Ortner (2003), the sacroiliac joint is the second most common area of TB involvement in the pelvis, which is in contrast to the findings of this study. Tuli (2010) also stated that because this joint is a synovial joint, it can be subject to tuberculous infection. In an early study by Kelley and El-Najjar (1980) showed an almost 8% incidence of the sacroiliac joint of confirmed TB cases from the Hamann-Todd Osteological Collection. In the current study the TB group had the lowest positive results for indicator ILI, and this condition was in fact most commonly present in the control cohort. This too may be explained by research that stated that isolated tuberculous lesions on the ilium are rare in comparison with other joints (Roberts and Buikstra, 2003) and its presence may suggest a cause that is not TB. Ankylosing spondylitis, rheumatoid disease, pyogenic infection and neoplastic

lesions should be considered in the differential diagnosis as these may lead to the involvement of this joint (Tuli, 2010).

5.1.4 Femur indicators

In general, femur lesions associated with TB are fairly common. The same mechanisms that allow for the invasion of tubercle bacilli into the acetabulum can result in the involvement of the proximal femur (Ortner, 2003). This in essence means that in addition to the haematogenous route, direct extension to the hip joint can result by contact in cases where there is an abscess from vertebral or pelvic tuberculosis (Ortner, 2003). The proximal and distal femur are some of three prime locations for extra-pulmonary TB involvement (Roberts and Buikstra, 2003). This was not found in the current study. Indicator PF2, clustered pits and cavities on the greater trochanter, was observed more often than PF1, Clustered pits and cavities on the femoral head. According to Tuli (2010) the femoral head is the most commonly affected location on the femur and the greater trochanter is the least common. Although the greater trochanter has been described as a rare location for TB involvement, a lesion in this area would be characteristic (Ortner, 2003).

The distal femur, DF, was expected to have frequent involvement associated with TB because the knee is sometimes reported as being the third most common area of TB involvement accounting for as much as 10% of all skeletal tuberculous lesions (Tuli, 2010; Uboldi *et al*., 2017). Uboldi *et al.* (2017) confirmed a case of TB that had resulted in formation of a lesion on the lateral femoral condyle. Only one such case was observed in the current study. The Dangvard Pedersen et al. (2019a) found lesions in this location in only 2% of the individuals documented to having died of TB. It is possible that the knee has increased in incidence in the post antibiotic era. Both

the current as well as the Dangvard Pedersen *et al*. (2019a) studies were conducted on individuals who died during the pre-antibiotic era.

5.1.5 Tibia indicators

The knee joint is considered to be a weight bearing area in the body and previous research has shown that such areas are terminus points for tubercle bacilli infiltration which subsequently leads to lesions in these areas (Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003; Roberts and Buikstra, 2003; Tuli, 2010). As with the distal femur, there were expectations of seeing a higher incidence of the involvement of the proximal tibia as it too is part of the knee joint. However, in the current study, the tibia indicators were second only to the ulna indicators for least number of positive results in the entire study. The Dangvard Pedersen study also had a very low incidence of the involvement of TB associated changes located on the tibia.

5.1.6 Humerus indicators

Two to five percent of cases of skeletal TB are said to be located at the elbow joint (Tuli, 2010). Tuli (2010) also explained that the area most affected in cases of elbow joint infiltration is the distal humerus. This is consistent with what was found in the current study, however, the lesions observed on the articular surface were not as common as those on the posterior condylar surface.

5.1.7 Radius and ulna indicators

Tuli (2010) suggested that tuberculous infiltration of the humerus was probably due to spread from the olecranon. This would suggest that the olecranon would show at least as much skeletal involvement of TB as the distal humerus. However, this was not what was found in the current study. There was much less involvement of the proximal ulna ‘PU’ as compared to the distal humerus (‘DH1’ and ‘DH2’). Instead,

more lesions were observed on the proximal radius ‘PR’ which might suggest a different route of infection.

5.1.8 Cranial indicators

The skull is not frequently involved in TB, but the current study may suggest that these are more common than previously reported.

Cranial lesions were found in higher incidences than expected. Roberts and Buikstra (2003) stated that only 0.1% of individuals with skeletal TB had involvement of the skull with others stating that any lesions in the skull were rare (Aufderheide and Rodriguez-Martin, 1998). Pathological alterations that are isolated to the endocranium have largely not captured the attention of palaeopathological studies (Hershkovitz *et al*., 2002). In South Africa, a study that was looking into skeletal manifestations of TB in modern remains revealed an unexpectedly high frequency (7-10%) of skull involvement in South African skeletal collections (Steyn and Buskes, 2016). The findings involving cranial indicators in the current study support the published data by Hershkovitz *et al*. (2002) and Steyn and Buskes (2016) that there are cranial lesions that have the potential to be of diagnostic value for TB in palaeopathology. These are particularly related to changes in the anterior and middle cranial fossa, as well as the superior endocranium. The possibility that these changes may be associated with TB meningitis should be considered.

5.2 Overall comparison with the Dangvard Pedersen *et al*. (2019a) study

Dangvard Pedersen and colleagues were aware of the importance of estimating disease prevalence such as TB in archaeological samples which assist in determining what happened in past populations. Their study was a quantifiable probabilistic assessment of TB prevalence in a skeletal sample. Comparing the

current study with the Dangvard Pedersen *et al*. (2019a) allowed for differences to be identified and inferences to be made.

Positive bone alterations were observed in the TB cohort statistically significantly more frequently than they were in the control cohort for 10 of the indicators in the Dangvard Pedersen *et al*. (2019a) study, as opposed to only two indictors in the current study. One of the distinct differences between the current study and that of Dangvard Pedersen *et al*. (2019a) is the sourcing of the research sample. These authors intentionally sought to find a control sample where the likelihood of TB was very low based on the context regardless of the disease not being recorded as the cause of death. This may have resulted in the frequencies of a high number of bony indicators being found to be statistically significant across skeletal samples. Finding a South African sample with the same low likelihood of TB would be very hard considering the level of infection in the country (van Rensburg *et al*., 2005), and the fact that the collections are mostly made up of individuals of lower SES in whom the presence of TB is more likely. The current study sourced both their TB and control cohorts from the same skeletal collection. The collection, as is the case with all skeletal collections in the country, was largely populated with unclaimed bodies from regional hospitals in and around the Transvaal (Callinicos, 1985; Kramer and Hutchinson, 2015). Individuals who were included in the study had to have died before 1950 which means that there was no effective treatment for TB at the time (Pfuetze *et al*., 1955). This means that the probability of people who may have had TB being classed into the control groups was inherently high. As such, the pulmonary as well as the control groups could have inadvertently had individuals who had TB and the indicators to which it may be associated with. This may explain why there was such a high frequency of TB indicators being found in control groups.

Both the current and the Dangvard Pedersen *et al*. (2019a) study found proliferation of bone with a woven appearance on the ventral part of lumbar and thoracic vertebral bodies (VEN2) to be the most sensitive bony alterations for the presence of TB. The specificity for the same indicator, however, was very different between the two studies. There was only a 4.1% chance of the presence of TB in the absence of these bony changes in the Dangvard Pedersen *et al*. (2019a) study which is a vast difference compared to the 43.5% chance of the presence of TB in the SA group when the same indicator is absent. This finding also substantiates the suggestion that control groups have a high probability of the presence of TB even in the absence of lesions in this skeletal collection.

Dangvard Pedersen *et al.* (2019a) found that there were ten other indicators with a sensitivity estimate that surpassed 0.05, which means that more than half of the indicators in that study were sensitive enough to positively identify TB with a 5% probability. The current study had 9 indicators (RIB1, RIB2, VEN1, VEN2, BOD, ACE1, ACE2, ILI, DH1 and PU) that has at least a 5% probability of identifying a true TB diagnosis. TB sensitivity is the presence of an indicator given that the individual did have TB (Dangvard Pedersen *et al.,* 2019a). The lower sensitivity in this study could have been due to indicators being found in large numbers in controls. This would render many indicators ineffective in positively predicting the probability of TB in the study as a result of controls unwittingly possessing individuals who had TB but not having died of it.

Only half of the indicators in the Dangvard Pedersen *et al*. (2019a) study had a close association with at least half of the other indicators. This means that half of the presence of these indicators increase the probability of the presence of at least half of the other indicators. For the current study, all but two indicators have a direct

correlation, when present, with more than half of the other indicators in the suite. Indicator VEN2 had a close association with 12 other indicators (RIB1, RIB2, VER, VEN1, BOD, ACE1, ACE2, ILI, DH1, DH2, PR, and PU'). This suggests that these lesions are often associated, and it can be surmised that if more than one lesion is present there will be a higher chance of a positive diagnosis.

Stringent criteria had to be met for lesions to be considered as good markers of TB. A third (6) of the indicators in the Dangvard Pedersen *et al*. (2019a) study met these criteria. These bone alterations were associated with a TB diagnosis that matched a diagnosis that had been made when they were alive. Only a single indicator from the current study met the three criteria (VEN2). This indicator has consistently been identified as a good TB indicator, meeting criteria in both studies. For the current study, however, this indicator warrants some caution as it was found more commonly in the pulmonary sample. More research is warranted to explore other possible pulmonary diseases that may lead to these skeletal alterations.

The criterion that was least often met was the first, which required that an indicator have a Fisher's Exact test p-value of ≤0.001, which would indicate that the frequency of that indicator was more common in the cases than it was in the controls. As discussed before, the failure of the majority of the indicators in the current study to meet this criterion could be indicative of control individuals, particularly those in the pulmonary group, to be affected / have been affected previously by TB. What may be worth considering in this setting, is allowing for an indicator to be considered as sufficiently useful when it has met at least 2 of the three outlined criteria. When this was done, nine other candidate indicators, as listed above, could be considered as potentially useful in identifying TB in skeletal samples. The results found in the

current study were not as conclusive as those described by Dangvard Pedersen *et al*. (2019a).

5.3 Inter and intra-observer reliability

Inter-observer reliability is an essential aspect of research especially in studies such as this one where the criteria are rather subjective. Not many studies in palaeopathology include measures of repeatability, but this is a practice that should be encouraged. A Cohen's Kappa value of 0.5 is considered to be moderate (McHugh, 2012). This means that there was an agreement of 50%. A possible reason that can account for the low level of agreement is one that is not uncommon in palaeopathology. Bony lesions are difficult to correctly identify. The study by Miller *et al*. (1996) that was discussed in Chapter 1 demonstrated how even seasoned professionals in the field may find it challenging to identify specific changes in skeletal remains. It is also important to keep in mind the information that is available to observers in a palaeopathological setting as opposed to a clinical one. The availability of patient history, list of symptoms and physical examination of said patient provides valuable information that corroborates what the observer is seeing. Working exclusively with skeletal remains provides less information and this perpetuates the possibility of disagreement between observers. The small sample size may exacerbate the tests tendency to lower the estimate of agreement.

The percentage agreement for this study was 0.91, which meant that in only 9% of the total number of observations there was a difference in agreement. This reflects the total number of observations – i.e., when there were no lesions and when lesions were deemed to be present. While not perfect, this is an accepted level of agreement but should be improved in future. The low level of agreement further illustrates the

necessity of studies such as the current one that seek to provide tangible data that make the diagnosis of disease on bone to be done with more ease.

Intra-observer reliability is also a good indicator of how the primary investigator scored lesions in this study. A Cohen's Kappa value of 0.8 is considered as a strong level of agreement. Differences between the first and second assessments only occurred 6 times. In four of these occasions, lesions that were initially considered as absent were scored as present the second time around. This could indicate an increased level of confidence and being less conservative. The more variations one sees the more skilled their eye can become in discerning, with greater conviction, the presence of absence of a lesion. Palaeopathology is highly subjective and this can be minimized when the observer is experienced (Bruzek, 2002).

5.4 Shortcomings and future directions

The use of cause of death as a basis of determining the sample groups is flawed due to the fact that a disease may have been present even when it was not listed as having been the result of an individual's death. The use of one skeletal collection may have also amplified the inadvertent misallocation of individuals with TB into the control groups. Future research should attempt to minimize these shortcomings by using controls where there is extensive medical history available. Alternatively, to the approach that was taken in this study, future researchers should analyse the TB and pulmonary group together, against the control group. As TB and other pulmonary disease may leave the same signs, and because of the overlap as many pulmonary individuals may in fact have had TB.

A total of only 436 skeletons were analysed in this study. This meant that other indicators which were found to be highly characteristic to TB associated changes

were not observed frequently enough to properly demonstrate their diagnostic value. For the six indicators that missed the $p<0.05$ criterion for their frequency across all sample groups, this could have been overcome if more skeletons were analysed. A bigger sample size will explore each candidate indicator thoroughly enough to show, more definitely, an indicators usefulness.

Observer repeatability was low for the current study, however, the results can assist in tackling the issues surrounding the difficulties related to the scoring of skeletal remains. Photos that depict the changes that were found to be of value can also be used by future researchers to lower the level of disagreement between observers. Intra-observer reliability found that during the initial analysis, during this study, the researcher was slightly more conservative in scoring some indicators as present. It can be extrapolated that the more an individual familiarises themselves with these changes, the more comfortable they become in identifying them.

Upon the completion of the study by Dangvard Pedersen *et al*. (2019), they identified a few indicators that were not good indicators of TB. They outlined possible flaws in the cut off points with several indicators. Some of these indicators were also found to be poor indicators for the current study as well and future studies should use the results from both studies and exclude these indicators and focus on the ones with promising results. For a lot of the indicators in this study, a correlation was found between them and the other indicators in the suite of 23. Exploring several indicators with the most favourable results in combination may provide more conclusive results. Their combined diagnostic efficacy might be more informative than when they are observed individually.

5.5 Summary

Lesions that are located on the vertebrae as well as the ribs were the most promising according to the current study. Alone these lesions will probably not be adequate to confirm a TB diagnosis but together they increase the probability of a true TB diagnosis. When attempting to diagnose any disease it always important to consider a differential diagnosis. There are several diseases that may affect the skeleton in a similar manner to that found in skeletal TB. Pyogenic infections often spread to the vertebral column, which is a characteristic location for characteristic TB associated changes. In the case of pyogenic infections, however, the changes are often not accompanied by spinal osteomyelitis as often seen where TB is concerned (Tuli, 2010). Syphilis also often results with spinal lesions, although it is seldom accompanied by rib involvement which can assist in excluding it from the list (Tuli, 2010). It is also important that researchers familiarise themselves with the features of primary malignant tumours especially those located in the spine as these may mimic changes often associated with TB.

TB continues to be endemic to South Africa and as such most South Africans will have been exposed to the disease and thus the lesions associated with TB will be present in these individuals even when TB is not the disease that ultimately killed them. This is a fact that must be kept in mind throughout the current studies and any subsequent studies conducted in South Africa and the implications thereof.

Chapter 6: Conclusion

In this study, 23 skeletal lesions were investigated for their association with TB. It was found that:

1. Lesions on the ventral surface of thoracic and lumbar vertebral bodies were observed significantly more in TB cases than in controls. It was also found to be the most valuable indicator with a high sensitivity and 55% probability of a true TB diagnosis if observed.
2. Proliferative and lytic lesions on the visceral surface of ribs were also more frequent in the TB cases than in controls. This was the second most valuable indicator because it was also relatively sensitive to TB.
3. An association between skeletal lesions and TB could only be found for rib and vertebral lesions. Many other lesions (VER, VEN1, BOD, ACE2, DH1, CRA2 and ENDO2), however, met most of the criteria and could thus also provide insight into how TB and other pulmonary disease present.
4. Distinct differences between this study and that of Dangvard Pedersen *et al*. (2019a) indicated that possible tuberculosis-related changes were likely to be observed in a South African skeletal sample even when individuals were not documented to have died of the disease.
5. Cranial lesions were found more often than what is mentioned in the classic literature and should be investigated in future.
6. Inter-observer repeatability was relatively low and demonstrates the difficulties in palaeopathology to consistently score observed lesions.

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Appendix 1

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor, Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor, Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za Fax +27 (0)11-717-1265

Ref: W-CJ-140604-1 25/01/2016

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: School of Anatomical Sciences (Head: Prof M Steyn - Previously Prof T J M Daly, initial approval 04/06/2014 – recertified 27/01/2016).

Project title: Research on Cadaveric Material.

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health, and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
29 JAN 2016
RESEARCH OFFICE HREC (Medical)

Appendix 2

A No:

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Rib2		
VER		
VEN1		
VEN2		
BOD		
ACE1		
ACE2		
PF1		
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CRA1		
CRA2		
CRA3		

Additional Notes:

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