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ORIGINAL ARTICLE

Muskuloskeletal

Subclinical synovial proliferation in patients with severe haemophilia A: The value of ultrasound screening and biochemical markers

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Abstract

Aim: Subclinical bleeding and inflammation play a role in progression of haemophilic arthropathy. Synovial proliferation is predictive of joint bleeding and its early detection may guide treatment changes and prevent arthropathy progression. This study evaluated the prevalence of active and inactive subclinical synovial proliferation and investigated potential biochemical blood/urine markers to identify patients with active subclinical synovial proliferation.

Methods: This cross-sectional study included patients with severe haemophilia A born 1970–2006 who were evaluated during routine clinic visits. Patients with (a history of) inhibitors or recent joint bleeding were excluded. Elbows, knees and ankles were examined for subclinical synovial proliferation by ultrasound and physical examination. Active synovial proliferation was distinguished from inactive synovial proliferation using predefined criteria. Blood/urine biochemical markers (serum osteopontin, sVCAM-1, Coll2-1, COMP, CS846, TIMP, and urinary CTX-II) were compared individually and as combined indexes between patients with and without active synovial proliferation.

Results: This cohort consisted of 79 patients with a median age of 31 years (range 16.5–50.8 years) with 62/79 (78%) of the patients using continuous prophylaxis. The annualized joint bleeding rate over the last 5 years was .6 (.2–1.1). Active (17/79, 22%) and inactive subclinical synovial proliferation (17/79, 22%) were both prevalent in this cohort. Biochemical markers were not correlated with active subclinical synovial proliferation.

Conclusion: Subclinical synovial proliferation, both active and inactive, was prevalent in patients with severe haemophilia A with access to prophylaxis and would be

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overlooked without routinely performed ultrasounds. Biochemical markers were unable to identify patients with active subclinical synovial proliferation.

KEYWORDS
biomarkers, haemophilia, synovitis, ultrasonography

1 | INTRODUCTION

Haemophilia is an X-linked inherited disease caused by a deficiency or dysfunction of factor VIII (haemophilia A) or factor IX (haemophilia B). This results in spontaneous and trauma-related bleeding, mainly in the large synovial joints.[1] These bleeds cause damage to all joint components. (Recurrent) joint bleeds overload the synovial capacity to resorb blood and trigger an inflammatory response, resulting in synovial proliferation, inflammation and the formation of new fragile blood vessels. This results in a vicious circle of recurrent (subclinical) bleeding, inflammation and joint degradation, ultimately leading to (irreversible) arthropathy.[2,3]

Early prophylactic clotting factor replacement therapy is effective in preventing joint bleeds.[4] However, bleeding rates and joint complaints may be insufficient to evaluate joint health. There is cumulating evidence of subclinical (non-observed) joint bleeding and inflammation. Several studies have demonstrated blood-related joint changes in clinically bleed-free joints.[5–9] Moreover, subclinical synovial proliferation on Magnetic Resonance Imaging (MRI) appears to predict an increased risk of joint bleeding over the next 5 years.[10]

Subclinical synovial proliferation can imply various pathologic processes. Based on the current knowledge of the pathophysiology, it is hypothesized that blood-induced synovial proliferation may indicate two different tissue types: 'Active' inflammatory synovial proliferation, which leads to arthropathy progression through production of pro-inflammatory cytokines, yet is considered to be potentially reversible.[11] In contrast, 'inactive' fibrotic synovial proliferation which is potentially irreversible.[11,12] These different soft tissue findings may require a different approach. Early detection of potentially reversible active synovial proliferation may guide treatment changes, such as intensification of prophylactic treatment and start of anti-inflammatory treatments, to preserve joint health. In addition, the detection of subclinical active synovial proliferation may become increasingly important as a measure of joint health. New treatment modalities such as emicizumab have annualized bleeding rates of <1 and sensitive joint outcome measures are required to demonstrate their efficacy in protecting joint health.

Ideally, patients with active subclinical synovial proliferation would be identified during routine follow-up with an easily accessible tool. Determining active synovial proliferation based on ultrasound-detected soft tissues findings can be difficult.[13] Biochemical markers of joint tissue turnover may be useful in differentiating ultrasound-detected synovial proliferation into active and inactive synovial proliferation. The dynamic biochemical markers increase shortly after a single joint bleed.[14] Therefore, we hypothesize that these markers may detect synovial inflammation.

In this study, we estimated the prevalence of active and inactive subclinical ultrasound-detected synovial proliferation in patients with severe haemophilia A and investigated whether biochemical markers in blood and urine could identify patients with active subclinical synovial proliferation.

2 | MATERIALS AND METHODS

2.1 | Patients

This cross-sectional study consecutively included patients with severe haemophilia A, aged 16 years and older, born after 1969, who had access to prophylaxis and were treated at the Van Creveldkliniek (UMC Utrecht, the Netherlands). Patients were assessed during routine clinic visits between December 2019 and March 2022. All patients had to be on the same treatment regimen (prophylaxis or on-demand treatment) for at least 1 year. Exclusion criteria were: a history of an inhibitor (≥5 Bethesda units (BU) at any time or 1–5 BU for ≥1 year), a history of a (self-reported) major joint bleed or ultrasound-confirmed joint bleed in the 3 months before assessment, or a (self-reported) minor joint bleed in the month before assessment. A major joint bleed was defined as a joint bleed causing a clearly reduced range of motion, severe pain and swelling, and required treatment with more than one infusion of clotting factor concentrate. A minor joint bleed was defined as a joint bleed causing slight reduction in range of motion, moderate pain and swelling, and resolved after a single infusion of clotting factor concentrate.[15] The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Review Board of the UMCU (19-273 – BEGIN). All study participants gave written informed consent.

2.2 | Data collection

Age, treatment history, history of intra-articular interventions, annualized joint bleeding rate over the past 5 years, and body mass index (BMI) were extracted from the electronic patient records. Treatment regimens were defined as continuous prophylaxis, intermittent prophylaxis (non-adherent to prophylaxis) and on-demand treatment. Treatment adherence was determined by actual clotting factor use, based on pharmacy dispensing records, divided by prescribed

clotting factor during the 12 months prior to assessment. Patients who used <75% of the prescribed clotting factor were considered non-adherent.[16] For baseline joint health characteristics, the most recent Haemophilia Joint Health Score (HJHS), Haemophilia Activities List (HAL) and Pettersson scores were extracted from the patient records. The HJHS (range 0–124, optimal score 0) assesses joint impairment based on body structure and function.[17] The HAL (0-100, optimal score 100) measures self-perceived functional ability.[18] Arthropathy severity on X-rays was interpreted according to the Pettersson score, which assesses osteochondral changes of haemophilia arthropathy in elbows, knees and ankles (range 0–13 points/joint; 0–78 points/patient, optimal score 0).[19]

2.3 | Physical examination

Physical examination of elbows, knees and ankles was performed and/or supervised by a single experienced physiotherapist (MT). Joint swelling was assessed according to the HJHS 2.1.[17] Warmth was subjectively assessed by palpation as being present or absent compared to the contralateral joint. Physical examination, ultrasound and blood/urine collection were performed on the same day. Physical examination was performed and scored before ultrasound assessment.

2.4 | Ultrasound

Ultrasound examination of elbows, knees and ankles was performed and/or supervised by an experienced physiotherapist (MT) according to the Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) score,[20] with additional Power Doppler examination of the synovium according to the Joint Tissue Examination and Damage Exam (JADE) protocol.[21] Joints with an arthrodesis received the highest cartilage/bone score. Synovial proliferation was scored as 0 in joints with an arthrodesis.

2.5 | Urine/blood sampling and biomarker assays

Blood was collected into Vacutainer tubes (1 × 20 mL serum, 1 × 9 mL citrate) and 30 mL of urine was collected. A total of 8 IU/10 mL clotting factor VIII was added to the serum tube and kept at room temperature for at least 1 h to ensure proper coagulation. Samples were centrifuged at 1500 g for 10 min. Citrate samples were kept at 4°C for at least 1 h and thereafter centrifuged at 1900 g for 10 min. Urine was kept at 4°C and centrifuged at 1500 g during 10 min. Samples were divided into aliquots and stored at −80°C. All samples were analysed at the same time to minimize variability.

Biochemical markers were measured using standard enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. The same plate was used for all patients to avoid inter-plate variance. Serum samples were assessed for soluble vascular cell adhesion molecule 1 (sVCAM-1, human sVCAM-1, Thermo Fischer, Vienna, Austria), osteopontin (human osteopontin, R&D Systems, Minneapolis, USA), tissue inhibitor of metalloproteinase 1 (TIMP-1, R&D Systems, Minneapolis, USA), chondroitin sulfate 846 (CS846, aggrecan chondroitin sulphate 846, IBEX, Montréal, Canada), cartilage oligomeric matrix protein (COMP, human COMP, Novateinbio, Woburn, USA) and type II collagen degradation (Coll2-1, human Coll2-1, Bio-Connect, Huissen, the Netherlands). Urine samples were assessed for urinary C-terminal telopeptide of type II collagen (uCTX-II, Urine Cartilaps, IDS Ltd., Boldon, UK) and creatinine.

2.6 | Definitions of subclinical synovial proliferation

In the absence of published standards for synovial findings in patients with haemophilia, consensus-based definitions were established prior to the study by a panel consisting of (paediatric) haematologists (K.F., L.V.), a physiotherapist (M.T.), a radiologist (W.F.) and two medical doctors (E.B., F.L.). Active synovial proliferation was defined as synovial proliferation on ultrasound (HEAD-US synovium score > 0) and the presence of at least one of the following criteria:

- Synovial hyperaemia on ultrasound (JADE score > 0);
- Presence of joint swelling or warmth on physical examination;
- Newly detected synovial proliferation (no history of synovial proliferation based on ultrasound assessments and medical records for the last 3 years);
- Current episode treated as synovitis (intensified prophylaxis combined with celecoxib according to the local protocol).

Synovial changes not meeting the definition above were considered to be inactive subclinical synovial proliferation.

2.7 | Analysis

Baseline characteristics were reported as medians with interquartile ranges (IQR) for continuous variables and as frequencies with percentages for categorical or dichotomous variables. The prevalence of active and inactive subclinical synovial proliferation at patient level was reported as a percentage with a 95% confidence interval (CI). The Exact method was used to calculate CIs of proportions.

The presence of synovial proliferation on ultrasound was compared to abnormalities on physical examination. The percentage of patients on continuous prophylaxis and the percentage of patients with a joint bleed in the year prior to inclusion were compared between the active synovial proliferation, inactive synovial proliferation, and no synovial proliferation groups to address the potential effect of treatment adherence and recent joint bleeding on the occurrence of (active) synovial proliferation.

Biochemical marker levels were compared between the active synovial proliferation, inactive synovial proliferation, and no synovial proliferation groups. Combined indexes of biochemical markers may improve discrimination between the presence and absence of

active synovial proliferation.[22,23] Therefore, Z-scores ((value—mean value)/standard deviation)) were calculated for all biomarkers to combine multiple biochemical markers into indexes summarizing inflammation and osteochondral activity. To compare differences between groups the Chi square test was used for dichotomous/categorical variables and the Kruskal–Wallis test for (skewed) continuous variables. Multivariate logistic regression was performed to adjust for potential influence of differences in age, HJHS and Pettersson score between the patients with and without active synovial proliferation. *P*-values < .05 were considered as statistically significant. All analyses were performed in RStudio (Version 1.3.1093).

3 | RESULTS

3.1 | Patients

Patient characteristics are available in Table 1. We included 79 patients with severe haemophilia A and a median age of 31 years (range 16.5–50.8 years). Fourteen patients were non-adherent to their prescribed prophylaxis and three patients received on-demand treatment. The other 62 patients received continuous prophylaxis. Joint health characteristics extracted from the patient records were ≤3 years old for 92% of HJHS, 91% of HAL and 82% of Pettersson scores.

3.2 | Occurrence of subclinical synovial proliferation

Figure 1 gives an overview of the proportion of patients with synovial proliferation, stratified by active and inactive proliferation. Active subclinical synovial proliferation in at least one joint was observed in 17/79 (22%, CI 13–32) of the patients (21/474 joints; 4% CI 3–7). Likewise, inactive synovial proliferation was observed in 17/79 (22%, CI 13–32) patients (27/474 joints; 6%, CI 4–8). Of the 17 patients with active synovial proliferation, 14 patients had abnormalities during physical examination and only seven patients showed hyperaemia on ultrasound. In 20 patients, synovial proliferation on ultrasound was not accompanied by warmth or swelling during physical examination.Table S1 shows the results of ultrasound and physical examination at joint level.

Table 2 shows that the proportion of patients on continuous prophylaxis in the active (71%) and inactive (71%) synovial proliferation groups was slightly lower than in the no synovial proliferation group (84%), although the differences were not statistically significant ($p = .333$). The percentage of patients with joint bleeding in the year prior to inclusion was higher in the active synovial proliferation group (53%) than in the inactive (35%) and no synovial proliferation (38%) groups. Also, these differences were not statistically significant ($p = .491$).

TABLE 1 Patient and joint characteristics.

	Median (IQR) or *n* (%)	Median years between score and study procedures (IQR)
(A) Patient characteristics (*n* = 79)		
Age (years)	31 (23–42)	–
AJBR	.6 (.2–1.1)	–
Continuous prophylaxis	62 (78%)	–
FVIII IU/kg/year	*1897 (1452–2439)*	–
Emicizumab	*4 (5%)*	–
BMI	25 (23–27)	–
HAL[a]	94 (81–100)	2 (1–3)
(B) Joint characteristics (patient level, *n* = 79)		
Total HJHS[b]	4 (0–16)	2 (1–2)
Total HEAD-US score	4 (1–13)	0 (0–0)
Total Pettersson score	3 (0–12)	3 (1–3)
History of intra-articular interventions	15[c] (19%)	–

Note: % might not add up exactly due to rounding.
Abbreviations: AJBR, mean Annualized Joint Bleeding Rate in the 5 years prior to inclusion; BMI, body mass index; HAL, Haemophilia activity list; HEAD-US, Haemophilia Early Arthropathy Detection with Ultrasound; HJHS, Haemophilia Joint Health Score.
[a]Available for 66/79 patients.
[b]Available for 75/79 patients.
[c]Ankle arthrodesis in three patients, ankle distraction in three patients, joint nettoyage in five patients, synovectomy in five patients and surgery after an intra-articular ankle fracture in one patient. All interventions were at least 3 years ago.

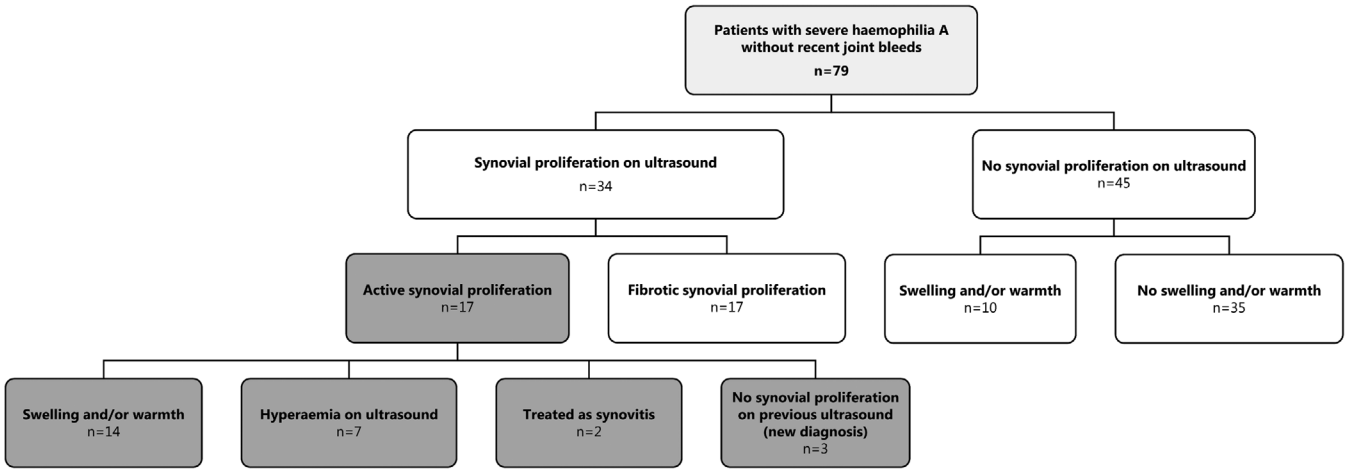

FIGURE 1 Flowchart showing the proportion of patients with synovial proliferation, stratified by active and inactive proliferation.

TABLE 2 Comparison of patient characteristics and biochemical marker levels between patients with active, inactive and no synovial proliferation.

	Overall ($n = 79$)	Patients with active synovial proliferation ($n = 17$)	Patients with inactive synovial proliferation ($n = 17$)	Patients without synovial proliferation ($n = 45$)	*p*-values of univariate analyses[a]
(A) Patient characteristics	Median (IQR) or *n* (%)	Median (IQR) or *n* (%)	Median (IQR) or *n* (%)	Median (IQR) or *n* (%)	
Age	31 (23–42)	43 (30–47)	30 (20–41)	29 (21–37)	.030[b]
Joint bleed ≤1 year	32 (41%)	9 (53%)	6 (35%)	17 (38%)	.491
Continuous prophylaxis	62 (78%)	12 (71%)	12 (71%)	38 (84%)	.333
BMI	25 (23–27)	25 (24–27)	25 (22–26)	24 (22–27)	.505
(B) Joint characteristics (patient level, $n = 79$)					
HJHS score	4 (0–16)	20 (5–31)	6 (2–14)	2 (0–5)	<.001[b]
Pettersson score	3 (0–12)	14 (4–24)	4 (0–12)	0 (0–6)	.002[b]
History of intra-articular interventions	15 (19%)[c]	6 (35%)	3 (18%)	6 (13%)	.143
(C) Biomarkers	Median ng/mL (IQR)	Median ng/mL (IQR)	Median (IQR) or *n* (%)	Median ng/mL (IQR)	
Inflammation					
Osteopontin	49 (41–61)	47 (38–58)	46 (39–49)	52 (43–65)	.157
sVCAM-1	384 (309–477)	384 (302–505)	409 (336–488)	369 (309–456)	.701
Inflammation z-score	*−.3 (−1.0–.6)*	*−.3 (−.9–.9)*	*−.9 (−1.2–.5)*	*−.1 (−1.0–.6)*	*.664*
Osteochondral					
Coll2-1	88 (64–133)	89 (71–120)	95 (59–131)	88 (65–136)	.787
COMP	22 (15–28)	23 (14–27)	24 (15–31)	22 (16–26)	.741
CS846	111 (89–139)	96 (80–113)	113 (102–132)	115 (89–150)	.208
uCTX-II	226 (159–336)	272 (199–348)	267 (170–369)	198 (141–283)	.130
TIMP	145 (133–165)	142 (129–159)	153 (138–171)	144 (132–157)	.387
Osteochondral z-score	*−.2 (−1.8–1.8)*	*−.2 (−1.9–1.6)*	*.4 (−.8–2.0)*	*−.6 (−1.9–1.7)*	*.267*

Note: % might not add up exactly due to rounding. BMI, body mass index; HJHS, Haemophilia Joint Health Score, available for 75/79 patients.

[a]For continuous variables the Kruskal–Wallis test and for categorical/dichotomous variables the Chi square test was used.

[b]Significant difference ($p < .05$).

[c]Ankle arthrodesis in three patients, ankle distraction in three patients, joint nettoyage in five patients, synovectomy in five patients and surgery after an intra-articular ankle fracture in one patient. All interventions were at least 3 years ago.

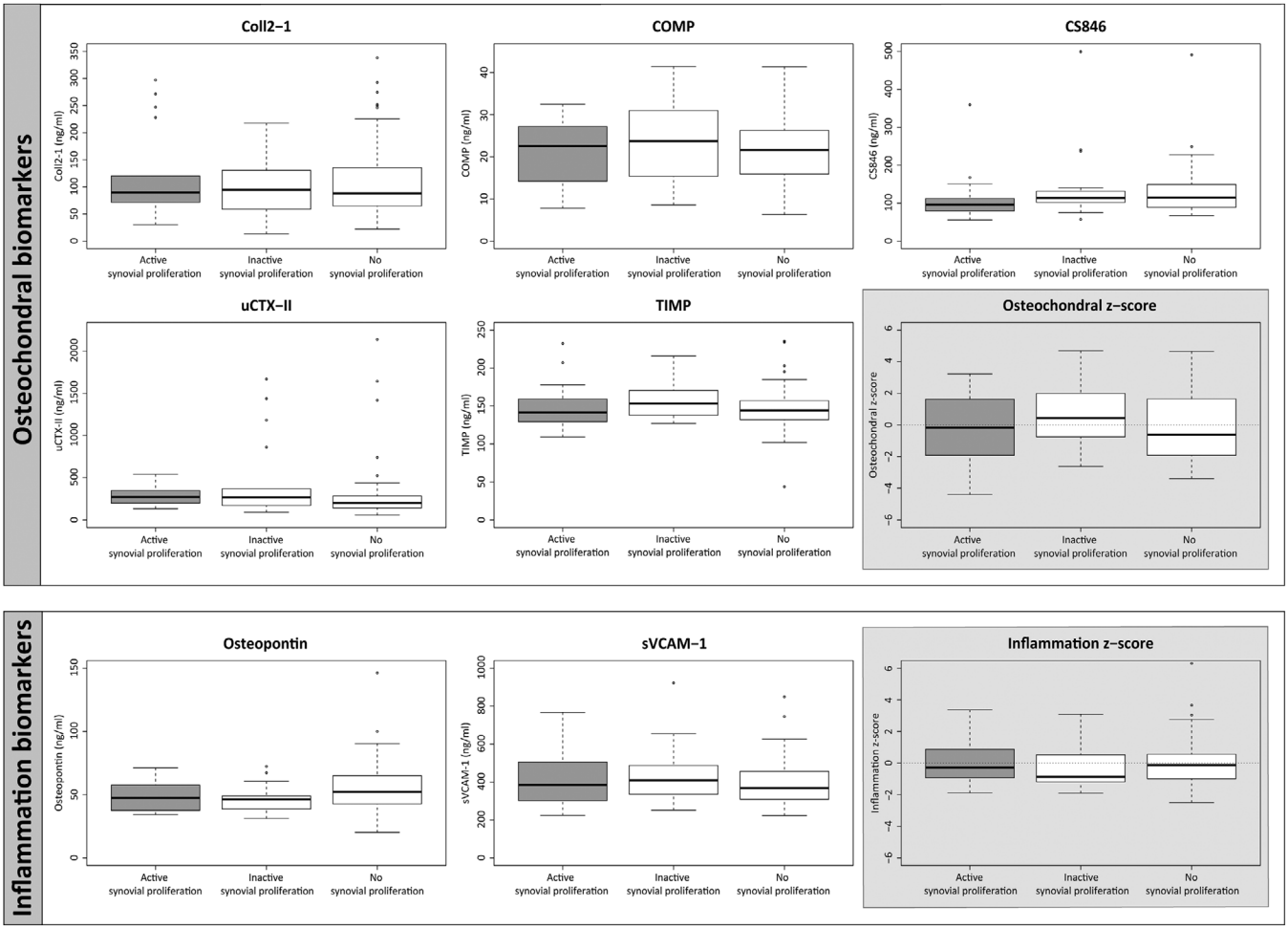

FIGURE 2 Boxplots comparing biochemical marker levels of patients with active, inactive or no synovial proliferation.

3.3 | Correlation of biochemical markers with active subclinical synovial proliferation

Biochemical marker levels of (synovial) inflammation and osteochondral damage were compared in patients with active synovial proliferation (n = 17), patients with inactive synovial proliferation (n = 17) and patients without synovial proliferation (n = 45). Neither the inflammatory markers osteopontin and sVCAM, nor the osteochondral markers Coll2-1, COMP, CS846, uCTX-II and TIMP showed a difference between patients with active synovial proliferation, patients with inactive synovial proliferation, and patients without synovial proliferation. The combined index of z-scores for the inflammatory markers osteopontin and sVCAM-1 did not differ between the groups. Similarly, the combined index of the osteochondral markers Coll2-1, uCTX-II, CS846 and COMP did not differentiate. Boxplots comparing biochemical marker levels between patients with active, inactive and no synovial proliferation are available in Figure 2. Table 2 shows the median biochemical levels with interquartile ranges (and p-values) in the groups. Multivariate logistic regressions, adjusting for age, HJHS and Pettersson score, showed that neither the individual biochemical marker levels nor the combined indexes were significant predictors of active synovial proliferation.

4 | DISCUSSION

This cross-sectional study showed that active and inactive subclinical synovial proliferation in a Dutch cohort of patients with severe haemophilia A both had a prevalence of 22% on patient level (4% and 6% on joint level). Almost 60% of the patients with synovial proliferation on ultrasound did not show warmth or swelling during physical examination. The (combined indexes of) biochemical markers osteopontin, sVCAM, Coll2-1, COMP, CS846, uCTX-II and TIMP were unable to identify patients with active subclinical synovial proliferation.

4.1 | Strengths and limitations

This study included a relatively large sample and almost all patients were treated at our clinic from childhood onwards, reducing

information loss and variability between treatment histories. Besides, all study examinations were performed on the same day. Regarding biochemical markers, all measurements were performed in the same batch, avoiding inter-plate variability.

A limitation of the current study is the use of ultrasound to assess synovial proliferation, instead of the reference standard MRI. However, the accuracy of ultrasound for detecting synovial proliferation is comparable to MRI[24,25] and its interrater reliability is high.[26] In clinical practice, routine examination of six joints with ultrasound is more feasible than with MRI. The use of ultrasound is limited by the fact that there is need for a specially qualified observer and the risk of interrater variability. To ensure high quality assessments and to reduce variability, the ultrasound examinations in our study were performed or supervised by one experienced observer and scored according to the HEAD-US protocol.

Another limitation is the lack of a previous ultrasound assessment in some patients. This complicated distinguishing between old and new findings of synovial proliferation. In the absence of previous ultrasound assessments, we considered synovial proliferation to be new if it had not been mentioned in the medical record in the previous 3 years. However, this cut-off point was chosen subjectively by the expert panel.

Finally, biochemical markers were measured systemically and compared to local joint conditions. This may explain why the biochemical markers could not detect active synovial proliferation. However, measuring biomarkers in the joint compartment is not feasible.

Furthermore, the cross-sectional design of the study limited the biochemical marker research. The observed high inter-individual differences in biochemical marker levels may have masked correlations. In our analyses we adjusted for differences in age, HJHS and Pettersson score between patients with and without active synovial proliferation. However, these analyses were underpowered due to the small number of patients with active synovial proliferation ($n = 17$). Inter-individual differences may be increased by the multifactorial pathophysiology of haemophilic arthropathy and heterogeneous phenotypes. Some patients have synovial inflammation, while others have more pronounced osteochondral damage.[27] A longitudinal study would allow differences in marker levels within patients to be examined over time, correcting for inter-individual baseline differences. Moreover, additional heterogeneity may be induced by diurnal variability, food intake or exercise, distribution volumes or renal/hepatic dysfunction.[28–30] We did not adjust for these factors. On the other hand, translation of biochemical markers into daily practice can only be achieved if they function under practically feasible circumstances.

4.2 | Relation to other studies

The prevalence of subclinical synovial proliferation in our study (43%) is in line with previous studies.[31,32] However, a slightly higher prevalence (55%)[33] and a lower prevalence (5%)[34] have also been described. These differences in prevalence may be explained by differences in age, the moment of treatment initiation, and prophylaxis adherence between the studies.

To our knowledge, no previous studies have aimed to identify active subclinical synovial proliferation in people with severe haemophilia using biochemical marker levels. Therefore, we analysed biochemical markers that we consider most relevant for answering our question based on the existing literature.[35,36] Osteopontin and soluble VCAM-1 are considered potential markers of inflammatory synovitis. In addition, we analysed osteochondral markers that correlated with joint status on imaging (ColI2-1, COMP, uCTX-II, TIMP), increased in overt joint bleeding (COMP and CS846), differed between patients on prophylaxis or on-demand treatment (TIMP), or could differentiate patients with slow and fast progression of haemophilic arthropathy (combined index of uCTX-II and CS846).

We found that neither synovial markers nor osteochondral markers and combined marker indexes were able to identify patients with active synovial proliferation. This is consistent with the results of a recent study in people with non-severe haemophilia that analysed a comparable set of biochemical markers and found poor correlations with MRI findings.[37]

4.3 | Clinical practice and future recommendations

Subclinical synovial proliferation, including active synovial proliferation, was prevalent in this Dutch cohort of patients with severe haemophilia A who visited the clinic for routine follow-up and reported no recent joint bleeds, emphasizing the importance of ultrasound screening to detect subclinical synovial proliferation.

The clinical relevance of ultrasound-detected subclinical synovial proliferation, as well as a diagnostic method to distinguish active from inactive synovial proliferation remain to be established. In this study, we have established a consensus-based definition of active synovial proliferation. However, this definition needs to be validated and may be reconsidered in future prospective studies. We hypothesize that we can interfere in active 'inflammatory' synovial proliferation by early and targeted treatment. We also hypothesize that inactive synovial proliferation contributes less to progression of arthropathy. However, the reversibility of inactive 'fibrotic' synovial proliferation and its contribution to joint damage progression are still unknown. Although it is unclear whether intensified prophylaxis can prevent progression of these subclinical findings, it is currently our only intervention available.

We advocate for routine ultrasound screening in order to have baseline and follow-up ultrasounds for all patients. Comparing these ultrasounds can support the diagnosis of newly developed, and potentially reversible, synovial changes and assist in determining the clinical relevance of these findings. The frequency of this screening needs to be determined. This is useful not only for clinical practice, but also for clinical trials to assess whether maximum joint protection is being achieved.

5 | CONCLUSION

This study highlights the importance of routine ultrasound screening to monitor joint health in people with haemophilia. Subclinical

synovial proliferation was observed in 43% (34/79) of patients with severe haemophilia A who had access to prophylaxis. Of the 34 patients with synovial proliferation, 17 (22%) were considered to have active synovial proliferation. Patients with active versus inactive synovial proliferation could not be discriminated by biochemical markers. Future studies should focus on determining the clinical relevance of ultrasound-detected subclinical synovial proliferation and a diagnostic method to distinguish active from inactive synovial proliferation.

AUTHOR CONTRIBUTIONS

E.D.P. van Bergen: Formal analysis, Investigation, Data Curation, Writing—Original Draft, Visualization. F.H.P. van Leeuwen: Formal analysis, Investigation, Data Curation, Writing—Review & Editing, Visualization. W. Foppen: Conceptualization, Investigation, Writing—Review & Editing, Supervision, Funding acquisition. M.A. Timmer: Conceptualization, Investigation, Writing—Review & Editing, Supervision. R.E.G. Schutgens: Resources, Writing—Review & Editing. S.C. Mastbergen: Resources, Writing—Review & Editing. F.P.J.G. Lafeber: Resources, Writing—Review & Editing. P.A. de Jong: Resources, Writing—Review & Editing. K. Fischer: Conceptualization, Resources, Writing—Review & Editing, Supervision. L.F.D. van Vulpen: Conceptualization, Resources, Writing—Review & Editing, Supervision.

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CONFLICT OF INTEREST STATEMENT

E.D.P. van Bergen, F.H.P. van Leeuwen, R.E.G. Schutgens, S.C. Mastbergen, and F.P.J.G. Lafeber, declare no conflicts of interest. P.A. de Jong declares a research collaboration with Vifor Pharma and Philips Healthcare. K. Fischer has received speaker's fees from Bayer, Baxter/Shire, Sobi/Biogen, CSL Behring and Novo Nordisk; has performed consultancy for Bayer, Biogen, CSL Behring, Freeline, Novo Nordisk, Roche and Sobi; and has received research support from Bayer, Baxter/Shire, Novo Nordisk, Pfizer and Biogen; all fees were paid to the institution. W. Foppen received research grants from Novo Nordisk and Pfizer, which were paid to the institution. M.A. Timmer received research grants from Novo Nordisk and SOBI, which were paid to the institution. L.F.D. van Vulpen received research grants from CSL Behring and Grifols, and consultancy for Sobi, CSL Behring and Tremeau. All fees paid to the institution.

ETHICS STATEMENT

The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Review Board of the UMCU (19-273 – BEGIN).

PATIENT CONSENT STATEMENT

All study participants gave written informed consent.

DATA AVAILABILITY STATEMENT

Data is available from the senior author (Dr. L.F.D. van Vulpen, L.F.D.vanvulpen-2@umcutrecht.nl) upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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