Published in final edited form as:

J Clin Densitom. 2018 ; 21(4): 485–492. doi:10.1016/j.jocd.2017.05.008.

A Candidate Imaging Marker for Early Detection of Charcot Neuroarthropathy

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Abstract

INTRODUCTION—Inflammation-mediated foot osteopenia may play a pivotal role in the etiology, pathogenesis and therapeutic outcomes in individuals with diabetes mellitus (DM), peripheral neuropathy (PN) and Charcot neuroarthropathy (CN). Our objective was to establish a volumetric quantitative computed tomography-derived, foot-bone measurement as a candidate prognostic imaging marker to identify individuals with DMPN who were at risk of developing Charcot neuroarthropathy.

RESEARCH DESIGN AND METHODS—We studied three groups: 16 young controls (27±5 yrs), 20 with DMPN (57±11 yrs), and 20 with DMPN and Charcot neuroarthropathy (55±9 yrs). Computed tomography image analysis was used to measure metatarsal and tarsal bone mineral density in both feet. The mean of 12 right (7 tarsals and 5 metatarsals) and 12 left foot bone mineral densities, maximum percent difference in bone mineral density between paired bones of right and left feet, and the mean difference of the 12 right and 12 left bone mineral density measurements were used as input variables in different classification analysis methods to determine the best classifier.

RESULTS—Classification tree analysis produced no misclassification of the young controls and individuals with DMPN and Charcot neuroarthropathy. The tree classifier found seven of 20 (35%) individuals with DMPN to be classified as Charcot neuroarthropathy (one participant developed Charcot neuroarthropathy during follow-up) and 13 (65%) to be classified as healthy.

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Duality of Interest: No potential conflicts of interest relevant to this article were reported.

CONCLUSIONS—These results indicate that a decision tree employing three measurements derived from volumetric quantitative computed tomography foot bone mineral density defines a candidate prognostic imaging marker to identify individuals with diabetes and peripheral neuropathy who are at risk of developing Charcot neuroarthropathy.

Keywords

Candidate Imaging Marker; Whole Bone Volume; Foot Bone Mineral Density; Diabetes Mellitus; Peripheral Neuropathy; Charcot neuroarthropathy

Introduction

In 2014 approximately 29.1 million Americans (9.3%) had diabetes mellitus (DM)[1]. Mild to severe forms of nervous system damage are common complications of DM and occur in 60% to 70% of individuals [1]. Early epidemiologic studies report the prevalence of acute Charcot neuroarthropathy (CN) in people with diabetes to be low, varying between 0.1% and 7.5%.[2] More recent reports (for which estimates are based upon evaluations performed at a specialty clinic) estimate the prevalence to be approximately 1% to 13% in people with DM and peripheral neuropathy (PN) [2]. Therefore, acute CN is increasingly being recognized as a serious complication of DM and PN that may be amenable to prevention by early recognition and prompt intervention [3]. CN can be difficult for untrained medical professionals to diagnose because presently there is no diagnostic test beyond radiographic changes evident only in well-established disease. Misdiagnosis, delayed recognition, or inappropriate treatment interventions typically lead to poor outcomes [4, 5] as well as higher than expected mortality [6, 7].

Medical treatment for CN is limited [8]. Operative (surgical) intervention for deformity correction is typically performed with the goal of achieving a stable foot for normal ambulation and promoting wound healing. Reconstructive surgery is often performed to salvage the limb but can further prolong and delay healing, resulting in a poor outcome [9]. To date there have been few published studies of non-operative, therapeutic treatments for individuals with DM, PN and acute CN. Two small studies [10, 11] used pharmacologic agents (intravenously administered pamidronate, a bisphosphonate) in combination with off-loading and immobilization to concurrently reduce inflammation and limit excessive bone resorption (inflammatory osteolysis). These studies used serum and urinary markers of bone turnover as their primary endpoints; however, these markers only detect systemic change in bone metabolism and are not reflective of local bone density that is associated with CN. Interventional studies could benefit from accurate, precise, and fully qualified imaging markers of CN activity in the foot.

It is important for such imaging markers of disease activity to contain information on all foot bones; however, there is a comprehensive study of foot osteopenia (including all foot bones) in individuals who have DM, PN and diabetic foot disease [12]. The reason for limited studies is due to limitations in the current imaging technologies of dual energy x-ray absorptiometry (DXA) and quantitative ultrasonography (QUS) [13]. DXA and QUS cannot currently measure bone mineral density (BMD) of the talus, navicular, cuneiforms, and

cuboid, where the majority of deformity and joint destruction occur in CN [14]. By contrast, volumetric quantitative computed tomography (VQCT) is a well-established and proven method of assessing volumetric bone density [15–19] despite its being less commonly used than DXA or QUS[20]. Recently, VQCT was used to study the capacity of bone quantity and bone geometric strength indices to predict ultimate force in the human second metatarsal (Met2) and third metatarsal (Met3). This investigation showed that geometric indices were more highly correlated to ultimate force than was BMD, and bone thickness and density-weighted minimum section modulus had the highest individual correlations to ultimate fracture force [21]. VQCT has also been used to characterize osteolytic changes related to development and progression of CN by measuring metatarsal bone mineral density (BMD) and geometric strength indices. Results showed BMD was lower in both involved and uninvolved feet of CN participants compared with DM+PN participants [22]. VQCT is ideally suited for the comprehensive study of volumetric BMD in all of the bones of the foot e.g., tarsal and metatarsal bones affected by CN because VQCT captures the entire volume of each bone. Currently, no VQCT or QCT imaging marker has been reported that is capable of early detection of CN.

In light of the accumulating evidence that in CN, there is an inflammation-mediated osteopenia that may play pivotal roles in the etiology, pathogenesis, and outcomes of therapeutic interventions in individuals with DM, PN and CN of ankles/feet[12, 23–29], imaging markers are needed: 1) for identifying individuals who have DM and PN and are at high risk of developing CN (prognostic marker) and 2) for predicting treatment outcomes in individuals with acute CN (predictive marker) [26, 27, 30]. Developing an image-based prognostic marker for CN onset is particularly important because such imaging markers could be used to identify individuals prior to the onset and preventing the well-known sequelae including deformity, plantar ulceration, infection and ultimately lower extremity amputation.

The objective of our study was to establish a candidate prognostic imaging marker for CN based on VQCT-derived, foot-bone measurements. The central hypothesis was that foot BMD-based measurements derived from CT imaging represent an imaging marker capable of early detection of CN. To assess this hypothesis, we built a classifier to identify individuals with DM and PN who were at risk of developing CN. Our *a priori* hypotheses were: 1) individuals with DM and PN who have low foot BMDs and 2) individuals with DM and PN who have the highest right-left foot-bone differences in BMDs have the highest probabilities of being classified as individuals with CN.

Materials and Methods

Participants

We recruited 16 young, healthy participants, 20 participants with DM and PN, and 20 participants with DM, PN and CN. The demographics for the 3 groups of participants are presented in Table 1. The young healthy controls did not have DM or PN or a history of known foot impairments. For the DM and PN group, inclusion criteria were Type 1 or Type 2 DM and PN diagnoses and no history or evidence of acute CN. Exclusion criteria for participants with DM and PN were history or current signs of foot disease (e.g., plantar

ulceration, pedal fractures or fixed foot deformities), metabolic bone disease (e.g., Paget's disease, rickets, primary hyperparathyroidism), history of kidney or liver disease (e.g. renal or hepatic osteodystrophy) or kidney or liver transplantation, currently taking immunosuppressive medications including prednisone, women on oral contraceptives or hormone replacement therapy, diagnosis of osteoporosis and taking bone anti-resorptive medications (e.g., bisphosphonates or selective estrogen receptor modulators) or a weight exceeding 350 lbs (CT and DXA scanner weight limits). Participants were included in the DMPN and CN group if they had diabetes mellitus, peripheral neuropathy and a diagnosis by a physician of acute Charcot neuroarthropathy. The exclusion criteria for the DMPN and CN group were participants with partial or complete tarsal or metatarsal bones but they could be missing part or all of the phalanges, anyone with a history or active evidence of osteomyelitis or a weight exceeding 350 lbs. The participants with DM and PN were recruited from the City, State and surrounding area and were age, body mass index (BMI) and sex-matched to the participants with CN. The young healthy participants were recruited from students and staff members of Blinded University. The participants with CN were recruited from a clinic where participants with foot ulcers and CN are treated. All research was conducted under a protocol approved by Blinded University School of Medicine's Institutional Review Board. Informed consent was obtained from participants before they participated in the study. PN in each foot was tested using previously published methods [31]. Briefly, a single thickness (5.07/10-gr) Semmes-Weinstein monofilament was used to test seven plantar surface locations. Participants with DM were included in the study if they were not able to sense the monofilament at any one of the seven locations.

Image Acquisition

A Siemens Sensation 64 CT scanner (Siemens Medical Systems, Inc., Iselin, NJ) was used to acquire the data using a standard QCT measurement protocol defined by our team [32]. Each participant was placed supine on the CT scanner table with the right foot positioned near the isocenter of the scanner and at an approximately 45-degree angle to the table (Figure 1 A and B). A QCT calibration phantom (QCT-Bone Mineral™ phantom; Image Analysis, Inc., Columbia, Kentucky, Serial No. 4225) was placed on the scanner table in front of the right foot. The foot was scanned from above the talus to beyond the toes including the phantom. After the right foot was scanned, the left foot and QCT calibration phantom were scanned in a similar manner. The phantom was used to convert the Hounsfield units to BMD (calcium hydroxyapatite, mg/cm³). The following CT parameters were used to acquire the images: 0.5 second rotation time, 38.4-mm table increment/gantry rotation (64 × 0.6 mm collimation), 220 mAs, 120 kVp, pitch of one, and 512 × 512 matrix. A B70f bone kernel was used to reconstruct the foot at the QCT scanner.

Bone Segmentation and Measurement

Digital imaging and communications in medicine (DICOM)-formatted, CT image data were transferred to a stand-alone image processing workstation. For each foot, the 7 tarsal and 5 metatarsal bones were segmented (isolated from surrounding tissues) using previously described methods (Figure 1 C) [33, 34]. Briefly, the processing pipeline consisted of 1) an edge detection method to locate the external boundary of each bone, 2) a graph-cut-based method to separate bones into discrete objects[35], 3) a morphological operator to remove

internal holes within a bone, 4) a region of interest assessment to obtain an average Hounsfield unit (HU) value for each bone, and 5) a calibration phantom to convert the average HU value for each of the 7 tarsals and 5 metatarsals to 12 BMDs given in mg hydroxyapatite/cm³. Steps 1, 2, 3 and 5 were performed using in-house software and step 4 was performed using Analyze software [36, 37].

Statistical analyses

Prior to the study, we conducted a power analysis to determine group sample size. For the DM, PN, and CN group, we used quantitative ultrasonography (QUS) data to estimate CT BMDs [38]. For participants with DM and PN, we had previous CT foot BMDs for three participants. Based on these values and a logistic regression model, we estimated a sample size of 33 participants in each group with diabetes. The availability of participants was less than expected, and age, gender, and BMI matching of participants of the two groups was more difficult than expected. We revised our sample size to 20 in each. Preliminary data distributions indicated more overlap in foot BMD values for the two groups with diabetes than we had anticipated. Because of this, we added a third group of young healthy control participants ($n = 16$) to our study. We used young healthy control participants and participants with DM, PN and CN to build our classification model and used this model to classify participants with DM and PN. We reasoned that participants with DM and PN who's BMDs most resembled those of participants with DM, PN and CN would be classified as having CN—that is, they would be at the greatest risk of having a new Charcot event.

Our *a priori* variables were (1) the mean of the 12 right and 12 left foot BMD measurements (Mn RL BMD) from Hypothesis 1 (Figure 2 A) and (2) the mean difference of the 12 right and 12 left foot BMD measurements (Mn BMD Diff) from Hypothesis 2 (Figure 2 B). An additional group of potential variables for use in developing the classifier were included based on our experience with segmenting and reviewing the data and to better account for localized BMD effects. Based on group separation in BMD values, homogeneity of variances, and normality of data distributions, three variables with the greatest separation between groups (Figure 2 C, D, and E) out of the potential additional variables were selected for possible inclusion in the classifier (1) the maximum absolute BMD difference between paired bones of right and left feet within a participant (Max_abs), (2) the maximum percent difference in BMD between paired bones of right and left feet within a participant (Max_per), and (3) the BMD value of the first metatarsal of the involved foot of Charcot participants compared to the average BMD of the first metatarsal from both feet for the other groups (Inv_Ave_Met1TotBMD).

For these 5 possible classifier inclusion variables (Mn RL BMD, Mn BMD Diff, Max_abs, Max_per, Inv_Ave_Met1TotBMD), box plots were created, the normality of the data distributions tested with the Shapiro-Wilk W Test, and the homogeneity of variances tested with Box M tests. Log transformations were used to reduce variance heterogeneity and to better normalize data distributions. Models were built with (1) logistic regression analysis, (2) discriminant analysis, and (3) classification & regression tree analysis. There are a number of advantages for using classification trees for analyzing our data [39]. First, if a classification tree has only a few branches, it is easy to interpret. Second, the use of

classification trees requires no assumption that the relationship between the dependent variable and the predictor variables is linear or that this relationship is described by a link function (required for generalized linear and nonlinear models). In sum, classification trees are non-parametric and nonlinear. In building our classification tree, we used v-fold cross-validation. A P value < 0.05 was considered a statistically significant difference. Statistical analyses were performed with Statistica Release 9 (StatSoft, Inc., Tulsa, OK), JMP Statistical Software Release 8.0.1 (SAS Institute, Inc., Cary, NC), and Power and Precision (Biostat, Inc., Englewood, NJ).

Results

Figure 2 contains the box plots of the BMD values for the variables that were used to develop the classifier. For all variables except for Inv_Ave_Met1TotBMD, data were non-normally distributed and variances were not equal (Shapiro-Wilk W , O'Brien, Brown-Forsythe, Levene, and Bartlett tests ($p < 0.01$)). The Box M test indicated extreme heterogeneity of variances/covariances ($p < 0.01$). After log transformation, data normality and homogeneity of variances was improved; however, some variables still had non-normal data distributions and variances were not homogeneous ($p < 0.01$). The Box M test indicated a reduction in heterogeneity but remained significant ($p < 0.01$). The Box M test is particularly sensitive to deviations from multivariate normality. With regard to our use of discriminant analysis, it has been pointed out that this method is robust to minor violations of the above assumptions, and the best guide for how harmful violations of assumptions are is how successful a model is in correctly classifying cases [40]. Values were not available for all variables of one participant with CN. For discriminant analysis, our error rate in classifying young healthy control participants and those with CN was 5.7% (2 misclassifications for 35 participants—19 with CN and 16 young healthy controls). This was the same error rate with logistic regression. Classification tree analysis resulted in no misclassification—an error rate of 0.0%. Because the classification tree resulted in no misclassification and because the resulting tree is easy to interpret and deploy, we selected it for our classifier. Figure 3 contains the resulting classification tree; the Figure 3 caption contains an explanation of the classification tree. This tree was used to classify participants with DM and PN. Seven of 20 participants (35%) were classified as CN and 13 (65%) were classified as healthy. One participant with DM and PN who was classified as CN had a Charcot event during our follow-up period. This is the only participant who at baseline had DM and PN and during the study had an acute onset of CN.

Discussion

We found foot BMD-derived measurements can be used to classify all of the healthy participants and participants with CN with no misclassification. To our knowledge, this is the first study that has used BMD-derived measurements of foot bones to classify participants with CN. We also were able to classify our 20 participants with DM and PN, and one of the seven participants classified as being CN did develop CN within the study period. The ability to correctly classify participants as being healthy or having CN is important because such a classification has the potential to identify DM and PN participants who are at the greatest risk of developing CN.

Jeffcoate and Sinacore have reported that inflammation plays a causative role in osteopenia and osteolysis in individuals with CN [12, 29]. We think our finding that there is a difference in BMD values between participants with DM and PN and participants with DM, PN and CN agrees with our previous studies where BMD comparisons between a selected tarsal bone and all tarsal and metatarsal bones were reported, although no classifier was developed in these studies [12, 38].

A foot BMD classifier could be used to help medical professionals determine a participant's risk for developing CN based on the measured foot BMD for the three variables (Mn RL BMD, Mn BMD Diff, and Max_per) used in our classifier tree. In Figure 2 A, the participants in the Charcot group had on average lower mean right left foot BMD compared to the DMPN and Healthy groups which indicates lower bone mass. Some of the causes of low bone mass and osteolysis are advanced age, menopause, vascular disease, drug and dietary deficiency related loss, disuse, reflex sympathetic dystrophy syndrome, osteomalacia, hyperparathyroidism, alcoholism, and chronic liver and kidney disease [41]. Any of these bone-loss disorders in conjunction with DM and PN, may cause an individual to be at high risk for CN, but more research into these disorders is needed. A BMD classifier could also be used by orthopedic surgeons, podiatrists, and physical therapists to determine CN risk in DM and PN participants for whom offloading and immobilization will be used to treat accompanying neuropathic foot ulcers. Though foot off-loading and immobilization have been shown to cause bone loss in the calcaneus [42], our VQCT-derived foot BMD methods may be used to monitor for further bone loss and recovery. In a separate study [43], we measured the BMD of 12 tarsal and metatarsal bones in each foot of a male participant with DM, PN and CN before and after total contact casting treatment. We found a decrease in BMDs for 10 of the 12 bones for the involved foot ranging from 1.1 (navicular) to 11.3% (cuneiform 1) with two of the bones increasing (cuneiform 3 and cuboid increasing by 1.4 and 0.3%, respectively) over a 12 week period of total contact casting. For the uninvolved foot, BMD changes ranged from 0.2 to 0.6% for the same 12 week period [43]. For offloading and immobilization with total-contact casting, extra precautionary procedures might be warranted for participants with low foot BMDs and classified as being at risk for developing CN particularly when full weight bearing reloading resumes.

A limitation of this study is the small sample size, but even with the small sample size, we were able to correctly classify all of the healthy participants and participants with CN. In addition, our classifier identified one of our DM and PN participants as being a DM, PN and CN participant. This participant did develop CN during the 1-year, follow-up period. Since the study only had a 1-year follow-up, we were unable to follow the other six participants that were classified to develop CN. Another limitation of the study is that CT utilizes ionizing radiation, but because many of the participants with DM and PN are beyond child bearing age and because the foot is far from reproductive organs, the ionizing-radiation risk is minimal. However, if multiple CT scans were to be required within a year, there would be an accumulative effect of the ionization-radiation risk. The cost of a CT scan is more than a plane radiograph, but BMDs for all foot bones can be determined from a CT scan unlike with a foot radiograph.

Currently, clinical methods, assessment techniques, and outcome measures such as DXA and QUS are incapable of providing sensitive and specific indicators of CN. In addition, they have limited usefulness in monitoring disease progression and identifying CN risk, which could be used in decision-making situations to possibly avoid devastating, long-term negative outcomes such as lower extremity amputation. Using specialized scanning protocols with high-resolution magnetic resonance imaging (MRI), it is possible to quantify porosity and mechanical properties of bone [44], however, these protocols are not available on clinical MR scanners and are more expensive than VQCT. Our VQCT, BMD classifier could provide the medical community with a new means of identifying participants who are at risk for developing CN. Additional research is necessary to test the candidate imaging marker in participants who are at high risk for developing CN.

In conclusion, we were able to successfully establish a decision tree employing 3 foot BMD-based parameters as a candidate prognostic imaging marker for CN. Once this imaging marker has been validated, it could help medical professionals identify individuals at risk for CN so they can provide the appropriate medical care including surgical stabilization, off-loading, and prescribing medications relating to bone health. The results support our *a priori* hypotheses that participants with DM and PN who have low foot BMDs and participants with DM and PN who have the highest right-left foot-bone differences in BMDs have the highest probabilities of being classified as CN. We achieved our objective by building a classifier that identifies participants with DM and PN who are at risk of developing CN.

Acknowledgments

The authors want to thank Jared and Karen, who were both students in the Blinded University DPT program at the time when the measurements were obtained and now have their DPT, for measuring the BMD for the bones in the feet for many of the participants.

Funding. Funding was supported by the National Institute of Diabetes and Digestive and Kidney Diseases, contract R21 DK.

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Figure 1. Image Analysis QCT calibration phantom and foot positioning on the CT scanner table (A – side view and B – Top view). Segmented tarsal and metatarsal bones, (C).

Figure 2. Box plots of bone mineral density values (BMDs) for the variables used to build a classifier to identify participants with DM and PN (DMPN) whose BMDs most resemble those of participants with Charcot neuroarthropathy (Charcot)

To better illustrate the distribution of the data points; they are spread horizontally to minimize their overlapping one another. The ends of the boxes are the 25th and 75th quantiles (quartiles). The (red) lines across the middles of the boxes are the medians. The interquartile range is the difference between the quartiles. The lines (whiskers) extend from the boxes to the outermost points that fall within the distance computed as 1.5 (interquartile range). The lines to the left and right of each box are the means. Healthy = healthy control participants; Mn RL BMD = mean of the right and left foot BMD measurements; Mn BMD Diff = the mean difference of the right and left foot BMD measurements; Max_abs = the maximum absolute BMD difference between paired bones of right and left feet within a participant; Max_per = the maximum percent difference in BMD between paired bones of right and left feet within a participant; Inv_Ave_Met1TotBMD = the BMD value of the first metatarsal of the involved foot of Charcot participants compared to the average BMD of the first metatarsal from both feet for the other groups.

Figure 3. Classification tree for classifying participants with Charcot neuroarthropathy (Charcot) and healthy control participants (Healthy)

This classification tree resulted in neither a misclassification of a participant with Charcot neuroarthropathy nor a healthy control participant. The node number is given in the upper left hand corner. For node 1, there were 20 participants with Charcot neuroarthropathy and 16 healthy controls. This tree model is based on 3 if-then statements. Under node 1, if the Max_per value (the maximum percent difference in BMD between paired bones of right and left feet within a participant) is ≤ 0.1038 the participant is classified as healthy; otherwise, the participant is classified as Charcot. At this step 14 of 16 Healthy participants were classified as Healthy. Under node 3 if the Mn BMD Diff value (mean difference between the right and left foot BMD measurements) is > 15.875 the case is classified as Charcot—18 of the 22 cases from node 3 were classified as Charcot in terminal node 5; otherwise, the case is classified as Healthy. Under node 4 if the Mn RL BMD (the mean of the right and left foot BMD) is ≤ 411.396 , the case is classified as Charcot; otherwise, it is classified as Healthy. Two cases each were classified in terminal nodes 6 and 7. No case was misclassified by this tree, which was subsequently used to classify participants with DM and PN into the classes of Charcot or Healthy

Table 1

Participant Demographics

	Young Healthy Controls		Participants with DM/PN		Participants with DM/PN & CN	
	Male (n=8)	Female (n=8)	Male (n=8)	Female (n=12)	Male (n=10)	Female (n=10)
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)
Age	27.1 (3.1)	27.8 (6.8)	62.0 (13.3)	54.7 (8.1)	56.0 (9.1)	54.4 (10.2)
Diagnosis Age			51.4 (9.1)	40.5 (13.4)	39.1 (12.6)	35.3 (16.6)
Height (cm)	178.1 (8.8)	166.5 (8.4)	176.6 (6.1)	168.4 (8.3)	181.9 (5.0)	168.2 (4.1)
Weight (kg)	87.2 (17.3)	64.5 (9.5)	103.7 (22.6)	89.0 (26.9)	125.9 (24.4)	100.0 (21.6)
BMI (kg/m ²)	27.5 (5.3)	23.2 (2.3)	33.6 (8.8)	31.0 (7.9)	37.9 (6.3)	35.5 (8.3)