Reduced bone mineral density in patients with haemophilia A and B in Northern Greece

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Summary

Haemophilia A and B has been associated with increased prevalence of low bone mass (67–86%). The aim of this study was to estimate the prevalence of bone disease in haemophiliacs and its association with potential risk factors. Adult patients with haemophilia A and B followed-up in the Haemophilia Centre of Northern Greece were included. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) in lumbar spine (LS), femoral neck (FN), total hip (TH) and great trochanter (GT). One-hundred four male patients (aged 45.8 ± 15.1 years) and 50 controls (aged 44.9 ± 12.8 years) were screened. Low BMD was diagnosed in 28 patients (26.9%) and 10 controls (20%) (p=0.0001). Patients had lower BMD in TH (p=0.007), FN (p=0.029) and GT (p=0.008) than controls, without differences in LS. BMD was positively associated with the severity of haemophilia, history of herpes virus C or human immunodeficiency virus and level of physical activity, and negatively with the level of arthropathy. In multiple-regression analysis, only the level of physical activity and 25-hydroxyvitamin D [25(OH)D] significantly predicted BMD. Half of the patients had vitamin D deficiency. In conclusion, our study showed increased prevalence of low BMD in haemophiliacs. The levels of physical activity and 25(OH)D independently predicted low BMD.

Keywords

Haemophilia, osteoporosis, osteopenia, bone mass

Introduction

Osteoporosis is a skeletal disorder characterised by decreased bone mass and microarchitectural deterioration predisposing a person to an increased risk of fracture (1). Fractures significantly affect quality of life. In particular, hip fractures are associated with a two-fold increase in mortality rate compared to that of the general population (2). Although its most common form is postmenopausal, osteoporosis constitutes also an important clinical issue for men, still under-recognised. Male osteoporosis can be separated into primary (which is further divided into age-related or idiopathic if the patient is over or below 70 years old, respectively) and secondary. The most common causes of secondary osteoporosis are: endogenous or exogenous glucocorticoid excess, hypogonadism, vitamin D deficiency, alcoholism, smoking, hyperthyroidism, hyperparathyroidism and type 1 diabetes mellitus (3, 4). Epidemiological data suggest that about 3–6% of men >50 years of age have osteoporosis and 30% of hip fractures and 20% of vertebral fractures occur in men (3, 4). Furthermore, in older men, fracture-related morbidity and mortality seem to be higher than women, associated with the presence of more age-related co-morbidities, indicating thus that men with osteoporotic fractures are frailer than women (5).

Haemophilia A and B are X-linked recessive bleeding disorders characterised by deficiency of factor VIII (FVIII) and IX (FIX), respectively. Due to the physiological deficiency of tissue factor in the synovial membrane, the additional coagulation disorder in haemophiliacs leads to spontaneous intra-articular bleeding episodes (haemarthrosis) (6). Haemarthrosis leads to various structural transformations of the joint, such as synovial and vascular cell proliferation and cartilage destruction, via mechanical distension of the joint capsule, inflammatory infiltration of synovial membrane and cartilage cells and iron deposition. This entity is termed “haemophilic arthropathy” (7, 8).

Reduced mobility due to haemophilic arthropathy and avoidance of weight-bearing exercise during childhood or adolescence negatively affect the acquisition of peak bone mass and may increase bone resorption in adult life (9). Indeed, a few studies (10–20) and a meta-analysis (21) have assessed the prevalence of bone disease in haemophiliacs demonstrating that haemophilia is associated with low bone mineral density (BMD) both in children (10–13) and adults (14–20). In addition to these factors, hepatitis...
C virus (HCV) (17, 19) and human immunodeficiency virus (HIV) (19, 20, 22) infections have also been implicated in the pathogenesis of osteoporosis in haemophilia.

The aim of this study is to estimate (i) the prevalence of low bone mass in haemophiliac patients of Northern Greece and (ii) its association with possible risk factors.

Patients and methods

Adult patients aged 18 years or older with haemophilia A and B, who have a regular follow-up in the haemophilia centre of Northern Greece of “Hippokration” Hospital of Thessaloniki, were included. The study was approved by the Local Ethics Committee and all the participants gave their informed consent. Severe haemophilia was defined as activity of FVIII FIX <1%, moderate as FVIII/FIX activity of 1–5% and mild as FVIII/FIX activity of 5–40%.

Patients were given a brief questionnaire classifying their physical activity into five general categories. More specifically, unrestricted school/work and recreational activities were scored as 5, full school/work with limited recreational activity levels, due to pain, loss of motion or weakness was scored as 4, limited school/work and recreational activity levels due to pain, loss of motion or weakness was scored as 3, limited school/work, recreational activity levels and self-care activity levels due to pain, loss of motion or weakness was scored as 2, while inability to participate in recreation due to pain, loss of motion or weakness and requirement of assistance from another person for school/work/self-care was scored as 1 (19). Body mass index (BMI) calculated as weight (kg)/height (m²), individual history (focusing on factors associated with increased fracture risk according to the recently developed FRAX model (23), such as alcohol, smoking, corticosteroids, rheumatoid arthritis, previous fracture and causes for secondary osteoporosis, such as vitamin D deficiency) and family history of osteoporosis and fractures were obtained. FRAX calculates the 10-year probability of hip and major osteoporotic fractures in both women and men using information on 10 clinically available risk factors for fracture (23).

All patients underwent the following blood tests: complete blood count, FVIII or FIX activity levels and inhibitor titres, serum creatinine, calcium, albumin, phosphorus, alkaline phosphatase, liver function tests, thyrotropin (TSH) levels, free T4, 25-hydroxy vitamin D [25(OH)D], parathyroid hormone and total testosterone. Vitamin D deficiency was diagnosed when 25(OH)D levels were below 20 ng/ml, low testosterone was defined as levels <200 ng/dl, and hyperthyroidism was diagnosed when TSH levels were <0.4 mIU/l with or without elevated FT4. Serological screening for HCV, hepatitis B virus and HIV, with viral loads if screened positive, was also carried out.

Bone mineral density (BMD) measurement

BMD was assessed by dual X-ray absorptiometry (DXA). All DXA measurements were performed by the same device (Challenger Envision osteodensitometer, Diagnostic Medical System, Montpellier, France), in “Hippokration” Hospital of Thessaloniki. Acquisition sites were the lumbar spine (LS) femoral neck (FN), great trochanter (GT) and total hip (TH). All DXA measurements were performed by a single experienced operator. Right FN was chosen if the left one was affected by severe arthropathy or when arthroplasty had been performed.

Although several new technologies measuring central and peripheral sites have been developed, DXA measurement at the hip and spine is the best predictor of hip and vertebral fractures, respectively. Areal BMD is expressed in absolute terms of grams of mineral per square centimetre scanned (g/cm²). It is also expressed as a relationship to two norms: compared with the expected BMD for the patient’s age and sex, which is called Z-score, or compared with “young normal” adults of the same sex, which is called T-score. The difference between the patient’s score and the norm is expressed in standard deviations (SD) above or below the mean (1, 3, 4, 24).

The World Health Organization has established the following definitions based on BMD measurements: in postmenopausal women and men >50 years of age, T-score –1 SD and above is defined as “normal”, values between –1 and –2.5 SD are defined as osteopenia, while values below –2.5 SD are defined as osteoporosis (1, 3, 4). According to the criteria for the definition of osteoporosis in males by the International Society of Clinical Densitometry (ISCD), for patients <50 years of age, Z-scores should be used and when they are < -2 SD or lower, BMD is defined as “below the expected range for age” (24).

BMD was also measured in age-matched healthy males, without a reported cause or severe risk factor for secondary osteoporosis (such as rheumatoid arthritis, treatment with corticosteroids, hormonal disorders associated with osteoporosis malignancies) served as controls. They were from the same geographical region with the patients, in order to obtain more accurate results. They gave their informed consent and their screening was approved by the ethical committee. None of the controls was infected by HBV, HCV or HIV. There was no restriction in their physical activity (level 5). Their risk profile according to FRAX score was low.

Assessment of haemophilic arthropathy

Plain radiographs of the lower limb joints (knees and ankles) were obtained by all patients and were examined by two expert radiologists. Two different score systems were used for this purpose: the Pettersson score (normal joints are scored as 0 and the highest score possible for a joint is 13, thus for four joints the maximum possible score is 52) and the Arnold-Hilgartner classification system (normal joint score is regarded as stage 0 and the highest as stage 5) (25).
Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL; USA) for Windows (version 17.0). All statistical tests were two-tailed; p-values <0.05 were considered statistically significant. Based on the distribution of data (checked by Kolmogorov-Smirnov test & Shapiro-Wilk test) non-parametric statistics were used. Descriptive statistics of demographic and clinical data are represented as frequencies, percentages, means, SD and minimum & maximum values.

Initial analyses examined the associations between BMD (either as a categorical or ordinal parameter) and severity of haemophilia, viral infection, level of physical activity, haemophilic arthropathy scores or number of affected joints, using Chi² and Mann-Whitney U-test, as appropriate. Spearman r correlation was conducted in order to indicate the association between BMD in four sites (LS, TH, FN, GT expressed in g/cm²) and severity of arthropathy assessed by Pettersson score and Arnold-Hilgartner classification system, number of affected joints, age and BMI. Moreover, Kruskal-Wallis H-tests were performed in order to find any significant correlation between BMD sites (LS, TH, FN, GI) and hormonal factors (25(OH)D, testosterone), level of physical activity, severity of haemophilia and other risk factors associated with low BMD.

Finally a series of hierarchical multiple regressions were used to test the predictability of various variables to BMD.

Results

One-hundred four male patients (89 with haemophilia A and 15 with haemophilia B) aged 45.8 ± 12.8 years, range 27–70) were screened. The patients' demographics are presented in Table 1. Low BMD was diagnosed in 28 patients (26.9%), with a significantly higher prevalence than in controls (20%) (p=0.0001). Patients had lower BMD in TH (p=0.007), FN (p=0.029) and GT (p=0.008) than controls, while no differences in lumbar BMD were observed between the two groups (p=0.794). Nineteen patients (68% of those with low BMD) demonstrated decreased BMD in LS, while 21 (75%) in TH and 14 (50%) in FN, respectively.

With respect to the severity of haemophilia, low BMD was observed in six (32%) of those with severe, in nine (43%) of those with moderate and in 13 (20%) of those with mild disease (Fig. 1). Regarding viral infections, none of the HBV, 12 (28%) of the HCV and three (42%) of the HIV patients had low BMD (Fig. 2). In 13 of 28 (46%) additional causes of secondary osteoporosis were identified, such as exogenous corticoid excess (n=1 or 3.6%), vitamin D deficiency (n=14 or 50%) and low testosterone with hyperthyroidism (n=1 or 3.6%). Ninety-nine patients of the whole were receiving on-demand coagulation factors, while only five were on prophylactic therapy 1–2 times/week.

BMD as a categorical parameter (defined as "normal" or "low") was positively associated with the severity of haemophilia [χ²(2) =37.29, p=0.0001], the history of HCV [χ²(1) =3.85, p=0.05] or HIV [χ²(1) =77.89, p=0.0001] and the level of physical activity [χ²(3) =71.62, p=0.0001], while it was negatively associated with the level of arthropathy assessed by both Pettersson score (U=463.5, p=0.039)-as a total score in knees and ankles- and Arnold-Hilgartner system in knees (U=489.0, p=0.019), in univariate analysis. These correlations were due to the association of BMD in hip (TH and FN) with these parameters, while no significant as-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number</th>
<th>%</th>
<th>Mean</th>
<th>Range</th>
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<td>104 A 90 B 14 Controls 50</td>
<td>87</td>
<td></td>
<td></td>
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<tr>
<td>Severity</td>
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<td>64</td>
<td>62</td>
<td>19</td>
</tr>
<tr>
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<td>20</td>
<td></td>
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</tr>
<tr>
<td>Severe</td>
<td>19</td>
<td>18</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>Patients</td>
<td>45.87 ± 15.15</td>
<td>44.9 ± 12.8</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>Patients</td>
<td>27.05 ± 4.51</td>
<td>28.49 ± 4.39</td>
<td>18.82–44.31</td>
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<tr>
<td>Number of affected joints</td>
<td>Patients</td>
<td>2.69 ± 2.31</td>
<td>0–8</td>
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</tr>
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<td>Total Pettersson score</td>
<td>Patients</td>
<td>9.61 ± 10.36</td>
<td>0–47</td>
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<td>HBV infection</td>
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<td>HIV infection</td>
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<td>Lumbar spine BMD (g/cm²)</td>
<td>Patients</td>
<td>1.047 ± 0.169</td>
<td>1.065 ± 0.156</td>
<td>0.637–1.626</td>
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<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td>Patients</td>
<td>0.938 ± 0.163</td>
<td>1.015 ± 0.176</td>
<td>0.549–1.368</td>
</tr>
<tr>
<td>Controls</td>
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<tr>
<td>Total hip BMD (g/cm²)</td>
<td>Patients</td>
<td>0.929 ± 0.161</td>
<td>1.018 ± 0.189</td>
<td>0.567–1.339</td>
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<tr>
<td>Great trochanter BMD (g/cm²)</td>
<td>Patients</td>
<td>0.757 ± 0.152</td>
<td>0.848 ± 0.167</td>
<td>0.464–1.177</td>
</tr>
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<tr>
<td>Low BMD</td>
<td>Patients</td>
<td>28</td>
<td>26.9</td>
<td>20</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
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</table>

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Association was found with LS BMD, except for the grade of arthropathy in knees assessed by Arnold-Hilgartner system ($r_s=-0.224$, $p=0.036$).

More specifically, BMD (expressed in g/cm$^2$) in TH was positively associated with the severity of haemophilia ($H=12.84$, $p=0.002$), the history of HCV ($U=947.5$, $p=0.025$) or HIV ($U=184.5$, $p=0.047$) and the level of physical activity ($H=22.25$, $p=0.0001$), in unadjusted analysis, whereas it was negatively associated with the number of affected joints ($r_s=-0.237$, $p=0.016$) and the level of arthropathy assessed by both Pettersson score ($r_s=-0.584$, $p=0.0001$) in knees and ankles, and Arnorld-Hilgartner system in knees ($r_s=-0.432$, $p=0.0001$) and ankles ($r_s=-0.432$, $p=0.0001$). Most of these correlations were detected both in FN and GT sites. No association was found between BMD at any site and age or BMI. With respect to age, 38 of 104 patients (37%) were >50 years of age, 12 of which (32%) had decreased BMD, while 66 (63%) were <50 years of age, 16 of which (24%) had decreased BMD.

Seventeen patients (16%) had a history of fracture, with three of them having sustained two episodes (total number of fractures: 20). Fractures had occurred in five of 28 (18%) patients with low BMD compared with 12 of 76 (16%) of those with normal BMD ($p=0.831$). The fractures were the consequence of a trivial fall in nine patients (53%). The mean age at which fractures happened was 27.7±19 years (range 3–65). Fracture sites were: wrist (n=5), elbows (n=4), tibia (n=2), femur (n=2), radius (n=2), lumbar vertebrae (n=2), patella (n=1), clavicle (n=1) and hip (n=1). Six patients with severe haemophilia (31%), four of those with moderate (19%) and seven of those with mild disease (11%) had sustained a fracture. History of fracture was positively associated with the severity of haemophilia [$x^2(2) =37.29$, $p=0.0001$], the number of affected joints [$U=543.0$, $p=0.043$], the level of physical activity [$x^2(3) =71.62$, $p=0.0001$] and HCV [$x^2(1) =4.65$, $p=0.031$] and HIV [$x^2(1) =77.86$, $p=0.0001$] infection.

In multiple regression analysis, after controlling for age, BMI, severity of arthropathy, number of affected joints, HCV/HIV infections, clinical risk factors associated with osteoporosis (such as smoking, alcohol >2 units/day, corticosteroids, rheumatoid arthritis, individual or family history for fracture), 25(OH)D and testosterone levels, only the level of physical activity and 25(OH)D levels significantly predicted BMD in TH, [$F_{1,72}=18.42$, $p=0.0001$, $\beta=0.496$, $\Delta R^2=0.156$, 95% confidence interval (CI): 0.052–0.142] for physical activity and [$F_{1,75}=4.95$, $p=0.029$, $\beta=0.216$, $\Delta R^2=0.041$, (95% CI:0.000–0.006) for 25(OH)D, respectively], FN ($F_{1,72}=9.25$, $p=0.003$, $\beta=0.367$, $\Delta R^2=0.086$ (95% CI: 0.025–0.121) for physical activity and $F_{1,73}=8.85$, $p=0.004$, $\beta=0.303$, $\Delta R^2=0.080$, (95% CI: 0.001–0.007), for 25(OH)D, respectively] and GT ($F_{1,72}=21.92$, $p=0.0001$, $\beta=0.539$, $\Delta R^2=0.185$, CI 0.057–0.143) for physical activity.)

Interestingly, 40 out of 86 patients (47%) with available 25(OH)D levels had vitamin D deficiency and 10 (12%) severe deficiency, that is 25(OH)D <10 ng/ml. Sixteen of 25 (64%) patients with low BMD and available 25(OH)D levels were vitamin D deficient. We further assessed any possible factors for the low vitamin D levels and found that there was a positive association between 25(OH)D and the level of haemophilic arthropathy assessed by Pettersson score ($r_s=-0.256$, $p=0.032$) and a negative association with the number of affected joints ($r_s=-0.219$, $p=0.045$).

**Discussion**

This is the largest study conducted so far evaluating the prevalence of low BMD in haemophilic patients and its association with possible risk factors. We found an increased prevalence of bone disease (either as osteopenia or osteoporosis or as “below the expected range for age”) in haemophilic patients of Northern Greece, compared with apparently healthy age- and sex-matched controls. However, this prevalence was lower than previously reported.
(17–20), although even rates of 7.5% (13) or 38% (11) have been described in paediatric studies. This difference may be attributed to two parameters. Firstly, the vast majority (or even the total number) of patients included in previous adult studies were suffering from severe haemophilia and the reported rates of low BMD were >67%, although some lacked a control group (17–20). In our study, the percentage of patients with low BMD amongst those with severe haemophilia was 32%. Another significant reason for the difference in prevalence is the fact that these studies used T-score for all patients irrespective of their age. Based on the criteria for the definition of osteoporosis in males by the ISCD, we used T-scores for patients older than 50 years and Z-scores for those younger than 50 (24). If we had used T-scores, the incidence of bone disease in our series would have been about 50% and about 74% in those with severe haemophilia (14 of 19).

In our study, we observed that only BMD in hip sites (TH, FN and GT) was significantly different from controls, in contrast to lumbar BMD. In studies using control groups, lower BMD values have been reported in both LS (9–15, 17, 19) and hip (14–16, 18, 20) compared with age and sex-matched healthy controls. Notably, lower BMD values and higher prevalence of low bone mass in hip compared with LS have also been reported (14–16, 18, 20). If we had used T-scores, the incidence of bone disease in our series would have been about 50% and about 74% in those with severe haemophilia (14 of 19).

Several parameters contributed to low BMD in the present study: severity of haemophilia, level of physical activity, severity of arthropathy, HCV and HIV exposure and vitamin D deficiency. The severity of haemophilia as a co-factor for decreased BMD was assessed by few studies, which found no association at all (13, 26). Nonetheless, the history of fracture was positively associated with the severity of haemophilia in our study. The level of physical activity, the number of affected joints and HCV and HIV infections were also positively associated with the history of fracture. Similar fracture rates with our study (12–20%) have also been demonstrated by previous studies (18, 19), although others have reported lower rates (4%) (27). However, it is not clear whether haemophilia increases or decreases fracture risk and, thus, a prospective case-control study of fracture incidence and prevalence is needed to better clarify this issue (28).

The level of physical activity (11, 19), as well as the severity of arthropathy in lower limb joints assessed either by clinical or radiological criteria (17–20), were confirmed as major factors for low BMD by previous reports. Physical inactivity has been recognised as a risk factor for osteoporosis and osteoporotic fractures in middle-aged and older individuals (29). On the other hand, exercise and, in particular, weight-bearing activity at childhood and adolescence is essential for acquisition of adequate peak bone mass and thus higher BMD at older ages (30). Although the exact mechanisms are not fully elucidated, it seems that physical activity and mechanical loading stimulate bone formation (9). Moderate and readily accessible weight-bearing exercise undertaken before puberty seems to increase femoral BMD by increasing cortical thickness (31). As mentioned above, haemophilic arthropathy and concomitant muscle atrophy attenuate mobility and participation in sport-activities (10, 11, 28). Prolonged immobility may also be associated with recovery from serious bleeding episodes. Haemophilic arthropathy leads also to avoidance of weight-bearing activ-

![Figure 3: Association between the level of physical activity and BMD.](image)

<table>
<thead>
<tr>
<th>Score</th>
<th>BMD LS (g/cm²)</th>
<th>BMD TH (g/cm²)</th>
<th>BMD Neck (g/cm²)</th>
<th>BMD GT (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.040 ± 0.297</td>
<td>0.973 ± 0.145</td>
<td>0.966 ± 0.150</td>
<td>0.810 ± 0.132</td>
</tr>
<tr>
<td>4</td>
<td>1.037 ± 0.135</td>
<td>0.952 ± 0.146</td>
<td>0.989 ± 0.144</td>
<td>0.795 ± 0.135</td>
</tr>
<tr>
<td>3</td>
<td>1.010 ± 0.159</td>
<td>0.790 ± 0.132</td>
<td>0.816 ± 0.147</td>
<td>0.622 ± 0.128</td>
</tr>
<tr>
<td>2</td>
<td>0.837 ± 0.282</td>
<td>0.725 ± 0.173</td>
<td>0.719 ± 0.241</td>
<td>0.593 ± 0.086</td>
</tr>
</tbody>
</table>

ity, due to the patients’ fear of precipitating a bleed (10, 11, 28). Another proposed mechanism linking haemophilic arthropathy with low BMD is the increased osteoclastic activity observed in haemophilic patients, judging by the increased levels of bone resorption markers (20). Haemophilic arthropathy shares the same pathogenesis with rheumatoid arthritis, in which several cytokines, such as interleukin-6 and the receptor activator of nuclear factor-kB (RANK) ligand (RANKL), play a key role in the induction of osteoclastogenesis (6, 32).

Some argue that arthropathy does not inevitably result in osteopenia and osteoporosis, since physical inactivity may lead to a secondary increase in body weight (28). The protective role of obesity on BMD has long been documented (33). However, we did not find any correlation between BMI and BMD, which is in line with previous reports (10) and with a recent meta-analysis of studies with haemophilia and osteoporosis assessing relevant predictors of decreased BMD (21). Notably, some authors did recognise low BMI as an additional risk factor for low BMD in haemophiliacs (17, 19). We also did not find any correlation between age and BMD, in contrast to previous reports which showed a negative correlation between age and BMD (17, 19). This is perhaps due to the fact that most patients in our study (63% of the whole) were <50 years, and thus differences in BMD were less likely emerge. In one study with available data regarding the association between the age of patients with haemophilia and BMD, 86% of those >50 years of age had decreased BMD (19), while this percentage in our study was 24% compared with the 32% of those <50 years of age.

Except for prolonged inactivity and avoidance of weight-bearing exercise, HCV and HIV infections have also been regarded as potential co-factors for decreased BMD in haemophilic patients. Some researchers have found significant association between HCV infection and BMD (17, 19), while others found no association at all (10, 15, 18, 21). The exact pathogenetic mechanisms regarding the role of HCV in bone disease have not been clarified. Hypogonadism, low levels of 25(OH)D and insulin-like growth factor-I (IGF-I), as well as high bilirubin levels, as a consequence of liver disease, may lead to attenuation of bone formation and enhancement of bone resorption (34). One study demonstrated low free androgen index in haemophilic patients with HCV and low BMD, although serum total and free testosterone were normal in another study (20). HCV has also been proposed to stimulate osteoclastic activity via RANK/RANKL/Osteoprotegerin pathway (35), an effect that has also been attributed to anti-HCV treatment (36).

Regarding HIV infection, high prevalence of low BMD and considerable progression to osteopenia/osteoporosis has been found in HIV patients (37, 38). HIV infection has also been associated with low BMD in studies with haemophiliacs (19, 20). Possible causes are low BMI, smoking, alcohol and HIV-related pathologies, such as kidney disease, muscular degeneration and hormonal disorders (including vitamin D, PTH, sex steroids) (39). Another proposed mechanism linking HIV with bone metabolism may be the HIV-induced bone loss via the RANKL pathway. The viral load has been positively associated with the concentrations of RANKL and specific molecules belonging to tumour necrosis factor (TNF) and the TNF-receptor family (40). Antiviral therapy has also been associated with increased markers of bone resorption (38). However, the association of HIV with low BMD was not confirmed by others (17).

Except for physical activity, 25(OH)D levels were independently associated with low BMD in the hip in the present series. About half of the patients (and 2/3 of those with low BMD) had vitamin D deficiency and 12% severe deficiency, despite living in a climate which provides adequate sun exposure and concomitant skin production of vitamin D. Few studies have assessed the association between vitamin D and BMD in haemophilic patients. In a recent study, all the patients studied had levels of 25(OH)D <30 ng/ml, being significantly lower in those with low BMD (19), while in others the levels of 1,25-dihydroxyviamin D3 (the active metabolite of vitamin D) did not differ between persons with haemophilia and healthy controls (14). We also found that the level of haemophilic arthropathy and the number of affected joints were significantly related to 25(OH)D levels. Although these findings can not suggest causality, it can be speculated that vitamin D deficiency contributes to a more severe form of haemophilic arthropathy. This has been shown by studies of patients with rheumatoid arthritis, in which disease activity and disability scores were inversely related to 25(OH)D levels (41, 42). Furthermore, low serum 25(OH)D concentrations have been associated with higher joint pain scores in postmenopausal women with osteoarthritis (43). On the other hand, decreased mobility may be lead to reduced sun exposure and thus low vitamin D levels.

In conclusion, this study confirms a high prevalence of low BMD (although lower than usually reported) in haemophilic patients in the largest cohort studied so far and adopting the latest definition of the international osteoporosis societies. Several factors seem to contribute to bone disease, such as severity of haemophilia, level of physical activity, severity of arthropathy, HCV and HIV exposure and vitamin D status. Further analysis showed that the level of physical activity and the 25(OH)D levels indepen-
dently predicted low BMD in haemophilic patients. These find-
ings necessitate the need for intervention trials focusing on ther-
apies for patients with haemophilia and low BMD.

**Conflict of interest**
None declared.

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