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Increased bone resorption is implicated in the pathogenesis of bone loss in hemophiliacs: correlations with hemophilic arthropathy and HIV infection

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Abstract Osteoporosis has been recently recognized as a severe comorbidity factor in hemophilia. However, its pathogenesis is still obscure. We evaluated the incidence of osteoporosis in 90 hemophilia patients and investigated possible correlations with clinical and laboratory data. Out of the 90 patients, 80 (89%) had severe hemophilia, and 35 (38.9%) were human immunodeficiency virus (HIV)-positive. Hemophilic arthropathy was assessed using World Federation of Hemophilia clinical score and Petterson radiological score. Bone mineral density of the lumbar spine (LS) and femoral neck (FN) were measured using dual-energy X-ray absorptiometry. Bone turnover was evaluated by the measurement of: (1) bone resorption markers [N-terminal cross-linking telopeptide of collagen type I (NTX), C-terminal cross-linking telopeptide of collagen type I (CTX), and tartrate-resistant acid phosphatase isoform-5b (TRACP-5b)], (2) bone formation markers [bone-alkaline phosphatase (bALP) and osteocalcin], and

(3) osteoclast stimulators (receptor activator of nuclear factor- κ B ligand, osteoprotegerin, and tumor necrosis factor- α). Osteopenia or osteoporosis was observed in 86% and 65% of the patients in FN and LS, respectively. Osteoporosis was more common among HIV-positive patients in both FN (65.3% vs 41.6%; $p = 0.007$) and LS (17.86% vs 5.41%, $p = 0.004$). The severity of osteoporosis in FN correlated with the patients' total clinical and radiological score ($p = 0.001$). Hemophilia patients showed increased osteoclastic activity (significant increase of TRACP-5b, NTX, and CTX), which was not accompanied by a comparable increased bone formation (reduced osteocalcin and borderline increase of bALP). In multivariate analysis, HIV infection ($p=0.05$) and total clinical score ($p=0.001$) were independent risk factors for osteoporosis development. We conclude that there is a high prevalence of osteoporosis among hemophiliacs, which is related to the severity of arthropathy and is enhanced by HIV infection. We report for the first time a high bone resorption that seems not to be balanced by a comparable bone formation.

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Keywords Hemophilia · Osteoporosis · Bone metabolism · HIV infection

Introduction

Osteoporosis is a disorder characterized by decreased bone mass and microarchitectural deterioration, resulting in loss of bone strength and fragility fractures [1, 2]. The fundamental pathogenetic mechanisms includes: (a) failure to achieve optimal strength during growth, (b) excessive bone resorption resulting in loss of bone mass, and (c)

failure to replace lost bone due to defects in bone formation [3, 4]. Increased incidence of osteoporosis has been observed in postmenopausal women; the elderly; and in patients with disorders such as hypogonadism, thalassemia syndromes, and hematologic malignancies [5, 6].

Hemophilia is a congenital bleeding disorder characterized by intra-articular bleeding leading to severe hemophilic arthropathy [7]. Recent studies have demonstrated that osteoporosis is an underestimated problem among hemophilia patients [8]. Prolonged immobilization and lack of weight-bearing exercise, as well as comorbid infections [hepatitis C virus (HCV) and human immunodeficiency virus (HIV)], are suggested as the main factors contributing to the pathogenesis of osteoporosis in hemophilia. Although there are a few studies where the prevalence of osteoporosis was investigated [9, 10], the exact pathogenetic mechanism has not yet been fully clarified.

This study was conducted to estimate the incidence of osteoporosis among a cohort of hemophilia patients in Greece and investigate possible correlations with clinical and laboratory data concerning bone metabolism in an attempt to better understand the pathogenetic mechanisms that are involved in the development of bone loss in hemophilia.

Patients and methods

Patients with severe and moderate hemophilia A and B (FVIII of FIX <3%) who had a regular follow up in the hemophilia center of “Laikon” Hospital in Athens (Greece) and had a history of hemarthrosis were included in the study. Patients with concomitant diseases that could affect bone metabolism and lead to secondary osteoporosis, such as vitamin D deficiency, cortisone intake, hypogonadism, and thyroid gland dysfunction, were excluded. Informed consent was obtained from all patients prior to inclusion in the study. The study was conducted with the approval of the respective ethical committees in keeping with the guidelines of Helsinki.

Assessment of hemophilic arthropathy The severity of hemophilic arthropathy was assessed in all patients in the six major joints—knees, ankles, and elbows—by the orthopedic consultant of the center, using the *clinical score* as described by the Orthopedic Advisory Council of the World Federation of Hemophilia [11]. This score is based on joint swelling, fixed flexion deformity, range of motion, crepitus, instability, axial deformity, and muscle atrophy. The degree of arthropathy was graded between 0 and 12 for the knees and ankles, and from 0 to 10 for the elbows; thus, the maximum score was 68.

The *Pettersson score* [12] for joints was assessed on plain radiographs. The six major joints were scored from 0

to 13 based on the extent of radiological deterioration, with a maximum possible score of 78.

Measurement of bone mineral density Bone mineral density was measured using the dual-energy X-ray absorptiometry (DEXA) technique. Both sites, lumbar spine (LS) (L2–L4) and femoral neck (FN), were evaluated. *T*-score values between -1.5 and -2.5 were considered as osteopenia, while values lower than -2.5 were considered as osteoporosis, according to the standard World Health Organization (WHO) criteria for postmenopausal women [13]. We used the same criteria for the definition of osteoporosis in males, as the International Society of Clinical Densitometry (2004) has applied the same bone mineral density (BMD) criteria for the definition of osteoporosis in males and recommended that a male database may be used for male patients and a Caucasian database for both Caucasian and non-Caucasians [14, 15]. Moreover, BMD *T* scores were derived by comparison to BMD values of normal persons from the Greek population [16].

Measurement of markers of bone remodeling and osteoclast regulation After veinpuncture, serum was separated within 4 h and stored at -70°C until the day of measurement. An enzyme-linked immunosorbent assay was used for the detection of serum (1) markers of bone resorption: N-terminal cross-linking telopeptide of collagen type I [NTX; Osteomark[®] NTx Serum, Ostex International, Seattle, WA, USA], C-terminal cross-linking telopeptide of collagen type I (CTX; serum CrossLaps[®], Nordic Bioscience Diagnostics A/S, Herlev, Denmark), and 5b isoenzyme of tartrate-resistant acid phosphatase (TRACP-5b; BoneTRAP[®], Immunodiagnostic Systems, Boldon, Tyne and Wear, UK); (2) markers of bone formation: bone-specific alkaline phosphatase (bALP; Metra[®] BAP, Quidel, San Diego, CA, USA) and osteocalcin (OC; N/MID[®] Osteocalcin, Nordic Bioscience Diagnostics A/S); and (3) markers of osteoclast regulation: soluble receptor activator of nuclear factor-kappa B ligand (sRANKL; ampli-sRANKL, Biomedica Medizinprodukte, Gesellschaft GmbH & Co KG, Wien, Austria), osteoprotegerin (OPG; Biomedica Medizinprodukte, Gesellschaft GmbH & Co KG), and tumor necrosis factor-alpha (TNF- α ; Diaclone, Bensacon, France), according to the manufacturers' instructions.

The above biochemical parameters were also evaluated in a control group of 18 male, age-matched, healthy blood donors (median age of 37 years, range: 18–59 years). Each control was examined to ensure that there was no evidence of bone disease (osteoporosis or osteoarthritis) and no receipt of medication that could alter the normal bone turnover during the last 6 months. Weight as body mass index (BMI), calculated as weight (kg)/height (m)², was also evaluated in patients and controls.

Statistical analysis Differences in quantitative characteristics between patients and controls and among the categories defining the magnitude of osteoporosis were examined using the Wilcoxon rank-sum test and the Kruskal–Wallis rank test, respectively. Correlations between quantitative characteristics with FN and LS *T*-score levels were measured using the Spearman rank correlation coefficient. Independence of HIV infection and magnitude of osteoporosis was tested by the chi-square test. Potential risk factors of disease were evaluated, applying multiple logistic regression analysis. Additionally, associations of *T*-score levels with patients' characteristics were examined using multiple linear regression models. In models for both FN and LS *T* scores, HIV status and age, as a possible confounder, were included regardless of their level of significance.

Results

Patients Ninety-three patients were included in the study. Three of them were excluded from the analysis, one because of pituitary adenoma and two because of hyperthyroidism. The 90 patients finally analyzed had a median age of 36 years (range 18–60), while 35 of them (38.9%) were HIV-positive and 86 (95.5%) were HCV-positive. All HIV-positive patients were under highly active antiretroviral therapy. None of the patients had decompensated liver cirrhosis. The patients' characteristics are shown in Table 1.

Bone density measurements Seventy eight patients underwent DEXA scan, three of them only in LS because of bilateral total hip arthroplasty. Reduced bone density was found in 86.1% and 65.3% of the patients in FN and LS, respectively. In FN, osteopenia was found in 22 patients (30.5%); the median score was -1.90 (range: -2.33 to -1.53), while osteoporosis was found in 40 patients (55.6%); the median score was -3.53 (range: -4.66 to -2.50). In LS, 39 patients (52%) had osteopenia, with a median *T* score of -1.87 (range: -2.38 to -1.54), and 10 patients (13.3%) had osteoporosis, with a median *T* score of -2.75 (range: -3.23 to -2.51 ; Table 2).

In univariate analysis, the BMD *T* score in FN was significantly associated with the severity of hemophilic arthropathy as measured by the total clinical and radiological score ($r^2 = -0.63$ and -0.62 , respectively, $p < 0.001$; Table 3). The median clinical scores in patients with normal BMD, osteopenia, and osteoporosis were 12.5, 23, and 32.5, respectively ($p = 0.011$). Similar correlations were found between BMD *T* score and the radiological score (Fig. 1).

Patients with HIV infection had higher magnitudes of bone loss compared to HIV-negative hemophilia patients.

Table 1 Patients' descriptive characteristics

Factor	N:90	%	Median	Range
Hemophilia				
A	72	80.0		
B	18	20.0		
Severity				
Severe	80	89		
Moderate	10	11		
HIV-Ab				
Positive	35	38.9		
Negative	55	61.1		
HCV Ab				
Positive	86	95.5		
Negative	4	4.5		
Age (years)			36	18–60
Affected joints (<i>N</i>)			4	1–6
Total clinical score			26	1–68
Total radiological score			32	1–78

This difference was found in both LS and FN. In FN, the median *T* score was -3.27 (range: -3.80 to -2.41) and -2.25 (range: -3.32 to -1.13) for HIV-positive and HIV-negative patients, respectively ($p = 0.006$). In this site, no HIV-positive patient had normal bone density, compared to 10 (24.3%) of the HIV-negative patients. In LS, the difference between the two groups of patients was more prominent as only 15.5% of the HIV-positive patients had normal BMD and 21.2% had osteoporosis, compared to the 50% and 7.14% of HIV-negative patients, respectively. The median *T* scores in the two groups were -1.83 (range: -2.38 to -1.16) and -0.98 (range: -1.50 to -0.29), respectively ($p < 0.001$).

Evaluation of bone metabolism markers Hemophilia patients had increased levels of bone resorption markers compared to the control group. Median NTX levels of hemophilia patients were 19.49 vs 16.13 nM BCE/mM creatinine in the control group ($p = 0.018$). The respective values for patients and controls were 0.61 vs 0.47 ng/ml for CTX ($p = 0.028$) and 2.75 vs 1.38 U/L for TRACP-5b levels ($p < 0.001$). In contrast, the osteoclast regulators sRANKL, OPG, and TNF- α did not differ significantly between patients and controls. Median 25-OHvit D3 levels and BMI were also similar in the two groups (Table 4). Markers of bone formation showed a contradictory picture: osteocalcin was reduced ($p = 0.023$), while bALP was slightly increased ($p = 0.043$) in hemophiliacs compared to controls.

Markers of bone resorption were not different between HIV-positive and HIV-negative patients, while bALP was found significantly higher in HIV-positive patients [25.8 U/L

Table 2 Incidence of BMD reduction in study patients in the two sites of measurement

	FN		LS	
	N	Median T score (IQR)	N	Median T score (IQR)
Normal BMD	10 (13.9%)	-0.69 (-1.39 to 0.85)	26 (34.7%)	-0.43 (-1.28 to 1.51)
Osteopenia	22 (30.5%)	-1.90 (-2.33 to -1.51)	39 (52.0%)	-1.87 (-2.38 to -1.54)
Osteoporosis	40 (55.6%)	-3.53 (-4.66 to -2.50)	10 (13.3%)	-2.75 (-3.23 to -2.51)
Total	72 (100%)		75 (100%)	

(range: 17.0 to 33.3 U/L) vs 17.0 U/L (13.9 to 20.9 U/L); $p < 0.001$]. In addition, median BMI was found to be higher in the HIV-negative patients (25.40 vs 23.80 kg/m², $p=0.007$; Table 5).

The factors that were strongly related to the severity of BMD reduction in univariate analysis, such as age, total clinical score, and HIV status, were further investigated in multivariate models. Multiple regression analysis has shown that the severity of hemophilic arthropathy and HIV infection had independent prognostic values for the development of osteoporosis (Table 6). In FN, the odds ratio (OR) for osteoporosis was 2.42 [95% confidence interval (CI): 1.436–4.102] per ten-unit increase of total clinical score ($p = 0.001$), while HIV infection had an OR of 3.765 (95% CI: 1.026–13.820; $p = 0.046$). Furthermore, in LS, HIV infection was a stronger predictor for BMD reduction with an OR of 7.294 (95% CI: 1.917–27.758; $p = 0.004$), followed by the severity of arthropathy, which had an OR of 3.22 (95% CI: 1.331–7.821) per ten-unit increase of total clinical score ($p = 0.01$).

Discussion

Osteoporosis is a major problem of social health, with severe implications on the subject's quality of life, known to usually affect women over 45 years old. Only recently has it been demonstrated that hemophilia-induced osteoporosis could severely affect the morbidity of these patients,

increasing the rate of fractures in adult life [10]. However, its pathogenetic mechanism has not been fully evaluated to date.

In our cohort of hemophilia patients, the largest according to our knowledge in the literature, we have confirmed the high incidence of osteoporosis that few published studies have reported [8–10, 17, 18]. Overall, 86% of the hemophilia patients with severe and moderate hemophilia who were included in the study and underwent DEXA had pathologic BMD in FN according to the WHO criteria. Among them, more than a half (55.6%) had osteoporosis and the rest (30.5%) had osteopenia. Gallacher et al. first reported a reduced BMD in 19 hemophilia patients [8], and recently, similar results had also been reported in a larger study with 62 hemophilia patients [9]. In that study, 70% of the patients had reduced BMD in the FN by DEXA evaluation.

The incidence of osteopenia/osteoporosis in FN was higher than in LS in our study. This pattern was also similar to that of previous studies concerning osteoporosis in hemophilia, but different from that observed in other high-risk groups, such as postmenopausal women, where LS is usually more heavily affected.

In our study, the presence of osteopenia/osteoporosis was positively related to the severity of hemophilic arthropathy, as evaluated by the total joint clinical and radiological score. More importantly, hemophilic arthropathy had an independent prognostic value for the development of bone loss in our patients. This relation could be explained by the reduced weight-bearing activity and prolonged immobilization in patients with more frequent bleeding episodes that lead to more severe arthropathy. Two recent reports [17, 18] demonstrated that hemophilic children have decreased bone density compared to gender- and age-matched control subjects. In the study of Tlacuilo-Parra et al., 77% of the children with hemophilia were considered to be inactive vs 51% in controls. This led to the hypothesis that lack of weight-bearing activity does not allow bone to reach the adequate peak of its density compared to healthy controls.

None of these previous studies had included an evaluation of bone metabolism that could explain the pathogenetic mechanism implicated in the high incidence

Table 3 Factors associated with BMD reduction in FN and LS

	FN r^2 (p)	LS r^2 (p)
TRACP-5b	-0.30 (0.021)	-0.20 (0.121)
b-ALP	-0.31 (0.017)	-0.19 (0.135)
Age	-0.44 (<0.001)	-0.25 (0.028)
N of joints affected	-0.52 (<0.001)	-0.19 (0.100)
Total clinical score	-0.63 (<0.001)	-0.34 (0.003)
Total radiological score	-0.62 (<0.001)	-0.30 (0.009)

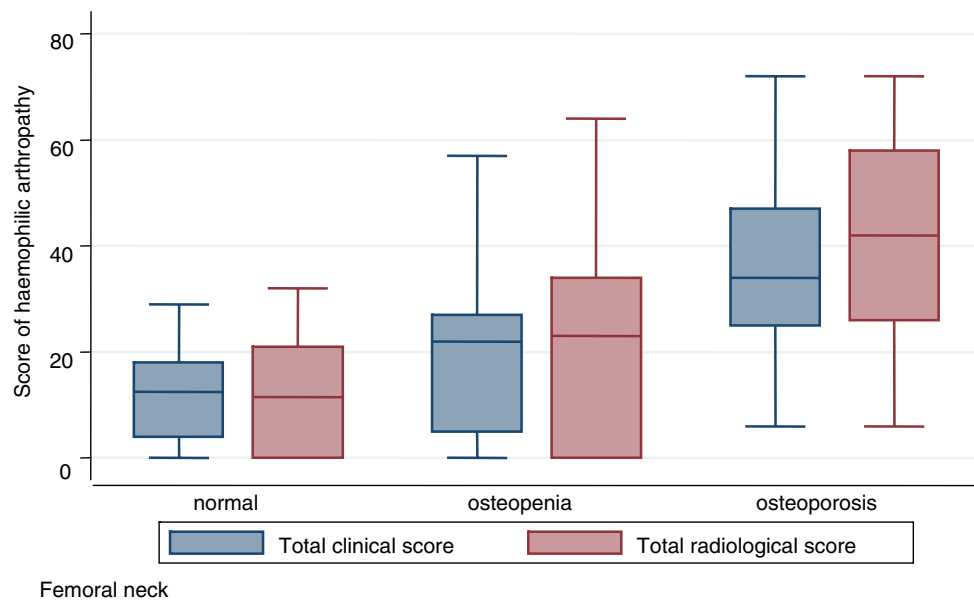


Fig. 1 BMD reduction is associated with the severity of hemophilic arthropathy, expressed as total clinical score (World Federation of Hemophilia score) and total radiological score (Pettersson score)

of osteopenia and osteoporosis observed in hemophilia patients. In the study of Gallacher and coworkers, there was a suggestion, based on serum calcium and PTH levels, that reduced bone density was not secondary to increased bone turnover. However, we clearly demonstrated that hemophilia patients have a higher rate of bone resorption compared to healthy controls, as assessed by the significantly higher levels of NTX, CTX, and TRACP-5b. This increased osteoclastic activity seems not to be balanced by a comparable increase in osteoblast function, as osteocalcin was reduced in hemophiliacs compared to controls and the increase of bALP was of borderline significance ($p = 0.043$). However, what is the cause of this increased osteoclast activity? The levels of osteoclast regulators, such

as sRANKL, OPG, and TNF- α , were not found significantly different in our patients compared with controls, suggesting that the RANKL/OPG system, which is the major stimulating pathway of osteoclast activity, seems not to be a direct contributor in the increased osteoclast function observed in our patients. Then, how can we explain the increased bone turnover in our hemophilia population? In a study by Hoots et al. [19], it is suggested that hemophilic arthropathy has similar pathogenesis to rheumatoid arthritis where the preinflammatory cytokines with the RANKL/RANK/OPG system induce the differentiation and activity of osteoclasts [20, 21]. In our study, the levels of RANKL and OPG did not differ between patients and controls. We can then speculate that other cytokines

Table 4 Levels of bone remodeling markers in patients and controls

	Patients Median (IQR)	Controls Median (IQR)	<i>p</i>
RANKL (pmol/L)	0.26 (0.10, 0.56)	0.27 (0.03, 0.38)	0.419
OPG (pmol/L)	3.84 (2.28, 11.24)	4.67 (2.66, 6.18)	0.948
RANKL/OPG ratio	0.05 (0.03, 0.09)	0.07 (0, 0.13)	0.903
TNF- α (pg/mL)	0 (0.448, 2)	0	0.063
NTX (nM BCE/mM creatinine)	19.49 (15.49, 24.02)	16.13 (12.17, 20.45)	0.018
CTX (ng/ml)	0.61 (0.45, 0.86)	0.47 (0.40, 0.58)	0.028
TRACP-5b (U/L)	2.75 (2.21, 3.32)	1.38 (1.14, 1.63)	<0.001
OC (ng/mL)	14.19 (8.73, 18.59)	15.72 (13.08, 20.98)	0.023
bAPL (U/L)	20.07 (14.52, 25.88)	16.97 (12.32, 21.71)	0.043
25-OHvitD3 (ng/ml)	17.00(10.80, 26.20)	20.20 (11.00, 32.00)	0.500
BMI (kg/m ²)	25.00 (23.40, 27.16)	26.35 (24.00, 28.50)	0.371

Table 5 Clinical and laboratory parameters in HIV+ and HIV- hemophilia patients

	HIV+	HIV-	<i>p</i>
	Median (IQR)	Median (IQR)	
RANKL (pmol/L)	0.15 (0.08, 0.39)	0.37 (0.12, 0.69)	0.073
OPG (pmol/L)	3.79 (2.27, 11.24)	3.89 (2.28, 12.08)	0.748
RANKL/OPG ratio	0.05 (0.02, 0.08)	0.05 (0.03, 0.13)	0.193
NTX (nM BCE/mM creatinine)	20.34 (15.27, 23.62)	19.08 (15.49, 24.37)	0.892
CTX (ng/ml)	0.59 (0.46, 0.85)	0.61 (0.41, 0.91)	0.771
TRAPC-5b (U/L)	3.00 (2.13, 3.70)	2.73 (2.37, 3.09)	0.479
OC (ng/mL)	14.18 (9.39, 19.06)	14.21 (8.46, 16.73)	0.527
bAPL (U/L)	25.88 (17.08, 33.39)	17.08 (13.99, 20.93)	<0.001
Age (years)	41.00 (35.50, 47.50)	32.00 (24.00, 41.00)	0.001
TCS	32.00 (15.50, 48.00)	24.00 (13.00, 39.00)	0.109
TRS	44.00 (18.00, 55.00)	28.00 (14.00, 41.00)	0.037
BMI (kg/m ²)	23.80 (23.40, 26.80)	25.30 (24.00, 27.80)	0.007

TCS total clinical score, TRS total radiological score

with osteoclast activation function, such as interleukin-6, may be the inducers of osteoclastic activity. Furthermore, the serum levels of sRANKL/OPG may not accurately reflect the abnormal process that takes place in the affected joints microenvironment and leads to bone loss.

In a recent study, a significant reduction in the relative amount of bone tissue in chronically bled male mice has been documented [22]. The authors concluded that chronic blood loss led to augmented production of the hematopoietic microenvironment. Enhanced production of growth-stimulating activities subsequently intensifies proliferation of hematopoietic progenitors and leads to numerical increase of hematopoietic cells including osteoclasts and

possible intensification of their function and extensive resorption of bone. If this hypothesis is confirmed by further studies, then it is possible that chronic bleeding that occurs in hemophilia patients could be the primary factor responsible for the increased osteoclastic activity observed in our study and the subsequent osteoporosis development.

Hepatitis C is a factor related to osteoporosis development. Hypogonadism, abnormalities of vitamin D metabolism, and increased bilirubin levels, situations usually found in chronic liver disease, may lead to either reduced bone formation or increased bone resorption [23–25]. The results from the studies that have investigated the impact of hepatitis C on osteoporosis of hemophilia patients are conflicting. In two studies [10, 17], no difference in the severity of osteoporosis between HCV-positive and HCV-negative patients was observed, while in the study of Wallny et al. [9], a significant difference in mean values of BMD was reported in the two groups. Almost all of the patients of our cohort were HCV-positive, and this could be considered as a factor implicated in the high incidence of osteoporosis observed in our study. Although we could not directly study the impact of HCV infection in the patients of our group, we examined several factors that could affect bone metabolism and are related to chronic liver disease, such as serum PTH and calcium, which were found to be normal, and testosterone and free-testosterone levels, which were not found to be low. In addition, the levels of 25OHvit D3 were not found to be significantly different from those of the control group.

We observed in our study an increased incidence of osteopenia and osteoporosis in HIV-positive patients in both studied sites (FN and LS). This was more prominent in LS, where 43% and 7% of HIV-negative patients had osteopenia and osteoporosis, respectively, compared to 64% and 21% of the HIV-positive patients. Furthermore, HIV infection had an independent prognostic value for the

Table 6 Multivariate model for the development of osteoporosis in FN (A) and LS (B)

	Odds ratio	95% C.I.	<i>p</i>
A			
HIV			
Negative	1		
Positive	3.765	(1.026, 13.820)	0.046
Age			
Per 5 years	0.818	(0.572, 1.171)	0.274
Total clinical score			
Per 10 units	2.427	(1.436, 4.102)	0.001
B			
HIV			
Negative	1		
Positive	7.294	(1.917, 27.758)	0.004
Age			
Per 5 years	0.779	(0.554, 1.096)	0.152
Total clinical score			
Per 10 units	3.226	(1.331, 7.821)	0.01

development of osteopenia/osteoporosis in our hemophilia patients. These results strongly support the role of HIV infection in accelerating the development of hemophilia-induced osteoporosis. During the past few years, the impact of HIV infection on bone metabolism has been extensively studied [26–28]. Several factors are implicated in the pathogenesis of increased bone resorption observed in HIV-positive patients. HIV infection itself has been considered as a cause, by increased production of cytokines [29] or impaired vitamin D absorption due to chronic diarrhea. In addition, antiretroviral treatment and mainly protease inhibitors are associated with reduced BMD, although the results of different studies are controversial [26, 30, 31]. Recently, the long-term adverse events from highly active antiretroviral therapy administration, especially lipid and glucose metabolism disorders, are studied, but the results are not consistent as well [32–34]. In our study, the exact mechanism for the more severe bone loss observed in the HIV-positive patients could not be explained by the measurement of bone markers, as we found no significant differences between HIV-positive and HIV-negative patients. A factor that could explain this difference is BMI, which has been proved in previous studies as an important risk factor for osteoporosis development [35]. Although BMI was not found to be different between patients and controls, HIV status was a factor associated with significant difference in BMI between patients. The long-term administration of antiretroviral therapy resulting in lipotrophy could be the cause of the lower BMI in HIV-positive patients

Despite the significant difference in age found between the two groups, this could not be considered as a cause for the increased severity of osteoporosis in HIV-positive patients as both groups had median ages that are not usually affected with osteoporosis. In addition, in multivariate analysis, it has been clarified that HIV infection was the strongest risk factor for the development of osteoporosis in LS, while age was not.

Conclusion

Osteopenia and osteoporosis are found in high frequency in hemophilia patients, and it is strongly related with the severity of hemophilic arthropathy. HIV infection seems to enhance the negative effects of hemophilia on bone metabolism. Although the inactivity from an early age due to chronic pain and impaired joint function is an important cause for reduced bone mass, in our study, we observed an increased osteoclastic activity, suggesting that an increased bone turnover is implicated in the pathogenesis of osteoporosis in hemophilia patients. This increased bone resorption seems not to be accompanied by a comparable increase in osteoblast function. The pathogenesis needs to be further

elucidated in future studies. Moreover, these findings may have important implications for the management of hemophilia patients, concerning the strategies that we should develop in order to prevent the development of osteoporosis from early childhood, and the evaluation of different therapies, including bisphosphonates, which are potent inhibitors of osteoclast function, for the improvement of BMD in adult patients.

References

- Genant HK, Cooper C, Poor G et al (1999) Interim report and recommendation of the World Health Organization Task-Force for Osteoporosis. *Osteoporos Int* 10:259–264
- National Institutes of Health (2000) NIH consensus statement osteoporosis prevention, diagnosis and therapy. *NIH Consensus Statement* 17:1–45
- Raisz LG (2005) Pathogenesis of osteoporosis: concepts, conflicts and prospects. *J Clin Invest* 115:3318–3325. doi:10.1172/JCI27071
- Sambrook P, Cooper C (2006) Osteoporosis. *Lancet* 367:2010–2018
- Voskaridou E, Terpos E (2004) New insights into the pathophysiology and management of osteoporosis in patients with beta thalassaemia. *Br J Haematol* 127:127–139. doi:10.1111/j.1365-2141.2004.05143.x
- Cummings SR, Melton LJ, 3rd (2002) Epidemiology and outcomes of osteoporotic fractures. *Lancet* 359:1761–1767. doi:10.1016/S0140-6736(02)08657-9
- Hoyer LW (1994) Haemophilia A. *N Engl J Med* 330:38–47. doi:10.1056/NEJM199401063300108
- Gallacher SJ, Deighan C, Wallace AM et al (1994) Association of severe hemophilia A with osteoporosis: a densitometric and biochemical study. *Q J Med* 87:181–186
- Wallny TA, Scholz DT, Oldenburg J et al (2007) Osteoporosis in haemophilia—an underestimated comorbidity? *Haemophilia* 13:79–84. doi:10.1111/j.1365-2516.2006.01405.x
- Nair AP, JiJina F, Ghosh K et al (2007) Osteoporosis in young haemophiliacs from Western India. *Am J Hematol* 82:453–457. doi:10.1002/ajh.20877
- Rodriguez-Merchan EC (2003) Orthopaedic assessment in haemophilia. *Haemophilia* 9(Suppl 1):65–74. doi:10.1046/j.1365-2516.9.s1.9.x
- Pettersson H (1994) Can joint damage be quantified? *Semin Hematol* 31(Suppl 2):1–4
- Nguyen TV, Pocock N, Eisman JA (2000) Interpretation of bone mineral density measurement and its change. *J Clin Densitom* 3:107–119. doi:10.1385/JCD:3:2:107
- Binkley NC, Schmeer P, Wasnich RD et al (2002) What are the criteria by which a densitometric diagnosis of osteoporosis can be made in males and non-Caucasians? *J Clin Densitom* 5 (Suppl):19–27. doi:10.1385/JCD:5:3S:S19
- Leib ES, Lewiecki EM, Binkley N et al (2004) Official positions of the International Society for Clinical Densitometry. *J Clin Densitom* 7:1–6. doi:10.1385/JCD:7:1:1
- Hadjidakis D, Kokkinakis E, Giannopoulos G et al (1997) Bone mineral density of vertebrae, proximal femur and os calcis in normal Greek subjects as assessed by dual-energy X-ray absorptiometry: comparison with other populations. *Eur J Clin Invest* 27:219–227. doi:10.1046/j.1365-2362.1997.980636.x
- Barnes C, Wong P, Egan B et al (2004) Reduced bone density among children with severe hemophilia. *Pediatrics* 114:177–181. doi:10.1542/peds.114.2.e177

18. Tlacuilo-Parra A, Morales-Zambrano R, Tostado-Rabago N et al (2008) Inactivity is a risk factor for bone mineral density among haemophilic children. *BJH* 140:562–567
19. Hoots WK (2006) Pathogenesis of hemophilic arthropathy. *Semin Hematol* 43(Suppl 1):S18–S22. doi:10.1053/j.seminhematol.2005.11.026
20. Manolagas S, Jilka R (1995) Bone marrow, cytokines and bone remodeling. *N Engl J Med* 3325:305–311. doi:10.1056/NEJM199502023320506
21. Boyce B, Li P, Yao Z et al (2005) TNF- α and pathologic bone resorption. *Keio J Med* 54:127–131. doi:10.2302/kjm.54.127
22. Gurevitch O, Khitrin S, Valitov A et al (2007) Osteoporosis of hematologic etiology. *Exp Hematol* 35:128–136. doi:10.1016/j.exphem.2006.09.010
23. Olsson R, Johansson C, Lindstedt G et al (1994) Risk factors for bone loss in chronic active hepatitis and primary biliary cirrhosis. *Scand J Gastroenterol* 29:753–756. doi:10.3109/00365529409092505
24. Schiefke I, Fach A, Wiedmann M et al (2005) Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection. *World J Gastroenterol* 11:1843–1847
25. Tsuneoka K, Tameda Y, Takase K et al (1996) Osteodystrophy in patients with chronic hepatitis and liver cirrhosis. *J Gastroenterol* 31:669–678. doi:10.1007/BF02347615
26. Landonio S, Quirino T, Bonfanti P et al (2004) Osteopenia and osteoporosis in HIV+patients, untreated or receiving HAART. *Biomed Pharmacother* 58:505–508
27. Mondy K, Yarasheski K, Powderly WG et al (2003) Longitudinal evolution of bone mineral density and bone markers in HIV-infected individuals. *Clin Infect Dis* 36:482–490. doi:10.1086/367569
28. Moore AL, Vashisht A, Sabin CA et al (2001) Reduced bone mineral density in HIV-positive individuals. *AIDS* 15:1731–1733. doi:10.1097/00002030-200109070-00019
29. Fakruddin JM, Laurence J (2005) HIV-1 Vpr enhances production or receptor of activated activity. *Arch Virol* 150:67–78. doi:10.1007/s00705-004-0395-7
30. Carr A, Miller J, Eisman JA et al (2001) Osteopenia in HIV-infected men association with asymptomatic lactic acidemia and lower weight pre-antiretroviral therapy. *AIDS* 15:703–709. doi:10.1097/00002030-200104130-00005
31. Nolan D, Upton R, McKinnon E et al (2001) Stable of increasing bone mineral density in HIV infected patients treated with nelfinavir of indinavir. *AIDS* 15:1275–1280. doi:10.1097/00002030-200107060-00009
32. Amiel C, Ostertag A, Slama L et al (2004) BMD is reduced in HIV infected men irrespective of treatment. *J Bone Miner Res* 19:402–409. doi:10.1359/JBMR.0301246
33. Brown TT, Ruppe MD, Kassner R et al (2004) Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. *J Clin Endocrinol Metab* 89:1200–1206. doi:10.1210/jc.2003-031506
34. Huang JS, Rietschel P, Hadigan CM et al (2001) Increased abdominal visceral fat is associated with reduced bone density in HIV infected men with lipodystrophy. *AIDS* 15:975–982. doi:10.1097/00002030-200105250-00005
35. Katzman DK, Bachrach LK, Carter DR, Marcus R (1991) Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. *J Clin Endocrinol Metab* 73:1332–1339