Quantitative computerized tomography for the diagnosis of osteopenia in prehistoric skeletal remains

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Abstract

In this study, we examine the value of bone density assessed by computed tomography (QCT) of the right tibia in the diagnosis of low bone mass in prehistoric bones. Trabecular bone mass (TBM) was assessed by histomorphometry in undecalcified bone sections of a small part of the proximal epiphysis of the right tibia of 78 prehispanic individuals. Bone density was also assessed in the proximal epiphysis of the right tibia, in an area of cancellous bone immediately adjacent to the wedge which was destined to histomorphometry, with the aid of a Tomoscan 60 TX/C212 (Philips Medical System, Eindhoven, The Netherlands) using a phantom of hydroxyapatite included in plastic resin at three known concentrations (50, 100 and 200 mg/cc) and ethanol as fat equivalent. Bone density (as bone hydroxyapatite concentration in milligram per cubic centimeter) was calculated by means of a specific software tool (QCT Bone Mineral Analysis System, Image Analysis, California). We compared bone density assessed with QCT with TBM in these individuals, and also, with the results obtained from a modern control group. We calculated the median TBM of the prehispanic sample and tested the sensitivity, specificity, and overall accuracy of QCT in diagnosing TBM values below the median. We later performed the same analyses on 24 more prehispanic individuals. Both QCT (t = 5.61, p < 0.001) and TBM (t = 3.79, p < 0.001) were significantly lower among the prehispanic individuals than among the control ones. QCT showed a significant relationship with TBM (r = 0.41, p < 0.001). QCT values below 100 mg/cc serve to establish a diagnosis of low TBM values with a sensitivity of 82.1% and a specificity of 41%. In the test group sensitivity was 83.3% and specificity, 50%.

In conclusion, in contrast with the results obtained in clinical studies, QCT serves only to obtain a rough estimate of TBM in prehistoric samples. The relatively low accuracy may be due to the lack of soft tissue and the air bubbles entrapped within the cancellous bone, which persist even after prolonged submersion of the bones in a water bath.

Keywords: Osteopenia; Computed tomography; Bone density; Bone-histomorphometry; Prehistoric Canary Islands; Osteoporosis

Osteoporosis is a systemic skeletal condition characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [44]. The term osteopenia denotes a decreased bone mass, whereas the term osteoporosis requires the presence of bone fractures due to bone fragility. Fractures occur because of qualitative and quantitative deterioration in the trabecular and cortical skeleton. Whereas bone quality cannot be measured in clinical setting, bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry (DEXA) accurately estimates bone mass [44]. A low BMD is a strong predictor of fracture risk, and therefore, the operational definition of osteoporosis is based on a BMD threshold indicated by a T-score of −2.5, i.e., a BMD value which is at least 2.5 standard deviations below the young-normal mean BMD [35]. This concept stresses the importance of osteopenia
in the pathogenesis of osteoporosis, although, as just mentioned, the quality of bone is also of paramount importance.

For any individual, the observed bone mass is a combination of peak bone mass and any subsequent bone loss. Although the presence of osteopenia in a single ancient individual is of paleopathological interest, but may be an anecdotical finding, assessment of the prevalence of osteopenia in a prehistoric population may inform about nutritional status. During the first three decades of life bone synthesis predominates, so bone mass progressively increases until it peaks towards the second half of the third decade [40]. Peak bone mass is under the influence of a variety of factors, including genetics [17,26], physical activity — especially weight — bearing exercise [13,41,48], diet [15], delayed menarche [2] and altered reproductive hormone concentrations [49]. After the age at which peak bone mass is achieved, bone synthesis and bone resorption are nearly in equilibrium but with a slight predominance of bone breakdown, so bone mass declines gradually, at a rate of less than 1%/year, except in women during the first 5—10 years after menopause, in whom bone loss is accelerated and bone mass decreases. Indeed, sexual hormones, especially estrogens, slow bone resorption and increase bone mineral density. Besides advanced age, several conditions, such as hyperthyroidism, hypogonadism, Cushing syndrome, hyperparathyroidism of any cause, including calcium deficiency, Addison’s disease, diabetes, malabsorption, severe liver disease, rheumatoid arthritis, ankylosing spondylitis, myeloma and other malignant hemopathies, chronic obstructive pulmonary disease, chronic alcohol consumption, and rare inherited disorders such as homocystinuria, Marfan syndrome, glycogen storage diseases, among others [29], may lead to reduced bone mass, but the overall prevalence of all these clinical entities in a non-selected “normal” population is low. Therefore, the finding of a high prevalence of osteopenia in a given population raises the possibility of protein-calorie malnutrition as the underlying cause. In this sense it is well known that both protein [50] or protein-calorie malnutrition [38] adversely affect bone development and bone mass. In these situations, bone synthesis is decreased [9], and although bone resorption is also decreased [8], an imbalance between synthesis and resorption ensues, leading to bone loss. These findings lend support to the classical “nutritional hypothesis”, which was widely used to explain the high proportion of osteopenia, both in ancient [1,14,34] and modern [22] population samples. The results of several experimental data and observational studies [33,37,39,46,47] support this hypothesis.

There are several methods available to determine bone mass. Some of them, such as bone histomorphometry, are destructive in nature and require a specialized laboratory. Currently, the most widely employed method in the assessment of bone mineral density (BMD) is double-energy X-ray absorptiometry (DEXA) [30,32,52], which is accurate, non invasive and relatively inexpensive. Other non invasive methods include the calculation of corticomедular indices on long bones such as tibiae [3], quantitative computerized tomography (QCT), and the assessment of radiological density [7]. All these procedures have shown a good relationship both with bone calcium content and histomorphometrically assessed trabecular bone mass (TBM) in clinical studies [10]. However, their validity in ancient samples has been scarcely tested [5,16,19,21,23,28,31]. QCT is based on the principle that the attenuation suffered by the X-ray photons is related to the thickness and composition of the tissues at the attenuation path [32]. This method allows the calculation of the X-ray attenuation coefficients in a cross section of the bone, providing a true and accurate density measurement, especially for cortical bone. Regarding trabecular bone, the presence of red marrow and marrow fat, which relative amounts varies with age, reduces the accuracy of QCT [10]. In general, methods assessing bone mineral density and/or bone mineral content in ancient samples are not as accurate as theoretically expected, due to the lack of soft tissue and/or the presence of air bubbles and, eventually, soil trapped within the trabeculae. In this sense, in a recent study on 95 tibiae, we concluded that DXA may serve to estimate the intensity of bone loss in a given ancient population, although it is useless for establishing the prevalence of osteopenia, since BMD cut-off values obtained for modern controls are not applicable to ancient samples [20], probably due to the lack of soft tissue and the distorting effect of the air entrapped within the cancellous bone.

In the present study, we analyze the sensitivity, specificity and overall accuracy of bone density assessed by QCT scan of the proximal epiphysis of the right tibia in the diagnosis of osteopenia, comparing it with histomorphometrically determined trabecular bone mass, also measured in the proximal epiphysis of the tibiae. The study was performed on 78 prehistoric individuals from Gran Canaria and El Hierro, and the results obtained were further applied to a test group which includes 24 prehistoric individuals from Gran Canaria.

1. Material and methods

1.1. Samples

The study was performed on 78 right tibiae belonging to prehispanic inhabitants from Gran Canaria and El Hierro (3 cases). As reported elsewhere the vast majority of these samples were included in a study comparing prevalence of osteoporosis between the prehistoric population of Gran Canaria and that of El Hierro, in which details about sample location and antiquity were provided [51]. In summary, samples from Gran Canaria were found in several mass burial caves in the central mountains of the island, such as Guayadeque (53 cases), or in coastal tumuli, such as Agujero (8) and Hormiguero (3), whereas those from El Hierro were found in volcanic caves from Punta Azul (3). The remaining cases from Gran Canaria were found in Dragonal, another in Charquitos, 3 in Santa Lucía, one in Tabacalate, and one in Tifaracas. Radiocarbon dating on some individuals buried in Guayadeque yielded a time depth ranging from 1405 ± 60 to 1213 ± 60 BP, and 875 ± 60 BP for samples from El Agujero.

Sex was estimated by adapting Iscan and Miller-Shaivitz discriminant functions analysis [24] to the population of
Gran Canaria, as previously described [51]; a total of 17 tibiae were classified as belonging to female individuals, and 60, to male ones. In one case, values obtained did not allow unambiguous sexing.

Age at death could not be estimated. However, in 11 cases, the epiphyseal closure line, which was still evident, allowed classification of these individuals as dead at a young age [4].

1.2. Methods of measurement

We performed the following analysis.

1.2.1. Bone histomorphometry

A small portion of the medial part of the posterior aspect of the proximal epiphysis was removed and processed for undecalcified bone sample analysis. Briefly, samples were embedded in methylmetacrylate (Sigma Chemicals, St Louis, Missouri, USA), stored for 24 h at 4 °C and later polymerized at 32–34 °C for 3–4 days. Embedded samples were then cut in 9–12 μm thick slices with a Reichert-Jung microtome — so that the resulting sections were perpendicular to the long axis of the tibiae — and stained with toluidine blue. Trabecular bone mass (TBM) was determined using an image analyzer equipped with the program “Image Measure 4.4a” (Microscience Inc.), at 40×. Results are given as % of total area.

1.2.2. Quantitative computer tomography

High resolution axial CT scan was performed to all the bones from which the aforementioned samples were obtained. These bones were introduced in a water bath during 50–90 min, in an attempt to eliminate air bubbles trapped within the bone, something which was incompletely achieved, as shown in Fig. 1. However, we succeeded in obtaining an area of trabecular bone of a diameter of at least 1.5 cm without air bubbles immediately adjacent to the wedge taken out for histomorphometry (Fig. 1). QCT was assessed with the aid of a Tomoscan 60 TX™ (Philips Medical System, Eindhoven, The Netherlands) using a phantom of hydroxyapatite included in plastic resin at three known concentrations (50, 100 and 200 mg/cc) and ethanol included in plastic resin as fat density. Bone density (as milligram per cubic centimeter hydroxyapatite) was calculated by means of a specific software tool (QCT bone mineral analysis System, Image Analysis, California). QCT measurements were made in the axial plane with a slice thickness of 10 mm.

1.3. Control group

In order to test the relation between QCT at the epiphysis of the right tibia and bone mineral density (BMD) assessed by DEXA we performed both procedures to 19 healthy sanitary workers aged 26–48, 10 women (mean age = 34.9 ± 7.55
years range 26–48) and nine men (mean age 38.2 ± 5.89 years, range 29–48), determining BMD at the lumbar spine (L2–L4) and at the hip. QCT values of the right tibia of these individuals served as control values. In these individuals QCT was also determined in an area with a diameter of 1.5–2 cm of cancellous bone located at the posterior lateral part of the tibial epiphysis (i.e., an area similar in location and size to that in which QCT had been measured in the prehispanic sample).

We compared TBM of the prehispanic population with that of our own control group, which consisted of 12 modern male individuals aged 17–44 years, from whom a bone specimen was obtained from tibiae during surgical operations on the right knee.

1.4. Methods of statistical analysis

We compared TBM and QCT values by single correlation analysis. Differences between controls and prehispanic people were analyzed by means of Student’s t-test.

We classified TBM and QCT values according to quartiles. Differences between mean values of TBM among the four quartiles of QCT, and of QCT among the four quartiles of TBM were analyzed using ANOVA and further SNK test. We further classified our population in those with TBM above the median and those with TBM below median values (low TBM values). We then calculated sensitivity, specificity and total accuracy of QCT to diagnose low TBM values, and depicted the corresponding ROC curves [18].

1.5. Test group

In order to test the validity of our results, the same procedure was applied to 24 additional tibiae (14 men, 9 women, 20 from Guayadeque, two from Aguero and two from Crucceitas), calculating sensitivity and specificity of QCT in the diagnosis of low TBM values (using the median value of TBM for the study group), and depicting the corresponding ROC curve.

2. Results

2.1. QCT in the ancient population compared with the control group

Mean QCT of the control population was 157.83 ± 38.63 mg/cc. No relationship existed between QCT and age or BMD at the lumbar spine, but there was a significant relationship between QCT and BMD at the hip (r = 0.58, p = 0.009).

The mean QCT value for the prehispanic population was 114.76 ± 30.39 mg/cc, significantly different from that of the controls (t = 5.61, p < 0.001). Statistically significant differences between prehispanic men and women were observed for QCT (t = 2.33, p = 0.023, Table 1). Differences were also statistically significant when prehispanic men were compared with modern men (t = 7.78, p < 0.001) and prehispanic women when compared with modern women (t = 2.24, p = 0.034). Also, statistically significant differences in QCT were observed between those who died at a young age (with epiphyseal closure line still evident in whom QCT was 136.18 ± 42.36 g/cc), and those who died at an older age (110.67 ± 26.54 mg/cc, t = 2.69, p = 0.009).

2.2. Relationships between QCT and TBM

The mean TBM values for the prehispanic population was 17.96 ± 5.11%, whereas that of the controls was 24.00 ± 5.28% (t = 3.79, p < 0.001). No statistically significant differences were observed between prehispanic men and women (Table 1, t = 1.63, p = 0.11). Statistically significant differences were observed when prehispanic men were compared with modern men (t = 2.83, p = 0.005) and prehispanic women when compared with modern women (t = 2.07, p = 0.042). Younger individuals showed higher TBM values (19.96 ± 5.11%) than the others (17.63 ± 5.07), but the differences were not statistically significant (t = 1.41, p = 0.161).

A significant correlation was observed between QCT and TBM (r = 0.41, p < 0.001, Fig. 2). In addition, the mean QCT was significantly different among the prehispanic individuals when they were classified according to TBM quartiles (Table 2, Fig. 3, F = 4.20, p < 0.01). Also, significant

<table>
<thead>
<tr>
<th>Table 1</th>
<th>QCT and TBM values in the prehispanic and control sample</th>
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<tbody>
<tr>
<td>QCT (mg/cc)</td>
<td>Modern controls</td>
</tr>
<tr>
<td>Men</td>
<td>186.83 ± 18.82</td>
</tr>
<tr>
<td>Women</td>
<td>126.18 ± 27.97</td>
</tr>
<tr>
<td>TBM (%)</td>
<td>Men 25.96 ± 7.05</td>
</tr>
<tr>
<td>Women</td>
<td>22.03 ± 1.54</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between bone density assessed by QCT and TBM in the study group.
Table 2
Mean QCT values of the samples classified in quartiles according to TBM values

<table>
<thead>
<tr>
<th>Quartile</th>
<th>1st Quartile</th>
<th>2nd Quartile</th>
<th>3rd Quartile</th>
<th>4th Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCT (mg/cc)</td>
<td>100.39 ± 34.64</td>
<td>105.67 ± 19.69</td>
<td>121.85 ± 25.21</td>
<td>128.95 ± 33.39</td>
</tr>
<tr>
<td>F</td>
<td>4.20</td>
<td>p &lt; 0.01</td>
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differences were observed in mean TBM values when the individuals were classified according to QCT quartiles (Table 3, \( F = 4.06, p = 0.01 \)). Similar differences were observed in QCT when individuals were classified in TBM below and above the 50th percentile (Table 4), and also in TBM when individuals were classified into those with QCT above or below the 50th percentile (Table 5).

2.3. Test group

In the test group mean TBM values were 18.77 ± 6.38%, and mean QCT values, 116.83 ± 36.13 g/cc. Differences with QCT \( (t = 2.50) \) and TBM \( (t = 2.45) \) were also significant \( (p < 0.02 \) in both cases). A significant correlation was also observed between TBM and QCT \( (r = 0.62, p = 0.001, \text{Fig. 4}) \).

As with the study group, statistically significant differences were observed in QCT when individuals were classified into TBM below the 50th percentile (98.83 ± 25.37) and above the 50th percentile (134.83 ± 37.13, \( t = 2.77, p = 0.001 \)) and also, in TBM, when individuals were classified into those with QCT over the 50th percentile (22.67 ± 5.71) or below the 50th percentile (14.87 ± 4.38, \( t = 3.75, p = 0.01 \)).

2.4. Sensitivity and specificity of QCT in the diagnosis of osteopenia

We also calculated sensitivity, specificity and overall accuracy of diverse QCT values in the diagnosis of TBM below the median value of our population (less than 17.06%). As shown in Fig. 5, the ROC curve has shifted significantly to the left, with an area under the curve of 0.723 ± 0.057 (95% confidence interval = 0.61 – 0.835, \( p < 0.001 \)). So, a QCT value less than 100 mg/cc detects low TBM values (TBM below 17.06%) with a 82.1% sensitivity and 41% specificity, whereas a QCT value below 115 mg/cc detects low TBM values with a 61.5% sensitivity and 66.7% specificity (Table 6). An even better result was observed in the test group (0.816 ± 0.093, 95% confidence interval = 0.634 – 0.998, \( p = 0.009 \), Fig. 6).

The sensitivity of a QCT value below 100 mg/cc for detecting low TBM was 83.33% and the specificity was 50%. QCT values below 114 mg/cc detect TBM values below 17–06% with a sensitivity of 83.3% and a specificity of 83.3% (Table 7).

3. Discussion

In this study, we tested the ability of QCT to diagnose low TBM values, assessed by histomorphometry, at the proximal right tibial epiphysis. QCT is related with TBM, and indeed, QCT may provide an approximate estimate of the severity of osteopenia. As shown in Tables 2 and 3 and in Fig. 3, low QCT values are associated with low TBM values. However, although the correlation between both measurements was highly significant, the sensitivity and specificity do not permit an accurate estimation of TBM using QCT in ancient samples. This result is in contrast with results reported for a living population. Several studies performed in the last 30 years have clearly shown that QCT accurately predicts bone mineral content with an error less than 1% [25,42], and the technique has been widely employed to estimate BMD at different sites of the skeleton, especially the vertebrae [11,12,36], and to assess the risk of fracture [27,53]. Even early studies performed on cadavers with technically obsolete devices have shown a high correlation coefficient between QCT and calcium content (following ashing) of the cortical bone of tibiae and fibulae [42,43]. Trabecular bone measurements with QCT are more problematic, since marrow tissue and fat distort the X-ray attenuation. Moreover, the relative contents of both kinds of tissue (i.e., red marrow and marrow fat) varies with age, so that the standard error of QCT increases with advancing age, 6–9 to 10–15% in older patients.

Table 3
Mean TBM values of the samples classified in quartiles according to QCT values

<table>
<thead>
<tr>
<th>Quartile</th>
<th>1st Quartile</th>
<th>2nd Quartile</th>
<th>3rd Quartile</th>
<th>4th Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBM (%)</td>
<td>15.29 ± 5.68</td>
<td>17.60 ± 3.67</td>
<td>18.03 ± 4.88</td>
<td>20.60 ± 4.83</td>
</tr>
<tr>
<td>F</td>
<td>4.06</td>
<td>p &lt; 0.01</td>
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with single energy CT and 3–6 and 6–10%, respectively, with double energy devices, [10]). However, our results on prehispanic samples are worse, but are consistent with those reported by others who have applied X-ray densitometry to the analysis of bare bones or bones from cadavers [16,28,45] and with our own results using DEXA [20] or X-ray density [21]. It is likely that attenuation differences caused by entrapped air bubbles may explain the relatively poor accuracy observed. Some other authors have overcome this problem, either using technical innovations [6], either by introducing the sample in a water tank [31], as we also did. However, as shown in Fig. 1, entrapped air bubbles still persist after more than 1 h under water and would, surely, constitute a major limitation for this kind of studies. If we add to this the time required to maintain the bones under water, the cost of the QCT procedure, the eventual deleterious effects which this procedure may have on bone preservation, and the relatively poor results, we can conclude that QCT is not an efficacious tool to analyze osteopenia in ancient bones. It is also possible that soil retained within the bone distorts QCT measurement. However, as shown in Fig. 1, this is not a relevant problem in our samples, due to the special inhumation procedures performed by the prehispanic inhabitants of the Canary Islands: they usually deposited their dead on stony or vegetal layers in volcanic caves, so the corpses were not covered with earth. This fact, together with the subdesertic climate of many part of the islands, has led to an excellent preservation of many skeletons, but, surely, in bones not subjected to these conditions, soil filling the trabecular spaces within the bones may distort QCT assessment of bone density. Otherwise, soil, which is radiopaque, is easily detectable with the Tomoscan — or with a plain X-ray film therefore allowing identification of samples not suitable for QCT analysis.

In any case, the results obtained with QCT are similar to those observed for DEXA, and the ROC curve shows that QCT may serve as a rough estimator of low TBM values osteopenia. Forty-four samples included in this study were also subjected to DEXA analysis, and 63, to plain X-ray and assessment of the corticomedullary index. The result of these works have already been published [20]. Correlation between QCT and DEXA in these 44 samples is statistically significant

\[
T = 3.44, \ p < 0.001
\]

\[
T = 2.63, \ p = 0.01
\]

### Table 4

<table>
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<tr>
<th>QCT values in individuals with TBM below or above the 50th percentile</th>
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<tr>
<td>TBM below the 50th percentile</td>
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<tr>
<td>QCT (mg/cc)</td>
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\[
T = 3.44, \ p < 0.001
\]

### Table 5

<table>
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<tr>
<th>TBM values in individuals with QCT below or above the 50th percentile</th>
</tr>
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<tbody>
<tr>
<td>QCT below the 50th percentile</td>
</tr>
<tr>
<td>TBM (%)</td>
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</table>

\[
T = 2.63, \ p = 0.01
\]
significant, especially between control men and prehispanic men. Thus, bone mass was clearly reduced among the prehispanic population from Gran Canaria, a result which has been interpreted as deriving from episodic nutritional stress, due to the high population density (20–30 inhabitants per square kilometer) of the island at least at the time of the Spanish conquest (towards the end of the 15th century), the great dependence on agriculture, the subdesertic climate with irregular rainfall, and the frequency of locust plagues arriving from the neighboring Sahara desert. As commented before, there are many other conditions associated with decreased bone mass (29). However, the prevalence of all these conditions in a non-selected population group is low, except perhaps for diabetes, which may affect 5–10% of the population. However, osteoporosis mainly affects chronically compensated type I diabetes, a rapidly fatal disease in the absence of insulin therapy. Therefore, as discussed elsewhere [51], episodic starvation, with low intake of protein, calcium, and other nutrients, may explain the high prevalence of osteopenia in the prehispanic population from Gran Canaria.

### References


### Table 6

<table>
<thead>
<tr>
<th>QCT (mg/cc)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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</thead>
<tbody>
<tr>
<td>&gt; 89</td>
<td>92.3</td>
<td>25.6</td>
</tr>
<tr>
<td>&gt; 99.5</td>
<td>82.1</td>
<td>41</td>
</tr>
<tr>
<td>&gt; 114.5</td>
<td>61.5</td>
<td>66.7</td>
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<tr>
<td>&gt; 125.5</td>
<td>41</td>
<td>87.2</td>
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### Table 7

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<tr>
<th>QCT (mg/cc)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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</thead>
<tbody>
<tr>
<td>&gt; 91</td>
<td>91.7</td>
<td>41</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>83.3</td>
<td>50</td>
</tr>
<tr>
<td>&gt; 114</td>
<td>83.3</td>
<td>83.3</td>
</tr>
<tr>
<td>&gt; 125.5</td>
<td>58.3</td>
<td>83.3</td>
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4. Conclusions

In summary, QCT may serve only to roughly estimate the trabecular bone mass, although several limitations derived from the cost of this time-consuming procedure, the difficulty in eliminating entrapped air bubbles, and the relatively poor correlation with histomorphometrically determined bone mass, preclude the utilization of this technique to assess prevalence of osteopenia in ancient population.


