ARTICLE

Clinical, instrumental, serological and histological findings suggest that hemophilia B may be less severe than hemophilia A

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ABSTRACT

Recent evidence suggests that patients with severe hemophilia B may have a less severe disease compared to severe hemophilia A. To investigate clinical, radiological, laboratory and histological differences in the arthropathy of severe hemophilia A and hemophilia B, 70 patients with hemophilia A and 35 with hemophilia B with at least one joint bleeding were consecutively enrolled. Joint bleedings (<10, 10-50, >50), regimen of treatment (prophylaxis/on demand), World Federation of Hemophilia, Pettersson and ultrasound scores, serum soluble RANK ligand and osteoprotegerin were assessed in all patients. RANK, RANK ligand and osteoprotegerin expression was evaluated in synovial tissue from 18 hemophilia A and 4 hemophilia B patients. The percentage of patients with either 10-50 or more than 50 hemarthrosis was greater in hemophilia A than in hemophilia B (P<0.001 and P=0.03, respectively), while that with less than 10 hemarthrosis was higher in hemophilia B (P<0.0001). World Federation of Hemophilia (36.6 *vs.* 20.2; P<0.0001) and ultrasound (10.9 *vs.* 4.3; P<0.0001) score mean values were significantly higher in hemophilia A patients. Serum osteoprotegerin and soluble RANK ligand were decreased in hemophilia A *versus* hemophilia B (P<0.0001 and P=0.006, respectively). Osteoprotegerin expression was markedly reduced in synovial tissue from hemophilia A patients. In conclusion, the reduced number of hemarthrosis, the lower World Federation of Hemophilia and ultrasound scores, and higher osteoprotegerin expression in serum and synovial tissue in hemophilia B suggest that hemophilia B is a less severe disease than hemophilia A. Osteoprotegerin reduction seems to play a pivotal role in the progression of arthropathy in hemophilia A.

Introduction

Hemophilia A (HA) and hemophilia B (HB) are X-linked recessive bleeding disorders caused by mutations in the genes encoding coagulation factor VIII (FVIII) and factor IX (FIX), respectively. Subjects with factor plasma levels less than 1 IU/dL are classified as severe hemophiliacs, whereas those with factor levels between 1 and 5 IU/dL and more than 5 IU/dL are affected by moderate and mild hemophilia.[1] Although the bleeding phenotype may be rather heterogeneous,[2,3] this classification reflects closely the severity of clinical symptoms.

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Traditionally, HA and HB have been considered clinically indistinguishable, with recurrent musculoskeletal bleeding, particularly joint bleeding, as hallmark of severe disease. Some evidence, however, suggests that patients with severe HB may have a less severe bleeding phenotype, a lower bleeding frequency, and better long-term outcomes compared to severe HA patients.[4,5]

More than 50 years ago, prior to the availability of clotting factor concentrates, Quick *et al*. noticed that severe HB was less handicapping than HA.[6] More recently, many studies demonstrated a higher use of continuous prophylaxis and greater factor consumption in severe HA patients compared with those with severe HB.[7-10] Moreover, Tagariello *et al*., in a retrospective survey of joint arthroplasty in the frame of the Italian Hemophilia Center Association, showed that patients with HA had a 3-fold higher risk of undergoing orthopedic arthroplasty, that is an indirect expression of severity of arthropathy.[11] Finally, Mannucci *et al*. suggested that HB is milder than HA also because of the different expression of the pathogenetic gene defects.[5] Indeed, the type of gene mutation does affect the residual coagulant activity of FVIII or FIX, so that gene defects that totally prevent the synthesis of the protein (referred to as null mutations) are usually associated with undetectable factor activity, whereas non-null mutations account for variable factor levels in the plasma, even when below 1 IU/dL. Null mutations are prevalent in severe HA, whereas missense mutations are prevalent in HB.[12,13] The fact that less severe gene mutations are more frequent in severe HB supports the view that some FIX activity may be present in the plasma of these patients, thus attenuating bleeding severity and frequency.

Recurrent joint bleeding leads to initially independent adverse changes in both the synovial tissue and the articular cartilage/subchondral bone which reciprocally influence each other. The synovial inflammatory changes enhance articular cartilage damage and *vice versa*, eventually resulting in arthropathy and disability.[14,15] The introduction into clinical practice of the ultrasound (US) evaluation coupled with the US score[16] allows frequent monitoring of the evolution of arthropathy in HA and HB.[17]

Another crucial parameter of bone biology is the molecular triad consisting of osteoprotegerin (OPG), receptor activator of nuclear factor-kB (RANK) and RANK ligand (RANKL), which tightly controls bone turnover and is involved in the severity of arthropathy, as demonstrated in HA.[18,19] OPG is a member of the tumor necrosis factor receptor superfamily, acts as a decoy receptor for RANKL, and competes with RANK for binding to RANKL.[20-22] By this mechanism, OPG down-regulates osteoclast differentiation, activity and survival both *in vivo* and *in vitro*.[23,24] Instead, RANKL is expressed by fibroblast-like synoviocytes (type B synoviocytes) and by activated T cells, and may induce osteoclastogenesis through a mechanism enhanced by several cytokines (e.g. tumor necrosis factor-α, interleukin-1 and interleukin-17) that promote both inflammation and bone resorption.[25]

With this as background, the aim of the present study was to investigate the differences in the severity of arthropathy in HA and HB by assessing clinical, imaging and biochemical markers.

Table 1. Clinical characteristics of hemophilia A and hemophilia B patient groups.

	Hemophilia A (n=70)	Hemophilia B (n=35)
Median age and range (years)	33.5 (3-69)	34.6 (2-69)
Primary and secondary prophylaxis treatment (n, %)	10 (15%)	5 (14%)
Tertiary prophylaxis treatment (n, %)	24 (34%)	8 (23%)
On demand treatment (n, %)	36 (51%)	22 (63%)
Viral infections		
HCV (n, %)	49 (70%)	19 (54%)
HCV-HIV (n, %)	7 (10%)	5 (14%)
None (n, %)	21 (30%)	16 (46%)

Table 2. Clinical and imaging findings of hemophilia A and hemophilia B patient groups.

	Hemophilia A (n=70)	Hemophilia B (n=35)	P
Hemarthrosis, n (%)			
<10	11 (15.7)	15 (42.9)	<0.0001
10-50	16 (22.8)	3 (8.5)	0.001
>50	43 (61.4)	17 (48.6)	0.03
Pettersson score, mean±SD	6.81±3.99	5.64±4.02	0.2
WFH score, mean±SD	36.6±21.6	20.2±14.6	<0.0001
US score, mean±SD	10.91±4.05	4.34±3.39	<0.0001
US score >5, n (%)	46 (65.7)	11 (31.4)	0.003

WFH: World Federation of Hemophilia; US: ultrasound.

Methods

Patients' characteristics

Seventy hemophilia A patients and 35 hemophilia B patients attending the Center for Bleeding Disorders of Careggi University Hospital in Florence, Italy, were consecutively enrolled in the study. At recruitment, all these patients had suffered from at least one joint bleeding. Clinical and demographic characteristics of the study population are shown in Table 1. All patients gave informed consent, and the study protocol was approved by the institutional medical ethics committees.

Hemophilia A group

The median age of HA patients was 33.5 years (range 3-69 years). All patients (100%) had severe HA (FVIII:C <1 IU/dL). Thirty-six out of 70 HA patients (51%) were treated on demand, and 10 of 70 (15%) and 24 of 70 (34%) with primary and secondary prophylaxis, respectively.

According to the last guidelines for management of hemophilia of the World Federation of Hemophilia (WFH),[26] the prophylaxis is defined as the long-term continuous factor replacement therapy two or three times per week at dosage of 25 U/kg. Primary prophylaxis is when it starts in the absence of documented osteochondral joint disease, as determined by physical examination and/or imaging studies, and before the second clinically evident large joint bleeding and the age of three years. Secondary prophylaxis is when it starts after two or more bleedings into large joints and before the onset of joint disease documented by physical examination and imaging studies.[26] The tertiary prophylaxis is when it starts in the presence of documented joint disease. Forty-nine out of 70 (70%) patients were HCV positive: HCV viremia

was present in 28 of 49 subjects (57%) and HCV-RNA was undetectable (<15 IU/mol) in the other 21 patients (43%); 25 of 29 patients who had received anti-HCV therapy were still HCV positive. Seven out of 70 (10%) patients were also HIV positive with undetectable viremia (HIV-RNA <20 cp/mL), and were all receiving antiretroviral therapy.

Hemophilia B group

Median age of HB patients was 34.6 years (range 2-69 years). All patients (100%) had severe HB (FIX:C <1 IU/dL). Twenty-two of 35 HB patients (63%) were treated on demand, 5 of 35 (14%) and 8 of 35 (23%) with primary and secondary prophylaxis, respectively. Nineteen of 35 (54%) patients were HCV positive: HCV viremia was present in 9 of 35 subjects (26%) and HCV-RNA was undetectable (<15 IU/mol) in the other 10 patients (29%) for sustained virological response to anti-HCV treatment. Five of 35 (14%) patients were also HIV positive with undetectable viremia (HIV-RNA <20 cp/mL), and were all receiving antiretroviral therapy.

Clinical and imaging score

The severity of arthropathy was measured using the WFH orthopedic joint scale score consisting of a physical examination and pain scale.[27] Knee X-ray was performed in all subjects over 14 years of age, while US was carried out and scored in each patient. X-ray score (Pettersson score) evaluates osteoporosis, enlarged epiphysis, irregular subchondral bone surface, narrowing of the joint space, subchondral cyst formation, erosions of the joint margins, gross incongruence of articulating bone ends, and deformity (angulation and/or displacement between articulating bones).[28] The joint score for a single joint varies between 0 (normal joint) and 13 (i.e. a totally destroyed joint). US was performed by an experienced sonographer (DM) blinded with regard to diagnosis using ESAOTE my LAB 70 (linear probe 13-4 MHz, Milan, Italy). For a single joint, US score with 9 items was applied: 1) joint effusion; 2) fibrotic septa; 3) synovial hyperthropy with flags on power Doppler US (pDUS) or hemarthrosis; 4) synovial hyperthropy without flags on pDUS; 5) hemosiderin deposition; 6) bone erosion; 7) osteophytes; 8) bone remodeling; and 9) cartilage modifications. US score is based on a range from 0-21 with a cut off less than 5.[16] Indeed, pDUS may identify synovial blood flow, synovitis or muscle hematoma in the extremities.

Patients were divided into three groups according to the total number of hemarthrosis in their life: 1) patients with less than 10 hemarthrosis (<10); 2) patients with hemarthrosis 10-50 (10-50); and 3) patients with hemarthrosis greater than 50 (>50).

Serum analysis of soluble RANKL and OPG

Blood samples were collected from all HA and HB patients. Thirty healthy subjects (median age 36.5 years, range 18-73 years) were used as controls. Serum levels of soluble RANKL (sRANKL) and OPG were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Ampli-

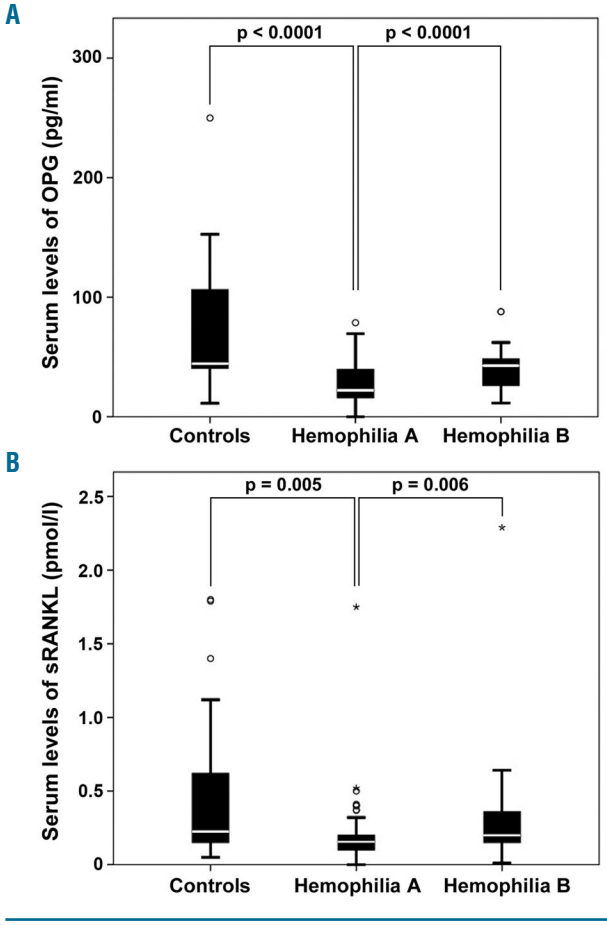

Figure 1. Circulating levels of osteoprotegerin (OPG) and soluble receptor activator of nuclear factor-κB ligand (sRANKL). Serum concentrations of OPG (A) and sRANKL (B) were determined by enzyme-linked immunosorbent assay in 70 patients with hemophilia A, 35 patients with hemophilia B and 30 healthy controls. Boxes show 25th and 75th percentiles. Vertical lines below and above boxes show 10th and 90th percentiles. Lines inside the boxes represent the medians, circles the outliers and asterisks the extreme values. Significant differences between patients with hemophilia A and healthy controls, as well as between hemophilia A and hemophilia B are indicated.

Table 3. US findings of hemophilia A and hemophilia B patient groups.

	Hemophilia A (n=70)	Hemophilia B (n=35)
Effusion, n (%)		
Small	16 (22.5)	13 (37.1)
Moderate	18 (25.4)	8 (22.9)
Large	22 (31)	2 (5.7)*
Fibrotic septa, n (%)	1 (1.4)	2 (5.7)
Hemarthrosis, n (%)	11 (15.5)	5 (14.3)
(>3 flags on pDUS)		
Synovial hypertrophy (without flags on pDUS), n (%)		
<1.5 mm	4 (5.6)	3 (8.6)
1.5-2.5 mm	8 (11.3)	2 (5.7)
>2.5 mm	24 (33.8)	13 (37.1)
Hemosiderin deposition, n (%)		
Small	10 (14.1)	2 (5.7)
Moderate	14 (19.7)	2 (5.7)
Large	9 (12.7)	2 (5.7)
Bone erosion, n (%)	6 (8.5)	1 (2.9)
Osteophytes, n (%)	27 (38)	5 (14.3)¥
Bone remodeling, n (%)	63 (88.7)	22 (62.9)**
Cartilage modifications, n (%)		
Hyperechogenicity	33 (46.5)	4 (11.4)*
Irregular profile	18 (25.4)	2 (5.2)
Calcification	17 (23.9)	3 (8.6)

*pDUS: power Doppler ultrasound; US: ultrasound. *P<0.0001* versus *hemophilia A large effusion and hyperechogenicity. **P=0.001* versus *hemophilia A bone remodeling. ¥P=0.01* versus *hemophilia A osteophytes.*

sRANKL, Biomedica Medizinprodukte GmbH & Co, Wien; Human OPG Instant ELISA, Bender MedSystems, Wien, Austria).

Synovial biopsy samples and immunohistochemistry

Eighteen HA and 4 HB patients suffering from severe knee arthropathy underwent arthroplasty and samples of synovial tissue obtained during surgery at the First Orthopedic Clinic in Florence were analyzed as described elsewhere.[19] Synovial samples from 16 osteoarthritis (OA) patients were included as controls. Each synovial specimen was cut into small pieces, fixed in 10% buffered formalin and, after standard processing, embedded in paraffin wax and used for light microscopy. Immunohistochemistry was performed using the following mouse monoclonal antibodies: anti-RANK (Abcam, Cambridge, UK), anti-RANKL (Abcam), anti-OPG (Santa Cruz Biotechnology, Santa Cruz, CA, USA), as described elsewhere.[19]

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) software for Macintosh (v. 19.0). Values are expressed as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate. χ^2 test was used to compare proportions. Student's *t*-test was used to compare two independent groups for normally distributed parameters, while Mann-Whitney U-test was used to compare two independent groups for non-normally distributed parameters. Spearman's rank correlation coefficient (r) was used to analyze the relationship between two continuous variables. $P<0.05$ was considered statistically significant.

Results

Clinical and imaging findings

The overall results of clinical and imaging findings are shown in Table 2. The percentage of patients with less than 10 hemarthrosis was significantly higher in the HB group compared with the HA group ($P<0.0001$). Conversely, the percentage of patients with either 10-50 or more than 50 hemarthrosis was significantly greater in HA group than HB group ($P<0.001$ and $P=0.03$, respectively). The mean WFH clinical score and US score were significantly worse for the HA group, while no difference was observed in terms of the Pettersson score.

The main results of the US findings are summarized in Table 3. Large joint effusion and cartilage modifications were more frequent in HA patients ($P<0.0001$ *vs.* HB).

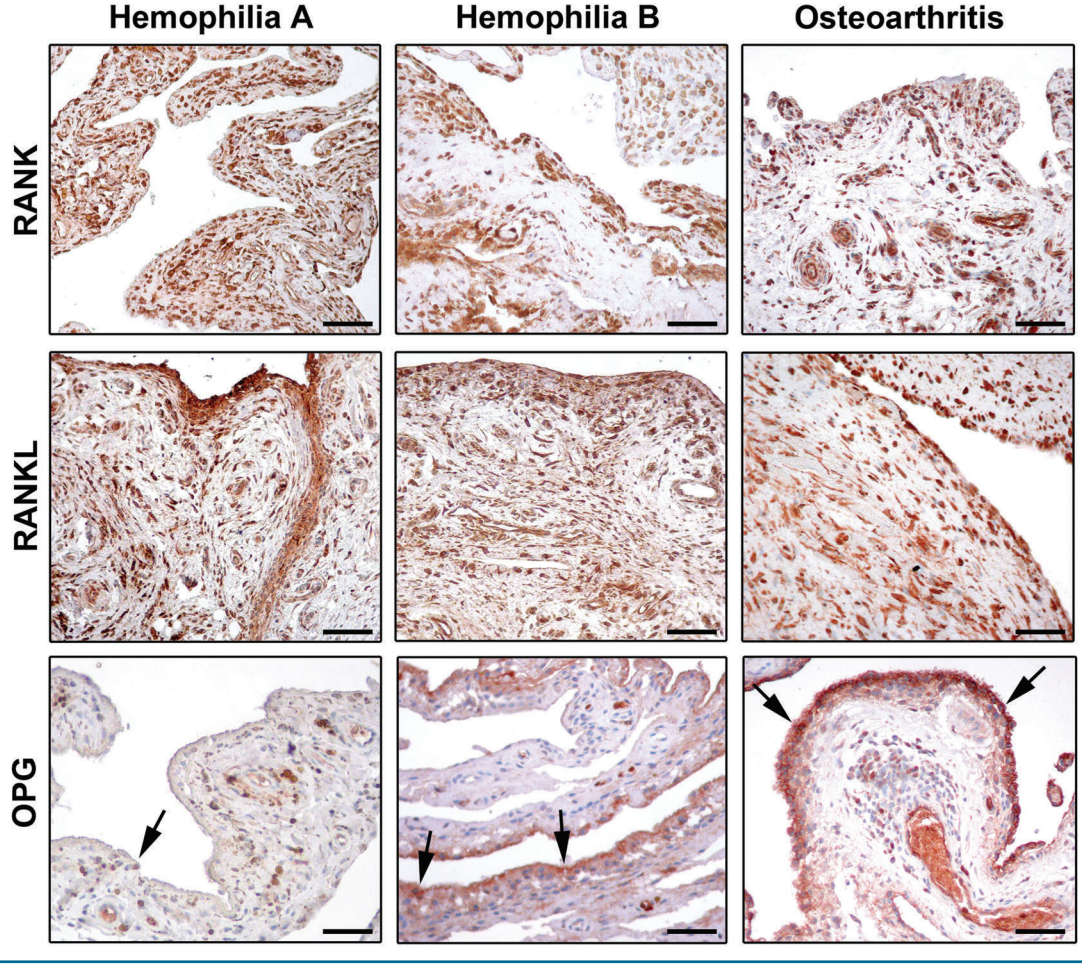

Figure 2. Expression of receptor activator of nuclear factor-κB (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) in synovial tissue from patients with hemophilia A, hemophilia B and osteoarthritis. Representative microphotographs of tissue sections subjected to immunoperoxidase staining for RANK, RANKL and OPG (brownish-red color) and counterstained with hematoxylin are shown. Arrows indicate OPG immunostaining in the synovial lining layer. Original magnification: x20. Scale bar: 100 μm.

Bone remodeling and osteophytes were also more frequent in HA patients (P=0.01 and P<0.01 *vs.* HB, respectively). There was no significant difference in hemarthrosis (>3 flags on pDUS), synovial hypertrophy without flags on pDUS, fibrotic septa, hemosiderin deposition and bone erosions between the two groups of patients.

Furthermore, we compared Pettersson, WFH and US scores between HA and HB patients according to the number of hemarthrosis. All mean score values were higher for all HA groups (i.e. <10, 10-50 and >50) compared to the respective HB groups. In particular, a remarkable significant increase in WFH score was observed in HA more than 50 hemarthrosis group *versus* HB more than 50 hemarthrosis group (P<0.0001). As far as the US score is concerned, significantly higher scores were found in the HA 10-50 group *versus* the HB 10-50 group (P<0.0001), as well as in the HA more than 50 group *versus* the HB more than 50 group (P=0.001) (Table 4).

Circulating levels of OPG and sRANKL

Serum OPG was significantly decreased in HA patients (median 22.15 pg/mL, IQR 15.83-39.63 pg/mL) compared both to controls (median 44.36 pg/mL, IQR 40.36-123.53 pg/mL) and HB patients (median 42.74 pg/mL, IQR 24.54-50.81 pg/mL) (P<0.0001 for both comparisons) (Figure 1A). In HB patients, circulating levels of OPG did not differ to those from healthy controls (Figure 1A).

When HA patients were stratified according to the number of hemarthrosis, OPG levels in less than 10 group (median 52.94 pg/mL, IQR 44.97-75.30 pg/mL) were significantly higher than in 10-50 (median 19.59 pg/mL, IQR 4.05-29.02 pg/mL) and in more than 50 (median 17.55 pg/mL, IQR 10.44-29.82 pg/mL) groups (P=0.004 and P<0.0001, respectively). In HB patients, serum OPG levels were higher, although not significantly, in less than 10 group (median 42.27 pg/mL, IQR 22.65-87.81 pg/mL) compared with both 10-50 (median 32.12 pg/mL, IQR 24.54-52.09 pg/mL) and more than 50 (median 29.69 pg/mL, IQR 20.19-43.59 pg/mL) groups.

Furthermore, we compared OPG levels between HA and HB patients according to the number of hemarthrosis. Interestingly, OPG levels were significantly higher in the HB more than 50 group compared with the HA more than 50 group (P=0.02).

In HA patients, circulating levels of OPG correlated inversely with WFH score (r=-0.44, P<0.0001), Pettersson score (r=-0.26, P=0.04) and US score (r=-0.39, P=0.001). In HB patients, a trend toward a significant inverse correlation between OPG levels and all three scores was observed, although not statistically significant.

Circulating levels of sRANKL were similar between HB patients and healthy controls (median 0.20 pmol/L, IQR 0.14-0.36 pmol/L *vs.* 0.23 pmol/L, IQR 0.15-0.68 pmol/L), while they were significantly lower in HA patients (median 0.16 pmol/L, IQR 0.09-0.20 pmol/L) compared both to controls (P=0.005) and HB patients (P=0.006) (Figure 1B). sRANKL levels did not correlate significantly with the number of hemarthrosis and scores in HA or HB patients (*data not shown*).

Expression of RANK, RANKL and OPG in synovial tissue

Both in HA and HB synovium, RANK was strongly expressed in the lining and sublining layers, especially in synoviocytes and vascular endothelium. In OA, RANK was less expressed in the lining layer, while a strong immunopositivity was observed in the inflammatory infiltrate of the sublining layer (Figure 2).

The expression of RANKL in the lining and sublining layers of HA and HB synovium was similar to that observed in OA (Figure 2).

In synovial tissue from HB patients, the expression of OPG was increased compared with HA patients, particularly in the lining layer and sublining vessels. In HA synovium, only a few cells of the sublining layer were positive for OPG. In OA tissue, OPG was strongly expressed in synovial lining cells, as well as in endothelial cells (Figure 2).

Discussion

In this study, we show that the WFH score and the US score are significantly worse in the HA group compared to the HB patient group when matched for age, even with a similar frequency of hemarthrosis. The lower mean US score observed in the HB group compared to the HA group (4.3 *vs.* 10.9) represents an important result for the follow up of these patients. US findings show that joint involvement is more marked in HA than in HB patients. Mainly, fewer large joint effusion and cartilage modifications (hyperechogenicity) and less bone remodeling were detected in HB patients. Similarly, the lower value of WFH clinical score in the HB group (20.2 *vs.* 36.6) indicates that the arthropathy is less severe in HB than in HA patients.

Table 4. Clinical, serological and imaging findings of hemophilia A and hemophilia B patient groups according to the number of hemarthrosis.

	HA	HB	HA	HB	HA	HB
Number of hemarthrosis	<10	<10	10-50	10-50	>50	>50
Patients, n (%)	11 (15.7)	15 (42.9)	16 (22.8)	3 (8.5)	43 (61.4)	17 (48.6)
Age, years (range)	26.6 (4-57)	28.7 (1-65)	34.62 (11-69)	56.33 (49-60)	35.5 (14-66)	35.9 (12-69)
Pettersson score, mean ± SD	4.2±2.7	2.6±1.9	5.9±3.8	4.3±1.1	9.6±8.6	7.5±3.6
WFH score, mean ± SD	10±5.7	9±5.8	21.5±13.8	16.3±13.5	48.6±16.2	22.6±16.4[¥]
US score, mean ± SD	5.8±3.7	3.6±3.2	6.9±2.7	1.7±0.6*	9.4±4.2	5.3±3.5**
US score >5, n (%)	6 (54.5)	3 (8.5)	9 (56.2)	0	31 (72)	8 (47)
OPG, median (range)	52.94 (44.97-75.30)	42.27 (22.65-87.81)	19.59 (4.05-29.02)	32.12 (24.54-52.09)	17.55 (10.44-29.82)	29.69[¥] (20.19-43.59)

HA: hemophilia A; HB: hemophilia B; WFH: World Federation of Hemophilia; US: ultrasound; OPG: osteoprotegerin. [¥]*P<0.0001* **versus** *HA hemarthrosis >50.* **P<0.0001* **versus** *HA hemarthrosis 10-50;* ***P=0.001* **versus** *HA hemarthrosis >50;* [¥¥]*P=0.02* **versus** *HA hemarthrosis >50.*

The lesser severity of HB with respect to HA is mostly supported by the fact that US and WFH scores were lower in HB than in HA patients matched for the number of hemarthrosis. Instead, the mean Pettersson score was 5.6 points for the HB group and 6.8 points for the HA group. These data may be explained both by the young patient age, also in the HA group, and because radiographic examination can detect abnormalities only in advanced stage, as demonstrated in previous studies.[16,29-31] Another very important aspect concerning the severity of hemophilia was the number of hemarthrosis as marker of arthropathy.[16] It is worthy of note that the percentage of the joint bleedings was lower in the HB group with respect to the HA group, also when matched for age. These results confirm the lower risk of bleeding and consequent arthropathy in HB, as also supported by the significant different distribution of patients according to the number of hemarthrosis between HA and HB groups.

Moreover, as expected, we observed a greater use of on demand treatment in HB patients (63%) with respect to HA patients (51%) and a different use of prophylaxis in the two groups (49% in HA patients, 37% in HB patients).

Our clinical data are in agreement with previous studies.[7-9] In a previous study, we provided evidence of a strong correlation between the severity of arthropathy in HA patients and the expression of the RANK/RANKL/OPG triad in synovial tissue, as well as circulating levels of sRANKL and OPG.[19] Therefore, in the present work we investigated for the first time the possible differences in the RANK/RANKL/OPG triad between HA and HB patients.

Assuming that these cytokines are involved in the progression of the arthropathy, the markedly reduced expression of OPG, which plays a protective role for the subchondral bone, in HA, confirms the more severe clinical outcome of these patients. As a further confirmation, RANK and RANKL, which play a pivotal role in osteoclast activation and bone erosions, were strongly expressed in the synovium of HA. On the contrary, a marked increase in OPG and sRANKL serum levels in the HB compared to the HA group was found. This behavior mirrored the OPG and sRANKL serum levels in healthy controls, thus strengthening the hypothesis that the arthropathy in HB may be less severe and exhibit different features compared to HA. This conclusion is further supported by the significantly higher serum levels of OPG found in the HB more than 50 hemarthrosis group compared with the HA patients of the same group. Furthermore, the histological analysis on synovial tissue of 4 HB patients underlined important differences in the expression of OPG compared with HA. Collectively, these data confirm that the arthropathy is less severe in HB patients, in keeping with the lower number of patients who went on to arthroplasty, and the increased expression of OPG compared to the HA group.

It has been demonstrated that even a single or a few episodes of joint bleeding are sufficient to initiate the arthropathy, since even microhemorrhages into the joint may cause articular deterioration in HA.[32] Furthermore, joint bleeding affects the synovial tissue, resulting in synovitis and subsequent articular cartilage damage, mainly caused by the excretion of tissue-destructive mediators, such as enzymes and cytokines.[14,15,33-35]

In conclusion, our results suggest that there are clinical differences between HB and HA and that the degree of arthropathy is more severe in HA patients, as supported by the higher number of hemarthrosis and lower levels of OPG both in serum and synovium. Our data suggest that the synovitis may play a crucial role in blood-induced arthropathy provoking an overreaction which subsequently becomes independent from bleeding, as postulated in other studies.[36,37]

In addition, on the basis of our findings, the reduction in OPG seems to play a pivotal role in the progression of arthropathy and could even serve in the future as a biomarker of disease severity. Thus, an early clinical, instrumental and serological screening of all hemophiliacs may be recommended. Further investigation of the mechanisms promoting and sustaining blood-induced synovial inflammation will be necessary to shed additional light on the pathogenesis of hemophilic arthropathy.

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