Premature changes in trabecular and cortical microarchitecture result in decreased bone strength in hemophilia

Adrienne Lee,1,2 Steven K. Boyd,3 Gregory Klime,1 and Man-Chiu Poon1,2

1Department of Medicine, University of Calgary, Calgary, AB, Canada; 2Southern Alberta Rare Blood and Bleeding Disorders Comprehensive Care Program, Foothills Medical Centre, Calgary, AB, Canada; and 3Department of Radiology, University of Calgary, Calgary, AB, Canada

Key Points
- Patients with hemophilia have abnormal bone microarchitecture and decreased biomechanical bone strength compared with normal healthy controls.
- Decreased BMD and bone strength are associated with severity of hemophilic arthropathy.

Introduction

With the increasing availability and affordability of safe factor concentrate replacement therapy, factor prophylaxis, and comprehensive care, persons with hemophilia (PWH) today should have a normal life expectancy, and more are living beyond age 65 years.1,2 However, comorbid complications associated with aging in this population are largely unknown, and background data on the clinical implications of these issues are lacking. Low bone mineral density (BMD) and associated osteoporosis is one of the emerging concerns that has been described, with an estimated prevalence of up to 70% in PWH.3-5 Although low BMD is well described in PWH, data remains limited on the clinical implications of these issues, and background data on the clinical implications of these issues are lacking. Low bone mineral density (BMD) and associated osteoporosis is one of the emerging concerns that has been described, with an estimated prevalence of up to 70% in PWH.3-5

Osteoporosis is a pathologic bone disorder characterized by low BMD and microarchitectural bone disruption that results in increased risk for fracture.9 The majority of studies investigating BMD in males with hemophilia have used areal BMD measurement by dual X-ray absorptiometry, which is unable to account for geometric differences affecting true volumetric BMD.10 Furthermore, dual X-ray absorptiometry provides no information on the microarchitecture of the cortical and trabecular compartments that ultimately underpin bone strength.11

In this pilot study, we used high-resolution peripheral quantitative computed tomography (HR-pQCT) to measure true BMD and detect changes in cortical and trabecular bone microarchitecture (to an isotropic voxel size of 82 μm) and used novel microfinite element analysis (μFEA) to assess the effect of skeletal alterations on bone strength compared with matched controls. Because inactivity and cytokine-induced bone mineral loss resulting from chronic hemophilic arthropathy are proposed mechanisms for BMD loss in PWH,12,13 Gilbert joint scores for severity of hemophilia arthropathy were measured for correlation analysis.14

Study design

Subjects

After obtaining University of Calgary Research Ethics Board approval, 18 patients with hemophilia A and hemophilia B (≥18 years old) with severe (factors VIII or IX, <1 U/dL) or moderate (factors VIII/IX, 1-5 U/dL) disease were recruited through the Southern Alberta Rare Blood and Bleeding Disorders Comprehensive Care Program. Subjects receiving corticosteroids for more than 3 months were excluded. HR-pQCT data on age- and sex-matched controls (at a 2:1 ratio to PWH) were obtained from the Canadian Multicentre Osteoporosis Study on healthy subjects.15

Protocol

All procedures were conducted at the Southern Alberta Rare Blood and Bleeding Disorders Program and the University of Calgary Bone Imaging Laboratory. For each subject, anthropomorphic measurements and Gilbert joint score were collected. Gilbert joint score, a measure of severity of joint arthropathy, reflects the combined score of 6 joints (elbows, knees, ankles), where a higher score indicates worse arthropathy, and was determined by a single, expert-trained examiner (A.L.) for all subjects.16 Bone microarchitecture and strength of the nondominant
Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Hemophilia (n = 18)</th>
<th>Control (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>18 (100)</td>
<td>36 (100)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>33.5 ± 3.1</td>
<td>33.7 ± 2.2</td>
<td>.961</td>
</tr>
<tr>
<td>Height, cm</td>
<td>167.2 ± 6.8</td>
<td>177.6 ± 1.2</td>
<td>.148</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.7 ± 5.6</td>
<td>78.1 ± 2.2</td>
<td>.160</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.5 ± 2.4</td>
<td>24.8 ± 0.8</td>
<td>.512</td>
</tr>
<tr>
<td>HIV-positive, n (%)</td>
<td>3 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia A/B, n (%)</td>
<td>15 (83)/3 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe/moderate hemophilia, n (%)</td>
<td>15 (83)/3 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis, n (%)</td>
<td>4 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary prophylaxis, n (%)</td>
<td>5 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive fracture history, n (%)</td>
<td>4 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D, nmol/L†</td>
<td>59.1 ± 6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilbert joint score†</td>
<td>15.7 ± 2.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†Gilbert joint score measured in 17 subjects.
*Vitamin D levels available in 17 subjects; 7 (41%) vitamin D deficiency (<50 nmol/L).

Table 2. Distal tibia and radius bone parameters and biomechanical bone strength (derived from μFEA of HR-pQCT images)

<table>
<thead>
<tr>
<th>HR-pQCT bone parameters</th>
<th>Tibia</th>
<th>Radius</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD, mg HA/cm³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia</td>
<td>288.9 ± 15.8</td>
<td>346.7 ± 7.3</td>
<td>.003</td>
</tr>
<tr>
<td>Control</td>
<td>313 ± 19.8</td>
<td>372.3 ± 8.9</td>
<td>.012</td>
</tr>
<tr>
<td>Trabecular parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.15 ± 0.008</td>
<td>0.18 ± 0.005</td>
<td>.004</td>
</tr>
<tr>
<td>Tb.N, 1/mm</td>
<td>1.7 ± 0.05</td>
<td>2.0 ± 0.05</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tb.Th, mm</td>
<td>0.1 ± 0.005</td>
<td>0.09 ± 0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Tb.Sp, mm</td>
<td>0.52 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cortical parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ct.BMD, mg HA/cm³</td>
<td>880 ± 19.9</td>
<td>928 ± 5.4</td>
<td>.032</td>
</tr>
<tr>
<td>Ct.Th, mm</td>
<td>1.3 ± 0.07</td>
<td>1.5 ± 0.04</td>
<td>.049</td>
</tr>
<tr>
<td>Ct.Po, %</td>
<td>5.9 ± 0.67</td>
<td>4.9 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Biomechanical bone strength (derived from μFEA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated failure load, N</td>
<td>6357 ± 377</td>
<td>7573 ± 217</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>2371 ± 162</td>
<td>3013 ± 90</td>
<td>.002</td>
</tr>
</tbody>
</table>

Unless otherwise specified, values are presented as percentage or mean ± SE. Comparisons of bone parameters are adjusted for age and sex.

BMD, total volumetric bone mineral density; BV/TV, trabecular bone volume to total volume ratio (a measure of trabecular bone mineral density); Ct.BMD, cortical bone mineral density; Ct.Po, cortical porosity; Ct.Th, cortical thickness; HA, hydroxyapatite; N, newton; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; μFEA, microfinite element analysis.

Results and discussion

As indicated in Tables 1 and 2, 18 patients with moderate or severe hemophilia were included in this pilot study, including 15 (83%) with hemophilia A. Among them, 15 (83%) have severe disease, 3 (17%) are HIV-positive, and 4 (22%) have documented liver cirrhosis. Only 5 (28%) had received primary prophylaxis. The control and hemophilia groups had similar average age (33.5 vs 33.7 years), whereas the controls had insignificantly higher height and weight and lower BMI. Vitamin D levels were available on 17 patients; 41% had vitamin D deficiency (<50 nmol/L). Compared with matched controls, PWH demonstrated significantly lower nondominant distal tibia and radius total BMD (288.9 and 313 mg HA/cm³; P = .003, P = .012), trabecular BMD (BV/TV: 0.15 and 0.14; P = .004, P = .014), and cortical BMD (880 and 916 mg HA/cm³; P = .032, P = .003). In addition, PWH had significantly lower Tb.N in the distal tibia, but not radius, and increased Tb.Sp at both tibia and radius. Ct.Th was significantly lower at both sites, but not Ct.Po.

The three-dimensional reconstructed image of the distal radius of a patient with severe hemophilia A illustrates fewer trabeculae that are less dense, as well as a thinner cortex compared with the matched healthy control (Figure 1A).

μFEA assessment of the reconstructed HR-pQCT images indicates that the lower BMD and microarchitectural deficits present in PWH translated into significantly lower failure load at the distal tibia (6357 vs 7573 N; P = .009) and radius (2371 vs 3013 N; P = .002) compared with matched controls (Table 2; Figure 1B).

There is a significant inverse correlation of Gilbert joint score to tibia BMD (R = −0.697; P = .002), tibia failure load (R = −0.635; P = .006), and radius failure load (R = −0.552; P = .022) (Figure 1C). An inverse relationship with Gilbert score is seen for radius BMD.
(R = −0.372; P = .142), although it is not statistically significant. Individuals who received primary prophylaxis appear to have higher failure load.

These compartmental alterations in microarchitecture in PWH are similar to those reported in postmenopausal women with osteoporosis and fragility fractures; specifically, lower trabecular BMD and fewer Tb.N. Decreased Ct.Po, described in postmenopausal women, was not seen in PWH.\textsuperscript{18,19} Cortical thinning and trabecular bone loss are known to be important contributors to bone fragility, whereas low cortical porosity appears to be the main contributor to low bone strength in patients with monoclonal gammopathy of unknown significance.\textsuperscript{20} Studies in fractured osteopenic women demonstrate that lower trabecular BMD (BV/TV) resulting from loss of Tb.N (as opposed to decreased Tb.Th) and increased Tb.Sp are important contributors to low bone strength (2-5 times by finite element method).\textsuperscript{21,22} Population studies using HR-pQCT and \( \mu \)FEA demonstrate progressive cortical

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Bone microarchitecture, bone strength, and correlation with joint score. (A) Representative HR-pQCT images at a nominal isotropic resolution of 82 \( \mu \)m of the distal radius in a 43-year-old male patient with severe hemophilia A (Tb.N, 1.82; Tb.Th, 0.061; Ct.Th, 0.94; Ct.Po, 2.23) and an age- and sex-matched control subject (Tb.N, 2.02; Tb.Th, 0.07; Ct.Th, 1.12; Ct.Po, 1.18). (B) Failure load (higher failure load = better mechanical bone strength) at the distal tibia in PWH and controls. The upper and lower boundaries of the box plot represent the interquartile range, the diamond represents the mean, the line dividing the box plot represents the median, and the whiskers indicate the maximum and minimum values. (C) Failure load at distal tibia vs total Gilbert joint score (higher joint score = more severe arthropathy) in 17 PWH (the remaining patient did not have Gilbert joint score available). Open square data points (○) indicate subjects who received primary prophylaxis (only 4 of 5 receiving primary prophylaxis shown, as 1 did not have joint score available) and as a whole appears to have lower Gilbert joint scores and higher failure load. The outlier (X) with the lowest tibial failure load has liver cirrhosis secondary to hepatitis C virus infection and HIV, which likely explains the lower tibial failure load and BMD. When the outlier is excluded from the correlation analysis, the statistically significant inverse correlation of Gilbert joint score and tibial BMD and failure load still stands (tibial failure load: \( R = −0.601 \) \( P = .0139 \); tibial BMD: \( R = −0.66 \) \( P = .005 \); \( R = \) Spearman \( r \)).}
\end{figure}
Concerning, considering their average age was only 33.5 years. Low trabecular BMD specifically at the distal radius has also been described in boys with hemophilia (aged 6.6–19.8 years), using lower-resolution peripheral quantitative computed tomography (pQCT). Taken together, these findings suggest microarchitectural deficits start at an early age in hemophilia, and poor bone strength can be expected to worsen over time.

Through the mechanism for low BMD and osteoporosis in hemophilia is multifactorial. One of the hypothesized contributors to this process is severity of hemophilic arthropathy, which may result in decreased axial loading caused by inactivity, and/or inflammatory cytokine-induced bone resorption. This study suggests that hemophilic arthropathy results in greater BMD loss, as shown by the inverse association of joint score with BMD and failure load at both the tibia and radius.

Limitations of this study are the sample size and inability to perform subgroup analysis. In addition, the HR-pQCT data from this study are unable to describe the dynamic processes underlying the bone pathophysiology and identify whether there is excessive osteoclast-mediated resorption, decreased osteoblastic new bone formation, or both. Future inclusion of bone remodeling indices may help clarify the mechanisms and be pertinent to proposed interventions. Our data, however, are the first to our knowledge to describe abnormal microarchitectural changes in both cortical and trabecular compartments and significantly reduced bone strength associated with severity of hemophilic arthropathy in adult PWH. Further studies are needed to determine whether estimated bone strength using HR-pQCT is predictive of future fracture risk as these patients age.

Acknowledgments

We thank Michelle Kan, Duncan Raymond, and Anne Cooke from the University of Calgary Bone Imaging Laboratory for performing HR-pQCT scanning and μFEA calculations. A.L. is a recipient of the Bayer International Hemophilia Fellowship Award.

The study was supported in part by the University of Calgary Division of Hematology and Hematologic Malignancies Research Fund.

Authorship

Contribution: A.L., S.K.B., G.K., and M.-C.P. designed the study; S.K.B. supervised the HR-pQCT scans and μFEA calculations performed at the University of Calgary Bone Imaging Laboratory and provided control data from the Canadian Multicentre Osteoporosis Study; A.L. performed the statistical analysis and wrote the manuscript for this article; and all authors participated in its editing and revision.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Man-Chiu Poon, Foothills Medical Centre, 1403 29th St NW, Calgary, AB, Canada T2N 2T9; e-mail: mcpoon@ucalgary.ca

References

Premature changes in trabecular and cortical microarchitecture result in decreased bone strength in hemophilia

Adrienne Lee, Steven K. Boyd, Gregory Kline and Man-Chiu Poon