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3D gait analysis, haemophilia joint health score, leg muscle laterality and biomarkers of joint damage: A cross-sectional comparative assessment of haemophilic arthropathy

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Abstract

Introduction: 3D gait analysis has been proposed as a reproducible and valid method to assess abnormal gait patterns and to monitor disease progression in patients with haemophilia (PWH).

Aim: This study aimed at comparing *Gait Deviation Index* (GDI) between adult PWH and healthy controls, and at assessing the agreement between outcome measures of haemophilic arthropathy.

Methods: Male PWH aged 18-49 years (prespecified subgroups: 18-25 vs 26-49 years) on prophylactic replacement therapy, and male healthy age-matched controls passed through a cross-sectional assessment panel. Besides the 3D gait analysis derived GDI, secondary outcomes included kinematic, kinetic and spatio-temporal gait parameters, the *Haemophilia Joint Health Score* (HJHS), electric impedance derived leg muscle laterality and inflammatory biomarkers.

Results: Patients with haemophilia (n = 18) walked slower, in shorter steps and accordingly with less functional range of motion in the hips and ankles, as compared to healthy controls (n = 24). Overall, PWH did not differ significantly in GDI and specific gait parameters. PWH had a higher mean HJHS (18.8 vs 2.6, $P = .000$) and leg muscle laterality (4.3% vs 1.5%, $P = .004$). A subgroup analysis revealed progressed gait pathology in PWH aged 26-49 years (not statistically significant). Leg muscle laterality was strongly correlated with HJHS ($r = .76$, $P = .000$), whereas GDI just moderately ($r = -.39$, $P = .110$). PWH had higher levels of the inflammatory markers CRP and IL-6.

Conclusion: Progressed gait pathology was found in PWH, mainly those aged 26-49 years. Leg muscle laterality correlated strongly with HJHS and was identified as a promising tool for detecting progression and physiological consequences of haemophilic joint arthropathy.

KEYWORDS

3D gait analysis, adult, biomarker, electric impedance, haemophilia, haemophilia joint health score

1 | INTRODUCTION

Haemophilia is categorized as 'severe' (factor FVIII or FIX level <1%, characterized by spontaneous bleedings), 'moderate' (1%-5%) or 'mild' (>5%).[1] The majority (80%-90%) of bleeding episodes occur in the joints, which induces progressive cartilage damage, leading to joint destruction and functional impairments.[2] 3D gait analysis has been proposed as a reproducible, valid and promising method to assess abnormal gait patterns and to monitor disease progression in patients with haemophilia (PWH).[3-6] In particular, 3D gait analysis was suggested for monitoring the progression of ankle arthropathy or the effects of therapeutic interventions in adult PWH.[7] Furthermore, its results facilitate to design individually tailored therapeutic regimens.[4] 3D gait analysis takes place under weight-bearing conditions, which is relevant in terms of weight-induced pain.[2] Elevated levels of biomarkers in serum (cartilage oligomeric matrix protein [sCOMP], cartilage cleavage product sC1,2C and chondroitin sulphate 846 [sCS846]) have been suggested to reflect general joint damage, and acute elevations of these biomarkers might be associated with acute joint bleedings.[8] Urinary C-terminal telopeptide of type II collagen (uCTX-II) has been shown to be associated with the prevalence and progression of radiographic osteoarthritis at the knee and hip, and this association seems to be stronger in subjects with joint pain.[9] Pro-inflammatory cytokines, such as interleukin 1 (IL-1), IL-6 and tumour necrosis factor (TNF), are major determinants in the progression of osteoarthritis in synovial joints.[10] High levels of matrix metalloproteinases, such as MMP-2, have been shown to be present in osteoarthritis, and once these MMPs are fully activated, they may contribute to the destruction of cartilage.[11] Reduced levels of vitamin D were observed in children with haemophilia.[12] The *Haemophilia Joint Health Score* (HJHS) was developed to assess joint damage in children with haemophilia. For teenagers and young adults, it correlates strongly with the x-ray-derived Pettersson score (rho = 0.86) and its inter-observer reliability was rated to be excellent.[13]

The primary aim of this study was to explore the applicability of the 3D gait analysis derived *Gait Deviation Index* (GDI) to assess functional impairments in adult PWH on prophylactic replacement therapy and without acute joint bleedings. Several indices have been proposed to evaluate data obtained during gait analysis. The GDI is a comprehensive quantitative gait pathology index that offers an alternative to the previously validated and widely used *Gillette Gait Index*.[14] In contrast to the *Gillette Gait Index*, GDI uses only kinematic variables and thus is a more general measure of gait pathology. GDI and *Gait Profile Score* (GPS) are considered alternative and closely related measures.[15] Secondary aims were to compare further outcomes, such as HJHS, biomarkers reflecting cartilage damage and leg muscle laterality of PWH to those of healthy age-matched controls. Besides established outcomes, leg muscle laterality was included as exploratory outcome, based on the assumption that one-sided target joints and gait asymmetry may cause laterality. Moreover, this study investigated correlations between joint health-related scores and assessed the status of inflammatory biomarkers and vitamin D levels. With respect to the natural progression of the disease and based on the assumption of more rigid prophylaxis having been implemented in those aged 25 years or younger today, a stratified analysis of age groups of 18-25 years and 26-49 years was foreseen.

2 | MATERIALS AND METHODS

2.1 | Study design, setting and participants

In this cross-sectional observational study, we studied male PWH aged between 18 and 49 years, and age-matched healthy male controls. This study was registered at ClinicalTrials.gov: NCT03541811. PWH were considered eligible, if they were (a) diagnosed with severe or moderate haemophilia A or B, (b) aged between 16 and 49 years, (c) able to walk without assistance, (d) treated with prophylactic factor replacement that had been initiated before the age of 18 years, (e) not treated with immune-tolerance therapy and (f) not suffering from functional impairments caused by other conditions than haemophilia. PWH were subsequently included, if no joint bleedings had occurred within 30 days prior to the examination. In summary, the test group represents adult PWH with a covered demand for substitution and not in the condition of acute joint bleedings. Control group participants were considered eligible if they were male, aged between 16 and 49 years, and having a physiological and symmetric gait pattern without using aids or assistance. PWH were recruited by contacting eligible patients and in the course of regular visits to the paediatric and adult haemophilia clinics at the *Medical University of Vienna*, Austria. For the recruitment of healthy aged matched controls, students and staff members of the *FH Campus Wien—University of Applied Sciences* were addressed with the study information via in-house corridor monitors and an email newsletter. Data collections were carried out in the movement laboratory of *FH Campus Wien—University of Applied Sciences*, in the time between July 2018 and July 2019. All data were recorded during morning time (between 8 AM and 12 AM). Age was used as matching criterion. To enable stratified evaluations, equal age groups of 18-25 years and 26-49 years were sampled, based on the assumption of more rigid prophylaxis having been implemented in those aged 25 years or younger today. The sample size was estimated based on a recent study[4] that reported on the primary outcome GDI for different types of treatment in children with severe, moderate and mild haemophilia. In that study, children with severe haemophilia (prophylactically treated, no inhibitor history; n = 15) scored worse in GDI than children with mild haemophilia (treated on demand, no inhibitor history; n = 9): 88.2 (SD 10.8) vs 101.7 (SD 4.0). Since no functional impairments were found in children with mild haemophilia, we took that group as a proxy for healthy controls in our sample size consideration. Based on the reported differences, we estimated a required number of nine subjects for one-sided comparisons in independent samples ($\alpha < 0.05$, power = 0.80) for our study. Considering a dropout rate of 20% results in 12 subjects to be included in each of the two groups. To allow for a stratified evaluation by two age groups, we aimed for 24 PWH and 24 healthy controls.

Participants completed all study-related procedures within one visit, except for wearing an accelerometer device, over a period of seven consecutive days. The *Ethics Review Board* of the *Medical University of Vienna* approved the study protocol and all participants provided written informed consent, ahead of the collection of data. Participants received their individual outcome reports upon request. In addition, shopping vouchers of €40 were offered as incentive for participants.

2.2 | Medical history and background information

Information on age, type of haemophilia, type of replacement therapy, age at the initiation of prophylaxis, target joints, age at first bleeding, inhibitor history, presence of musculoskeletal injuries, musculoskeletal surgeries, preferred leg and use of analgesics were obtained via a structured interview. Annualized joint bleeding rates (ABR) were derived retrospectively from the frequency of joint bleedings recorded within the past year prior to the examination date. For this purpose, participants were asked to bring their personal documentation booklet.

2.3 | Gait analysis

The system consisted of a 10-camera T40S Vicon system (Vicon) with two floor-mounted AMTI OR6/7-2000 force plates (AMTI). The extended *Cleveland Clinical Markerset*[16,17] with 14 mm retroreflective skin-mounted markers was applied and processed in the capturing software VICON NEXUS 2.2 (Vicon). Markers were placed by one experienced examiner at all participants. The centre of the hip joint was defined by the method of Davis.[18] Prior to the collection of data, anthropometrical parameters were recorded, in order to individualize the biomechanical model. During a static trial, markers on medial and lateral epicondyle of the femur and markers on medial and lateral malleolus determined the knee joint axis and ankle joint axis. Time normalization and parameter extraction were done with MATLAB R2015B (The MathWorks, Inc). Subjects were instructed to walk a 10 m walkway, barefooted at self-paced walking speed, until five right and five left valid first force plate strikes were captured.

Gait Deviation Index was calculated by means of the spreadsheets provided in the supplementary material of the GDI introductory paper. A GDI value of 100 indicates absence of gait pathology (as defined by the control group mean) and every 10 points that the GDI falls below 100, corresponds one standard deviation away from the mean.[14] *Gait Symmetry Index* was calculated for step length, step duration, stance duration, loading response, single support, preswing duration and swing duration in order to quantify gait asymmetry, where a value of 0% indicates full symmetry.[19] Besides overall gait scores and symmetry indices, functional range of motion in the sagittal plane of hips (°), knees (°) and ankles (°), first peak load (N), timing of first peak load (%), average loading rate (N/s), walking velocity (m/s), cadence (steps/min), step length (m), stance phase duration (% gait cycle) and swing phase duration (% gait cycle) were evaluated.

2.4 | Haemophilia Joint Health Score (HJHS)

To assess impairments in body structure and function, two physiotherapists performed the HJHS 2.1 on all subjects. The physical examination assessment tool HJHS focuses on three joints most commonly affected in PWH, the elbows, knees and ankles. It assesses pain, range of motion and strength, as well as the functional tasks of walking, single leg jumps, stair ascent and descent. Outcome measures were transformed into score points according to the summary score sheet provided online[20] by the *International Prophylaxis Study Group* (IPSG), a non-profit collaborative group of healthcare professionals involved with the assessment and care for PWH. A higher HJHS indicates more impairments. Besides the overall HJHS, subscores were calculated for elbows, knees and ankles, as mean values of the right and left side.

2.5 | Biomarkers from blood and urine samples

Blood samples were collected into *Vacuette* serum-separating tubes (Greiner Bio-One) and were kept at room temperature for 30 minutes to ensure proper coagulation. Samples were centrifuged at 1500 *g* for 10 minutes and serum aliquots were stored at −80°C until bulk examination. Urine samples were collected into *Vacuette* tubes containing no additive from non-fasted individuals. Urine samples were kept at 4°C for a maximum of 8 hours and were then aliquoted and stored at −80°C. All biomarkers (except for vitamin D and CRP) were measured in duplicates using standard enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. All samples were tested on the same plate to avoid inter-assay variation, with all plates purchased from the same batches. Serum samples were assessed for the levels of the cartilage degradation markers sCOMP (Novatein Biosciences), sC1,2C and sCS846 (IBEX), and the inflammatory markers IL-1b, IL-6 (both eBioscience), sTNFR1 and sTNFR2 (both R&D Systems). Vitamin D and CRP were measured using automated immunoassay (Roche cobas e411). Urine samples were assessed for uCTX-II (CartiLaps; IDS Ltd.), and these levels were corrected for creatinine levels (Jaffé method, in-house validated method). In a prespecified exploratory approach, a combined joint damage biomarker score was calculated by merging outcomes of CTX-II, COMP, C1,2C and CS846. For this purpose, the average of these four biomarkers was calculated after z-standardization and transformation to percentile.

2.6 | Body composition

Body height was measured with a stadiometer Seca 213 (Seca Vogel&Halke) to the next 0.5 cm. Body size and weight were measured without shoes and outerwear. A correction of 1 kg was subtracted for clothing. Body weight and body composition were assessed with the stationary medical body composition analyser

Seca mBCA 515 (Seca Vogel&Halke) based on bioelectric impedance. Participants were asked to empty their bladder ahead of the anthropometric data collections. *Body Mass Index* (BMI) was calculated as kg/m^2 and the outcomes body fat rate (%), left leg muscle mass (kg) and right leg muscle mass (kg) were generated via the bioelectric impedance analysis. In a prespecified exploratory approach, a leg muscle laterality ratio was calculated as percentage deviation from the stronger leg.

2.7 | Physical activity assessment

After passing through the site-based examinations, participants were instructed to wear a *wGT3X-BT* tri-axial accelerometer (ActiGraph LLC) permanently over a period of seven consecutive days, except for sleeping and water activities. The device was worn with an elastic band on the subjects' right hand sided hip. Data were processed with the software ACTILIFE VERSION 6.13 (ActiGraph LLC). The widely applied cut-offs of at least 10 hours daily wear-time and a minimum of three valid weekdays and one valid weekend day [21] were applied for data cleaning. The count-sampling epoch was set at one minute. The so-called *Freedson Adult VM2 Cut-Offs*[22] were applied to categorize physical activity intensities. BMI, body fat rate and physical activity were assessed, in order to characterize the samples and to correct for confounding.

2.8 | Statistical methods

Normality of data was tested by *Shapiro-Wilk tests* and additional graphical inspections of q-q plots. Outcomes were reported descriptively as mean with corresponding 95% confidence intervals (CI 95%). For non-normally distributed parameters, median values and interquartile range (IQR) were reported. Differences between PWH and the control group were tested with *t* tests for independent samples, or Mann-Whitney *U* tests, respectively. Violation of the homogeneity of variance was assumed in *t* tests, when the associated *Levene* test *P*-value was smaller .05. Effect sizes *r* were calculated from the test statistic *t*, or *z*, respectively. Effect sizes were interpreted as small when $r \geq .1$, medium when $r \geq .3$ and large when $r \geq .5$.[23] For the joint health-related scores, partial eta squared was calculated as effect size. This provided a basis for assessing the independence of these effects from BMI, body fat rate and moderate-to-vigorous physical activity (MVPA), by means of analysis of covariance (ANCOVA). Pearson's correlation coefficients (*r*) were calculated to express the strength of association between the joint health-related scores. Statistical testing was limited to comparing the outcomes of interest between the group of PWH and the control group and for correlations between the joint health-related scores. Furthermore, a prespecified subgroup analysis compared the joint health-related scores in PWH between the two age groups. Statistical analysis was performed with SPSS VERSION 26 (IBM Corp.). Alpha was set at 0.05. Exact two-sided *P*-values were reported. We used Bonferroni correction to account for 76 statistical tests performed on the data set, and a *P*-value <.0007 was consequently considered statistically significant. The reporting of findings followed the *STROBE* checklist for cross-sectional studies.[24]

3 | RESULTS

3.1 | Characteristics of study participants

We recruited 18 patients into the PWH group and 24 subjects into the control group. For PWH, we did not reach the intended number of patients due to exhausted capacities of subjects willing to participate. Table 1 summarizes background information on age, anthropometrics and joint bleedings of all PWH and controls. Mean ages were 29.5 years (ranging from 18 to 49) in PWH and 29.2 years (ranging from 20 to 48) in healthy controls. Participants were equally represented in the age groups 18-25 years (9 PWH, 12 controls) and 26-49 years (9 PWH, 12 controls). All data of all participants were analysed, except for the confounding variable MVPA, where five PWH and seven controls were excluded in the course of a wear-time validation that assessed whether the accelerometer device had been carried adequately (see methods section on physical activity assessment). Moreover, Table 1 presents the participant characteristics stratified by the two age groups, corresponding with the age-subgroup analysis presented in Table 4. PWH aged 26-49 years had, on average, a higher annualized joint bleeding rate (6.0 vs 3.7) and a higher age at the initiation of prophylaxis (10.3 vs 3.9 years) as compared to those PWH aged 18-25 years.

3.2 | Joint health scores

A summary of joint health-related scores of PWH and healthy controls is shown in Table 2. Besides the gait scores GDI and GPS and the HJHS, a combined joint damage score based on four biomarkers and an assessment of leg muscle laterality were explored. PWH had significantly higher HJHS and higher leg muscle laterality. Assessment debriefings revealed that major leg muscle asymmetries had independently been noticed by the physiotherapists, in the course of assessing the HJHS. PWH had slightly less favourable gait scores (ie, lower GDI and higher GPS), but effect sizes were small and not statistically significant. As for the four single joint damage biomarkers, their averaged exploratory panel score did not result in relevant differences between PWH and controls.

ANCOVA was used to quantify the effect sizes for the group comparisons (PWH vs control) of HJHS and leg muscle laterality, adjusted for BMI, body fat rate and MVPA as covariates. In terms of HJHS, the variance explained by the group allocation (PWH vs control) was somewhat smaller (38%, $P = .001$) after that adjustment. For leg muscle laterality, this effect was also smaller (19%, $P = .030$) after adjustment. Corresponding partial eta squared effect sizes before adjustment are shown in Table 2. Due to the negligible variance explained by the group allocation (0.01 to 0.03), no such confounder correction was carried out for the scores GDI, GPS and joint damage score.

TABLE 1 Participant characteristics of patients with haemophilia and healthy age-matched controls, indicated as mean (with standard deviation, SD)

	PWH (n = 18)		Controls (n = 24)			
	Mean	SD	Mean	SD	Mean diff.	CI 95%
Age (y)	29.5	9.2	29.2	8.2	0.3	−5.1, 5.8
Body mass index (kg/m^2)	25.7	4.7	24.6	4.6	1.1	−1.8, 4.0
Body fat rate (%)	23.4	10.0	17.4	8.1	6.0	0.3, 11.6
MVPA[b] (min/d)	33.2	18.9	66.2	24.1	−32.9	−49.6, −16.3
ABR[c] (n/y)	4.8	5.8	–	–	–	–
Age at initiation of prophylaxis (y)	7.3	6.0	–	–	–	–
Prespecified subgroup aged 18-25 y						
	PWH (n = 9)		Controls (n = 12)			
	Mean	SD	Mean	SD	Mean diff.	CI 95%
Age (y)	22.4	2.9	22.7	1.9	−0.2	−2.6, 2.2[a]
Body mass index (kg/m^2)	23.7	3.7	23.9	2.5	−0.2	−3.0, 2,6
Body fat rate (%)	16.6	7.6	13.5	6.0	3.2	−3.0, 9.4
MVPA[b] (min/d)	33.6	18.2	70.5	26.1	−36.9	−66.4, −7.4
ABR[c] (n/y)	3.7	3.4	–	–	–	–
Age at initiation of prophylaxis (y)	3.9	6.2	–	–	–	–
Prespecified subgroup of 26-49 y						
	PWH (n = 9)		Controls (n = 12)			
	Mean	SD	Mean	SD	Mean diff.	CI 95%
Age (y)	36.6	7.7	35.7	6.8	0.9	−5.8, 7.5
Body mass index (kg/m^2)	27.8	4.8	25.4	6.0	2.4	−2.8, 7.5
Body fat rate (%)	30.2	7.1	21.4	8.3	8.8	1.6, 16.0
MVPA[b] (min/d)	33.0	20.6	62.3	23.1	−29.3	−52.1, −6.5
ABR[c] (n/y)	6.0	7.5	–	–	–	–
Age at initiation of prophylaxis (y)	10.3	4.1	–	–	–	–

[a]Confidence interval based on the assumption of unequal variances (Levene test $P < .05$).

[b]Moderate-to-vigorous physical activity (MVPA) measured objectively by Actigraph wGT3X-BT tri-axial accelerometers; incomplete outcome data for this parameter due to exclusions after wear-time validation (PWH: n = 13, healthy controls: n = 17).

[c]Annualized joint bleeding rate (ABR) as number of retrospectively self-indicated bleedings within the past year.

3.3 | Agreement between joint health scores

Table 3 presents a matrix of correlations between the five joint health-related scores. HJHS was found to be strongly correlated with leg muscle laterality ($r = .76$) and GPS ($r = .63$), but only moderately with GDI ($r = .39$). Among the subscores, *HJHS knee* showed the strongest correlation with GPS ($r = .51$), and *HJHS elbow* with GDI ($r = .40$). PWH with an HJHS of up to a 19 had a GDI (mean 100.2, SD 4.5, n = 12) that was not different from healthy controls. Those PWH with a HJHS of 20 or more showed an impaired overall gait pattern (mean GDI 91.5, SD 10.3, n = 6). These findings were independent of age.

3.4 | Age-subgroup analysis

A subgroup analysis revealed that, in PWH, all scores except for the biomarker based panel score were less favourable in the older group aged 26-49 years (n = 9), as compared to those aged 18-25 years (n = 9), whereas there were no age differences among healthy controls

TABLE 2 Comparison of joint health-related scores between patients with haemophilia and healthy age-matched controls, indicated as mean (with standard deviation, SD) and test statistics

	PWH (n = 18)		Controls (n = 24)					
	Mean	SD	Mean	SD	Mean diff.	CI 95%	Partial eta squared	*P*-value[a]
Gait deviation index (GDI)	97.3	9.6	100.0	10.0	−2.7	−8.9, 3.5	.02	.383
Gait profile score (GPS)	4.84	1.20	4.46	1.01	0.38	−0.31, 1.07	.03	.272
Haemophilia joint health score (HJHS)	18.8	12.0	2.58	1.32	16.2	10.2, 22.2[b]	.52	.000
Joint damage score[c]	47.0	14.4	48.7	8.7	−1.71	−9.59, 6.16[b]	.01	.658
Leg muscle laterality[a] (%)	4.34	3.47	1.53	1.33	2.82	1.02, 4.61[b]	.25	.004

[a]2-sided *P*-values derived from *t*-tests for independent samples.

[b]Confidence interval based on the assumption of unequal variances (Levene test $P < .05$).

[c]Average of four biomarkers (sCOMP, sC1,2C, sCS846, uCTX-II) after *z*-standardization and transformation to percentile.

[d]Difference between leg muscle masses of the two legs as percentage deviation from the stronger leg, measured by bioelectric impedance.

TABLE 3 Correlation matrix (Pearson's *r*) of joint health-related scores in patients with haemophilia (n = 18)

	GDI	GPS	HJHS	HJHS Elbow	HJHS Knee	HJHS Ankle	JDS	LML
GDI	–							
GPS	***r* = −.89**, *P* = .000	–						
HJHS	***r* = −.39**, *P* = .110	***r* = .63**, *P* = .005	–					
HJHS Elbow	***r* = −.40**, *P* = .097	***r* = .42**, *P* = .083	***r* = .66**, *P* = .003	–				
HJHS Knee	*r* = −.21, *P* = .399	***r* = .51**, *P* = .032	***r* = .73**, *P* = .001	***r* = .53**, *P* = .024	–			
HJHS Ankle	*r* = −.21, *P* = .415	***r* = .39**, *P* = .112	***r* = .69**, *P* = .002	*r* = .01, *P* = .976	*r* = .20, *P* = .437	–		
JDS	*r* = .00, *P* = .991	*r* = −.05, *P* = .848	*r* = .22, *P* = .388	***r* = .33**, *P* = .187	*r* = .06, *P* = .825	*r* = .08, *P* = .745	–	
LML	*r* = −.08, *P* = .753	***r* = .35**, *P* = .154	***r* = .76**, *P* = .000	***r* = .63**, *P* = .005	***r* = .48**, *P* = .043	***r* = .49**, *P* = .037	*r* = .16, *P* = .518	–

Note: **Bold figures** indicate at least medium-sized effects

Abbreviations: GDI, Gait Deviation Index; GPS, Gait Profile Score; HJHS, Haemophilia Joint Health Score; JDS, Joint Damage Score: average of four biomarkers (sCOMP, sC1,2C, sCS846, uCTX-II) after *z*-standardization and transformation to percentile; LML, leg muscle laterality: difference between leg muscle masses of the two legs as percentage deviation from the stronger leg, measured by bioelectric impedance.

(Figure 1). In PWH aged 26-49 years, average GDI was lower (i.e. less favourable) and, correspondingly, GPS was higher (i.e. less favourable) in PWH, than in healthy controls. These differences were not statistically; however, they represented small to medium effect sizes. In the older age groups, HJHS was on average higher in PWH (27.7) than in healthy controls (2.7). This difference was statistically significant ($P = .000$) and represented a large effect size, $r = .86$. Furthermore, in the older age groups, leg muscle laterality was on average higher PWH (5.93) than in healthy controls (1.27). This difference was not statistically significant ($P = .011$); however, it represented a medium-sized effect, $r = .49$ (Table 4).

3.5 | 3D gait analysis

Results of preselected kinematic, kinetic and temporal-spatial gait parameters are shown in Table 5. PWH walked slower, in shorter steps and accordingly with less range of motion in the hips and ankles, where effect sizes ranged from small to medium. However, PWH did not differ significantly in any of the gait parameters. Regarding symmetry indices, PWH and healthy controls showed only negligible to small differences at group level (Table 6). However, two PWH had outlying values in multiple symmetry indices (e.g. 8% in step duration).

3.6 | Biomarkers reflecting general joint damage

Table 7 shows results of biomarkers reflecting general joint damage, inflammation and vitamin D levels. PWH did not differ significantly, in the four biomarkers expressing general joint damage. However, the median level of CTX-II was in PWH 72% higher (not statistically significant). CTX-II was proposed for early detection and as a severity marker of osteoarthritis.[25,26] Levels of the pro-inflammatory

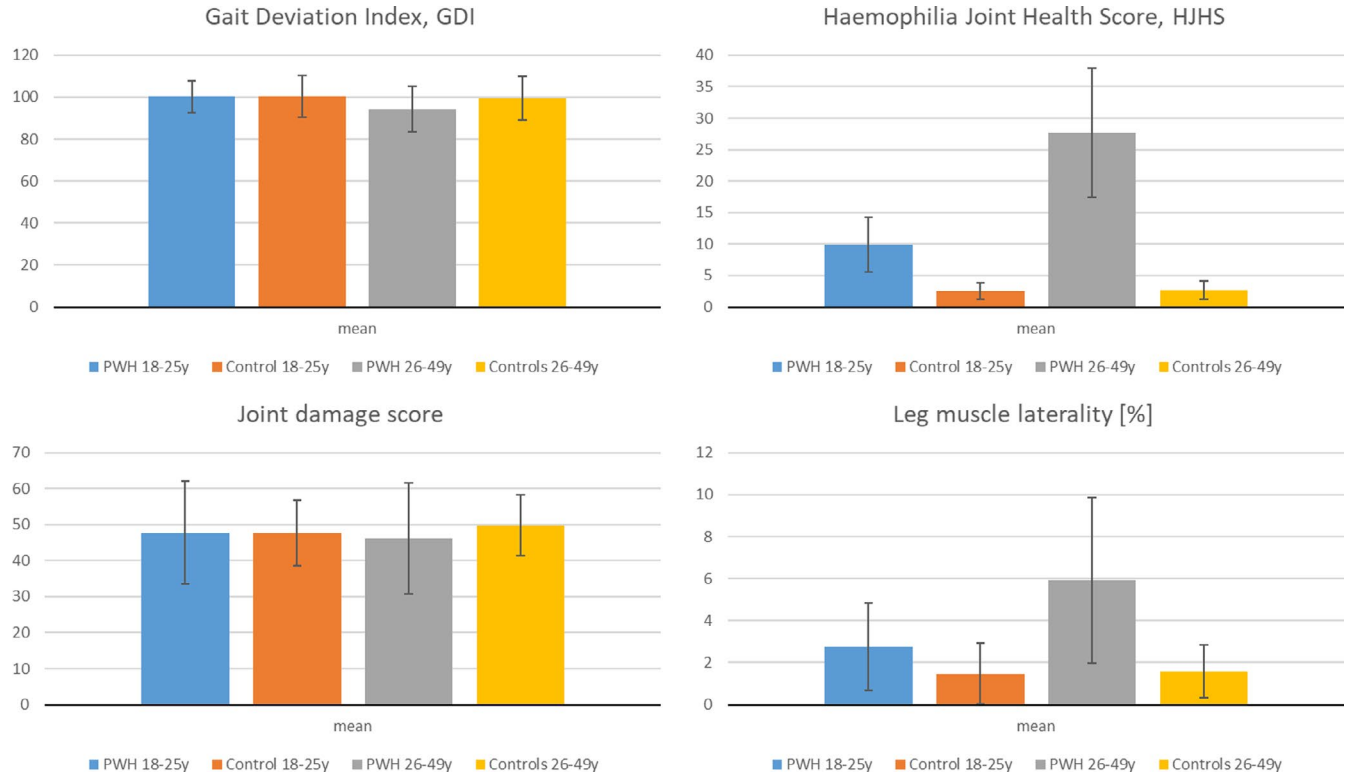

FIGURE 1 Overview of haemophilic arthropathy scores (mean, SD) for PWH aged 18-25 y (n = 9), healthy controls aged 18-25 y (n = 12), PWH aged 26-49 y (n = 9) and healthy controls aged 26-49 y (n = 12) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Comparison of joint health-related scores between patients with haemophilia and healthy age-matched controls and test statistics, by age groups, i.e. age-subgroup analysis of scores shown in Table 2

	PWH (n = 9)		Controls (n = 12)					
	Mean	SD	Mean	SD	Mean diff.	CI 95%	Effect size *r*	*P*-value[b]
18-25 y								
Gait deviation index (GDI)	100.2	7.6	100.4	10.0	−0.2	−8.5, 8.2	.01	.968
Gait profile score (GPS)	4.29	0.76	4.32	1.18	−0.03	−0.97, 0.92	.02	.953
Haemophilia joint health score (HJHS)	9.9	4.4	2.5	1.3	7.4	4.0, 10.8[c]	**.75**	.001
Joint damage score[d]	47.8	14.2	47.7	9.1	0.1	−10.5, 10.8	.00	.979
Leg muscle laterality[a] (%)	2.76	2.09	1.47	1.45	1.29	−0.33, 2.90	**.34**	.111
26-49 y								
Gait deviation index (GDI)	94.4	10.9	99.6	10.4	−5.2	−15.0, −4.6	.24	.277
Gait profile score (GPS)	5.39	1.34	4.61	0.82	0.79	−0.20, 1.78	**.33**	.113
Haemophilia joint health score (HJHS)	27.7	10.3	2.7	1.4	25.0	17.0, 33.0[c]	**.86**	.000
Joint damage score[d]	46.2	15.5	49.8	8.5	−3.6	−14.6, 7.5	.14	.507
Leg muscle laterality[a] (%)	5.93	3.94	1.59	1.27	4.35	1.28, 7.42[c]	**.60**	.011

[a]2-sided *P*-values derived from *t*-tests for independent samples, with effect size *r*, where bold figures indicate at least medium-sized effects.
[b]Confidence interval based on the assumption of unequal variances (Levene test $P < .05$).
[c]Average of four biomarkers (sCOMP, sC1,2C, sCS846, uCTX-II) after *z*-standardization and transformation to percentile.
[d]Difference between leg muscle masses of the two legs as percentage deviation from the stronger leg, measured by bioelectric impedance.

cytokine IL-6 and the common inflammatory marker CRP were higher in PWH. In contrast, levels of MMPs were higher in the group of healthy controls. PWH and controls did not differ significantly regarding vitamin D levels. However, severely diminished levels below 25 nmol/L[27] occurred more frequently in PWH (7, 39%) than in controls (5, 21%).

TABLE 5 Gait parameters (average of both legs) of patients with haemophilia and healthy age-matched controls, indicated as mean (with SD) and test statistics

	PWH (n = 18)		Controls (n = 24)					
	mean	SD	mean	SD	Mean diff.	CI 95%	Effect size *r*	*P*-value[b]
fROM[c] hip (°)	40.2	3.6	42.2	3.7	−2.0	−4.3, 0.3	.26	.081
fROM[c] knee (°)	64.5	5.5	65.8	4.1	−1.3	−4.3, 1.7	.13	.398
fROM[c] ankle (°)	27.7	7.0	31.1	4.7	−3.4	−7.1, 0.2	.27	.063
First peak load (N)	114.5	8.8	114.8	8.6	−0.4	−5.8, 5.1	.02	.894
Timing of first peak load (%)	14.5	1.2	14.1	1.3	0.5	−0.3, 1.3	.16	.240
Average loading rate (N/s)	4822	976	4968	1107	−146	−809, 518	.07	.659
Walking velocity (m/s)	1.26	0.14	1.35	0.12	−0.09	−0.17, −0.01	**.33**	.036
Cadence (steps/min)	115.2	7.1	115.0	6.7	0.20	−4.14, 4.53	.01	.927
Step length (m)	0.66	0.06	0.70	0.04	−0.05	−0.08, −0.01	**.37**	.010
Stance phase duration (% gait cycle)	60.49	1.11	60.45	1.22	0.04	−0.70, 0.78	.02	.907
Swing phase duration (% gait cycle)	39.51	1.11	39.55	1.22	−0.04	−0.78, 0.70	.02	.907

[a]2-sided *P*-values derived from *t* tests for independent samples, with effect size *r*, where **bold figures** indicate at least medium-sized effects.
[b]Functional range of motion in the sagittal plane.

TABLE 6 Gait symmetry indices of patients with haemophilia and healthy age-matched controls, indicated as and median (with interquartile range, IQR) and test statistics

	PWH (n = 18)		Controls (n = 24)			
	Median	IQR	Median	IQR	Effect size *r*[d]	*P*-value[d]
Symmetry index step length (%)	2.85	1.63, 4.99	2.01	0.80, 3.04	.23	.144
Symmetry index step duration (%)	1.50	0.71, 3.07	1.03	0.43, 1.96	.18	.247
Symmetry index stance duration (%)	1.45	0.50, 2.73	0.99	0.61, 2.03	.07	.638
Symmetry index loading response (%)	5.37	1.73, 9.16	6.15	2.95, 10.48	.04	.780
Symmetry index single support (%)	2.09	0.74, 4.02	1.47	0.45, 2.66	.19	.222
Symmetry index preswing duration (%)	4.98	1.99, 9.52	6.74	3.13, 11.01	.06	.703
Symmetry index swing duration (%)	2.21	0.83, 4.14	1.63	0.88, 3.05	.08	.611

P-values derived from Mann-Whitney *U* tests, with effect size *r* calculated as z/sqrt(n).

4 | DISCUSSION

Our results show that overall the primary outcome GDI did not differ significantly between adult PWH on prophylactic replacement therapy and without acute joint bleedings compared with healthy age-matched controls. However, a subgroup analysis revealed that the small effect towards unfavourable gait physiology observed, originates from progressed gait pathology in PWH aged 26-49 years (not statistically significant), while younger PWH aged 18-25 years had scores similar to those of healthy controls. Forneris et al found abnormal GDI and GPS derived gait patterns in children with severe haemophilia which were worst in PWH with a history of inhibitors and those receiving on-demand therapy, but no abnormalities in children with mild haemophilia.[4] A similar age progression was observed for HJHS and leg muscle laterality. These scores where already somewhat elevated in PWH aged 18-25 years. Independent of age, differences observed between PWH and controls regarding HJHS and leg muscle laterality remained after adjusting for BMI, body fat rate and MVPA.

We observed no significant differences between PWH and healthy controls for the joint health-related biomarkers assessed in urine (CTX-II) and serum (COMP, cartilage cleavage product C1,2C, CS846). Albeit not statistically significant, levels of CTX-II were markedly higher in PWH, as similarly observed by Hua et al who also found levels of COMP to be elevated.[27] While median CTX-II values were 468 (ng/mmole of urinary creatinine) in PWH and 272 healthy controls, the COBRA study on patients with early rheumatoid arthritis (mean age 49 years) reported a median of 352.[28] The exploratory approach of merging these biomarkers to a panel score did not result in relevant differences between PWH and controls. These markers reflect general joint damage and van Vulpen et al suggested that spontaneous elevations of these biomarkers might be associated with acute joint bleedings.[8] In contrast, our study examined PWH when they had no acute joint bleedings. The second exploratory approach of assessing leg muscle laterality emerged as a promising tool for detecting progression and physiological consequences of haemophilic joint arthropathy. Other than for leg muscle laterality, PWH did not differ

TABLE 7 Biomarkers reflecting general joint damage, inflammation and vitamin D levels of patients with haemophilia and healthy age-matched controls, indicated as median (with IQR) and test statistics

	PWH (n = 18)		Controls (n = 24)			
	Median	IQR	Median	IQR	Effect size *r*[a]	*P*-value[a]
sCOMP (ng/mL)	199.5	134.0, 292.7	212.7	162.9, 242.0	.04	.819
sC1,2C (µg/ml)	0.37	0.34, 0.42	0.41	0.37, 0.44	.22	.162
sCS846 (ng/mL)	1794	1596, 2008	2011	1755, 2252	.23	.134
uCTX-II (mg/mmol creat.)	467.8	193.4, 692.0	271.8	170.6, 524.4	.18	.253
sTNFR1 (pg/mL)	1086	1015, 1136	1067	957, 1198	.02	.899
sTNFR2 (pg/mL)	2179	1919, 2438	2094	1902, 2377	.09	.542
IL-1b (pg/mL)	0.00	0.00, 0.00	0.00	0.00, 7.12	.28	.067
IL-6 (pg/mL)	1.79	0.00, 2.77	0.00	0.00, 1.13	**.39**	.012
MMP2 (ng/mL)	25.3	21.8, 27.3	29.4	24.3, 32.9	**.42**	.007
MMP8 (pg/mL)	4428	2680, 5864	5527	3580, 6432	.23	.140
CRP (mg/L)	1.61	0.42, 3.83	0.49	0.21, 1.11	**.31**	.046
Vit D (ng/mL)	30.2	20.50, 37.08	29.3	25.93, 38.37	.08	.585

Abbreviations: C1,2C, cartilage cleavage product; COMP, cartilage oligomeric matrix protein; CRP, c-reactive protein; CS, chondroitin sulphate; IL, interleukin; IQR, interquartile range; MMP, matrix metalloproteinase; s, serum; TNFR, tumour necrosis factor receptor; uCTX-II, urinary C-terminal telopeptide of type II collagen; vit, vitamin.

P-values derived from Mann-Whitney *U* tests, with effect size *r* calculated as z/sqrt(n), where bold figures indicate at least medium-sized effects.

significantly from controls in the 3D gait analysis derived symmetry indices. Levels of the pro-inflammatory cytokine IL-6 and the common inflammatory marker CRP were higher in PWH and severely diminished vitamin D levels below 25 nmol/L[29] occurred more frequently in PWH than in controls.

Gait Profile Score, and to a lesser extent GDI, resulted in a strong association with HJHS. A strong correlation was also found between HJHS and leg muscle laterality.

Our data support the concept that PWH, in order to maintain a normal gait pattern, are able to compensate a certain extent of joint arthropathy, as indicated by a HJHS below 20. PWH aged 26-49 years have more progressed joint arthropathy, explained by later onset of prophylaxis and the natural progression of the disease.

4.1 | Clinical implications

Several PWH participating in our study may benefit from gait training aiming at increasing step length and active use of range of motion. Gait analysis can detect asymmetry, assess its patterns and consequently facilitate individually tailored gait training. However, noteworthy asymmetry was rarely present in PWH participating our study. Besides strengthening, resistance exercising may also focus on utilizing the full range of motion available. This may be achieved by focussing on the final joint positions, with 50%-60% resistance of the one-repetition maximum and a submaximal number of repetitions. Muscular atrophy (e.g. in the calf) may be detected by objective measures but, beyond a certain extent, also by trained physiotherapists. In affected PWH, exercising with higher resistance (70%-80% of the one-repetition maximum), without going to the final joint positions may additionally be considered. Aquatic exercising or other types of bodyweight support should be considered in case of pain and swelling.

4.2 | Strengths and limitations of the study and implications for future research

This cross-sectional study represents an initial step in assessing 3D gait analysis for PWH. Long-term longitudinal 3D gait assessments might prove to be even more useful to assess chronic haemophilic arthropathy but such data are not yet available. Consequently, the main implication for future research is to carry out a confounder controlled long-term cohort study on gait physiology in PWH. While the HJHS has been shown to have excellent reliability[30] and strong construct validity[31] in children, it has not yet been adequately studied for use in adults. Our study systematically evaluated the HJHS in adult PWH by comparing it with 3D gait analysis and a panel of other measures. The gait scores GDI and GPS have actually been developed for use in patients with infantile cerebral paresis, where the subjects of interest deviate substantially from healthy controls. Thus, these scores may not be as responsive to mild haemophilic arthropathy. While gait scores can serve as an overall assessment of gait pathology, only the appraisal of specific spatio-temporal, kinematic and kinetic gait parameters facilitates to design individually tailored therapeutic regimens. Finally, our study did not achieve the intended sample size for PWH due to difficulties in motivating enough young adult PWH to participate. Although the panel of investigations was not too burdensome, the time required separate from routine follow-up may have been a hurdle to recruitment.

5 | CONCLUSION

In our sample of adult PWH on prophylactic replacement therapy and without acute joint bleedings, progressed general gait pathology (GDI and GPS) was found in those aged 26-49 years, but not in those aged 18-25 years. Consequently, our data do not support GDI as an early detection marker of haemophilic joint arthropathy. PWH may benefit from individually tailored physiotherapy based on 3D gait analysis at a progressed stage of the disease that appears to correspond with a HJHS of at least 20. Such gait training and resistance exercising may aim at individual goals, such as increasing step length, active use of range of motion and strength.

Haemophilia Joint Health Score and leg muscle laterality were markedly elevated in PWH aged 26-49 years, and these scores were already somewhat elevated in younger PWH aged 18-25 years. In terms of biomarkers reflecting general joint damage, we found levels of CTX-II to be higher in PWH than in controls, but not so for COMP, C1,2C and CS846. Leg muscle laterality emerged as a promising approach in detecting progression and physiological consequences of haemophilic joint arthropathy.

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