Beyond the surface: Imaging of (sub)clinical joint changes in haemophilia

F.H.P. van Leeuwen

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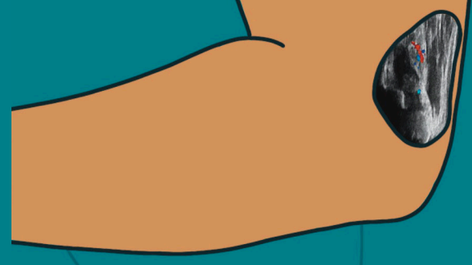

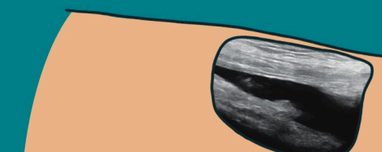

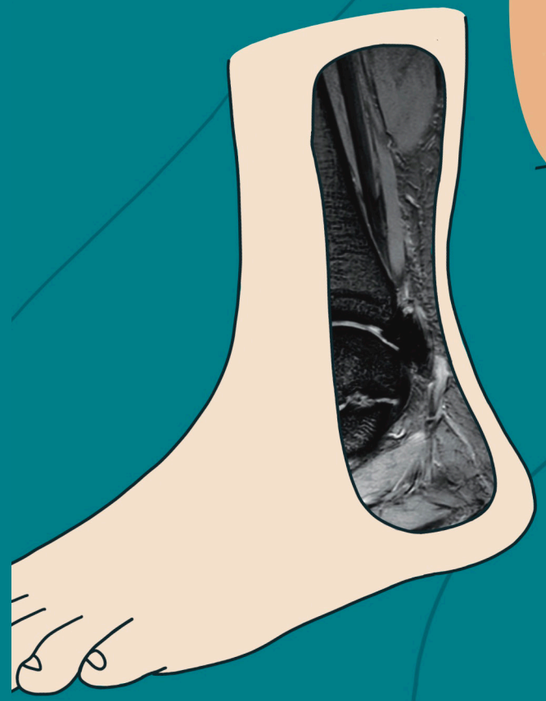

F.H.P. van Leeuwen

UITNODIGING

Voor het bijwonen van de openbare verdediging van het proefschrift

Beyond the surface:
Imaging of (sub)clinical joint changes in haemophilia

door

F.H.P. van Leeuwen

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om 14:15 uur

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Beyond the surface:
Imaging of (sub)clinical joint changes in haemophilia

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Onder de oppervlakte:
beeldvorming van (sub)klinische gewrichtsveranderingen bij hemofilie
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
woensdag 10 juli 2024 des middags te 2.15 uur

door

Flora Hendrica Pieternella van Leeuwen

geboren op 25 februari 1995
te Nijmegen

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Prof. dr. ir. Y.T. van der Schouw (voorzitter)

TABLE OF CONTENTS

CHAPTER 1

Introduction

HAEMOPHILIA

Haemophilia A and B are rare X-linked recessive inherited coagulation disorders. Haemophilia A affects approximately 13:100,000 men and haemophilia B 3:100,000 men. The (functional) deficiency of clotting factor VIII in haemophilia A and clotting factor IX in haemophilia B increase the bleeding tendency of people with haemophilia. Haemophilia severity is categorized according to the residual clotting factor activity as mild (>5% factor VIII or IX), moderate (1-5% factor VIII or IX) or severe (<1 % factor VIII or IX)[1]. Bleeding can occur throughout the whole body, however mostly occurs in joints (60-80%) followed by muscles (10-30%)[2,3]. Bleeding into the ankles, knees and elbows (the index joints) are most frequent[2,4,5]. Specifically severe haemophilia is characterized by traumatic or spontaneous joint bleeding. Recurrent joint bleeding eventually leads to painful and disabling joint damage known as haemophilic arthropathy.

PATHOPHYSIOLOGY AND CONSEQUENCES OF HAEMOPHILIC ARTHROPATHY

The pathophysiology of haemophilic arthropathy is not fully elucidated yet. When joint bleeding occurs, intra-articular blood affects different joint components in a multifactorial way, as shown in Figure 1. The blood has a direct damaging effect on the cartilage by inducing chondrocyte apoptosis and reducing proteoglycan synthesis[6–8]. These damaging effects of intra-articular blood can already be observed after a short period of exposure to small amounts of blood[9]. Furthermore, blood induces synovial inflammation with synovial hypertrophy and neoangionesis. Iron from the degraded erythrocytes accumulates as haemosiderin deposits in the synovium, which is an additional trigger for synovial inflammation. Synovial inflammation leads to cartilage damage through the production of cytokines and enzymes. In addition, the hypertrophied, highly vascularized synovium increases the risk of rebleeding, leading to a repetitive cycle of joint bleeding. The initial blood-induced joint changes, i.e. synovial hypertrophy, may be reversible. However, cartilage and bone damage are irreversible. End-stage haemophilic arthropathy is characterized by cartilage destruction, osteoporosis, bone erosion, subchondral cysts and sclerosis, osteophytes and joint deformity. This end-stage joint degeneration causes pain and functional limitations, leading to a reduced quality of life[6,7].

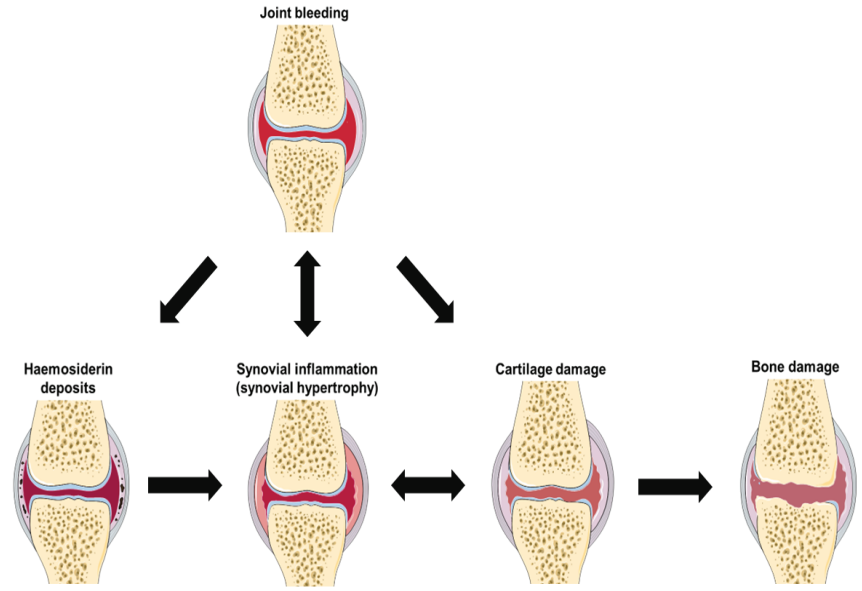

Figure 1. Multifactorial pathophysiology of haemophilic arthropathy.

TREATMENT OF HAEMOPHILIA

In the 1960s, intravenously administered clotting factor concentrates became available as replacement therapy for haemophilia[10]. Treatment of haemophilia aims to prevent or stop bleeding and thereby prevent development of haemophilic arthropathy[1,3]. Patients can be treated with clotting factor replacement therapy prophylactically (prophylaxis) and/or on demand in case of a bleed. Prophylaxis has been shown to be very effective in preventing bleeding and arthropathy[11]. Early prophylaxis, preferably continued throughout life, is therefore the recommended treatment for people with severe haemophilia[3]. Downsides of prophylaxis are the burdensome regular intravenous injections and high costs[12,13]. In clinical practice, prophylaxis is continuously adjusted based on reported (joint) bleeding, clinical joint assessment (according to the Hemophilia Joint Health Score[14,15]), and measured FVIII/IX (trough) levels. Despite prophylaxis, Dutch people with severe haemophilia A still experience approximately 1 joint bleed per year[12]. New treatments such as extended half-life clotting factor concentrates, non-replacement therapy and gene therapy may further improve treatment and reduce joint bleeding rates in the coming years[1,16–20].

Treatment options for haemophilic arthropathy are limited to prevention of joint bleeding and treatment of synovitis at one end of the treatment spectrum, and surgical joint fixation or joint replacement on the other end. Treatment of haemophilia therefore focuses on preventing the irreversible arthropathy and/or treating early synovial inflammation[3].

SUBCLINICAL JOINT BLEEDING AND INFLAMMATION IN HAEMOPHILIA

Previous studies have shown that joint changes occur and progress in the absence of overt joint bleeding[21–24]. These observations have led to hypotheses about the occurrence and contribution of subclinical joint bleeding and subsequent subclinical inflammation to the development of haemophilic arthropathy. Microscopic joint bleeding or minimal synovial inflammation may go unnoticed because they likely do not cause clinical symptoms. In addition, joint bleeding and inflammation can cause non-specific symptoms that may not be recognized as such by patients or their physicians. In either case, the bleeding and inflammation are not treated and haemophilic arthropathy can develop. With current treatment advances, overt joint bleeding is becoming rare. The contribution of subclinical joint bleeding and inflammation to the progression of arthropathy is becoming more important, and subclinical bleeding and inflammation are becoming the target of treatment.

ROLE OF IMAGING IN (SUB)CLINICAL HAEMOPHILIC ARTHROPATHY

Although haemophilia treatment monitoring is mainly clinical and laboratory based, imaging has become an important tool for monitoring joint health[3,25]. X-rays and the corresponding Pettersson score[26] are most widely used for diagnosis of haemophilic arthropathy. Late arthropathic changes, such as subchondral bone abnormalities, can be observed on X-rays. However, X-rays are not useful for detecting early joint changes[27]. Other imaging modalities, such as magnetic resonance imaging (MRI) and ultrasound, can detect early joint changes and may therefore be able to detect (previous) subclinical joint bleeding and/or inflammation. MRI can accurately detect early joint changes such as synovial hypertrophy, haemosiderin deposition and small cartilage defects. It can also provide detail on more advanced joint changes such as large cartilage defects, bone erosions and subchondral cysts. Hence, MRI is the current gold standard for diagnosis of haemophilia-related joint changes in clinical practice and research[3,28,29]. Ultrasound is a less expensive, faster and more accessible alternative to MRI which is increasingly used in haemophilia care[30,31]. It has comparable accuracy to MRI in detecting early soft tissue changes[32,33]. However, it is less useful for assessing central osteochondral changes and image quality is operator dependent[28]. Ultrasound is used in research and clinical practice for assessing joint health, yet can also diagnose joint bleeding[3,30].

OUTLINE OF THIS THESIS – BEYOND THE SURFACE

This thesis will go beyond the surface of haemophilic arthropathy. The focus will be on imaging, not only of clinical, but also of subclinical joint changes in people with haemophilia. Furthermore, the role of imaging in clinical decision-making in haemophilia care will be investigated. Being able to detect subclinical joint changes is the first step towards targeted treatment of these changes. Therefore, this thesis is divided into three parts: 1) detection of subclinical joint bleeding, 2) screening for subclinical joint inflammation and 3) the role of ultrasound in management of acute joint episodes.

Part 1 focuses on the use of MRI to detect subclinical joint bleeding. In a phantom study in **Chapter 2**, we investigated whether MRI T1 and T2 relaxometry can quantitatively differentiate synovial fluid from haemorrhagic joint effusion with varying blood concentrations. In **Chapter 3**, we subsequently investigated *in vivo* whether MRI T2 relaxometry of joint effusion is feasible and reproducible in haemophilia patients. The aim of **Chapter 4** was to investigate whether there is evidence of previous subclinical bleeding on conventional MRI of joints without a history of bleeding in severe haemophilia patients on prophylactic treatment.

Part 2 focuses on screening for subclinical synovial proliferation as a proxy for subclinical joint inflammation. **Chapter 5** includes a review and meta-analysis of the existing literature on joint swelling and ultrasound-detected synovial hypertrophy. The diagnostic accuracy of joint swelling for ultrasound-detected synovial hypertrophy was determined to estimate the added value of ultrasound to physical examination in detecting synovial hypertrophy. **Chapter 6** provides estimates of the prevalence of active and inactive synovial proliferation in people with severe haemophilia A. This chapter also investigated whether biochemical markers in blood and urine can identify patients with active synovial proliferation.

Part 3 focuses on the role of ultrasound in management of acute joint episodes. In **Chapter 7**, we evaluated the impact of point-of-care ultrasound in addition to clinical assessment for the diagnosis and treatment of acute musculoskeletal episodes in a heterogeneous cohort of children and adults with haemophilia and von Willebrand disease (VWD). The research in **Chapter 8** aimed to evaluate the duration of full recovery after joint bleeding and to determine how recovery differed between physical examination and ultrasound.

Chapter 9 summarizes the findings of this thesis and discusses their implications for clinical care and future research.

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PART 1

Detection of subclinical joint bleeding

CHAPTER 2

Detecting low blood concentrations in joints using T1 and T2 mapping at 1.5, 3, and 7 T: an *in vitro* study

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ABSTRACT

Background

Intra-articular blood causes irreversible joint damage, whilst clinical differentiation between haemorrhagic joint effusion and other effusions can be challenging. An accurate non-invasive method for the detection of joint bleeds is lacking. The aims of this phantom study were to investigate whether magnetic resonance imaging (MRI) T1 and T2 mapping allows for differentiation between simple and haemorrhagic joint effusion and to determine the lowest blood concentration that can be detected.

Methods

Solutions of synovial fluid with blood concentrations ranging from 0 to 100% were scanned at 1.5, 3, and 7 T. T1 maps were generated with an inversion recovery technique and T2 maps from multi spin-echo sequences. In both cases, the scan acquisition times were below 5 min. Regions of interest were manually drawn by two observers in the obtained T1 and T2 maps for each sample. The lowest detectable blood concentration was determined for all field strengths.

Results

At all field strengths, T1 and T2 relaxation times decreased with higher blood concentrations. The lowest detectable blood concentrations using T1 mapping were 10% at 1.5 T, 25% at 3 T, and 50% at 7 T. For T2 mapping, the detection limits were 50%, 5%, and 25%, respectively.

Conclusions

T1 and T2 mapping can detect different blood concentrations in synovial fluid *in vitro* at clinical field strengths. Especially, T2 measurements at 3 T showed to be highly sensitive. Short acquisition times would make these methods suitable for clinical use and therefore might be promising tools for accurate discrimination between simple and haemorrhagic joint effusion *in vivo*.

BACKGROUND

Haemarthrosis may be caused by (repeated) trauma or by bleeding disorders like haemophilia and Von Willebrand disease. The intra-articular blood has harmful effects on different joint components [1, 2]. These harmful effects already occur after relatively short exposure to small amounts of intra-articular blood as shown *in vitro* [3].

Clinical symptoms of joint bleeding, such as pain, swelling, and reduced functionality, are not specific for joint bleeding as these are observed in different joint conditions like arthritis, osteoarthritis, and other arthropathies as well [4,5,6]. Identification of joint effusion by imaging studies for example is not specific for joint bleeds, because effusion is also observed in healthy joints [7], arthropathy flare-ups, and arthritis [4, 8,9,10]. Nevertheless, being able to distinguish between a joint bleed and another cause of joint effusion is clinically relevant, especially in patients with bleeding disorders like haemophilia, since the different diagnoses require different treatments [4, 11, 12].

The current reference standard for the analysis of joint effusion is a joint aspiration. However, it is an invasive method that could introduce the potential risk of infection or a haemorrhagic procedure which could result in false-positive outcomes. Therefore, joint aspiration might not be desirable in all cases, e.g. in patients with an increased bleeding risk [11, 13].

Ultrasound is increasingly used in addition to physical examination since it is a non-invasive method that can accurately assess joint effusion and changes in synovial tissue [11, 14,15,16], it is widely available, and provides real-time information [11, 14,15,16]. Moreover, previous studies have shown that ultrasonography is sensitive to differentiate between simple and complex joint effusion [17, 18]. However, limitations of ultrasound are the operator dependency and therefore a substantial inter-observer variation of findings [11, 19].

Magnetic resonance imaging (MRI) is a reliable tool for the assessment of inflammatory and degenerative joint changes, due to its excellent contrast in tissues and fluids [10, 14]. Large haemarthroses may show fluid-fluid levels or thrombi on MRI. However, the accuracy of standard MRI protocols for the detection of early or minor joint bleeding has not been established yet [17, 18]. Therefore, an accurate non-invasive reference standard to detect joint bleeding is lacking.

Whilst standard MRI protocols are unable to accurately detect limited amounts of intra-articular blood, dedicated MRI sequences might be able to detect and quantify small amounts of blood within the synovial fluid. Since synovial fluid and iron-containing blood have different relaxation properties, quantification of longitudinal (T1) and transverse (T2) relaxation times with T1 and T2 mapping should in principle be able to assess the presence of blood in the synovial fluid.

In this phantom study, we investigated the potential of quantitative T1 and T2 relaxometry MRI protocols as non-invasive tools to differentiate between simple and haemorrhagic joint effusion at

1.5, 3, and 7 T. Specifically, we aimed to establish the minimal blood concentration in the synovial fluid which can be detected using these quantification methods.

METHODS

Sample preparation

All patients or their legal guardians approved the use of their remnant samples for method development, validation, and research purposes, in agreement with the institutional regulations (Art. 8, University Medical Center Utrecht Biobank Regulations; version June 19, 2013). The synovial fluid used consisted of pooled residual synovial fluid from clinical joint aspirations in four patients with rheumatoid arthritis (n = 1), juvenile arthritis (n = 1), and unknown disease (n = 2). The synovial fluid residuals were visually inspected to confirm clear, non-purulent, non-haemorrhagic aspirates, kept frozen at − 80 °C, and were defrosted 24 h before being pooled to one volume of synovial fluid. The blood used was venous whole blood (haemoglobin 9.8 mmol/L), from a healthy male volunteer, collected into lithium-heparin tubes. The freshly withdrawn blood was mixed with the pooled synovial fluid residuals.

Solutions with blood concentrations of 0, 2.5, 5, 10, 25, 50, 75, and 100% were prepared, and a volume of 3 mL was injected into nuclear magnetic resonance tubes (Wilmad LabGlass, WG-1000-8, Vineland, NJ, USA) (Fig. 1a). To ensure the blood was deoxygenated before starting the scanning sessions, the samples were kept at 37 °C for 24 h. Outside the scanner, the samples were kept in a stove at 37 °C and were manually shaken prior to scanning to maintain homogeneous mixtures.

Phantom

The samples were horizontally placed in batches of six samples in a cylindrical holder filled with water at 1.5 T and 3 T or mark-oil at 7 T, to reduce magnetic susceptibility effects around the samples. Details can be found in the supplementary materials (Fig. S1). The temperature inside the phantom was monitored using a fiberoptic thermometer (Luxtron Corp., Santa Clara, CA, USA), and was kept stable around 37 °C during the scanning session using a heating system based on heat exchange between water in the heating system and the water or oil volume in the phantom (Fig. 1b).

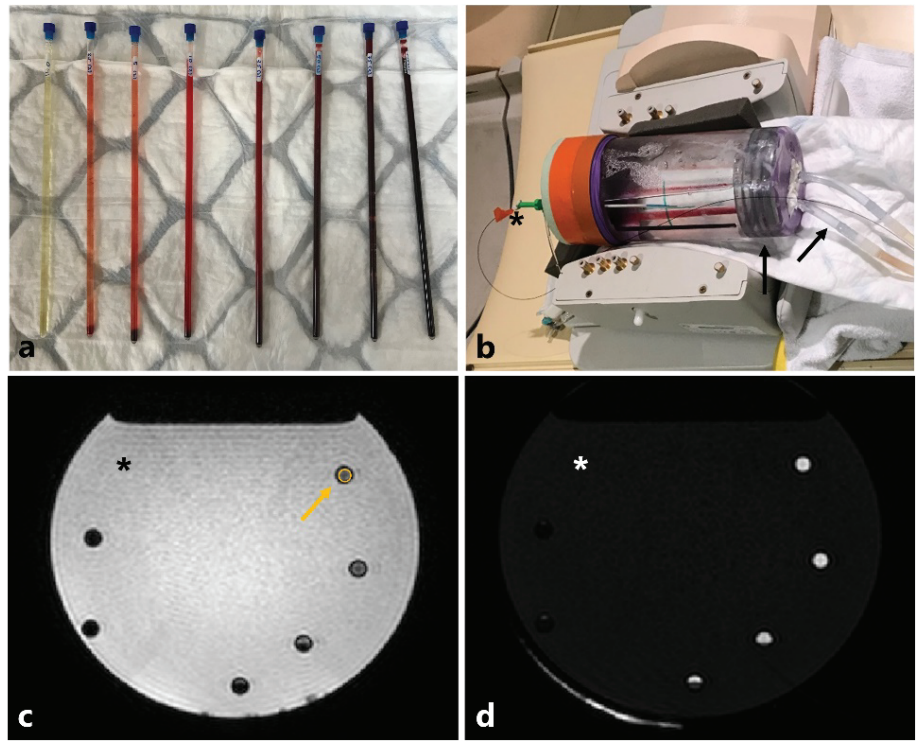


Figure 1. a Nuclear magnetic resonance tubes with different ratios of synovial fluid and blood. *Blood percentage from left to right: 0%, 2.5%, 5%, 10%, 25%, 50%, 75%, and 100%.* b Phantom setup: a cylindrical water-filled holder with six samples, horizontally placed in a 16-channel knee coil using a 3-T MRI system. *The phantom was equipped with a fiberoptic thermometer (the asterisk in all images) and a heating system based on heat exchange between water in the heating system (black arrows) and the water or oil volume in the phantom.* c Scan image from the T1 mapping sequence. *For each tube, a region of interest was manually placed within the tube region (example in yellow).* d Scan image from the T2 mapping sequence.

MRI data acquisition

The experimental data were acquired at 3 field strengths, using 1.5, 3, and 7 T systems (Philips Achieva, Best, The Netherlands). An 8-channel head coil and a 16-channel knee coil (Philips Achieva, Best, The Netherlands) were used at 1.5 and 3 T, respectively. A 32-channel head coil (Nova Medical, Wilmington, MA, USA) was used at 7 T. All acquisitions were performed in a time span of 25 h using the 1.5-T, the 3-T, and the 7-T scanners, in that chronological order.

T1 mapping

Images for the T1 measurements were obtained using a multislice inversion recovery technique with a gradient-echo echo-planar imaging readout (Fig. 1c) [20]. The T1 mapping strategy consists of inverting the longitudinal magnetisation in the imaging volume using an adiabatic inversion pulse and subsequently sampling the recovery curve by changing the order in which the slices are acquired after the inversion. The details of the MRI protocols are reported in Table 1.

Table 1. MRI protocols for T1 mapping and T2 mapping with turbo-spin echo and sensitivity encoding acceleration, at 1.5, 3 and 7 T

	T1 mapping			T2 mapping*		
Field strength	**1.5 T**	**3 T**	**7 T**	**1.5 T**	**3 T**	**7 T**
Number of slices	11	11	11	5	5	5
Number of inversions	11	11	11	32	32	32
Voxel size (mm)	0.8	0.8	0.8	0.8	0.8	0.8
Field of view (mm)	128 × 128	128 × 128	128 × 128	128 × 128	128 × 128	128 × 128
Slice thickness (mm)	5	5	5	5	5	5
Inversion time spacing (ms)	315	315	907	-	-	-
Repetition time (ms)	10,000	10,000	10,000	3,000	3,000	7,800
Echo time (ms)	7.8	7.8	4.33	20–950	20–950	30–960
Flip angle	15°	15°	15°	90°	90°	90°
EPI frequency encoding bandwidth (Hz)	614.6	614.6	1,179	-	-	-
Readout Bandwidth (Hz/pixel)	77.1	77.1	144.2	227	227	208
EPI factor	5	5	5	-	-	-
Parallel reduction factor in-plane	2	2	2	2	2	2
Scan duration (s)	220	220	220	252	168	632

EPI Echo-planar imaging, *with turbo-spin echo and sensitivity encoding acceleration

T2 mapping

T2 mapping was performed using a multi-slice multiple spin-echo sequence, where images are acquired as the signal decays after the initial slice excitation (Fig. 1d). The different echo times (TEs) are separated by the echo spacing. To limit the scan duration, different acceleration techniques were applied: turbo-spin echo (TSE), echo-planar imaging, and a combination of TSE acceleration with sensitivity encoding (SENSE). The details of the sequences are reported in Table 1.For T2 measurements, a parameter of particular significance in the pulse sequence is the echo spacing [21]. To verify that measured T2 values were independent of the echo spacing, four additional scans with different echo spacing (echo spacing 10, 20, 30, and 40 ms, respectively) were acquired at 1.5 T (information available in the Supplementary material).

Image analysis

T1 and T2 fitting

Image processing was done offline using MatLab 2018a (MathWorks, Natick, MA, USA). T1 and T2 maps were obtained by voxelwise fitting the fit functions to the signal intensity (S) from the data points using a Levenberg-Marquardt non-linear least squares method. To obtain T1, the signal was fitted as $S = S0\ (1 - 2\ e^{-TI/T1})$, where S0 is the spin density and TI is the inversion delay. To obtain T2, the signal was fitted as $S = S0\ (e^{-TE/T2})$, where TE is the echo time [22].

Postprocessing

The quantitative evaluation was based on the mean T1 or T2 values from regions of interest (ROIs) within each tube. The T1 and T2 standard deviation was assessed using the spatial standard deviation over the voxels inside the ROIs placed on the maps. Normally distributed data were reported as mean values with standard deviations. The normality of the data was evaluated by the Kolmogorov-Smirnov test. Finally, for each field strength, the mean T1 and T2 across the scan batches were calculated for different blood concentrations. To determine the lowest blood concentration that could be reliably measured by the T1 and T2 mapping, a method based on the guideline EPI17, by Clinical and Laboratory Standards Institute [23], was used. The synovial fluid, i.e. 0% blood concentration sample, was used as a blank sample. The limit of blank (LoB) was determined according to the guideline EPI17. The limit of detection (LoD) definition used by the Clinical and Laboratory Standards Institute was adapted to allow the discrimination of the highest apparent T1 or T2 likely to be reliably distinguished from the LoB among different blood concentrations. The LoD was determined by the following: $LoD=\mu_{x\%blood}+1.645\ \sigma_{x\%blood}$ where $\mu_{x\%blood}$ is the mean T1 or T2 and $\sigma_{x\%blood}$ is the standard deviation of T1 or T2 for a given blood concentration of x%, fulfilling the condition of LoD < LoB. The blood concentration in percent corresponding to the LoD was defined as the detection threshold.

Interrater reliability and agreement

To estimate the reproducibility of the measurements on the obtained T1 and T2 maps, all ROIs were independently determined by two raters, both relatively inexperienced in clinical radiology (FL, 4 months clinical radiology; BL, 4 years investigative radiology). Interrater reliability was assessed by intraclass correlation coefficient (ICC) for T1 and T2 mapping at the clinically available field strengths (1.5 and 3 T). ICC values range from 0 to 1, where values < 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and > 0.90 indicate poor, moderate, good, and excellent reliability, respectively. ICC estimates and their 95% confidence intervals (CI) were calculated using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, version 26.0.0.1, Armonk, NY, USA) based on a single rater, absolute agreement, 2-way random effects model. Interrater agreement was assessed by the interrater limit of agreement (LoA) and graphically displayed in the Bland-Altman method with plots [24] for the previously mentioned sequences and field strengths.

RESULTS

Imaging was successful at all field strengths. Scan images for T1 mapping presented Gibbs ringing artefacts at 7 T, which hampered the analysis of the 75% and in part of the 100% blood concentration samples. Moreover, it was not possible to measure T2 values for blood concentration above 25% at 7 T because of the complete signal loss due to the short T2 of blood. A temperature between 35 and 38 °C was maintained in the phantom during the scanning sessions. Blood clot formation and sedimentation were observed to some extent in the samples during scanning.

T1 measurements

The mean T1 values as a function of blood concentration for each field strength are shown in Fig. 2. Overall, the estimated T1 values showed an inverse dependence on the blood concentration for all field strengths. The blood detection thresholds for T1 were ≥ 10% at 1.5 T, ≥ 25% at 3 T, and ≥ 50% at 7 T. The T1 estimates for blood and synovial fluid, the LoB (ms), the LoD (ms), and the blood detection threshold (blood percentage) are reported in Table 2.

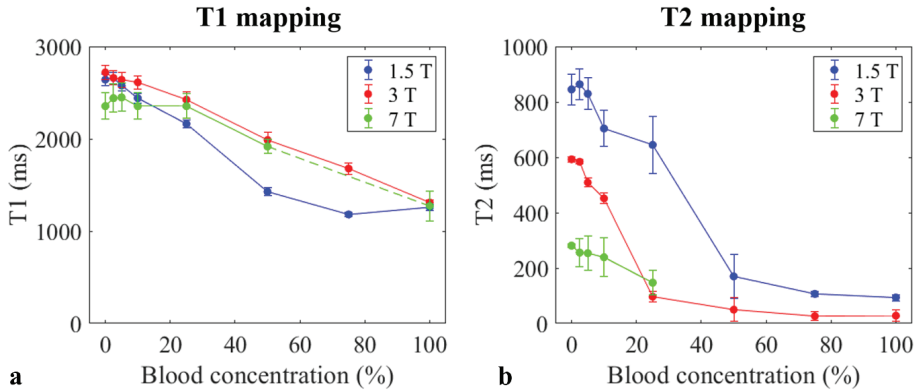


Figure 2. Mean T1 and T2 value estimates for the different blood concentrations, at 3 field strengths.
For 7 T, some of the estimates were not available, because artefacts or relaxation times were too short to be measured.

Table 2. Experimentally estimated T1 and T2 values for synovial fluid and blood, LoB, LoD and blood detection threshold at 1.5, 3, and 7 T

	Field strength	Relaxation time synovial fluid (ms)	Relaxation time blood (ms)	LoB (ms)	LoD (ms)	Blood detection threshold
T1	**1.5 T**	2,641 ± 60	1,258 ± 33	2,541	2,526	≥ 10%
	3 T	2,719 ± 72	1,310 ± 33	2,600	2,560	≥ 25%
	7 T	2,355 ± 145	1,272 ± 160	2,117	2,040	≥ 50%
T2	**1.5 T**	845 ± 56	93 ± 11	753	298	≥ 50%
	3 T	592 ± 13	28 ± 20	579	546	≥ 5%
	7 T	281 ± 5	Not measurable	272	218	= 25%

Results are reported as mean values and the standard deviations as data were normally distributed (Kolmogorov-Smirnov test, $p > 0.05$), *Blood* Blood concentration 100%, *Blood detection threshold* The blood concentration corresponding to the LoD, *LoB* Limit of blank, *LoD* Limit of detection, *Synovial fluid* Blood concentration 0%

T2 measurements

At 1.5 T, different protocols for T2 mapping were employed (Fig. 3). With all the protocols, the T2 exhibited an inverse dependence on the blood concentration. The three accelerated T2 mapping methods showed good agreement with the reference scan (echo spacing 30 ms) in almost all cases. A large T2 standard deviation was identifiable for the 5% blood concentration with the EPI acceleration technique.

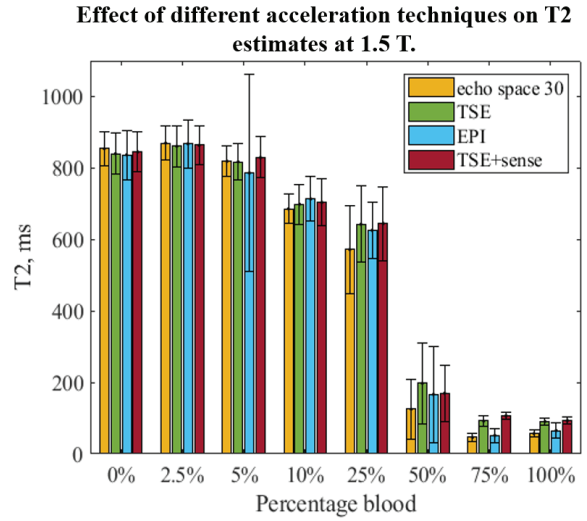
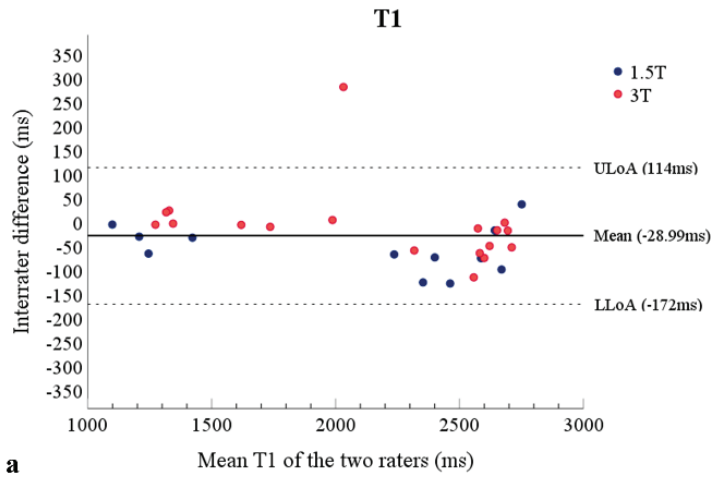


Figure 3. Effect of different acceleration techniques on T2 estimates at 1.5 T.
T2 exhibited an inverse dependence on the blood concentration with all acceleration techniques. The three accelerated T2 mapping methods showed good agreement with the reference scan (echo spacing 30 ms), except from the 5% blood concentration measurement using the EPI acceleration technique. EPI, echo-planar imaging; TSE, turbo spin-echo; TSE+SENSE, turbo spin-echo with sensitivity encoding.

The mean T2 values for different blood concentrations using multi-slice TSE/SENSE-accelerated sequences at each field strength are shown in Fig. 2. The blood detection thresholds for T2 were ≥ 50% at 1.5 T, ≥ 5% at 3 T, and equal to 25% at 7 T. The T2 estimates for the blood and synovial fluid, the LoB (ms), the LoD (ms), and the blood detection threshold (blood percentage) are reported in Table 2.

Interrater reliability and agreement

Interrater reliability as assessed by the ICC was excellent for both T1 (0.991, 95% CI 0.980–0.996) and T2 (0.969, 95% CI 0.937–0.985) measurements at 1.5 and 3 T. The interrater LoA, which reflects the deviation of the measurements performed by the two observers compared to the mean value, was 143.15 ms for T1 and 147.51 ms for T2 (Fig. 4).



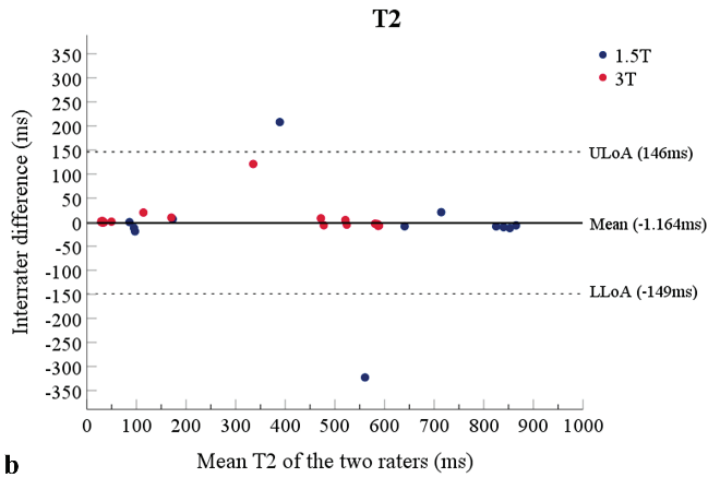


Figure 4. Bland-Altman plots for interobserver agreement regarding T1 and T2 measurements at 1.5 and 3 T. *Horizontal dashed lines indicate the upper and lower limit of agreement from the mean by the two observers (ULoA/LLoA). For T1 measurements, the mean was − 28.99 ms, with a limit of agreement (LoA) equal to 143 ms. For T2 measurements, the mean was − 1.164 ms with LoA equal to 148 ms.*

DISCUSSION

Our study presented T1 and T2 mapping MRI protocols aimed at detecting low blood concentrations in joints. We showed that different concentrations of deoxygenated blood and synovial fluid can be identified *in vitro* using quantification of the relaxation times at 1.5, 3, and 7 T. At all three field strengths, T1 and T2 relaxation times decreased as the blood concentration increased. Moreover, we were able to determine the detection thresholds of the T1 and T2 mapping approaches, i.e. the lowest blood concentration likely to be reliably distinguished from synovial fluid. For T1, the detection threshold was 10% at 1.5 T, 25% at 3 T, and 50% at 7 T. For T2, the detection threshold was 50% for 1.5 T, at 5% for 3 T, and is at least equal to 25% for 7 T.

We were unable to identify quantitative studies differentiating T1 and T2 for blood and synovial fluid at 1.5, 3, and 7 T. However, previous studies have reported on the relaxation times of blood and/or synovial fluid at various magnetic field strengths in *in vitro* and *in vivo* settings (Table 3). Our estimates of synovial fluid were in line with the relaxation times reported by previous works [25, 28]. Variations can be attributed to the intersubject variability of relaxation times in the synovial fluid [32, 33] or to the differences in the pulse sequence parameters, e.g. type of sequence, acquisition techniques, T1 and T2 estimation method, and echo times [25, 26, 28]. As for blood relaxation times, previous studies reported higher values than ours at 1.5, 3, and 7 T [26, 27, 30, 31]. This discrepancy probably results from a higher blood oxygen level [29, 31]: up to 95% oxygenated blood for reported values in the other studies [26], compared to fully deoxygenated blood in this study. At 7 T, estimates in the blood appeared to be different. However, the study at 7 T should only be considered as a proof of concept.

Table 3. T1 and T2 relaxation times of blood and synovial fluid at different field strengths: a comparison of the experimental values with literature values

	Field strength	Synovial fluid				Blood			
		Experimental values	Literature values	Author		Experimental values	Literature values	Author
T1 (ms)	1.5 T	2,641 ± 60	2,850 ± 280	Gold[24]		1,258 ± 33	1,440 ± 120	Stanisz[28]
							1,480 ± 61	Zhang[29]
	3 T	2,719 ± 72	3,620 ± 320	Gold[24]		1,310 ± 33	1,932 ± 85	Stanisz[28]
			2,564 ± 270	Jordan[25]			1,649 ± 68	Zhang[29]
							1,584 ± 5	Lu[32]
	7 T	2,355 ± 145	4,813 ± 700	Jordan[25]		1,272 ± 160	2,212 ± 53	Dobre[30]
							2,087 ± 131	Zhang[29]
T2 (ms)	1.5 T	845 ± 56	1,210 ± 140	Gold[24]		93 ± 11	290 ± 30	Stanisz[28]
	3 T	592 ± 13	767 ± 50	Gold[24]		28 ± 20	275 ± 50	Stanisz[28]
			652 ± 113	Jordan[25]			60 ± 6	Krishnamurty[31]
	7 T	281 ± 5	324 ± 60	Jordan[25]		Not measurable*	21 ± 4	Krishnamurty[31]

Experimental values Values measured in this study in region of interests within the synovial fluid (0% blood) and 100% blood tubes reported as means and standard deviations, *Literature values* Mean values found in other studies with corresponding standard deviations, * T2 times too short to be measured.

Furthermore, we observed a lower blood detection threshold with T1 mapping at 1.5 T than at 3 T and the opposite for T2 mapping. This behaviour can be related to the compartmentalisation of paramagnetic haemoglobin in the blood cells, which is reminiscent of the way superparamagnetic iron oxide particles are distributed. The relaxation effects of these iron oxide contrast agents are strongly dependent on the physical characteristics of the individual nanoparticles and are influenced by their local concentration as well as the applied field strength [34]. In our case, a higher detection threshold is caused by a higher mean value and a bigger standard deviation. T1 measurements at 1.5 T were more precise than at 3 T, and the T1 relaxivity (the inverse of the relaxation time, i.e. 1/T1) of clustered paramagnetic compounds is higher at 1.5 T than at 3 T [35, 36]. Thus, due to a combination of stronger T1 effect and smaller T1 standard deviation at 1.5 T compared to 3 T, the LoD for T1 is lower at 1.5 T. The opposite can be observed for T2, where the effect of paramagnetic compartments, such as blood cells or iron oxide, increases with field strength [34, 36].The current study reports on the performance of the developed MRI protocols in a phantom. Therefore, these results cannot be readily translated into patient settings. However, the observed trends and values give useful indications of T1 and T2 quantification in patients since the phantom mimics the conditions in a human joint. Firstly, the small volume of the scanned samples resembles the volume of synovial fluid in joints without large effusion. Secondly, the phantom was scanned at a constant temperature of 37 °C to simulate the intra-articular temperature. Thirdly, deoxygenated blood was used for the preparation of biological samples to ensure a stable oxygenation level throughout the scan session. Deoxygenated intra-articular blood is also expected in clinical and research settings after the expected patient delay between bleed onset and presentation at the clinic.

Nevertheless, the nature of the biological fluids employed in this study introduces some challenges on the experimental side. The blood used showed clot formation and sedimentation in the samples during scanning, despite heparinisation of the blood and the manual mixing before scanning. As a result of an inhomogeneous distribution of blood, observed T1 and T2 relaxation times might not fully correspond to values of homogeneous mixtures. However, clot formation and sedimentation can be expected to some extent *in vivo* as well. Therefore, imprecision of measured values *in vivo* can be expected too and cannot be prevented either. Furthermore, the reported T1 and T2 relaxation times were measured for blood with a haemoglobin level of 9.8 mmol/L, and relaxation times could vary with differences in haemoglobin levels.

The synovial fluid used came in part from patients with known inflammatory diseases. This might have increased the count of inflammatory cells in the synovial fluid mixture compared to the synovial fluid of patients without ongoing inflammatory joint processes. However, the concordance of our results to the values in the literature suggests that the effect of this increase in inflammatory cells on the relaxation time measurements can be considered minimal [25, 28].

From a technical perspective, the methods presented in this phantom study could be suitable for clinical settings, since a scan duration below 5 min of one T1 or T2 mapping sequence allows the use in clinical research and practice as part of the MRI protocol.

Additionally, the MRI sequences were shown to be sufficiently robust to be translated to different field strengths. The MRI protocols were initially developed at 1.5 T. Translation to 3 T was straightforward and effective. However, this choice led to compromises at 7 T. The echo times and spacing chosen for T2 measurements turned out to be suboptimal for estimating the short T2 at high blood concentrations at 7 T. Therefore, this study only shows a proof of concept of T2 measurements at 7 T.

At the clinically relevant magnetic field strengths, 1.5 and 3 T, the high ICC coefficient showed a high level of interrater reliability for the measurements. As supported by interrater agreement analysis, the T1 and T2 values estimated for each sample were independent from the rater as well.

What might be the potential use of these techniques in future patient studies or clinical practice? After *in vivo* validation, T1 and T2 mapping might be used for non-invasive differentiation between minor joint bleeding and no joint bleeding. The developed MRI sequences might be used to accurately detect low concentrations of blood in case of joint bleeding, whereas simple effusions suggest a non-bleeding aetiology. Therefore, the results of this study might eventually be used for research purposes or even in clinical cases as an objective reference standard to verify or rule out recent joint bleeding.

However, factors of variability should be investigated further, and some precautions need to be adopted for establishing the optimal usage of these sequences in clinics. Since the relaxation times of synovial fluid and blood depend on different conditions [25, 26, 28, 37,38,39], the effect of changes in variables as field strength and MRI pulse sequences, inter-patient differences in synovial fluid consistency and haemoglobin level, and time between bleed onset and scanning—and thereby differences in the blood oxygenation level—should be taken into account when interpreting the results.

To summarise, this study is providing different tools for the differentiation between simple and haemorrhagic joint effusion *in vitro*. *In vivo* validation of the MRI protocols is needed to establish the use in patients. A study in persons without joint bleeding may determine reference values for T1 and T2 of the synovial fluid *in vivo* that will enable interpretation of T1 and T2 measurements in patients with suspected joint bleeding. Evaluation of a joint with (suspected) bleeding and the contralateral joint without bleeding as a reference may be useful for comparison. Furthermore, in daily practice, the MRI protocol should be adapted according to the MRI field strength and sequence availability. If a 3-T scanner is available, our results indicate that quantitative T2 mapping allows detection of the lowest blood concentration (equal to 5%). In case a 3-T scanner is not available, T1 mapping at 1.5 T allows for the detection of blood concentrations as low as 10%.

CONCLUSION

This study shows that MRI T1 and T2 mapping techniques may differentiate between simple and haemorrhagic effusion *in vitro*. The relaxometry protocols were able to detect low blood concentrations in the synovial fluid at clinically available field strengths (1.5 and 3 T). Especially T2 measurements at 3 T MRI appeared to be highly sensitive with the lowest detectable blood concentration of 5%. With limited acquisition times below 5 min, these T1 and T2 mapping techniques might be promising for accurate non-invasive discrimination between simple and haemorrhagic joint effusion *in vivo*, for example, in patients with bleeding disorders.

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SUPPLEMENTARY MATERIALS

Samples were scanned in batches of 6 samples. Detailed information on the positioning of the samples in the phantom in the different batches is provided in Fig. S1.

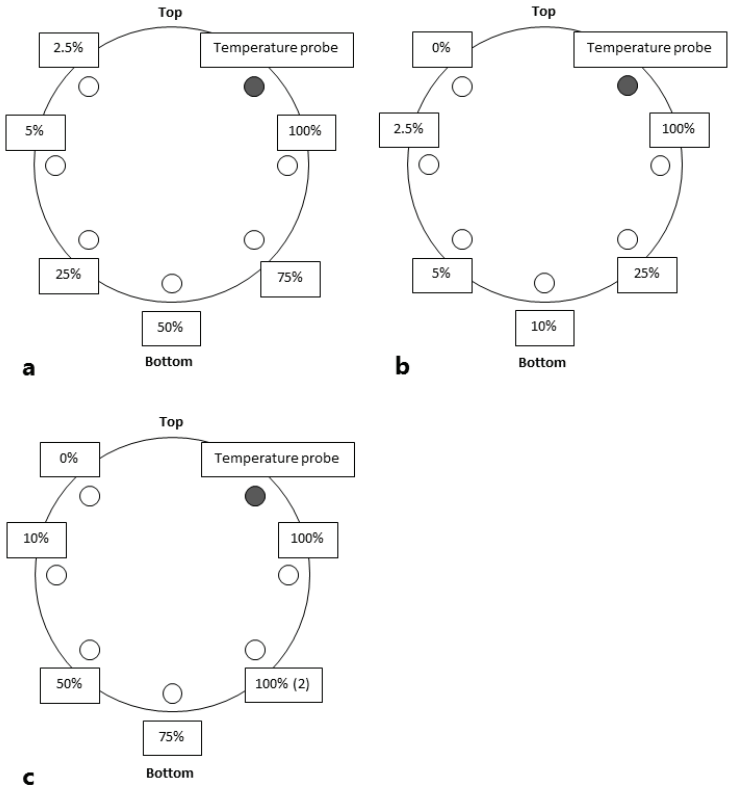

Figure S1. Detailed description of the position of the samples in the phatom in the different batches scanned. *Percentages correspond to the blood percentage in the sample. a) First batch scanned at all field strenghts. b) Second batch scanned at all field strengths. c) Third batch scanned at 3T.*

Fig. S2 shows the plot of the signal intensities for the 0% blood tube, from the second batch scanned at 3T versus the IR delays: the data were fitted for estimating T1. A plot of the residuals is also reported: all the values rely in the interval between -0.2 and 0.15.

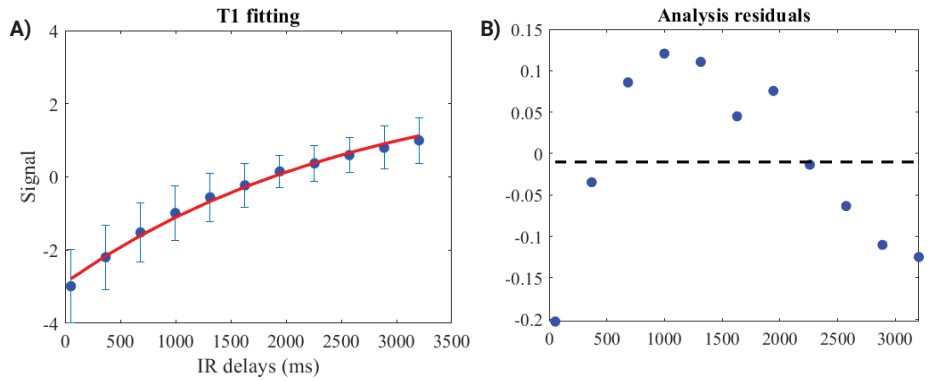

Figure S2. (A) Signal intensity in the ROI vs IR delays for the 0% blood tube at 3T: the experimental data are indicated with blue • and the fitting with a red line.
(B) Analysis of the residuals: each • represents the residual of each experimental data point from the one predicted with the fitting. The mean of the residual is also reported as dotted line.

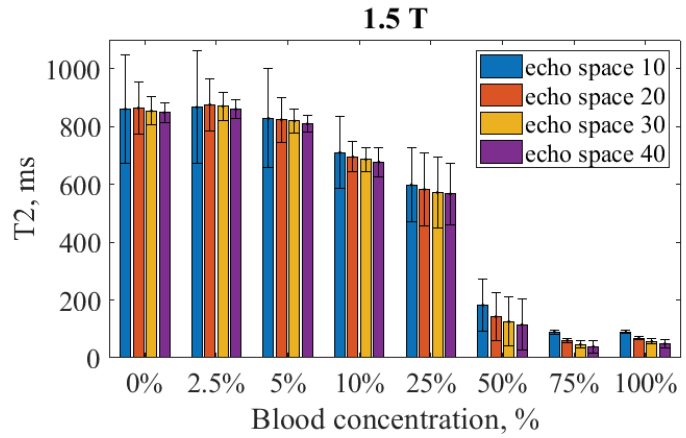

Figure S3. T2 mapping at 1.5T with different echo spacing on dependence on the blood concentration.

At 1.5 T, different MRI parameter setup were adopted for T2 mapping, as shown in Fig. S3. With all the setups, the T2 exhibited an inverse dependence on the blood concentration. The performances of the T2 mapping with different echo spacings showed in almost all cases good agreement with the reference scan (echo space 30ms). Although the T2s estimated with echo space 10 showed most deviation compared to those obtained with echo space. 30, the dependence of T2 on blood concentration was similar for all echo spaces, suggesting that the actual echo space is not of primary interest, but should be the same between subjects (controls vs. patients).

The accelerated TSE+SENSE multi-slice T2 mapping at 1.5T and 3T were compared with the single slice reference mapping without acceleration. The performances of the T2 mapping for both field strength showed comparable results with and without acceleration.

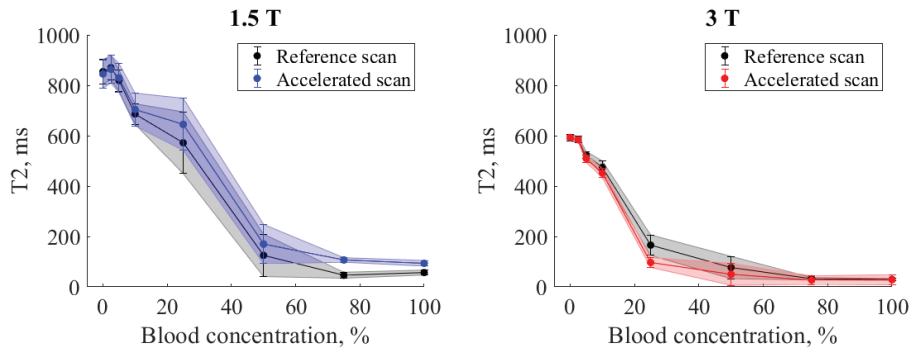

Figure S4. T2 mapping at 1.5T (A) and 3 T (B) with a multi-slice accelerated scans and single slice non accelerated scans.

The blood detection thresholds for T2 were:
≥50% with accelerated scans and ≥10% with reference scans at 1.5T;
≥5% with both accelerated scans and reference scans at 3T.

CHAPTER 3

Quantitative MRI assessment of joint effusion using T2-relaxometry at 3 Tesla: a feasibility and reproducibility study

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Submitted

ABSTRACT

Objective

T2-relaxometry could differentiate between physiological and haemorrhagic joint effusion (≥5% blood) *in vitro*. We evaluated the feasibility and reproducibility of quantitative T2-relaxation time measurements of synovial fluid *in vivo* in clinically bleed-free joints of men with haemophilia.

Materials and Methods

In this cross-sectional study, we measured T2-relaxation times of synovial fluid in clinically bleed-free ankles, knees or elbows of men with severe haemophilia A using a T2-mapping sequence (duration ≤7 min) at 3 Tesla MRI. Manual and circular regions of interest (ROI) were drawn in the synovial fluid of each joint by two independent observers to measure T2-relaxation times. Measurement feasibility was expressed as the success rate of the measurements by both observers. The interobserver and intraobserver reproducibility of the measurements were evaluated by the intraclass correlation coefficient of absolute agreement (ICC) and the limits of agreement (LoA) from Bland Altman analysis.

Results

We evaluated 39 clinically bleed-free joints (11 ankles, 12 knees, 16 elbows) of 39 men (median age: 24 years, range 17-33) with severe haemophilia A. The success rate of the T2-measurements was ≥90%. Interobserver reliability was good to excellent (manual ROI: ICC=0.92, 95% CI:0.76-0.97; circular ROI: ICC=0.82, 95% CI:0.66-0.91) and interobserver agreement was adequate (manual ROI: LoA=71ms; circular ROI: LoA=146ms). Intraobserver reliability was good to excellent (manual ROI: ICC=0.78, 95% CI:-0.06-0.94; circular ROI: ICC=0.99, 95% CI:0.98-0.99) and intraobserver agreement was good (manual ROI: LoA=63ms; circular ROI: LoA=41ms).

Conclusion

T2-relaxometry of synovial fluid in haemophilia patients is feasible with good interobserver and intraobserver reproducibility.

INTRODUCTION

Haemarthrosis can occur after trauma, or spontaneously in bleeding disorders such as von Willebrand disease and haemophilia[1,2]. Haemarthrosis can induce joint damage, even after brief exposure to a small amount of intra-articular blood[3–6]. Recurrent haemarthroses can lead to irreversible arthropathy[3–5], causing pain and impaired joint function that reduce quality of life[1,3,7].

Large traumatic haemarthroses are usually diagnosed based on clinical symptoms as pain, swelling and function loss[7]. Additionally, haemarthrosis is characterized by joint effusion on imaging. The haemorrhagic effusion may have a complex appearance, sedimentation of blood cells may be seen, and fluid-fluid levels may be observed with extensive intra-articular soft tissue damage or fractures (lipohaemarthrosis)[8,9].

Diagnosing small haemarthroses, haemarthroses without observed trauma, and haemarthrosis in joints with arthropathy can be difficult. Clinical symptoms are not specific to haemarthrosis and also occur in other joint conditions such as arthropathy[10–13]. Joint effusion on imaging is not specific either and may be observed in arthropathy flare-ups and arthritis, as in healthy joints[10,14–17]. Furthermore, the nature of smaller effusions may be difficult to determine with conventional imaging methods. Conventional T1 and T2 weighted magnetic resonance imaging (MRI) is not sensitive to detecting early small haemarthroses[18,19]. Therefore, T2*-weighted gradient echo (GRE) sequences are used to visually assess acute haemarthrosis and synovial haemosiderin deposition after haemarthrosis[20]. However, they cannot quantify or may not identify minor or subclinical haemarthroses.

Differentiation between haemarthrosis and other diagnoses is important because they require different treatment[7,10,21,22]. Specifically, people with bleeding disorders need clotting factor replacement therapy to stop haemarthrosis and prevent progression to arthropathy[7].

The current reference standard is joint aspiration, an invasive procedure with a risk of intra-articular infection and bleeding, particularly in people with bleeding disorders[7]. An induced haemarthrosis would result in a false-positive outcome. Therefore, joint aspiration is an imperfect reference standard and a non-invasive alternative would be preferable.

T2-relaxometry MRI could quantitatively differentiate between small volumes of physiological and haemorrhagic joint effusion with blood concentrations of ≥5% blood *in vitro*[23]. Differentiation was based on differences in T2-relaxation times caused by the T2-shorting effect of iron-containing blood. [24]. However, *in vivo* validation of the experimental T2-relaxometry method is required before its use in patients. Establishing good feasibility and reproducibility of the T2-relaxometry method in bleed-free joints is the first step in *in vivo* validation.

The primary study objective was to evaluate the feasibility and reproducibility of experimental MRI T2-relaxometry of joint effusion *in vivo* in clinically bleed-free joints of men with severe haemophilia A. Second, we tested robustness of the T2-measurements and determined normal values for T2-relaxation of synovial fluid without blood.

MATERIALS AND METHODS

Study design and population

The MRI T2-maps in this study were obtained as part of the Detecting Subclinical Joint BlEedinG and INflammation in Haemophilia study (BEGIN study). The BEGIN study was approved by the institutional medical ethical review board (19-273 – BEGIN) and all study participants gave written informed consent. This cross-sectional study investigated signs of subclinical bleeding and inflammation in adolescent and adult men with severe haemophilia A between December 2019 and March 2022[25,26].

In the BEGIN study, haemophilia patients born after 1988 who received prophylactic treatment reducing the (joint) bleeding risk were screened for a joint (elbow, knee or ankle) without a clinical history of bleeding. In the subgroup of patients with clinically bleed-free joints, one life-long clinically bleed-free joint per patient was examined using MRI. Clinical MRI images were scored for presence of joint effusion, haemosiderin and synovial hypertrophy by a musculoskeletal radiologist (WF) with >10 years of experience using the additive International Prophylaxis Study Group (IPSG) MRI score for haemophilic arthropathy[27]. The additive IPSG MRI score grades effusion, synovial hypertrophy and haemosiderin on a scale from 0 to 3 (0=absent, 1=minimal, 2=moderate or 3=large). The effusion grade was determined using previously described cut-off values[14]. In addition to the clinical MRI images, T2-maps were obtained in 40 joints for use in the current study. Figure 1 shows a flowchart summarizing the inclusion of patients in the current study.

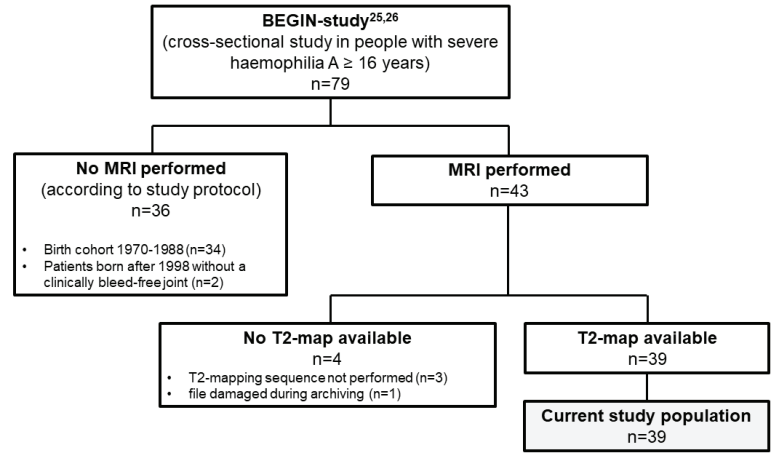

Figure 1. A Flowchart summarizing patient inclusion in the current study.

Magnetic Resonance Imaging

MRI examinations were performed on 3T MRI systems (Philips Achieva, Best, The Netherlands) using joint-specific coils (8-channel small extremity coil for elbows, 16-channel knee coil, 8-channel ankle coil). Detailed MRI protocols are available in Table 1. MRI protocols included sequences to assess soft tissue (effusion and synovial tissue) and osteochondral (cartilage and bone) pathology, and a GRE sequence to assess haemosiderin deposition according to the IPSG MRI score; these results have been reported elsewhere[25]. In addition, an experimental multi-slice Turbo Spin Echo (TSE) sequence with 32 echoes and SENSitivity Encoding (SENSE) acceleration was included in the imaging protocol for T2 mapping[23]. T2-mapping sequences contained 5 slices for knees and 3 slices for elbows and ankles. T2-mapping sequence duration was 7min in knees and 4min12s in elbows and ankles.

Table 1. Details of the used sequences in the MRI protocols per joint.

	Sequence	Plane	TR (ms)	TE (ms)	Flip angle	FOV (mm)	Slice thickness (mm)	Slice interval (mm)	Pixel size (mm)	Scanning time (s)
Elbow	T1_TSE	Transversal	685	20	90	140	2.5	2.5	0.22	146
	PD	Coronal	2817	25	90	120	2.5	2.5	0.23	163
	T2_SPAIR	Sagittal	3642	60	90	120	2.5	2.5	0.31	226
	T2_SPAIR	Coronal	3626	60	90	120	2.5	2.5	0.31	239
	T2_FFE	Sagittal	520	9.21	25	120	2.5	2.5	0.28	187
	T2_Q	Sagittal	3000	24-1140	90	128	4	4.5	0.50	252
Knee	3D_PD_SPAIR	Sagittal	1000	125	90	161	0.8	0.8	0.40	252
	3D_PD	Sagittal	1000	36.1	90	159	0.52	0.52	0.37	351
	T2_SPAIR	Sagittal	6384	62	90	150	3	3.3	0.29	202
	T2_FFE	Sagittal	514	11.5	20	150	3	3.3	0.29	234
	T2_Q	Transversal	3000	20-950	90	128	5	5.5	0.50	420
Ankle	3D_PD_SPAIR	Coronal	1100	183	90	200	0.8	0.8	0.8	358
	PD_Dixon	Sagittal	2558	20	90	200	2.5	2.5	0.35	373
	T2_Dixon	Transversal	8374	100	90	150	2.5	2.5	0.39	352
	T2_FFE	Sagittal	512	9.21	25	200	2.5	2.5	0.28	190
	T2_Q	Sagittal	3000	24-1140	90	128	4	4.5	0.50	252

TSE: Turbo Spin Echo, PD: Proton Density, SPAIR: SPectral Attenuated Inversion Recovery, FFE: Fast Field Echo (Gradient Echo), T2_Q: multi-slice TSE sequence with 32 echoes and SENSitivity Encoding (SENSE) acceleration used for the T2 mapping, FOV: Field Of View, TR: repetition time, TE: echo time

T2 mapping and T2 measurements

T2-maps were obtained by image processing in Matlab version R2021a (The MathWorks, Inc. Natick, MA, USA). The signal intensity (S) from the data points was voxel-wise fitted using a Levenberg-Marquardt nonlinear least squares method with the fit function $S = S0\ (e^{-TE/T2})$, where S0 represents the spin density, TE the echo time and T2 the T2-relaxation time[23,28].The T2 measurement of synovial fluid was based on the mean T2-relaxation time of a region of interest (ROI) in the synovial fluid. Two types of ROIs were investigated: manual delineation of visible synovial fluid (manual ROI) and placement of a circular ROI within the synovial fluid (circular ROI) as illustrated in Figure 2. ROIs were placed on the slice of the T2-map showing the most synovial fluid by 2 independent observers (FL: medical doctor with 4 months training in clinical radiology, JK: medical student with no radiology experience). One observer (JK) placed the ROIs twice in each joint with an interval of 2 weeks between measurements. Minimising the observer's learning effect of ROI placement on T2-relaxation times, the second round of ratings (JK2) was used to determine interobserver reproducibility and mean values. To verify ROI placement by the relatively inexperienced observers, a musculoskeletal radiologist (WF) reviewed all manual ROIs for correct placement within the synovial fluid.

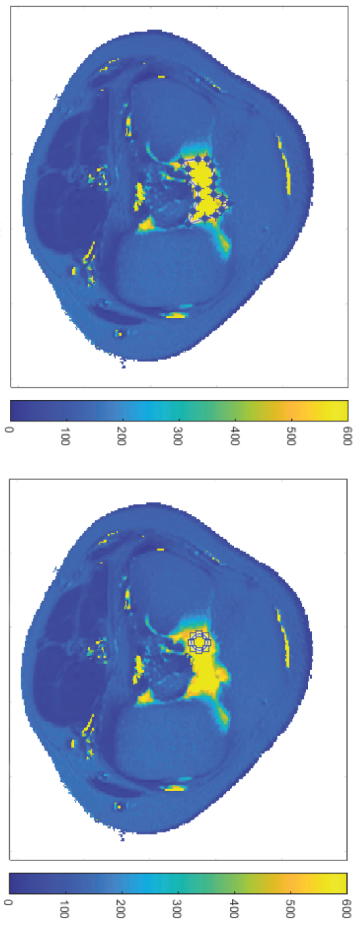

Figure 2. Two examples of a transversal T2-map of a knee with a manual ROI and a circular ROI drawn in the synovial fluid.

Statistical analysis

Patient and joint characteristics were reported as median values with ranges for continuous variables and as number with frequencies for dichotomous/categorical variables.

To determine the feasibility of T2 measurements *in vivo*, we calculated the proportion of joints where both observers successfully performed the measurement (success rate). Corresponding 95% confidence intervals (CI) were calculated using the Clopper-Pearson Exact method[29].

Interobserver and intraobserver reliability of T2 measurements was assessed using the intraclass correlation coefficient of absolute agreement (ICC)[30]. ICC values <0.5 indicated poor reliability, values between 0.5 and 0.75 indicated moderate reliability, values between 0.75 and 0.9 indicated

good reliability, and values >0.90 indicated excellent reliability. The interobserver and intraobserver limits of agreement (LoA) were determined using Bland Altman analysis[31]. To evaluate interobserver reliability and agreement, the measurements of the first observer (FL) were compared with the second measurements of the second observer (JK2). The smallest detectable change (SDC) was determined to quantify how large the difference between two measurements must be to be detected by the current measurement procedure. The SDC was calculated as $SDC=1.96*\sqrt{2}*SEM_{agreement}$. The $SEM_{agreement}$ refers to the standard error of measurement, which equals the square root of the sum of the interobserver/intraobserver variance and the residual variance[30].

Mean T2-relaxation times were calculated from the measurements of the first observer (FL) and the second measurements of the second observer (JK2) and were reported in ms with standard deviations (sd). The mean T2-relaxation times of the manual and circular ROIs were compared using a paired t-test. To investigate the robustness of the T2-relaxometry method, we investigated whether different joint types and varying amounts of effusion affected the T2 measurements and whether the presence of haemosiderin depositions in the synovial membrane caused significant T2 shortening. Correlations between T2-relaxation time and joint type, the amount of effusion (IPSG MRI scores no/minimal effusion versus moderate/large effusion) and the presence of haemosiderin were determined using multivariate linear regression. All analyses were performed in RStudio version 2022.12.0+353 (Posit Software, Boston, MA, USA).

RESULTS

Patients

A flowchart summarizing patient inclusion in the current study is available in Figure 1. T2-maps were obtained for 40 clinically bleed-free joints. One joint was excluded from evaluation because its MRI data was irreparably damaged during file archiving. Therefore, we ultimately measured T2-relaxation time of synovial fluid in 39 clinically bleed-free joints of 39 patients. All 39 patients had severe haemophilia A and were all male because of the recessive X-linked nature of haemophilia. They all received prophylactic treatment reducing the risk of spontaneous and traumatic (joint) bleeding. The median age was 24 years (range 17-33). Joint characteristics of the evaluated joints are available in Table 2. The 39 joints evaluated included 16 elbows (41%), 12 knees (31%) and 11 ankles (28%). None had a history of overt haemarthrosis. However, 6/39 joints (15%) had haemosiderin deposits in the synovium, indicating previous subclinical bleeding[25]. Concomitant synovial hypertrophy was observed in 1 ankle with haemosiderin deposits. A physiological amount of synovial fluid (no effusion) was observed in 19/39 joints (49%). There was minimal effusion in 12 joints (31%), moderate effusion in 7 joints (18%), and large effusion in 1 joint (3%).

Table 2. Joint characteristics

	Total (n=39) *n (%)*	**Elbows (n=16)** *n (%)*	**Knees (n=12)** *n (%)*	**Ankles (n=11)** *n (%)*
Effusion*				
Absent	19 (49%)	13 (81%)	3 (25%)	3 (27%)
Minimal	12 (31%)	3 (19%)	3 (25%)	6 (55%)
Moderate	7 (18%)	0 (0%)	6 (50%)	1 (9%)
Large	1 (3%)	0 (0%)	0 (0%)	1 (9%)
Haemosiderin present	6 (15)	1 (6%)	0 (0%)	5 (45%)
Synovial hypertrophy present	1 (3%)	0 (0%)	0 (0%)	1 (9%)

**Scored according to the additive IPSG MRI score[27]. Percentages might not add up to 100% due to rounding.*

Feasibility

For all 39 joints, correct placement of the manual ROIs within the synovial fluid was verified by a musculoskeletal radiologist (WF). The success rate of the T2-relaxation measurements was 100% in joints with moderate or large effusion (n=8/8, CI 69-100%). In joints with no or minimal effusion, the success rate was 87% using the manual ROI (n=27/31, CI 73-95%) and 94% using the circular ROI (n=29/31, CI 81-99%). Unsuccessful measurements were due to absence of joint effusion (n=1) or minimal joint effusion (n=3), which hampered placing the ROI.

Reliability

The interobserver and intraobserver reliability results are summarized in Table 3. Interobserver reliability of the T2 measurements was good to excellent (manual ROI ICC=0.92, CI 0.76-0.97; circular ROI ICC=0.82, CI 0.66–0.91). Intraobserver reliability was good to excellent (manual ROI ICC=0.78, CI -0.06-0.94; circular ROI ICC=0.99, CI 0.98-0.99).

Table 3. Inter- and intraobserver reproducibility of T2 relaxation measurements

	Interobserver reproducibility			**Intraobserver reproducibility**		
	Reliability	**Agreement**		**Reliability**	**Agreement**	
	ICC (CI)	LoA	SDC	ICC (CI)	LoA	SDC
Manual ROI	0.92 (0.76-0.97)	71 ms	84 ms	0.78 (-0.06-0.94)	63 ms	154 ms
Circular ROI	0.82 (0.66–0.91)	146 ms	155 ms	0.99 (0.98-0.99)	41 ms	40 ms

ROI: region of interest, ICC: intraclass correlation coefficient of absolute agreement, CI: 95% confidence interval, LoA: limit of agreement, SDC: smallest detectable change

Agreement

The interobserver and intraobserver agreement results are summarized in Table 3. Figures 3 and 4 show the Bland-Altman plots of interobserver and intraobserver agreement.

Interobserver agreement was adequate (manual ROI LoA=71ms; circular ROI LoA=146ms), without large systematic interobserver differences (manual ROI mean difference=23ms; circular ROI mean difference=29ms). The SDC based on the interobserver variance ranged from 84ms to 155ms.

Intraobserver agreement was good (manual ROI LoA=63ms; circular ROI LoA=41ms). A systematic intraobserver difference (mean difference: -72ms) was observed for the manual ROI measurements. The second measurements of the second observer (JK2) were systematically lower than the first measurements (JK1) which might indicate a slight learning effect after minimal experience with the T2 measurements. For the circular ROI method, there was no systematic intraobserver difference (mean difference: 0ms). The SDC based on the intraobserver variance ranged from 154ms to 40ms.

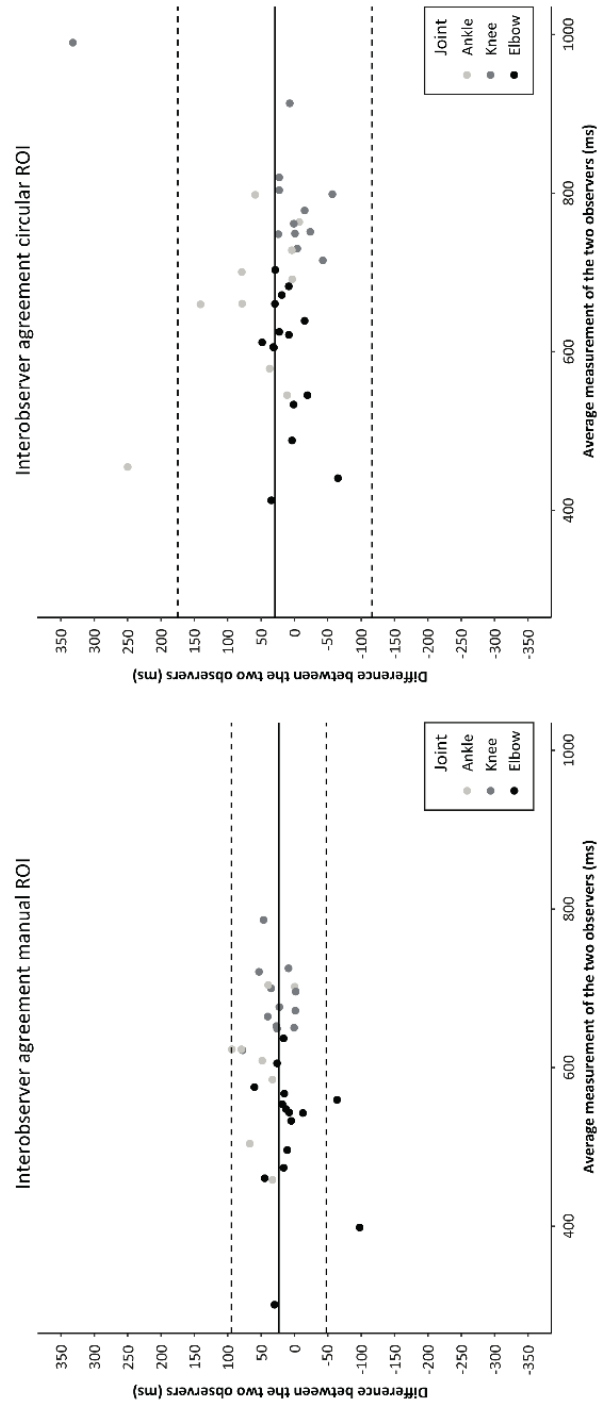

Figure 2. Bland-Altman plots regarding the interobserver agreement of T2-relaxation measurements for the manual and circular ROIs.

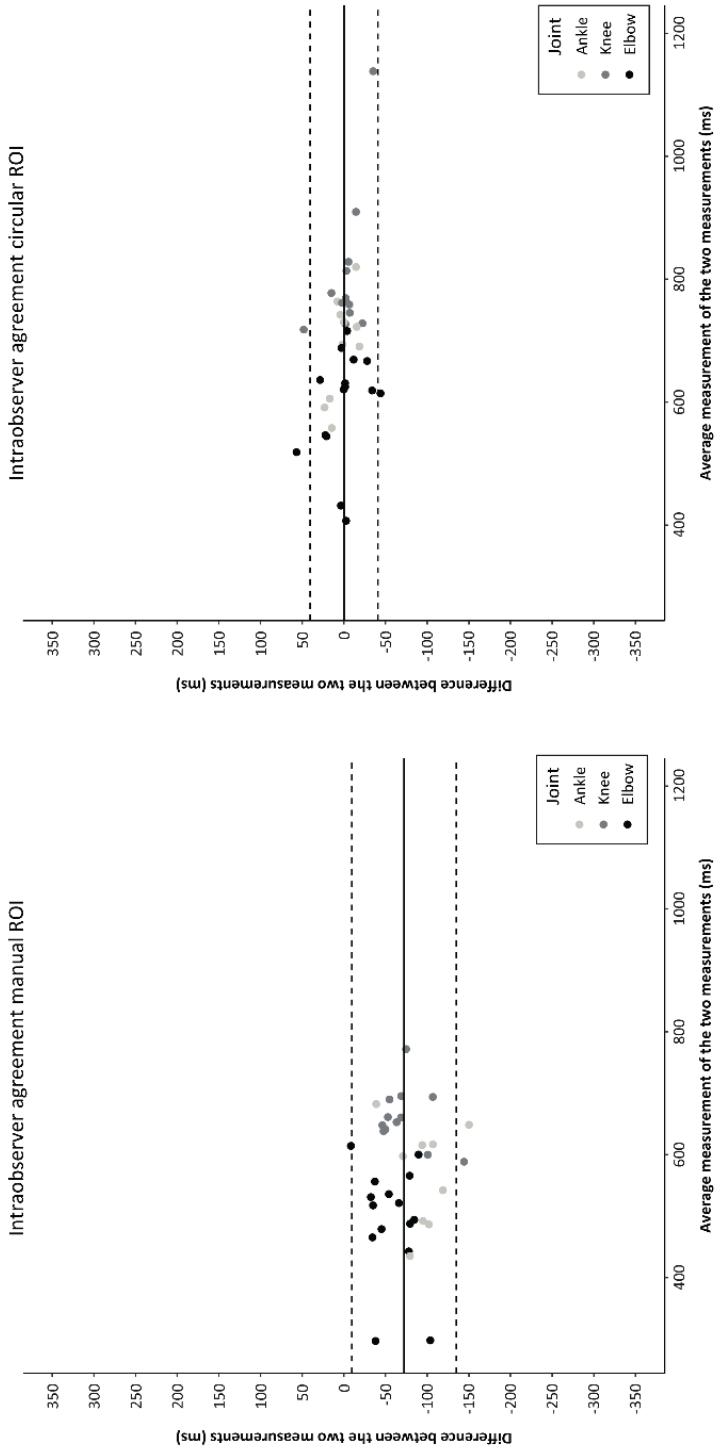

Figure 3. Bland-Altman plots regarding the intraobserver agreement of T2-relaxation measurements for the manual and circular ROIs.

T2-relaxation times

Mean T2-relaxation times of the synovial fluid are available in Table 4. Circular ROI measurements of T2 relaxation were higher than manual ROI measurements (p<0.00). Joint type (elbow, knee, ankle) significantly affected T2 measurements, even when corrected for the amount of effusion in the joint and presence of haemosiderin deposits in the synovial membrane. Figure 5 shows boxplots of T2-relaxation time for different joints. For manual ROIs, T2-relaxation times in the elbows were significantly shorter than T2-relaxation times in ankles and knees. For circular ROIs, T2-relaxation times were significantly different for all joint types (p≤0.04). T2-relaxation times were shortest in elbows, followed by ankles and knees.

Presence of haemosiderin showed a non-significant T2 shortening compared to absence of haemosiderin (manual ROI: -57ms, p=0.20; circular ROI: -74ms, p=0.13). T2-relaxation times were not correlated with the amount of effusion (manual ROI p=0.79; circular ROI p=0.97).

Table 4. Mean T2 relaxation times of synovial fluid per joint

T2 relaxation times (ms)	Total (n=39) *mean (±sd)*	Elbows (n=16) *mean (±sd)*	Knees (n=12) *mean (±sd)*	Ankles (n=11) *mean (±sd)*
Manual ROI	595 (±102)	520 (±85)	684 (±44)	601 (±86)
Circular ROI	675 (±125)	590 (±88)	797 (±80)	658 (±105)

ROI: region of interest; sd: standard deviation

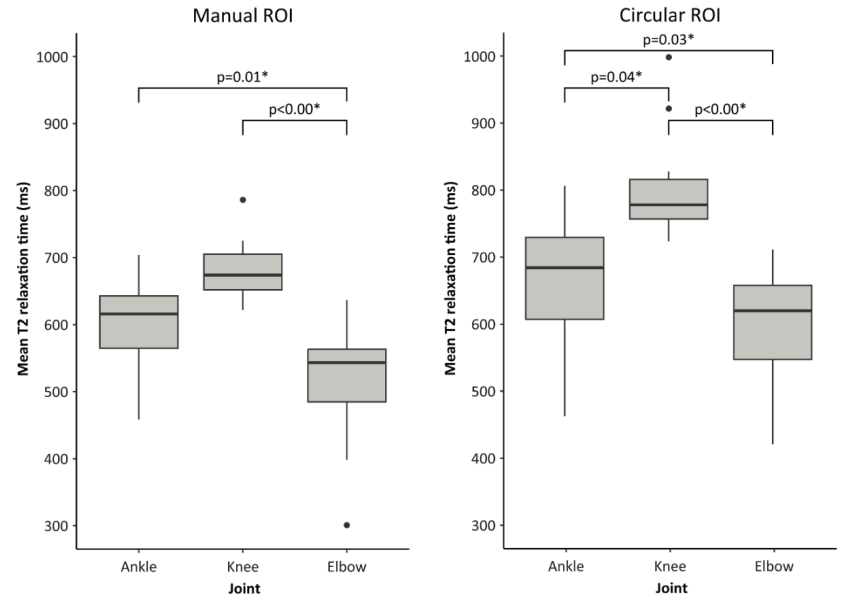

Figure 4. Boxplots showing mean T2-relaxtion times measured with manual and circular ROIs in the different joint types.
**significant p-values from multivariate linear regression adjusted for amount of effusion and presence of haemosiderin deposits in the synovial membrane.*

DISCUSSION

We evaluated feasibility and reproducibility of MRI T2-relaxometry of non-haemorrhagic joint effusion *in vivo* using MRI data from 39 clinically bleed-free joints in men with severe haemophilia A. T2-relaxometry measurements were feasible in all joints with moderate or large effusion and ≥87% of joints with no or minimal effusion. Both manual and circular ROIs showed good-excellent inter- and intraobserver reliability and agreement. Additionally, we obtained normal values of T2-relaxation time of synovial fluid without blood. Mean T2-relaxation times of synovial fluid were 595ms (±102) when effusion was manually delineated and 675ms (±125) when a circular ROI was placed within the effusion. T2-relaxation times appeared to vary among different joint types.

Differences in T2-relaxation times for different joints may be due to anatomical difference between joints. For example, knees are larger and have a physiologically larger volume of synovial fluid. This makes it easier to place a ROI in the synovial fluid without including surrounding tissue, making measurements less susceptible to partial volume effects. Because of significant differences in T2-relaxation times between joints, joint-specific normal values for synovial fluid T2-relaxation times should be used.

Comparison to previous publications

T2-relaxation times in the current study are consistent with reported T2-relaxation times of synovial fluid in both *in vitro* and *in vivo* studies. A previous *in vitro* study reported a mean T2-relaxation time of 592ms (±13) for synovial fluid at 3 Tesla MRI[23], comparable to the mean T2-relaxation times measured with manual ROI (595ms ±102) and circular ROI (675ms ±125) in the current *in vivo* study. Two studies measuring T2-relaxation times of synovial fluid in knees of healthy volunteers reported T2-relaxation times of 767ms (±49)[32] and 653ms (±113)[33] at 3 Tesla MRI. These are similar to the 684ms (±44) in knees using manual ROI and the 797ms (±80) in knees using circular ROI in the current study.

We acknowledge important work of previous investigators showing conventional T1 and T2 weighted MRI could not qualitatively discriminate physiological synovial fluid or haemorrhagic joint effusion. They report preliminary evidence (mainly *ex vivo*) that ultrasound is sensitive to small amounts of intra-articular blood[18,19]. Moreover, GRE sequences are used for visual qualitative differentiation of haemarthrosis in current clinical practice[20]. However, T2-relaxometry may potentially be of interest for detecting small subclinical haemarthrosis in research settings. The previous *in vitro* results[23] combined with good feasibility and reproducibility of the experimental T2-measurements *in vivo* suggest the experimental T2-relaxometry method allows quantitative differentiation of physiological synovial fluid from haemorrhagic joint effusion (with low blood concentrations).

Future research and applications

We demonstrated good feasibility and reproducibility of T2-relaxometry in bleed-free joints. T2 measurements failed in a few joints with no or minimal effusion. However, T2-relaxometry will

only be relevant in joints suspected of bleeding. This suspicion requires at least some effusion, increasing the probability of a successful measurement. T2 measurements were not significantly affected by haemosiderin deposits. Therefore, T2 measurements remain useful in joints with a history of bleeding.

The 3-slice T2-relaxometry sequence of 4m12s may be feasible in clinical or research settings. The 5-slice sequence for the knee may be shorted by scanning 3 slices at the effusion site only.

Demonstrating good feasibility and reproducibility was the first step in validating the T2-relaxometry method for differentiation between physiological and haemorrhagic joint effusion *in vivo.* The next step would be confirming T2 shortening of haemorrhagic joint effusion *in vivo.* This requires a future study including patients with (suspected) haemarthrosis. After confirmation of T2 shortening of haemorrhagic effusion *in vivo*, T2-relaxometry can serve as a non-invasive alternative to joint aspiration for the diagnosis of haemarthrosis. The T2-relaxation times obtained in the current study can then be used as normal values for synovial fluid without blood.

Limitations

Measuring T2-relaxation times of non-haemorrhagic synovial fluid in men with haemophilia could be seen as a limitation, as they do not reflect the healthy general population. However, there is no (patho)physiological indication to assume differences in synovial fluid between our study patients and healthy volunteers. In addition, haemophilia patients are a large part of the target population for non-invasive diagnosis of haemarthrosis. Therefore, it is logical to perform the study in this population.

Lack of joint aspiration as a reference standard for the diagnosis of join bleeds can be considered another limitation. However, as the patients had no symptoms or history of bleeding in the examined joint, likelihood of haemarthrosis prior to or during MRI is negligible. Furthermore, a false-positive aspiration due to induced haemarthrosis is possible in people with a bleeding disorder and joint aspiration has the risk of inducing intra-articular infection[7]. Therefore, in addition to the ethical concerns, performing joint aspiration in this cohort would have been contraindicated.

ROI placement by relatively inexperienced observers could be seen as a third limitation. However, reliability and agreement were good to excellent despite the lack of experience and a musculoskeletal radiologist verified accurate ROI placement for all joints. Therefore, no prior knowledge or extensive training seems required to perform measurements reliably. Circular ROIs are probably easiest to implement, as circles are easy and quick to place while maintaining good reproducibility. Manual ROIs showed better interrater reproducibility. However, manual delineation is more time-consuming and has a higher risk of interference from the surrounding (haemosiderotic) synovial membrane. Furthermore, its slightly worse intraobserver reproducibility showed potential dependency on observer's experience, indicating automated joint effusion segmentation can improve value of manual ROI.

Finally, heterogeneity in joint types, effusion grades and hemosiderin presence in our study population could have confounded results. Yet, only joint type affected T2 measurements significantly. Therefore, we reported joint-specific T2-relaxation times of synovial fluid.

Quantitative MRI T2-relaxometry can be used to measure T2-relaxation times of joint effusions in patients with good inter- and intraobserver reliability and agreement. Both manual delineation of the effusion and placement of a circular ROI within the effusion are feasible and reproducible. Mean T2-relaxation times obtained in this study may be used as reference values for synovial fluid in elbows, knees and ankles without bleeding.

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CHAPTER 4

Magnetic resonance imaging evidence for subclinical joint bleeding in a Dutch population of people with severe hemophilia on prophylaxis

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ABSTRACT

Background
Previous studies suggest that subclinical bleeding occurs in persons with hemophilia.

Objectives
The aim of this study was to investigate whether patients with lifelong access to prophylaxis showed signs of previous subclinical bleeding on magnetic resonance imaging (MRI) in joints without a history of joint bleeding.

Methods
This single-center cross-sectional study included persons with severe hemophilia A on prophylaxis, aged 16 to 33 years, with lifetime bleeding records available. Per participant, 1 index joint without a history of joint bleeding was evaluated with 3-Tesla MRI, including hemosiderin sensitive sequences. MRI scans were reviewed according to the International Prophylaxis Study Group (IPSG) additive MRI scale (range, 0-17/joint). Hemosiderin deposits with/without synovial hypertrophy were considered signs of previous subclinical bleeding. Additionally, physical examination was performed, followed by ultrasound examination according to the Hemophilia Early Arthropathy Detection with Ultrasound protocol.

Results
In 43 patients with a median age of 23.5 years, 43 joints (16 elbows, 13 knees, 14 ankles) without reported bleeds were evaluated with MRI. The median IPSG MRI score was 1 (range, 0-9). Signs of previous subclinical bleeding were observed in 7 of 43 joints (16%; 95% CI, 7-30): 7 of 7 joints showed hemosiderin deposits, with concomitant synovial hypertrophy in 2 of 7 joints. MRI changes were accompanied by swelling and ultrasound-detected synovial hypertrophy in 1 ankle only. None of the other joints showed abnormalities at physical examination and ultrasound.

Conclusion
In this study, 16% of the joints without reported bleeds showed signs of previous subclinical bleeding, providing evidence for subclinical bleeding in people with severe hemophilia with lifelong access to prophylaxis.

INTRODUCTION

Hemophilia is a rare X-linked genetic disorder, caused by a (functional) deficiency of coagulation factor VIII in hemophilia A and coagulation factor IX in hemophilia B. This deficiency results in an increased bleeding tendency. The hallmark, especially in severe hemophilia, is spontaneous or traumatic bleeding into the large synovial joints (mainly ankles, knees, and elbows). These recurrent provoked and spontaneous joint bleeds lead to joint damage, so-called hemophilic arthropathy, and cause considerable disease burden [1].

Multifactorial mechanisms of blood-induced joint damage have been described. When blood enters the joint, the synovial lining cells clear it from the joint cavity. In case of recurrent or ongoing bleeding, the synovial capacity to remove blood is exceeded. Subsequently, erythrocyte-derived iron accumulates as synovial hemosiderin deposits. The hemosiderin deposits induce synovial inflammation and proliferation, which are known predictors of recurrent bleeding and subsequent hemophilic arthropathy development [2,3].

Hemophilia treatment aims at preventing (joint) bleeds. Factor (trough) levels and records of bleeds are used to monitor and tailor treatment. The joint status is traditionally assessed by clinical outcome measures such as patient-reported outcomes and physical examination [4]. Since the introduction of prophylactic clotting factor replacement therapy, the number of clinically overt joint bleeds has decreased. Nijdam et al. [5] compared patients that stopped and continued prophylaxis and observed long-term joint deterioration in patients that had stopped their prophylaxis, despite comparable low bleeding rates and patient-reported outcomes. We hypothesize that this joint damage progression is because of subclinical bleeding and/or inflammation and underreporting of bleeds by the patient. This subclinical bleeding and inflammation theory is based on the observation of (presumably blood-induced) joint changes in the absence of clinically evident joint bleeds in the past [6–8].

In addition to clinically overt bleeds, subclinical bleeding or ongoing inflammation after a bleed may negatively affect joint outcome. The current outcome measures are unable to detect subclinical joint damage and may give the false suggestion of the absence of early joint changes [9–12]. Magnetic resonance imaging (MRI) is the reference standard for imaging of the synovium and iron/hemosiderin deposits [4,9]. Especially gradient echo sequences are known to be sensitive for the detection of hemosiderin because of the susceptibility artefacts that the iron-containing heme creates on the MR images [13]. As such, MRI is useful to evaluate joint health in studies with new treatment modalities (eg, emicizumab and gene therapy), as more sensitive joint outcome measures are required with the low bleeding rates reported [14,15].

This study aimed to provide evidence for subclinical bleeding in people with severe hemophilia with lifelong access to prophylaxis. We investigated the occurrence of signs of previous subclinical bleeds on MRI in joints without a history of joint bleeding in Dutch adolescents and adults with severe hemophilia A on prophylaxis.

METHODS

Study design and population

The Detecting Subclinical Joint BlEedinG and INflammation in Hemophilia study (BEGIN study) is a cross-sectional study evaluating signs of subclinical bleeding and inflammation in people with severe hemophilia A of 16 years and older, born after 1969, without recent joint bleeds nor (a history of) factor FVIII inhibitors who attended the Van Creveldkliniek (UMC Utrecht) for a routine follow-up visit from December 2019 until March 2022. The BEGIN study was approved by the institutional medical ethical review board (19-273 – BEGIN). All study participants gave written informed consent. For the present study, a subset of patients was evaluated with lifelong data on bleeding and treatment available.

Patients of the BEGIN study were eligible for inclusion in the present substudy if they had severe hemophilia A, were 16 years or older, and born after January 1, 1988. Patients had to have lifelong access to prophylaxis and had to be on prophylaxis for at least 12 months before inclusion [16,17]. They were included if they had at least 1 elbow, knee, or ankle without a history of joint bleeding according to their lifetime bleeding records. Patients were excluded if they had a history of a FVIII inhibitor (≥5 Bethesda Units [BU] at any time or 1–5 BU for ≥1 year), or contraindications for MRI (eg, claustrophobia, metal, or electronic implants that were incompatible with MRI).

Study procedures

Patients were assessed by MRI, and additionally by physical examination and ultrasound within 24 hours of the MRI scan. For all patients, lifetime bleeding records were retrospectively searched to identify 1 joint (elbow, knee, or ankle) without a history of joint bleeding to assess with MRI. In case a patient had multiple joints without a history of joint bleeding, assessing an ankle was preferred over assessing a knee, and assessing a knee was preferred over assessing an elbow, since ankles are the most affected joints, followed by knees and the elbows [18,19]. We used a 3-Tesla MRI scanner (Philips Achieva) with joint-specific coils to assess knees and ankles (Philips Achieva), and a small extremity coil (Philips Achieva) to assess elbows. The MRI examination was performed without gadolinium contrast administration and included gradient echo sequences for optimal hemosiderin detection. Total scanning time was ~30 minutes. Detailed MRI protocols are available in Supplementary Table S1. MRI scans were reviewed according to the International Prophylaxis Study Group (IPSG) additive MRI scale by a single musculoskeletal radiologist with 10 years of experience with imaging of hemophilic arthropathy (W.F.). The IPSG additive MRI scale consists of a soft tissue domain, scoring effusion, synovial hypertrophy, and hemosiderin on a scale from 0 to 3, and a osteochondral domain, scoring surface erosions (0-2), subchondral cysts (0-2), and cartilage degradation (0-4) [20]. Additional to the conventional IPSG scoring based on the assessment of the elbows, knees, and tibiotalar ankle joints, the subtalar ankle joints were assessed separately using the items of the IPSG. The radiologist was blinded for all clinical information and the outcomes of the other study procedures. Hemosiderin deposits with or without synovial hypertrophy were considered signs of previous subclinical bleeding.

Additional physical examination assessed joint swelling and warmth of elbows, knees, and ankles (index joints). Joint swelling was reported according to the Hemophilia Joint Health Score (HJHS) 2.1 [21]. Warmth of the joint was reported as present or absent compared to the contralateral side. Ultrasound assessment of the index joints was performed according to the Hemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) protocol [22], with additional assessment of synovial hyperemia using power Doppler assessment that was reported according to the Joint tissue Activity and Damage Exam protocol [23]. Physical examination and ultrasound assessment were performed or supervised by a physiotherapist trained in the use of the HJHS and ultrasound assessments with 9 years of experience in hemophilia care (M.T.). Physical examination and ultrasound assessment were performed without knowledge of the MRI scan findings.

Data collection

At inclusion, age, annualized joint bleeding rate over the 5 years before inclusion (AJBR), type of prophylaxis (clotting factor replacement therapy or emicizumab), and the last reported total HJHS were extracted from the electronic patient records. Adherence to prophylaxis was determined as actual clotting factor consumption compared to prescribed clotting factor consumption during 1 year before inclusion. Patients who used <75% of the prescribed dose were considered as nonadherent. The most recent X-rays of all 6 index joints in each of the 43 patients (median time window 3 years; range, 0-7) were evaluated according to the Pettersson scores by 1 radiologist (W.F.) using a reference atlas for scoring [24,25]. For all joints that were assessed with MRI, patient records were checked for reports of peri-articular bleeds and joint complaints not defined as intraarticular bleeding.

Analysis

Patient and joint characteristics were reported as medians with ranges for continuous variables and as frequencies with percentages for categorical or dichotomous variables. Signs of previous subclinical bleeding on MRI were dichotomized as present or absent for all MRI scans. The proportions of joints with signs of previous subclinical bleeding on MRI and joints with osteochondral abnormalities were calculated. Occurrence of abnormalities at physical examination and ultrasound were compared between groups with and without signs of previous subclinical bleeding on MRI. Patients on continuous prophylaxis 12 months before inclusion were compared with patients on intermittent prophylaxis 12 months before inclusion for signs of subclinical joint bleeding, IPSG MRI scores, and AJBRs. After obtaining informed consent and inclusion in the study, the patient's records could be thoroughly reviewed. This review identified 2 included patients who had a history of a transient high FVIII inhibitor that had been missed in screening for study eligibility. To evaluate the effect of 2 inadvertently included patients who turned out to have had transient high inhibitor titers (titer > 5BU), we compared the prevalence of signs of previous subclinical bleeding with and without these 2 patients. There is a possibility that minor bleeds were misclassified as peri-articular bleeds or nonjoint bleed-related complaints. Therefore, we compared the prevalence of signs of previous subclinical joint bleeding in all examined joints with the prevalence when leaving out any joints with reported peri-articular bleeds and/or complaints. The Exact method

was used to calculate 95% CI of proportions. The Fisher's exact test or Man–Whitney U test was used to compare groups. All analyses were performed in RStudio (Version 1.3.1093).

RESULTS

Study population

Patients characteristics are available in Table 1. This study included 43 participants with severe hemophilia A. Data were complete except from X-rays of 2 elbows in 1 patient, in which an ankle was studied with MRI. Two patients with transient inhibitors were inadvertently included. The median age of the participants was 23.5 years (range, 16.5-33.2). The majority used prophylaxis with FVIII (n = 40, 93%), of which 32 were adherent and 8 were nonadherent to their prophylaxis. Three patients were on emicizumab prophylaxis; 1 patient was on emicizumab prophylaxis for 1 year and 2 patients switched from FVIII to emicizumab prophylaxis in the year before inclusion (4 and 6 months before inclusion). Joint bleeds were rare with an AJBR of 0.4. Joint status was good, with a median total HJHS score of 0, a median total HEAD-US score of 1, and a median total Pettersson score of 0. In these 43 patients, 16 elbows, 13 knees, and 14 ankles without a history of joint bleeding were selected and scanned. The joint characteristics of the joints that were evaluated with MRI are available in Table 2. The median IPSG MRI joint score of the 43 assessed joints was 1 (range, 0-9).

Table 1. Patient characteristics

	Median (range) or n (%)
A) Patient characteristics (n=43)	
Age (years)	23.5 (16.5-33.2)
AJBR	0.4 (0-6.8)
Prophylactic treatment	43 (100%)
FVIII	40 (93%)
Adherent to FVIII	*32 (74%)*
Non-adherent to FVIII (<75% of prophylaxis)	*8 (19%)*
Emicizumab	3 (7%)
B) Joint characteristics at patient level (sum of 6 index joints, n=43)	
HJHS (range 0-124)	0 (0-17)
HEAD-US score (ultrasound, range 0-48)	1 (0-18)
Pettersson score (X-ray, range 0-78)	0 (0-19)

% might not add up to 100% due to rounding; AJBR: Annualized Joint Bleeding Rate over a 5 year time period prior to inclusion; Non-adherent: clotting factor consumption <75% of the prescribed dose

Table 2. Joint characteristics of joints without a history of bleeding that were evaluated with MRI

	Median (range) or n (%)
A) Joints (n=43)	
Elbows	16 (37%)
Knees	13 (30%)
Ankles	14 (33%)
B) Joint status at joint level (n=43)	
HJHS (range 0-20)	0 (0-1)
HEAD-US score (ultrasound, range 0-8)	0 (0-1)
Pettersson score (X-ray, range 0-13)	0 (0-0)
IPSG MRI score (range 0-17)	1 (0-9)

% might not add up to 100% due to rounding

MRI findings

The MRI findings are summarized in Figure 1 and Supplementary Table S2. Signs of previous subclinical bleeding on MRI were observed in 7 of 43 joints (16%; 95% CI, 7-30). Ankles were most often affected (43%; 95% CI, 18-72), followed by elbows (6%; 95% CI, 0-30). The examined knees in this study showed no signs of previous subclinical bleeding.

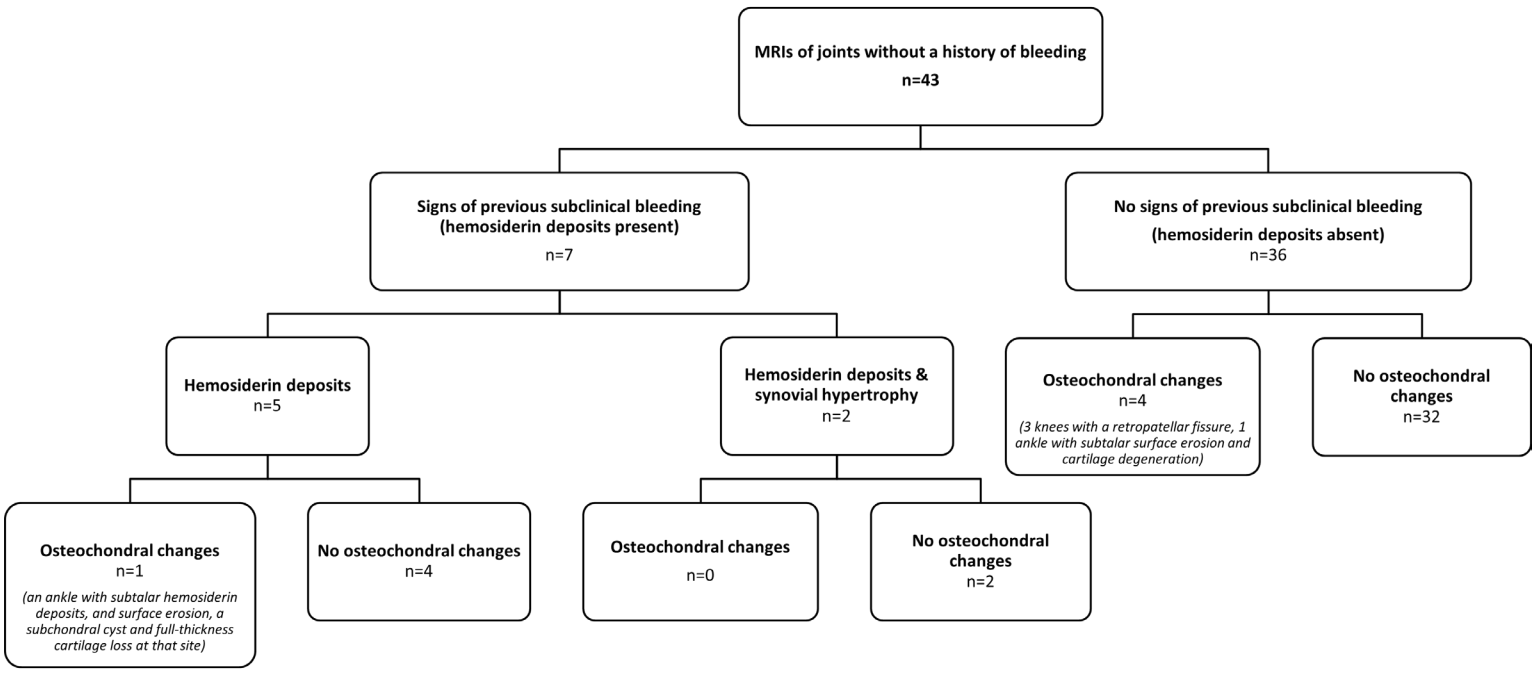

Figure 5. Flowchart summarizing magnetic resonance imaging findings in 43 joints without a history of joint bleeding.

Details on the joints with signs of previous subclinical bleeding on MRI are summarized in Table 3 and Figure 2 shows examples of observed signs of previous subclinical on MRI (hemosiderin deposits and synovial hypertrophy). Four ankles and 1 elbow showed small hemosiderin deposits, 1 ankle showed moderate hemosiderin deposits, and 1 ankle showed large deposits. Concomitant synovial hypertrophy was observed in 2 ankles (2 of 7 joints). In 1 ankle with subtalar hemosiderin deposits (1 of 7 joints), osteochondral changes were observed at the middle talocalcaneal facet. These osteochondral changes corresponded with the location of the hemosiderin deposits in this ankle. The other joints with signs of previous subclinical bleeding showed no osteochondral changes.

The MRI findings in joints without signs of previous subclinical bleeding are also included in Figure 1. In 4 joints without signs of previous subclinical bleeding, osteochondral changes were observed (9%; 95% CI, 3-22). The osteochondral changes in these joints were retropatellar fissures without other abnormalities in 3 knees and subtalar cartilage loss grade 2 with surface erosion grade 1 in 1 ankle. Additionally, simple joint effusion, which is a known nonhemophilia-specific item of the IPSG MRI scale [26], was observed in 23 of 43 joints (54%; 95% CI, 38-69). The amount of effusion varied from small (15 of 23 joints), to moderate (7 of 23 joints) to large effusion (1 of 23 joints).

Table 3. Details of joints with signs of previous subclinical bleeding on MRI.

		MRI findings				Physical examination	Ultrasound examination
Age (years)	Joint	Hemosiderin deposits	Synovial hypertrophy	Effusion	Osteochondral changes	IPSG MRI score[1]	
25	Left elbow	Small	Absent	Small	Absent	2	Normal
18	Right ankle	Moderate	Small	Absent	Absent	3	Normal
21	Left ankle	Large	Large	Large	Absent	9	Mild swelling	Mild synovial hypertrophy without hyperemia
24	Left ankle	Small	Absent	Small	Absent	2	Normal	Normal
25	Left ankle	Small	Absent	Small	Absent	2	Normal	Normal
26	Left ankle	Small	Absent	Small	Absent	2	Normal	Normal
28	Left ankle	Small*	Absent	Small	Surface erosion: grade 1* Subchondral cysts: grade 1* Cartilage degeneration: grade 3*	1	Normal	Normal

**Joint changes at the subtalar joint,* [1] *The International Prophylaxis Study Group (IPSG) MRI score is based only on tibiotalar joint changes of the ankle.*

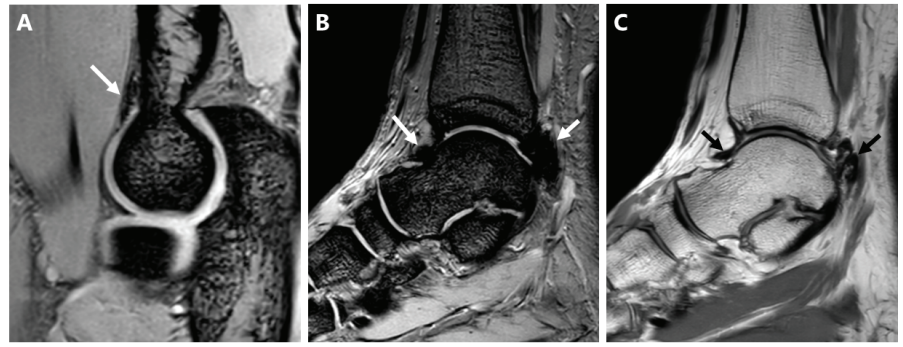

Figure 6. Examples of signs of previous subclinical bleeding on 3-Tesla magnetic resonance imaging scans of joints without a history of joint bleeding.
(A) Sagittal T2 weighted (T2w) gradient echo (GE) image of an elbow showing subtle blooming artefacts because of hemosiderin deposits (white arrow). (B) Sagittal T2w GE image of an ankle with evident blooming artefacts because of larger hemosiderin deposits (white arrows). (C) Sagittal PD Dixon image of the same ankle showing that the hemosiderin deposits are located within a hypertrophied synovial membrane (black arrows) on the anterior and posterior side of the tibiotalar joint.

Comparison of MRI with physical examination and ultrasound

Abnormalities at physical examination and ultrasound were similar between the groups with and without signs of previous subclinical bleeding on MRI (1/7 versus 0/36, p = .16). Of the 7 joints with signs of previous subclinical bleeding, the ankle with large synovial hypertrophy, large hemosiderin deposits, and large effusion on MRI showed minimal swelling at physical examination and minimal synovial hypertrophy without hyperemia on ultrasound. The other 6 joints with signs of previous subclinical bleeding showed no abnormalities at physical examination nor on ultrasound. The findings of physical examination and ultrasound in the joints with signs of previous subclinical bleeding are available in Table 3. Of the 36 joints without signs of previous subclinical bleeding on MRI, none showed abnormalities at physical examination nor on ultrasound.

Sensitivity analysis according to patient characteristics

Although all scanned joints were without intra-articular bleeds according to the lifetime bleed records, there is a possibility that minor bleeds were misclassified as peri-articular bleed or nonjoint bleed-related complaints. Therefore, we investigated whether hemosiderin deposits were present in joints with previous peri-articular complaints. We found previous peri-articular complaints in 5 of 43 joints (3 subcutaneous bleeds, 1 contusion, 1 tendinopathy). Hemosiderin was present in only 1 of 5 joints; an ankle with a registered subcutaneous bleed. The sensitivity analysis, leaving out the 5 joints with reported peri-articular complaints, resulted in a prevalence of signs of previous subclinical bleeding in 18% (95% CI, 7-36) of the joints. This was similar to the prevalence in all joints (16%; 95% CI, 7-30). Furthermore, the sensitivity analysis leaving out the 2 patients with a history of inhibitors resulted in a prevalence of previous subclinical bleeding (6 of 41 joints; 15%; 95% CI, 6-29) that did not significantly differ from the prevalence in all patients. Lastly, the sensitivity analyses on adherence, comparing patients who were adherent and nonadherent to FVIII prophylaxis during

the last 12 months, showed that the prevalence of signs of previous subclinical bleeding (5/32 versus 2/8, p = .611), IPSG MRI scores (median 1 versus 1.5, p = .556) and AJBR (median 0.4 versus 0.4, p = .986) were similar.

DISCUSSION

In this cross-sectional study, we used MRI, ultrasound, and physical examination to investigate signs of previous subclinical bleeding in adolescents and adults with severe hemophilia A on prophylaxis. Signs of previous subclinical bleeding on MRI were observed in 16% (95% CI, 7-30) of the 43 examined joints without a history of joint bleeding. These MRI findings support the hypothesis that subclinical bleeding may occur despite prophylaxis. Noteworthy, abnormalities during physical examination and ultrasound were observed in only 1 joint (2%). This joint showed signs of previous subclinical bleeding on MRI.

Strengths and limitations

Strengths of our study are parallel assessment of the joints with MRI, ultrasound, and physical examination, combined with 3-Tesla MRI scanning using protocols with gradient echo sequences. The relatively high field strength of the scanner combined with the properties of gradient echo sequences results in images that are highly sensitive for detecting small hemosiderin deposits [13]. Furthermore, all included patients had lifelong access to prophylaxis. Therefore, our study gives an indication of the occurrence of subclinical bleeding despite prophylactic treatment.

A limitation of our study is the retrospective review of patient records to determine which joint had no history of bleeding. Although lifetime bleed records were available, minor bleeds might have stayed unreported or might have been misclassified as nonjoint bleeding episode. However, missed bleeds can be considered subclinical bleeds by definition and therefore did not influence the evidence for subclinical joint bleeding in our study. Yet, misclassification of a joint bleed as peri-articular bleed or nonbleed related complaint might have led to overestimation of the prevalence of subclinical bleeding. However, a sensitivity analysis leaving out joints with any reported peri-articular complaints showed similar results.

A second limitation is that lifetime adherence to prophylactic treatment cannot be verified. We determined adherence in the 12 months before inclusion to get an impression about the adherence in our population. The prevalence of subclinical joint bleeding was comparable in adherent and nonadherent patients. However, adherence to treatment remains a relevant point for future research and therapies.

Lastly, we evaluated only a single joint without a history of bleeding per patient and not every (subclinical) joint bleed may cause hemosiderin deposits or osteochondral changes. Therefore, the current study provides further evidence for the subclinical bleeding theory, without providing the prevalence of previous subclinical joint bleeding in all joints without a history of joint bleeding.

Comparison with previous publications

Following the first description of MRI abnormalities in joints without reported bleeds by Manco-Johnson et al. [6], this phenomenon was reported in the Canadian CHPS study in children on tailored primary prophylaxis and a study in Dutch adults with nonsevere hemophilia [7,8,27]. The latter 2 studies reported the prevalence of hemosiderin deposits in joint without reported bleeds which allows comparison with our study. A MRI substudy of the CHPS study in 24 participants reported hemosiderin deposits in 17 of 65 joints without a history of joint bleeding (26%; 95% CI, 16-39) [7], which is comparable or slightly higher than the 16% (95% CI, 7-30) observed in 43 joints of 43 participants in the present study. This trend toward more joints with hemosiderin deposits may (partly) be explained by a higher risk of (subclinical) bleeding by the slower introduction of full prophylaxis in the CHPS study compared to the Dutch prophylaxis regimen [17]. Our findings were similar to those of the recent Dutch study in 51 adults with nonsevere (mild and moderate) hemophilia A that showed hemosiderin deposits in 21 of 149 joints without a history of joint bleeding (14%; 95% CI, 9%-21%) [8]. In this study, 88% of patients with soft tissue or osteochondral changes in joints without reported bleeds had mild hemophilia, but severity-specific data on hemosiderin deposits were not provided. Interestingly, only 19/80 joints (24%; 95% CI, 15-36) with a history of bleeding in this study showed hemosiderin deposits, especially those with recent bleeding. This could be because of misclassification of bleeding or suggest total absorption of hemosiderin several years after joint bleeding.

In contrast to these 2 studies, the present study only scanned a single joint per patient, selecting the joint with the highest risk of subclinical bleeding. This may have led to an overestimation of the prevalence of hemosiderin in joints without reported bleeds in the present study. Besides, we cannot estimate the prevalence of subclinical bleeds that did not leave hemosiderin deposits in the joint, since such subclinical bleeds cannot (yet) be demonstrated. Furthermore, hemosiderin can also be observed in other diagnosis such as rheumatoid arthritis, ankylosing spondylitis, and Pigmented Villonodular Synovitis [28,29,30]. Although it is highly unlikely that this has influenced our results, since there was no evidence for these diagnoses in our patients.

The occurrence of osteochondral changes on MRI in patients without signs of previous subclinical bleeding in the current study is comparable to the occurrence of asymptomatic osteoarthritis in the general population. In our study, none of the 13 knees investigated showed signs of previous subclinical bleeding, whereas 3/13 knees showed cartilage defects (23%; 95% CI, 5-24). A meta-analysis of MRI features of osteoarthritis in asymptomatic uninjured knees of patients with a mean age <40 years reported a comparable prevalence estimate of cartilage defects in 11% of knees (95% CI, 6-17) [31]. This illustrates that cartilage abnormalities occur in the general population and therefore aging or common pathology should be considered when patient history, clinical presentation, or imaging findings do not match with blood-induced damage. The same applies to joint effusion on MRI, which is not hemophilia specific and also observed in healthy controls [26].

Relevance for clinical practice and future research

This study identified that subclinical bleeding in patients on prophylaxis occurs and therefore supports the subclinical bleeding hypothesis. However, implementing MRI as a screening method in daily practice is difficult because of high costs, limited availability, time constraints, and the need for sedation in young children. Besides, signs of previous subclinical bleeding may not have direct clinical consequence. Nevertheless, detecting (previous) subclinical bleeding with MRI is important with the currently emerging novel therapies (emicizumab, gene therapy) and the clinically overt bleeds becoming increasingly rare [14,15]. Especially since physical examination and ultrasound were unable to detect signs of previous subclinical bleeding. Therefore, we would like to argue that comparing effectiveness of these new therapies based on outcome measures such as bleeding rates, factor levels, physical examination, and ultrasound is insufficient and that MRI-based outcomes should be considered to prove maximal joint protection in these trials.

CONCLUSION

This study provides evidence for subclinical bleeding in adolescents and adults (age range 16.5-33.2 years) with severe hemophilia A on prophylaxis. We observed signs of previous subclinical bleeding in 16% of the examined joints without a history of bleeding. Signs of previous subclinical bleeding were not associated with abnormalities at physical examination or ultrasound. MRI-based outcome measures should therefore be considered in the outcome assessment of novel nonfactor replacement therapies.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Details of the used sequences in the MRI scanning protocols per joint.

	Sequence	Plane	TR (ms)	TE (ms)	Flip angle	FOV (mm)	Thickness (mm)	Interval (mm)	Pixel (mm)	Scanning time (s)
Elbow	T1_TSE	Transversal	685	20	90	140	2.5	2.5	0.22	146
	PD	Coronal	2817	25	90	120	2.5	2.5	0.23	163
	T2_SPAIR	Sagittal	3642	60	90	120	2.5	2.5	0.31	226
	T2_SPAIR	Coronal	3626	60	90	120	2.5	2.5	0.31	239
	T2_FFE	Sagittal	520	9.21	25	120	2.5	2.5	0.28	187
Knee	3D_PD_SPAIR	Sagittal	1000	125	90	161	0.8	0.8	0.40	252
	3D_PD	Sagittal	1000	36.1	90	159	0.52	0.52	0.37	351
	T2_SPAIR	Sagittal	6384	62	90	150	3	3.3	0.29	202
	T2_FFE	Sagittal	514	11.5	20	150	3	3.3	0.29	234
Ankle	3D_PD_SPAIR	Coronal	1100	183	90	200	0.8	0.8	0.8	358
	PD_Dixon	Sagittal	2558	20	90	200	2.5	2.5	0.35	373
	T2_Dixon	Transversal	8374	100	90	150	2.5	2.5	0.39	352
	T2_FFE	Sagittal	512	9.21	25	200	2.5	2.5	0.28	190

TSE: Turbo Spin Echo, PD: Proton Density, SPAIR: SPectral Attenuated Inversion Recovery, FFE: Fast Field Echo, FOV: Field Of View, TR: repetition time, TE: echo time

Supplementary Table 2. Summary of MRI findings of joints without a history of joint bleeding

	Median (IQR) or n (%)
Joints	43
Elbows	16 (37%)
Knees	13 (30%)
Ankles	14 (33%)
IPSG joint score	1 (0-2)
Effusion	
Absent	*20 (47%)*
Small	*15 (35%)*
Moderate	*7 (16%)*
Large	*1 (2%)*
Synovial hypertrophy	
Absent	*41 (95%)*
Small	*1 (2%)*
Moderate	*0 (0%)*
Large	*1 (2%)*
Hemosiderin	
Absent	*36 (84%)*
Small	*5 (12%)*
Moderate	*1 (2%)*
Large	*1 (2%)*
Surface erosions	
Absent	*41 (95%)*
Any surface erosion	*2 (5%)**
Half or more of the articular surface eroded in at least one bone	*0 (0%)*
Subchondral Cysts	
Absent	*42 (98%)*
At least one subchondral cyst	*1 (2%)**
Subchondral cysts in at least two bones, or cystic changes involving a third or more of the articular surface in at least one bone	*0 (0%)*
Cartilage degeneration	
Absent	*38 (88%)*
Any loss of joint cartilage height	*2 (5%)*
Loss of half or more of the total volume of joint cartilage in at least one bone	*2 (5%)**
Full-thickness loss of joint cartilage in at least some area in at least one bone	*1 (2%)**
Full-thickness loss of joint cartilage including at least one half of the joint surface in at least one bone	*0 (0%)*

**Scoring based on joint changes at the tibiotalar and subtalar joint of the ankles, including subtalar findings in 2 ankles.*

PART 2

Screening for subclinical joint inflammation

CHAPTER 5

Screening for subclinical synovial proliferation in haemophilia: A systematic review and meta-analysis comparing physical examination and ultrasound

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ABSTRACT

Introduction

Ultrasound is increasingly used as addition to physical examination for detection of subclinical joint changes in haemophilia. However, the added value of ultrasound to physical examination for detecting synovial proliferation is not fully established.

Aim

To determine the diagnostic accuracy of swelling at physical examination for ultrasound-detected synovial proliferation in haemophilia.

Methods

PubMed and EMBASE were searched up to 2 August 2022. Studies reporting original data on occurrence of swelling at physical examination and synovial proliferation on ultrasound of index joints in persons with haemophilia were included. Risk of bias and applicability were assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool. Diagnostic accuracy parameters of swelling at physical examination for ultrasound-detected synovial proliferation were determined. Summary sensitivity and specificity were calculated using a bivariate random-effects model.

Results

Fifteen studies reporting on swelling at physical examination and synovial proliferation on ultrasound in 2890 joints of 627 patients were included. Prevalence of subclinical synovial proliferation ranged between 0% and 55%. Sensitivity of swelling was low [summary estimate .34; 95% confidence interval (CI) .24-.46], while specificity was high (summary estimate .97; CI .92-.99). Predictive values varied widely due to inter-study differences in prevalence of synovial proliferation.

Conclusion

Joint swelling has low sensitivity for presence of ultrasound-detected synovial proliferation in haemophilia, suggesting underestimation of synovial proliferation by physical examination alone. Consequently, ultrasound screening may generate important information on synovial changes which would otherwise remain undetected.

INTRODUCTION

Haemophilic arthropathy, caused by recurrent intra-articular bleeds in the ankles, knees and elbows (index joints), still causes major disease burden in persons with haemophilia[1-4]. Haemophilic arthropathy results from a multifactorial process in which synovial inflammation plays an important role. Several studies have reported that synovial proliferation is associated with increased bleeding and progression of arthropathy[4-8]. Through termination of the vicious circle of joint bleeding and inflammation, early detection and treatment of synovial proliferation might enable adaptation of treatment and improve outcome.

Traditionally, physical examination, according to, for example the Haemophilia Joint Health Score (HJHS)[9], is used to monitor joint health[10]. During physical examination, synovial proliferation may be characterized by painless swelling and subtle range of motion limitations. Yet, physical examination is thought to be relatively insensitive for detection of early joint changes such as synovial proliferation[10-12]. More sensitive methods for monitoring joint health are needed since progression of arthropathy is observed despite low joint bleeding rates[13]. These findings suggest that subclinical bleeding and inflammation may contribute to joint deterioration. With the expected reduction in joint bleed rates following intensive haemophilia treatment, the detection of early, subclinical cases of synovial proliferation[14] becomes even more relevant for clinical management.

Imaging techniques such as magnetic resonance imaging (MRI) and ultrasound may offer this increase in diagnostic accuracy. Although MRI is the current gold standard for detection of joint changes in haemophilia, routine MRI assessments of multiple joints is challenging due to duration, high costs and limited availability[10]. Ultrasound is a low-cost, widely available modality that provides real time information, which makes it easy to implement in clinical practice. Moreover, high to excellent agreement between ultrasound and MRI has been reported for detecting soft tissue abnormalities in joints of people with haemophilia[15-21]. The high accuracy makes ultrasound a good screening tool for presence of abnormal soft tissue. If clinically relevant, MRI can be used subsequently to differentiate the nature of the tissue. Over the past years, ultrasound is increasingly used as point-of-care addition to the existing check-up routine[22, 23].

Several studies have compared the accuracy of ultrasound and physical examination for detecting (early) joint abnormalities in people with haemophilia during routine assessment. Most literature on this topic has focused on detecting haemophilia arthropathy as a whole and less on detecting specific parameters such as synovial proliferation[11, 12, 15, 24-28]. Only one study specifically compared physical examination and ultrasound for detection of synovial proliferation[29]. However, the ability of physical examination compared to ultrasound to detect synovial proliferation is of particular interest, as synovial proliferation is potentially reversible and timely treatment may prevent further damage.

Our hypothesis is that swelling at physical examination is not highly sensitive for synovial proliferation and therefore ultrasound may have added value in screening for subclinical synovial proliferation. The aim of this study was to systematically review and meta-analyse the existing literature on swelling at physical examination and synovial proliferation on ultrasound in joints of people with haemophilia. We determined the diagnostic accuracy of swelling at physical examination for ultrasound-detected synovial proliferation. The diagnostic accuracy of swelling for ultrasound-detected synovial proliferation quantifies the underestimation of synovial proliferation based on swelling alone, providing an estimate of the added value of ultrasound for screening for subclinical synovial proliferation.

MATERIALS AND METHODS

The conduct of this systematic review was guided by the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy[30] and was reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses for Diagnostic Test Accuracy (PRISMA-DTA) guideline[31].

Literature search

PubMed and EMBASE were searched up to 2 August 2022 for relevant publications. Search queries were built with the help of an experienced librarian (mentioned in acknowledgements) and included synonyms, MeSH and Emtree terms for 'haemophilia', 'physical examination' and 'ultrasound'. The complete search strategy is provided in Supplement S1. Additionally, reference lists of included studies were checked for relevant publications.

Eligibility criteria and study selection

Observational studies reporting original data from routine physical examinations and ultrasound of index joints in children and/or adults with haemophilia A or B and related diseases, regardless of disease severity, were included. Data on presence of swelling at physical examination and synovial proliferation on ultrasound for individual joints had to be available from the publications or had to be provided by the authors upon request. Only publications written in English or Dutch and published in peer-reviewed journals were considered. To avoid overlap in study populations, publications reporting on the same cohort were identified and only the study providing the most complete description of the cohort was included. Studies on acute painful (bleeding) episodes or intra-articular interventions were excluded, since these studies were not considered to represent routine joint assessment and were therefore beyond the scope of this review. All publications were screened for eligibility based on title and abstract and subsequently relevant publications' full text were independently assessed by two reviewers (FL and MT). Discrepancies of evaluations were discussed upon consensus between the two reviewers or resolved by a third reviewer (WF).

Quality assessment

Assessment of the risk of bias and applicability of the studies to our research question was performed according to the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2)

tool[32]. The tool was tailored as described in the QUADAS-2 background document to specifically fit the current review question. The main refinement was no downgrading for unblinded ultrasound examinations as blinding for joint swelling seemed unfeasible and not in-line with clinical practice. The risk of bias assessment was categorized into four key domains: 'patient selection', 'index test', 'reference standard' and 'flow and timing'. Concerns about applicability were assessed for the first three domains. Per domain risk of bias and applicability concerns were scored as 'low', 'high' or 'unclear'. The tailored QUADAS-2 tool is available in Supplement S4. Two reviewers (FL and MT) independently assessed all studies, and discrepancies in the judgements were discussed upon consensus or resolved by the third reviewer (WF).

Data extraction and data analysis

Data on study design, patient characteristics, conduct of physical and ultrasound examinations and occurrence of swelling and synovial proliferation at joint level were extracted from the publications or requested from the authors by one reviewer (FL) using the data extraction form in Supplement S3. Authors were given a 2-month response period for providing additional data with a reminder sent after 2–4 weeks. Swelling was defined as 'absent' (HJHS/Gilbert score swelling = 0) or 'present' (HJHS/Gilbert score swelling > 0) and definitions used for synovial proliferation were 'absent' (ultrasound synovial proliferation score = 0, according to the HEAD-US score, Doria et al. or Klukowska et al.) or 'present' (ultrasound synovial proliferation score > 0)[9, 18, 33-35]. Diagnostic accuracy parameters (sensitivity, specificity, positive and negative predictive values) on joint level were calculated for each individual study using the original study data. The parameters' 95% confidence intervals (CI) were calculated according to the Clopper-Pearson 'exact' method. Heterogeneity between studies' sensitivities and specificities was assessed visually in forest plots and by Higgin's I2 statistics. Based on the judgement for the 'patient selection' domain of the of QUADAS-2, the included studies were divided into two groups: 'screening of index joints' where the evaluated joints were not preselected based on joint status and were therefore considered to represent a routine screening setting (e.g. assessment of all six index joints or a random selection of a subset of index joints) or 'preselected joints' where evaluated joints were selected based on joint status (e.g. preselection based on available HJHS/radiological Pettersson scores[36] or assessment of the most/least affected joint only). To estimate the diagnostic accuracy of swelling for ultrasound-detected synovial proliferation in routine screening, summary estimates for sensitivity and specificity with their CI were calculated using a bivariate random-effects model adjusting for between-study heterogeneity[37]. In addition, subgroup analyses for treatment modality, disease severity, age and risk of bias were performed, and forest plots of the diagnostic accuracy parameters of the studies sorted by prevalence of synovial proliferation and joint status were visually inspected for trends in the parameters based on these study characteristics. Analyses were performed in RStudio (version 1.3.1093), using the GenBinomApps (version 1.2), meta (version 6.0-0) and mada (version .5.11) packages.

RESULTS

The process of literature search is shown in Figure 1 and yielded a total of 1814 individual publications. Cross-reference searching did not identify additional publications. After title and abstract screening, full text publications of 188 studies were assessed for eligibility. The reasons for excluding studies based on full text are summarized in Supplement S2. Eventually, 15 studies[11, 12, 15, 24, 29, 38-47] were included in the systematic review. The 15 included studies reported on occurrence of swelling and synovial proliferation in 2890 joints of 627 patients. The study populations varied between children (n = 7), adults (n = 4) and mixed populations (n = 4) and patients investigated mostly had severe haemophilia A. Physical examination was performed according to the HJHS in 14/15 studies and the Gilbert score[33] in the remaining study. Ultrasound examinations were mainly performed according to the HEAD-US protocol (12/15 studies). In two studies an ultrasound protocol described by Zukotynski et al.[48] was used. Prevalence of synovial proliferation ranged between 4.8% and 95%. A detailed summary of the included studies is presented in Table 1.

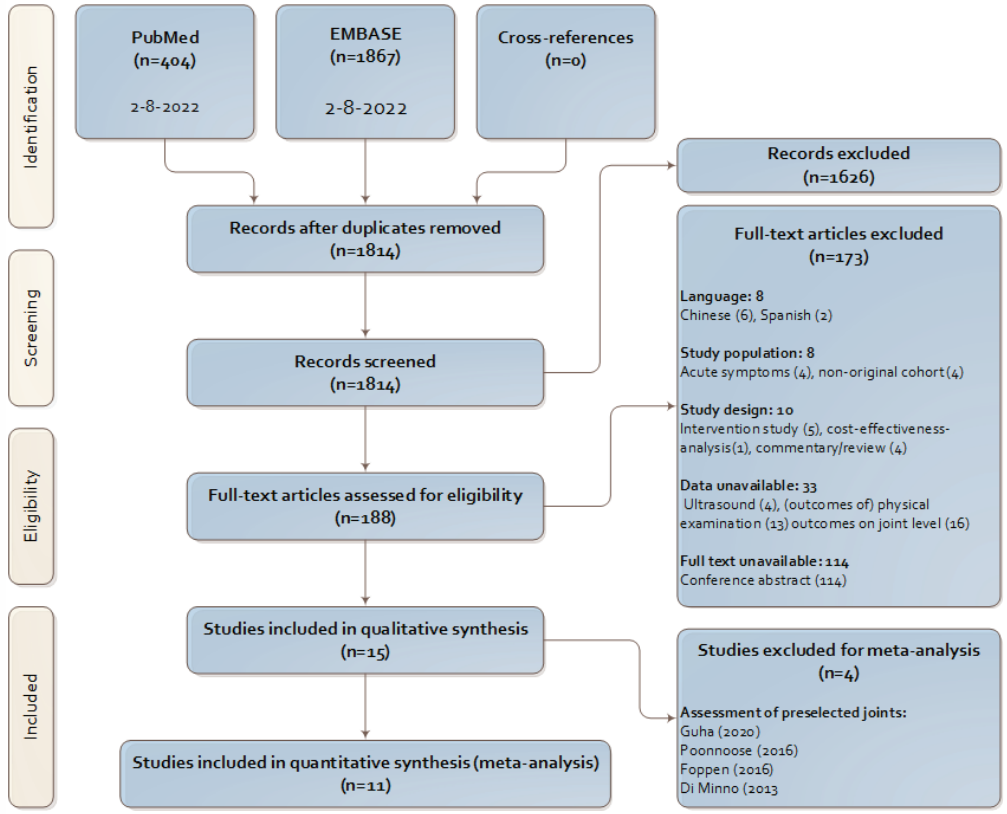

Figure 1. Flow chart of literature screening and study selection.

Table 1. Summary of studies included in the analysis.

	Study	Population							Examinations					
		N (joints)	Age (years)		Haemophilia A	Severe	Prophylaxis	Joint status (HJHS)	Physical examination		Ultrasound		Time window PE-US
				Median/mean				Median	Operator(s)	Score	Operator(s)	Protocol	
Screening of index joints	Adramerina (2022)	120	Children	11.5	90%	80%	100%	0	1 PT	HJHS	1 Physician	HEAD-US	<24h
	De la Corte-Rodriguez (2022)	361	Mixed	34	85%	80%	92%	na	1 RP	HJHS	1 RP	HEAD-US	<24h
	Roussel (2022)	59†	Adults	39.4	87%	80%	83%	21†	1 PT	HJHS	1 PT	HEAD-US	<24h
	Daffunchio (2021)	480†	Children	10.8	89%	100%	42.5%	0	1 PT	HJHS	1 PT & 1 Orthopaedist	HEAD-US	<24h
	Kavakli (2021)	438†	Mixed	18	85%	93%	100%	3	PTs & Haematologists†	HJHS	2 Radiologists†	HEAD-US	<24h
	Måseide (2021)	693	Mixed	28	61%	0%	38%	4	PTs	HJHS	PTs & Physicians	HEAD-US	<24h/ <1year§
	Plut (2021)	168	Mixed	33	100%†	100%	100%	1	2 Haematologists†	HJHS	1 Radiologist	HEAD-US	<24h
	Prasetyo (2021)	120	Children	9.35	100%	100%	0%	3	1 PT & 1 RP	HJHS	1 Radiologist	HEAD-US	<24h
	Stephensen (2018)	63	Adults	29.14	100%†	100%	100%†	na	3 PTs†	HJHS	6 PTs	HEAD-US	<3months
	Timmer (2017)	76	Adults	53	80%	53%	33%	0	1 PT	HJHS	1 Physician	HEAD-US	<24h
	Altisent (2016)	124	Children	8.3	100%	100%	100%	0	1 RP	HJHS	2 Radiologists	HEAD-US	<24h
Preselected joints	Guha (2020)	30	Children	7.4	70%	na	10%	na	1 Physician†	HJHS	1 Radiologist†	HEAD-US	<24h
	Foppen (2016)	63	Children	11.5	88%	94%	100%	0	2 PTs†	HJHS	1 Physician	HEAD-US	<24h
	Poonnoose (2016)	55	Children	15	88%	100%	na	na	Experienced investigators	HJHS	Radiologists	Zukotynski	<36h
	Di Minno (2013)	40	Adults	22.45	100%	100%	70%	0	1 Orthopaedic surgeon	Gilbert	1 Haemophilia physician†	Zukotynski	<24h

†Details provided by the author upon request, na: not available, Gilbert: Gilbert score[29], HJHS: Haemophilia Joint Health Score[9], PT: physiotherapist, RP: Rehabilitaion Physician, HEAD-US: Haemophilia Early Arthropathy Detection with UltraSound protocol[30], Zukotynski: ultrasound protocol as described by Zukotynski et al.[46], PE: Physical examination, US: Ultrasound, §In 8/118 examined patients time window >24h.

Quality assessment

A summary of the risk of bias assessment and the applicability concerns is presented in Table 2, additional details are available in Supplement S5. In 4 out of 15 studies[11, 12, 15, 42], preselected joints were included resulting in a high risk of selection bias with corresponding applicability concerns in the patient selection domain. Results of these studies were not generalizable to a routine screening setting and were not included in the meta-analysis. The conduct of the physical examination was assessed with 'high risk of bias' in 3 of 15 studies because of non-blinded operators (n = 2)[12, 47] and operators who were not experienced/trained in the use of the HJHS (n = 1)[38]. For Di Minno et al.[15], the applicability concerns of the physical examination performance were 'high' due to the use of the Gilbert score instead of the HJHS. Although use of the Gilbert score by Di Minno et al. was scored as a concern regarding the applicability in the quality assessment, its impact on the generalisability of the results to a setting in which the HJHS will be used is considered minimal. The performance of ultrasound examinations did not raise high risk of bias nor applicability concerns. A time window > 24 h between physical examination and ultrasound assessment introduced a high risk of bias in the 'flow and timing' domain in three studies[11, 40, 47]. Three studies scored 'high risk of bias' in the latter domain because examinations were not performed in all eligible joints[41, 44] or not all data from the examined joints was available for analysis[12].

Table 2. Tabular presentation of the results of the QUADAS-2 assessment.

Study	Risk of Bias				Applicability Concerns		
Author (year)	Patient selection	Index test	Reference standard	Flow & Timing	Patient selection	Index test	Reference standard
Adramerina (2022)	L	L	L	L	L	L	L
De La Corte-Rodriguez (2022)	L	L	L	L	L	L	L
Roussel (2022)	L	L	L	H	L	L	L
Daffunchio (2021)	L	L	L	L	L	L	L
Kavakli (2021)*	L	L	L	L	L	L	L
Måseide (2021)*	L	H	L	H	L	L	L
Plut (2021)*	L	H	L	L	L	L	L

Table 2. Tabular presentation of the results of the QUADAS-2 assessment. (continued)

Study	Risk of Bias				Applicability Concerns		
Author (year)	Patient selection	Index test	Reference standard	Flow & Timing	Patient selection	Index test	Reference standard
Prasetyo (2021)	?	L	L	L	L	L	L
Stephensen (2018)*	L	L	L	H	L	L	L
Timmer (2017)	L	L	L	L	L	L	L
Altisent (2016)	L	L	L	H	L	L	L
Guha (2020)*	H	?	L	L	H	?	L
Foppen (2016)*	H	H	L	H	H	L	L
Poonnoose (2016)	H	L	L	H	H	L	L
Di Minno (2013)*	H	L	L	L	H	H	L

L *Low Risk* H *High Risk* ? *Unclear Risk*

QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies 2

**Judgement based on additional data provided by the authors.*

Diagnostic accuracy of swelling for detection of synovial proliferation

The prevalence of subclinical synovial proliferation ranged from 0% to 55%. Calculated sensitivity, specificity, positive and negative predictive values (PPV, NPV) of swelling for detection of synovial proliferation from the individual studies are available in Figure 2 and Tables 3 and 4. Sensitivity was mostly low to moderate, with a few outliers ranging from .00 to 1.00. The overall low sensitivity indicates that only a small proportion of the joints with synovial proliferation on ultrasound showed swelling at physical examination. Specificity was mainly high, yet outliers were observed as well (range .14-1.00). The mostly high specificity of swelling indicates that absence of synovial proliferation on ultrasound was likely to correspond to absence of swelling in the included studies. Heterogeneity of included studies was considerable for sensitivity (Higgins' I2 79.7%, CI 67.3-87.4) and specificity (Higgins' I2 85.9%, CI 78.3-90.8). For positive predictive values, large variation was observed (range .48-1.00), where studies with a high prevalence of synovial proliferation showed

higher PPVs. Negative predictive values varied widely as well (range .13-1.00) with increasing NPVs with decease of the synovial proliferation prevalence in the study population. The wide ranges in predictive values indicate that the probability that presence or absence of swelling truly corresponds with presence or absence of synovial proliferation on ultrasound respectively varied widely between studies, depending on the prevalence. Subgroup analyses for treatment, disease severity, age and risk of bias showed no significant differences between sensitivity and specificity in the subgroups. Visually, sensitivity and specificity of swelling appeared not to be associated with joint status, nor with prevalence of synovial proliferation. Results of subgroup analyses are available in Supplement S6.

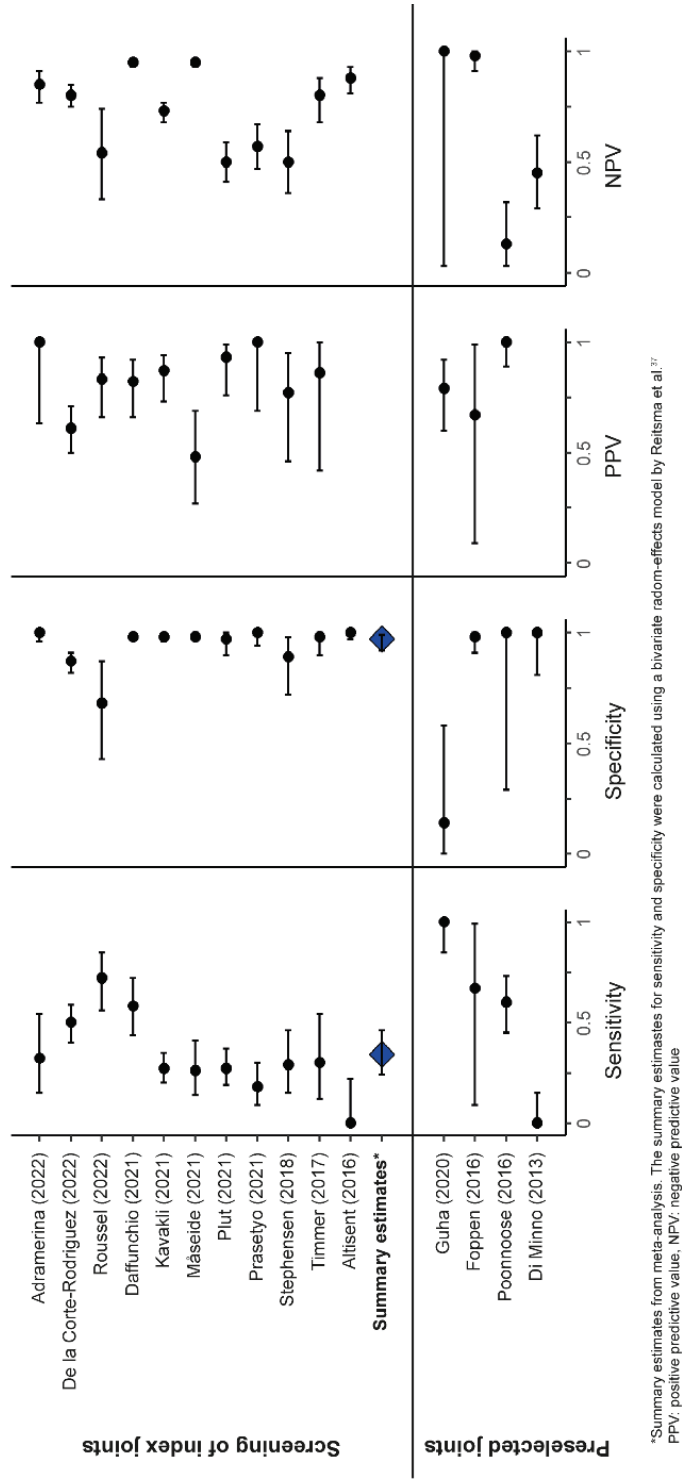

Figure 2. Diagnostic accuracy parameters of swelling at physical examination compared to ultrasound for detection of synovial proliferation.

Table 3. Diagnostic accuracy parameters of swelling at physical examination compared to ultrasound for detection of synovial proliferation in screening of index joints.

Study	Population							Diagnostic accuracy parameters					
	Age	Severe	Prophylaxis	Joint status (HJHS) Median	Synovial proliferation Prevalence	N (joints)	TP	FP	FN	TN	Sensitivity [CI]	Specificity [CI]	PPV [CI]	NPV [CI]
Adramerina (2022)	Children	80%	100%	0	20.8%†	120†	8†	0†	17†	95†	0.32 [0.15-0.54]	1.00 [0.96-1.00]	1.00 [0.63-1.00]	0.85 [0.77-0.91]
De la Corte-Rodriguez (2022)	Mixed	80%	92%	na	29.6%†	361	53†	34†	54†	220†	0.50 [0.40-0.59]	0.87 [0.82-0.91]	0.61 [0.50-0.71]	0.80 [0.75-0.85]
Roussel (2022)	Adults	80%	83%	21†	67.8%†	59†	29†	6†	11†	13†	0.72 [0.56-0.85]	0.68 [0.43-0.87]	0.83 [0.66-0.93]	0.54 [0.33-0.74]
Daffunchio (2021)	Children	100%	42.5%	0	11.0%	480	31†	7†	22†	420†	0.58 [0.44-0.72]	0.98 [0.97-0.99]	0.82 [0.66-0.92]	0.95 [0.93-0.97]
Kavakli (2021)	Mixed	93%	100%	3	33.3%†	438†	39†	6†	107†	286†	0.27 [0.20-0.35]	0.98 [0.96-0.99]	0.87 [0.73-0.94]	0.73 [0.68-0.77]
Måseide (2021)	Mixed	0%	38%	4	6.2%	693	11	12	32	638	0.26 [0.14-0.41]	0.98 [0.97-0.99]	0.48 [0.27-0.69]	0.95 [0.93-0.97]
Plut (2021)	Mixed	100%	100%	1	57.1%†	168	26†	2†	70†	70†	0.27 [0.19-0.37]	0.97 [0.90-1.00]	0.93 [0.76-0.99]	0.50 [0.41-0.59]
Prasetyo (2021)	Children	100%	0%	3	47.5%†	120	10†	0†	47†	63†	0.18 [0.09-0.30]	1.00 [0.94-1.00]	1.00 [0.69-1.00]	0.57 [0.47-0.67]
Stephensen (2018)	Adults	100%	100%†	na	55.6%†	63	10†	3†	25†	25†	0.29 [0.15-0.46]	0.89 [0.72-0.98]	0.77 [0.46-0.95]	0.50 [0.36-0.64]
Timmer (2017)	Adults	53%	33%	0	26.9%†	76	6†	1†	14	55†	0.30 [0.12-0.54]	0.98 [0.90-1.00]	0.86 [0.42-1.00]	0.80 [0.68-0.88]
Altisent (2016)	Children	100%	100%	0	12.1%	124	0†	0†	15†	109†	0.00 [0.00-0.22]	1.00 [0.97-1.00]	na	0.88 [0.81-0.93]
Summary estimates from meta-analysis*											0.34 [0.24-0.46]	0.97 [0.92-0.99]	-	-

*†Details provided by the author upon request, HJHS: Haemophilia Joint Health Score, na: not available, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: 95% confidence interval, PPV: positive predictive value, NPV: negative predictive value. *Summary estimates were calculated using a bivariate random-effects model by Reitsma et al[37].*

***Interpretation:** Reported diagnostic accuracy parameters reflect the performance of swelling at physical examination compared to ultrasound for detection of synovial proliferation. Swelling at physical examination was the test under investigation, with ultrasound serving as reference standard.*

Table 4. Diagnostic accuracy parameters of swelling at physical examination compared to ultrasound for detection of synovial proliferation in preselected joints.

Study		Population						Diagnostic accuracy parameters				
	Joint selection	Age	Severe	Prophylaxis	Joint status (HJHS)	Synovial proliferation Prevalence	N (joints)	TP	FP	FN	TN	
					Median							Sensitivity [CI]	Specificity [CI]	PPV [CI]	NPV [CI]
Guha (2020)	Most affected joint	Children	na	10%	na	76.7%†	30	23†	6†	0†	1†	1.00 [0.85-1.00]	0.14 [0.00-0.58]	0.79 [0.60-0.92]	1.00 [0.03-1.00]
Foppen (2016)	High risk & contralateral joint	Children	94%	100%	0	4.8%	63	2	1	1	59	0.67 [0.09-0.99]	0.98 [0.91-1.00]	0.67 [0.09-0.99]	0.98 [0.91-1.00]
Poonnoose (2016)	Negligible - severe HA	Children	100%	na	na	94.5%†	55	31†	0†	21	3	0.60 [0.45-0.73]	1.00 [0.29-1.00]	1.00 [0.89-1.00]	0.13 [0.03-0.32]
Di Minno (2013)	Healthy joints	Adults	100%	70%	0	55.0%	40	0	0	22	18	0.00 [0.00-0.15]	1.00 [0.81-1.00]	na	0.45 [0.29-0.62]

Healthy joints: clinically asymptomatic joints never involved by overt bleeding events with Gilbert score = 0, Most affected joint: most affected joint based on HJHS v2.1 assessment, Negligible – severe HA: preselected joints based on Pettersson score, representing a spectrum ranging from negligible to severe haemophilic arthropathy, High risk & contralateral joint: Joint with the highest risk of arthropathy based on life-time bleed reports and the contralateral joint for side-to-side comparison, †Details provided by the author upon request, na: not available, HJHS: Haemophilia Joint Health Score, TP: true positive, FP: false positive, FN: false negative, TN: true negative, CI: 95% confidence interval, PPV: positive predictive value, NPV: negative predictive value.

Meta-analysis of screening studies

Eleven out of 15 included studies were considered 'screening of index joints' studies[24, 29, 38-41, 43-47] and were thus included in a bivariate random-effects model to determine summary estimates for sensitivity and specificity. Subclinical synovial proliferation was found in 4.6-41.7% of the joints in these studies. The diagnostic accuracy parameters of the 11 individual studies are available from Table 3. The summary estimates obtained in the meta-analysis were .34 for sensitivity (CI .24-.46) and .97 (CI .92-.99) for specificity, indicating that in routine assessment no synovial proliferation on ultrasound corresponded to no swelling in most cases, yet only a part of the ultrasound-detected synovial proliferation cases showed swelling at physical examination.

Diagnostic accuracy in selected joints

Four out of 15 studies investigated preselected joints. The diagnostic accuracy parameters of these studies are summarized in Table 4. Di Minno et al.[15] investigated subclinical arthropathy in 40 'healthy joints' of Italian adults with severe haemophilia A and found subclinical synovial proliferation on ultrasound in 55% of joints. The diagnostic accuracy parameters based on this study were similar to the estimates found in the screening studies. PPV could not be determined due to the absence of swelling in this study. Guha et al.[42] conducted a study including the most affected joints, according to HJHS, of 30 Indian children and did not observe any subclinical synovial proliferation (0%). In contrast to the other studies, this study showed high sensitivity (1.00, CI .85-1.00) and low specificity (.14, CI .00-.58) of swelling for ultrasound-detected synovial proliferation. Lastly, two studies investigated heterogeneous groups of joints in which the prevalence of haemophilic arthropathy was dependent on joint selection. Foppen et al.[12] performed a side-to-side comparison of the joint with the highest risk of arthropathy with its contralateral joint in 32 Dutch children. Poonnoose et al.[11] preselected joints based on their x-ray Pettersson scores to establish the full spectrum of arthropathy severity including 50% of joints with negligible to mild haemophilic arthropathy. The difference in synovial proliferation prevalence of 4.8% in the study by Foppen and 94.5% in the study by Poonnoose was reflected in the obtained predictive values, with a high NPV (.98, CI .91-1.00) and moderate PPV (.67, CI .09-.99) based on Foppen et al. as opposed by low NPV (.13, CI .03-.32) and high PPV (1.00, CI .89-1.00) based on Poonnoose et al.

DISCUSSION

This systematic review and meta-analysis estimated the diagnostic accuracy of swelling for ultrasound-detected synovial proliferation based on 15 studies reporting on 2890 joints of 627 patients. Prevalence of subclinical synovial proliferation in the studies ranged between 0% and 55%. Overall, sensitivity of swelling was low indicating an underestimation of ultrasound-detected synovial proliferation based on swelling alone. Specificity of swelling was high, indicating that joints without ultrasound-detected synovial proliferation usually show no swelling. NPV and PPV varied widely corresponding to the variation in prevalence of synovial proliferation in the various studies. Summary estimates of sensitivity and specificity for studies performed in a routine screening setting showed low sensitivity (.34; CI .24-.46) and high specificity (.97; CI .92-.99) of swelling for ultrasound-detected synovial proliferation. These summary estimates indicate fair evidence for the added value of ultrasound in screening for subclinical synovial proliferation.

Strengths and limitations

A strength of this review is the systematic literature search with the retrieval of additional data from authors, combined with evaluation according to the Cochrane guidelines. As a result, this review provides the most complete overview possible of currently existing published and unpublished data on the occurrence of swelling at physical examination and synovial proliferation on ultrasound during routine joint assessment in study populations varying in age, disease severity, treatment, and/or joint status. However, using unpublished data also introduces a risk of information bias since the data provided by authors cannot be fully checked for accuracy. A possible limitation of this review is the focus on swelling as only clinical indicator for synovial proliferation, while disregarding minimal pain and slight loss of range of motion as potential signs of synovial proliferation[10]. However, synovial proliferation on ultrasound only shows weak correlation with pain ($r < .3$), and no significant correlation with range of motion[26]. A focus on assessment of swelling alone is therefore expected to be of minimal influence on the results of the current study. Lastly, we compared swelling on physical examination with ultrasound for detecting synovial proliferation. The ultrasound was serving as the reference standard in this regard. However, ultrasound is an imperfect reference standard, since the established gold standard for diagnosing synovial proliferation is MRI[49]. As a result, the diagnostic accuracy parameters presented only reflect performance of swelling relative to ultrasound. Due to the possible misclassification of outcome by ultrasound, the diagnostic accuracy parameters cannot be viewed as the performance of swelling for detecting the true disease status as determined by MRI.

Quality of evidence

Limitations for drawing high-quality evidence conclusions from this review are the risk of selection and information bias, the considerable between-study heterogeneity of the included studies, and their limited sample sizes. For 12 of 15 included studies, additional data on the presence of swelling and synovial proliferation for individual joints were provided by the authors upon request. In addition, the majority of studies were not specifically designed to compare physical examination and ultrasound for detection of synovial proliferation specifically, resulting in potential selection and/or information bias in 9 of 15 studies. While 12 of 15 included studies reported on the correlation between HJHS and ultrasound[11, 12, 46, 47, 24, 29, 39-42, 44, 45] and 7 of 10 aimed at investigating the correlation between HJHS and ultrasound[11, 29, 39, 40, 44, 45, 47], only De la Corte-Rodriguez et al.[29] aimed at comparing the separate items of the HEAD-US and HJHS. The large between-study heterogeneity made pooling of all studies to obtain summary estimates of diagnostic accuracy inappropriate. However, with use of bivariate random-effects modelling, heterogeneity-corrected summary estimates were generated for studies performed in a routine screening setting, thus providing evidence for the added value of ultrasound for screening of subclinical synovial proliferation.

Clinical implications and future research

This review provides fair evidence that there is added value of ultrasound to routine physical examination for screening of subclinical synovial proliferation: absence of swelling does not represent absence of synovial proliferation on ultrasound. The clinical relevance of ultrasound

screening may be highest in (relatively) healthy joints, since detection of subclinical synovial proliferation in these joints may have the largest impact. Early treatment of synovitis may limit bleeding and joint deterioration, and thereby prevent progression to haemophilic arthropathy in these relatively healthy joints. From a treatment perspective, it is important to distinguish between reversible synovial inflammation and irreversible fibrotic synovial changes. As fibrotic synovial changes may occur in joints following recurrent joint bleeding[50], ultrasound-detected synovial proliferation may not reflect active synovial inflammation in all joints affected by previous bleeding or haemophilic arthropathy. MRI might be used to differentiate between active synovitis and fibrotic synovial changes[21]. Increased synovial vascularisation detected by Power/Colour Doppler imaging on ultrasound might help in distinguishing between active inflammation and fibrotic synovium, yet its diagnostic accuracy in haemophilic joints is a topic of debate and has not been established[51]. Longitudinal ultrasound studies are needed to establish the clinical relevance of ultrasound-detected synovial proliferation with or without increased vascularisation by monitoring the effect of treatment alterations after diagnosis of synovitis.

CONCLUSION

Studies evaluating swelling at physical examination and synovial proliferation on ultrasound show large heterogeneity, causing variability in the observed diagnostic accuracy parameters. Overall low sensitivity of swelling for ultrasound-detected synovial proliferation suggests underestimation of synovial proliferation by physical examination alone. This review provides fair evidence that ultrasound has added value in a routine setting for detecting subclinical synovial proliferation. Future studies may identify patient subgroups in which ultrasound examination is most clinically relevant. Additionally, the clinical consequences of detection of synovial proliferation by ultrasound need to be established.

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SUPPLEMENTARY MATERIAL

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

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CHAPTER 6

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

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.

MATERIALS AND METHODS

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.

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].

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.

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.

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.

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.

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).

RESULTS

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.

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	0.6 (0.2-1.1)	-
Continuous prophylaxis	62 (78%)	-
FVIII IU/kg/year	*1897 (1452-2439)*	-
Emicizumab	*4 (5%)*	-
BMI	25 (23-27)	-
HAL¶	94 (81-100)	2 (1-3)
B) Joint characteristics (patient level, n=79)		
Total HJHS*	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[§] (19%)	-

*% might not add up exactly due to rounding; 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; ¶Available for 66/79 patients; *Available for 75/79 patients; [§]Ankle arthrodesis in 3 patients, ankle distraction in 3 patients, joint nettoyage in 5 patients, synovectomy in 5 patients and surgery after an intra-articular ankle fracture in 1 patient. All interventions were at least three years ago.*

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.

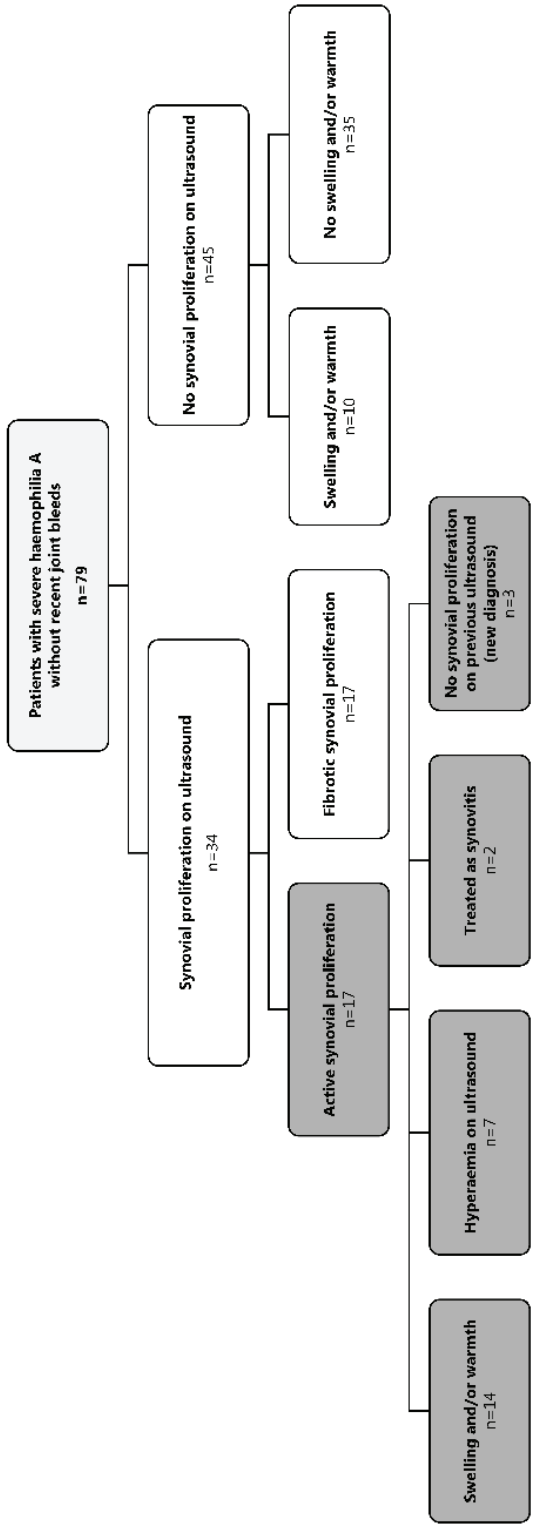

Figure 1. Flowchart showing the proportion of patients with synovial proliferation, stratified by active and inactive proliferation.

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 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) 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)	0.030*
Joint bleed ≤1 year	32 (41%)	9 (53%)	6 (35%)	17 (38%)	0.491
Continuous prophylaxis	62 (78%)	12 (71%)	12 (71%)	38 (84%)	0.333
BMI	25 (23-27)	25 (24-27)	25 (22-26)	24 (22-27)	0.505
B) Joint characteristics (patient level, n=79)					
HJHS score	4 (0-16)	20 (5-31)	6 (2-14)	2 (0-5)	<0.001*
Pettersson score	3 (0-12)	14 (4-24)	4 (0-12)	0 (0-6)	0.002*
History of intra-articular interventions	15 (19%)[¶]	6 (35%)	3 (18%)	6 (13%)	0.143
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)	0.157
sVCAM-1	384 (309-477)	384 (302-505)	409 (336-488)	369 (309-456)	0.701
Inflammation z-score	*-0.3 (-1.0-0.6)*	*-0.3 (-0.9-0.9)*	*-0.9 (-1.2-0.5)*	*-0.1 (-1.0-0.6)*	*0.664*
Osteochondral					
Coll2-1	88 (64-133)	89 (71-120)	95 (59-131)	88 (65-136)	0.787
COMP	22 (15-28)	23 (14-27)	24 (15-31)	22 (16-26)	0.741
CS846	111 (89-139)	96 (80-113)	113 (102-132)	115 [89-150)	0.208
uCTX-II	226 (159-336)	272 (199-348)	267 (170-369)	198 (141-283)	0.130
TIMP	145 (133-165)	142 (129-159)	153 (138-171)	144 (132-157)	0.387
Osteochondral z-score	*-0.2 (-1.8-1.8)*	*-0.2 (-1.9-1.6)*	*0.4 (-0.8-2.0)*	*-0.6 (-1.9-1.7)*	*0.267*

*[$]For continuous variables the Kruskal-Wallis test and for categorical/dichotomous variables the Chi square test was used; * significant difference (p<0.05); % might not add up exactly due to rounding; BMI: body mass index; HJHS: Haemophilia Joint Health Score, available for 75/79 patients. [¶]Ankle arthrodesis in 3 patients, ankle distraction in 3 patients, joint nettoyage in 5 patients, synovectomy in 5 patients and surgery after an intra-articular ankle fracture in 1 patient. All interventions were at least 3 years ago.*

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.

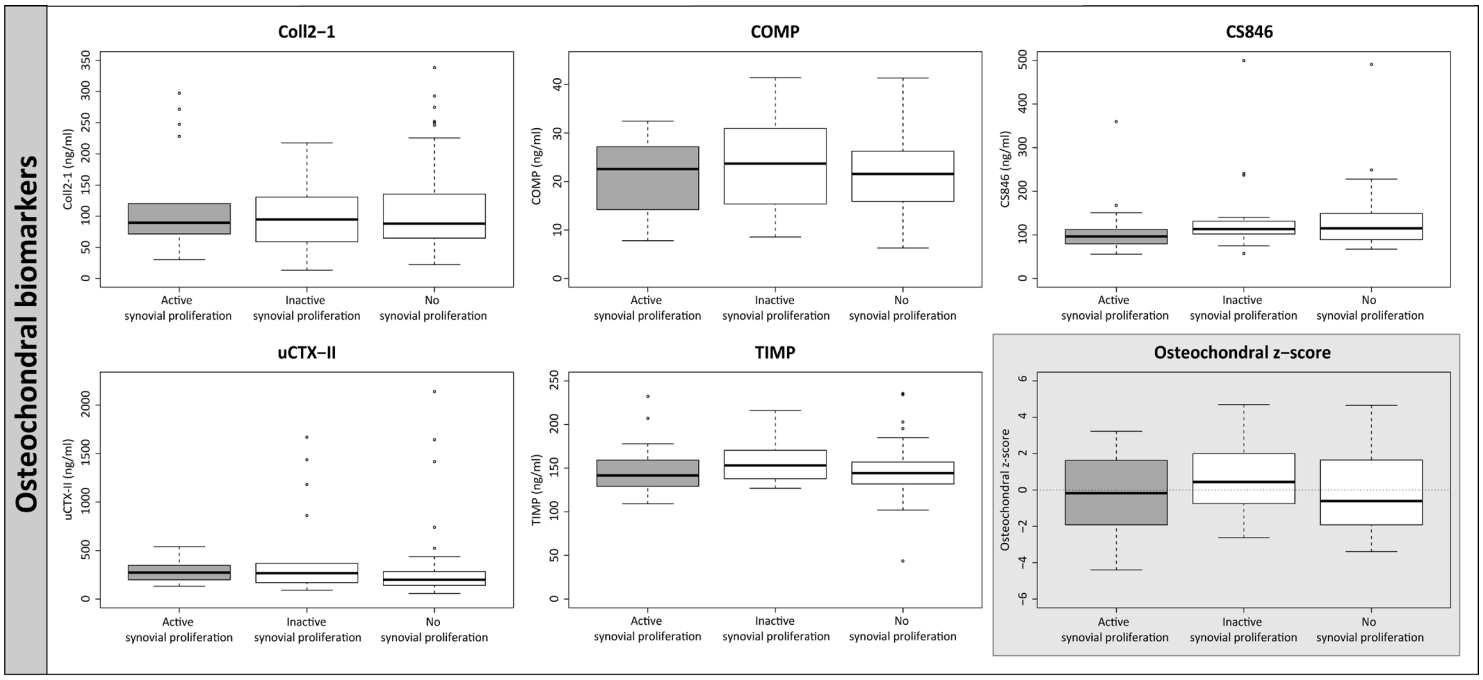

Figure 2. Boxplots comparing biochemical marker levels of patients with active, inactive or no synovial proliferation.

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.

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.

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 (Coll2-1, 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].

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.

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.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1: Clinical symptoms and ultrasound findings at joint level

	Elbows (n=158)	**Ultrasound**				
		Synovial proliferation absent	Synovial proliferation present			
Physical examination	Swelling and/or warmth absent	135	17	152	NPV	89% [83-93]
	Swelling and/or warmth present	0	6	6	PPV	100% [61-100]
		135	23	158		

	Knees (n=158)	**Ultrasound**				
		Synovial proliferation absent	Synovial proliferation present			
Physical examination	Swelling and/or warmth absent	145	5	150	NPV	97% [92-99]
	Swelling and/or warmth present	2	6	8	PPV	75% [35-97]
		147	11	158		

	Ankles (n=156)*	**Ultrasound**				
		Synovial proliferation absent	Synovial proliferation present			
Physical examination	Swelling and/or warmth absent	126	9	135	NPV	93% [88-97]
	Swelling and/or warmth present	16	5	21	PPV	24% [8-47]
		142	14	156		

**Two ankles were excluded due to arthrodesis, NPV: negative predictive value, PPV: positive predictive value, predictive value [95% confidence interval]*

PART 3

Ultrasound in management of acute joint episodes

CHAPTER 7

Ultrasound in addition to clinical assessment of acute musculoskeletal complaints in bleeding disorders: impact on patient management

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Submitted

ABSTRACT

Background
Ultrasound is increasingly used for musculoskeletal assessment in haemophilia care.

Objectives
To evaluate the impact of point-of-care ultrasound added to clinical assessment for diagnosis and treatment of acute musculoskeletal episodes in a heterogeneous cohort of children and adults with haemophilia and von Willebrand disease (VWD).

Methods
This prospective cross-sectional study consecutively included children and adults with haemophilia or VWD who visited the outpatient clinic with acute musculoskeletal complaints between March 2020 and May 2023. For all episodes, initial diagnosis and treatment determined by clinical assessment were recorded on a case report form. Subsequently, a physiotherapist with knowledge of the clinical diagnosis performed point-of-care ultrasound. After ultrasound, updated diagnosis and treatment were recorded. Diagnosis and treatment before and after ultrasound were compared and proportions of change with 95% confidence intervals (CI) were determined.

Results
We evaluated 77 episodes in 67 patients (median age: 24 years, IQR 13-42). Before ultrasound 37 joint bleeds, 13 muscle bleeds, and 27 other diagnoses were diagnosed. After ultrasound 33 joint bleeds, 11 muscle bleeds, and 33 other diagnoses were confirmed. The diagnosis changed in 28/77 episodes (36%, CI 26-48%). Nine joint bleeds and two muscle bleeds were missed by clinical assessment. Ultrasound findings changed treatment strategy in 30/77 episodes (39%, CI 28-51%).

Conclusions
Ultrasound in addition to clinical assessment of acute musculoskeletal complaints in people with haemophilia and VWD has an impact on diagnosis (36%) and treatment (39%), which supports the use of ultrasound in acute musculoskeletal complaints in haemophilia and VWD.

INTRODUCTION

Haemarthrosis and intra-muscular bleeding are characteristic for haemophilia[1] and occur to a lesser extent in von Willebrand disease (VWD)[2]. Approximately 60-80% of bleeding in people with haemophilia occurs in the joints, followed by 10-30% in muscles[3,4]. Both joint and muscle bleeds cause acute pain, swelling and reduced function[5]. Furthermore, intra-articular blood damages the joint in a multifactorial way. Recurrent joint bleeding can ultimately lead to irreversible and invalidating joint damage known as haemophilic arthropathy[1,6–8]. Muscle bleeding that is not well managed can lead to compartment syndrome, muscle contracture, and necrosis[3]. In rare cases, muscle bleeding can be complicated by pseudotumor formation[9,10].

In the acute phase, joint and muscle bleeds are treated with clotting factor concentrate to stop the bleeding, prevent rebleeding, and prevent the progression to either haemophilic arthropathy, or permanent muscle contractures and pseudotumor formation. When the bleeding has stopped, the comprehensive treatment focusses on functional rehabilitation[3].

Joint and muscle bleeds are usually diagnosed and treated based on clinical assessment. However, clinical symptoms of musculoskeletal bleeding such as pain, swelling and limited range of motion, are not specific to bleeding episodes[11–14]. Especially the differentiation between a haemarthrosis and a painful arthropathy flare-up can be difficult because of overlapping symptoms[11]. As treatment for bleeding episodes and arthropathy flare-ups/other musculoskeletal diagnoses is different, it is important to accurately distinguish between these diagnoses.

Ultrasound is increasingly used to evaluate joint health in haemophilia care[15–17]. It is a fast, non-invasive, relatively inexpensive, and accurate modality to assess blood-related changes in the musculoskeletal system with good reproducibility[18–21]. Ultrasound can also be used in acute settings as it is sensitive for detecting joint and muscle bleeding[3,14,22,23]. Most diagnostic studies assess differences between diseased and non-diseased, and some test diagnostic accuracy. However, for ultrasound to be useful when added to clinical assessment it needs to impact diagnosis and treatment. Evidence on the diagnostic and therapeutic impact of ultrasound in acute musculoskeletal episodes in bleeding disorders is limited. Three previous studies in adult patients, the majority of whom had pre-existing joint damage, have reported discrepancies between clinical diagnosis and ultrasound findings in 16-65% of painful musculoskeletal episodes, indicating an added diagnostic value of ultrasound[14,24,25]. It remains unknown whether clinical misdiagnosis occurs as frequently in a more heterogeneous and younger patient population.

In this cross-sectional study, we evaluated the diagnostic and therapeutic impact of point-of-care ultrasound in addition to clinical assessment in a heterogeneous cohort of children and adults with haemophilia or VWD, with and without pre-existing joint damage, who presented with an acute musculoskeletal episode. Our secondary aim was to explore if the clinical symptoms suggested in previous studies[26,27] can identify joint bleeding.

METHODS

Study design and population

The study design is summarized in Figure 1. This cross-sectional study included consecutive children and adults with haemophilia or VWD who attended our outpatient clinic with an acute musculoskeletal complaint between March 2020 and May 2023. Patients were included if they attended the outpatient clinic within 1 week of the onset of symptoms. Participants could contribute multiple episodes of musculoskeletal complaints. These could be complaints in different joints or muscles, or complaints in the same joints or muscles when the previous complaints were considered fully recovered by the multidisciplinary team. An a priori sample size of at least 60 episodes was planned based on feasibility. No actual sample size calculation was performed.

For all episodes, participants first underwent clinical assessment. Based on the clinical assessment, an initial diagnosis and treatment plan were recorded on a case report form (CRF). Participants were then assessed using point-of-care ultrasound. After the ultrasound, the final diagnosis and treatment plan were reported on the CRF. Clinical assessment and ultrasound assessment were performed on the same day. Ethical approval for the study was waived by the Institutional Medical Ethical Review Board (20-089/C) as ultrasound and clinical assessment were part of daily clinical practice at our center. All patients gave informed consent for use of their data.

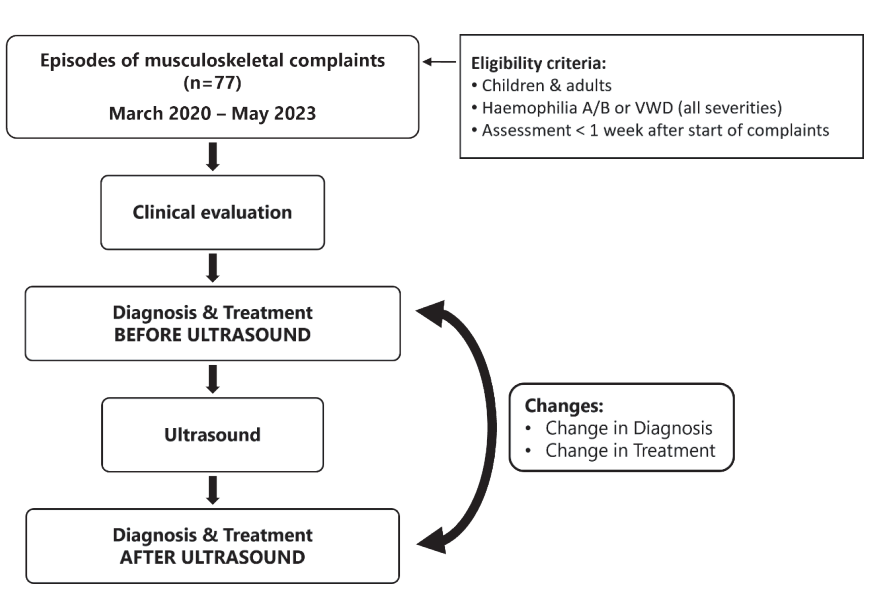

Figure 1. A Flowchart summarizing the study design.

Clinical assessment

The clinical assessment consisted of history and physical examination and was performed by physicians and physiotherapists of our Haemophilia Treatment Center. Established symptoms to differentiate between joint bleeds and arthropathy flare-ups[27] were collected: the cause of the musculoskeletal complaints (trauma/overexertion/unknown), pain (yes/no), pain localization (local/diffuse), type of pain (stabbing/pressing), pain at rest (yes/no), sleep disruption due to pain (yes/no), response of pain to treatment with factor replacement therapy (yes/no), pain with active range of motion (yes/no), and the course of the pain (constant/increasing/increasing with motion/start pain decreasing with motion). For lower extremity episodes, participants were additionally asked about pain on weightbearing (yes/no).

The physical examination included assessment of swelling (yes/no), swelling localization (local/diffuse), discoloration (none/red/blue), warmth on palpation (yes/no), and active range of motion (AROM). AROM and swelling were assessed according to the Haemophilia Joint Health Score (HJHS) 2.1[28–30]. For lower extremity episodes, participants' gait (no weightbearing/asymmetric gait/limited stability/no abnormalities) was also assessed. All the results of the clinical assessment were reported on the CRF.

Ultrasound assessment

Joint and/or muscle ultrasound were performed after clinical assessment by two physiotherapists (MT, JB) who were trained and experienced in point-of-care musculoskeletal ultrasound in haemophilia care. Ultrasound assessments were performed using a Esaote MyLab 25 Gold ultrasound scanner (Genova, Italy) with a 7.5–12 MHz linear transducer or a Mindray TE7 ultrasound scanner (Shenzhen, China) with a 6-14 MHz linear transducer. For joint episodes, effusion, synovial hypertrophy and synovial hyperaemia were assessed. For muscle episodes, fluid collection was assessed. Both joint effusion and muscle fluid collection were graded as 0 (absent/minimal), 1 (moderate) and 2 (large). Synovial hypertrophy in joints was graded as 0 (absent/minimal), 1 (mild/moderate) and 2 (severe) according to the HEAD-US protocol[31]. Synovial hyperaemia in joints was assessed by power Doppler and graded as 0 (no signal), 1 (small spots), 2 (confluent vessels in <50% tissue of interest), 3 (confluent vessels in ≥50% tissue of interest) according to the Joint Tissue Activity and Damage Examination (JADE) ultrasound protocol[32]. All ultrasound findings were reported on the CRF.

Definitions of diagnosis and treatment

Before and after ultrasound assessment the diagnosis and treatment were determined and reported on a CRF for all episodes. Diagnoses were categorized as one of the following options: intra-articular bleeds (joint bleeds), intra-muscular bleeds (muscle bleeds), arthropathy, synovitis, distortion, tendinopathy/tendinitis, muscle tear/strain, or other diagnosis.

For the treatment plans we recorded: factor replacement therapy (dose and duration in days), prescription of anti-inflammatory medication (yes/no), mobilization advise (unload/limited loading/limit intensive loading/mobilization guided by pain/no mobilization restriction), follow-up (in days),

referral to a healthcare professional (primary care physiotherapist/orthopaedic surgeon/ both. Referral for additional imaging was also recorded.

Data collection and analysis

Age, gender, disease type and severity, use of prophylaxis, inhibitor status, bleeding episodes in the year prior to inclusion, and the Haemophilia Joint Health Score (HJHS) closest to inclusion were extracted from the electronic patient records. For joint episodes in which recent joint-specific HJHS scores were not available, the osteochondral status of the joint was retrospectively estimated based on the ultrasound images or previous x-rays. Osteochondral status on ultrasound was scored according to the HEAD-US score[31] by two trained observers (FL and MT). X-rays were scored according to the Pettersson score[33] by a radiologist with >10 years of experience in imaging of haemophilic arthropathy (WF).

Patient, joint and episode characteristics were reported as medians with interquartile ranges (IQR) for continuous variables and as frequencies with percentages for categorical variables.

Diagnosis and treatment before and after ultrasound were compared and the proportion of changes with Clopper-Pearson 'exact' 95% confidence intervals (CI) were determined. Changes in diagnoses before and after ultrasound were visualized in a Sankey diagram. Additionally, the number needed to scan (NNS) for a change in diagnosis or treatment alteration was calculated. The NSS describes how many patients need to be scanned by ultrasound in order to change the diagnosis or treatment for one patient[34]. The NNS was calculated as NNS=1/(ARR), where the ARR (absolute risk reduction) was the proportion of changed diagnoses or treatment plans. Furthermore, the prevalence of misdiagnosis in 'healthy joints' versus arthropathic joints (HJHS/ HEAD-US cartilage and bone sum score/ Pettersson score >0) and children versus adults was compared using Fisher's exact test. To examine the effect of patients contributing multiple episodes, a sensitivity analysis was performed that included only the first episode of each patient.

Finally, to explore the diagnostic value of clinical symptoms in identifying joint bleeding, the positive predictive value (PPV) and negative predictive value (NPV) with CIs were calculated for clinical symptoms associated with joint bleeding. All analyses were performed in RStudio 2023.09.1+494.

RESULTS

Patients and episodes

Characteristics of the patients and acute musculoskeletal episodes are available in Table 1. We assessed 77 acute musculoskeletal episodes in 67 patients. Five patients were included twice with joint episodes in different joints. Two patients were included twice with two distinct joint episodes in the same joint. One patient was included four times with two muscle episodes in different muscles and two episodes in the right ankle.

The study cohort included episodes in 34 children and 43 adults. The median age at the time of assessment was 24 years (IQR 13-42). Most patients had haemophilia (94%). A total of 25/26 patients with severe haemophilia, 3/12 with moderate haemophilia and 3/25 with mild haemophilia received prophylactic treatment.

Approximately half of the musculoskeletal episodes (55%) were traumatic. Most episodes involved joints (79%), followed by muscles (14%). The majority of joint episodes (42/61, 69%) occurred in joints without signs of haemophilic arthropathy (HJHS=0/HEADUS cartilage and bone sum score=0/Pettersson=0). In 40/61 joint episodes, a joint-specific HJHS score was available from the patient records, with a median 1 year (IQR 0-2) between the HJHS score and study inclusion. The median HJHS joint score for these joints was 0 (IQR 0-1). Osteochondral status in 19 of the 21 remaining joints could be estimated retrospectively from ultrasound (n=16) or previous x-rays (n=3). The median sum of the HEADUS cartilage and bone score was 0 (IQR 0-1). X-rays showed 2 "healthy joints" (Pettersson score 0) and 1 joint with haemophilic arthropathy (Pettersson score 11). For only 2 joints, HJHS scores were not available and osteochondral status could not be estimated retrospectively.

Table 1. Patient and episode characteristics

	Median or n	IQR or %
A) Patients	**(n=67)**	
Age (years)	24	13-42
Male	66	99%
Disease		
Haemophilia A	57	85%
Haemophilia B	6	9%
Von Willebrand Disease	4	6%
Haemophilia severity		
Severe	26	39%
Moderate	12	18%
Mild	25	37%
Prophylactic treatment†	31	46%
Age at start prophylaxis	3.6	1.7-11.6
Emicizumab	11	16%
Positive inhibitor status	2	3%
Bleeding episodes ≤ 1 year	1	1-3
B) Episodes	**(n=77)**	
Extremity		
Upper	15	19%
Lower	62	81%
Location		
Joint	61	79%
Muscle	11	14%
Other	5	6%
Cause		
Trauma	42	55%
Overexertion	13	17%
Unknown	22	29%

% might not add up to 100% due to rounding, † including one patient with two episodes who received no demand treatment when included with the first episode and received prophylaxis when included with the second episode

Clinical impression

The clinical symptoms that patients presented with during the 77 acute musculoskeletal episodes are summarized in Supplementary Table 1. Pain (99%), painful AROM (90%), limited AROM (82%) and swelling (82%) were the most common symptoms. In addition, weight bearing was painful in almost all lower limb episodes (59/62, 95%). Based on the clinical symptoms only, joint bleeding was suspected in 37 episodes, muscle bleeding in 13 episodes, arthropathy in 4 episodes, synovitis in 1 episode, and other diagnoses were suspected in 22 episodes. A detailed list of suspected diagnoses before ultrasound based on the clinical impression is available in Table 2.

Table 2. Diagnoses before and after ultrasound assessment (n=77)

	Before ultrasound n (%)	**After ultrasound n (%)**
Joint bleed	37 (48%)	33 (43%)
Muscle bleed	13 (17%)	11 (14%)
Arthropathy	4 (5%)	2 (3%)
Synovitis	1 (1%)	1 (1%)
Muscle, tendon, ligament injuries	16 (21%)	20 (26%)
Distortion	*15 (19%)*	*18 (23%)*
Tendinopathy/tendinitis	*1 (1%)*	*1 (1%)*
Muscle tear/strain	*0 (0%)*	*1 (1%)*
Other	6 (8%)	10 (13%)
Subcutaneous bleed	*3 (4%)*	*4 (5%)*
Trauma without bleeding	*1 (1%)*	*2 (3%)*
Overexertion without anatomical damage	*1 (1%)*	*2 (3%)*
Sinus tarsi syndrome	*1 (1%)*	*0 (0%)*
Thrombophlebitis	*0 (0%)*	*1 (1%)*
No diagnosis	*0 (0%)*	*1 (1%)¶*

% might not add up to 100% due to rounding; ¶ Unclear diagnosis based on ultrasound, either synovitis or arthropathy

Ultrasound findings

Ultrasound findings are summarized in Supplementary Table 2. Effusion on ultrasound was present in 44/77 episodes. Synovial hypertrophy was observed in 9 joints and in 6 of them synovial hyperaemia was visible with Power Doppler. Ultrasound findings confirmed clinically suspected joint bleeding in 24 episodes. An additional 9 episodes were reclassified as joint bleed based on ultrasound findings. Ultrasound findings confirmed clinically suspected muscle bleeding in 9 episodes and diagnosed 2 additional muscle bleeds. Furthermore, after the ultrasound two cases of arthropathy related pain, one case of synovitis, 20 muscle/tendon/ligament injuries and 10 other diagnoses were established. A detailed list of final diagnoses after ultrasound is available in Table 2.

Changes in diagnosis

The diagnoses before and after ultrasound assessment are visualized in Figure 2 and summarized in Table 2. The ultrasound findings changed the diagnosis in 28/77 musculoskeletal episodes (36%; CI 26-48%). Therefore, the NNS for a change in diagnosis was 3 musculoskeletal episodes. Based on the clinical impression alone, 9 musculoskeletal bleeds would have been missed and in 2 episodes the type of bleeding would have been misclassified. In addition, 15 episodes would have been incorrectly treated as musculoskeletal bleeding. Misdiagnosis occurred with a similar prevalence in healthy joints (38%; CI 24-54%) versus arthropathic joints (29%; CI 10-56%, p=0.76) and children (47%; CI 30-65%) versus adults (28%; CI 15-44%, p=0.10). Patients contributing multiple

episodes had little effect on the results, as the proportion of misdiagnoses was similar (34%; CI 23-47%) when only the first episode of each patient was analysed. Figure 3 shows ultrasound images from two example cases where ultrasound findings contributed to a change in diagnosis.

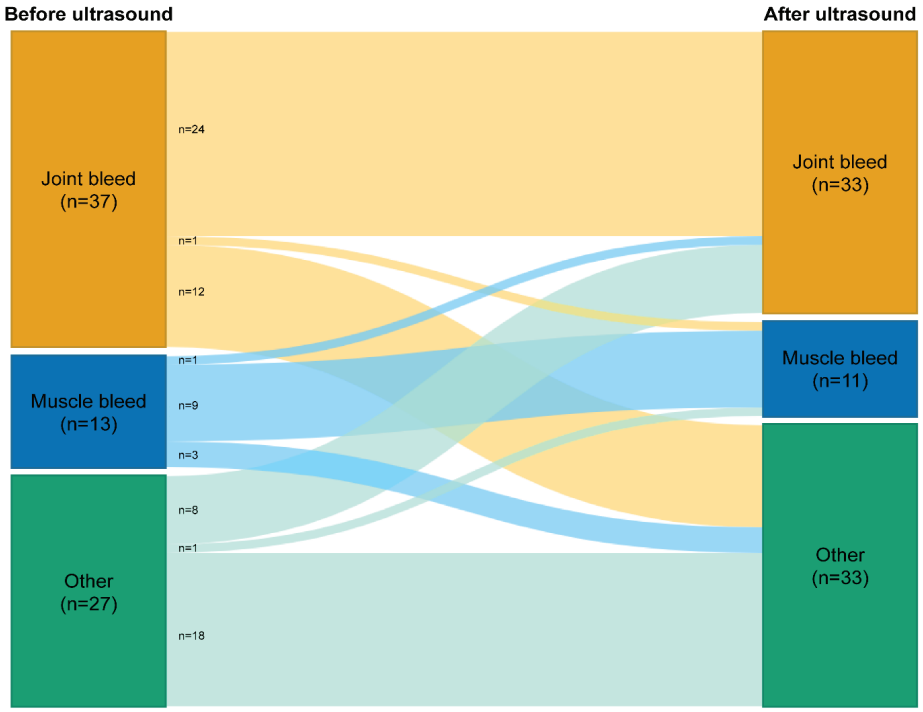

Figure 2. Sankey diagram visualizing the change in diagnoses before and after ultrasound assessment
Ultrasound findings changed the diagnosis in 28/77 episodes (36%; CI 26-48%).

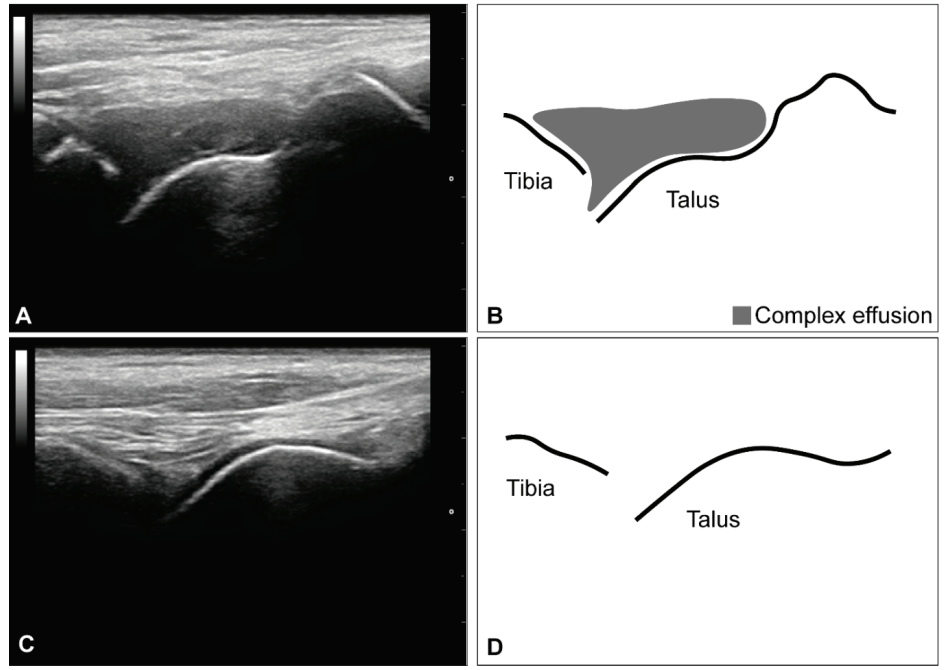

Figure 3. Midsagittal ultrasound images of the tibiotalar joint from two example cases illustrating discrepancies between clinical impression and ultrasound findings.
Case 1: A traumatic painful episode of the left ankle (HJHS=1) in a 10-year-old boy with severe haemophilia A on prophylaxis, suspected of ligament injury based on clinical impression. After ultrasound, the final diagnosis was joint bleeding. The patient presented with constant diffuse pressing pain on weightbearing and active range of motion (AROM). Despite the pain, AROM and gait were normal. Pain decreased after clotting factor concentrate. Patient had no pain at rest or during sleep. The ankle was not swollen and had a normal colour and temperature. A) Ultrasound showed complex joint effusion. B) Graphic representation of the anatomical bony landmarks (distal tibia and talus) and the complex effusion on the ultrasound image in A. Case 2: A traumatic painful episode of the left ankle (HJHS=0) in a 15-year-old boy with moderate haemophilia A treated on demand, suspected of joint bleeding based on clinical impression. After ultrasound, the final diagnosis was ligament injury. The patient presented with localized stabbing pain on weightbearing and AROM. AROM was limited and gait was asymmetric. Pain decreased with motion and after clotting factor concentrate. Patient had no pain at rest or during sleep. The ankle was diffusely swollen with normal colour and temperature. C) Ultrasound showed no joint effusion. D) Graphic representation of the anatomical bony landmarks (distal tibia and talus) on the ultrasound image in C.

Change in treatment

Changes in treatment plans after ultrasound are visualized in Figure 4 and summarized in Supplementary Table 3. The ultrasound findings led to treatment alterations in 30/77 episodes (39%; CI 28-51%). This corresponded to a number needed to scan of 3 for any type of treatment alteration. The dose and/or duration of the factor replacement therapy was adjusted in 27/77 episodes (35%; CI 25-47%). The total amount of IU factor prescribed decreased in 16 episodes and increased in 11 episodes. The net effect was a reduction (total: -96879 IU) of the total amount of IU factor prescribed in the study population. The NNS for a change in dose and/or duration of

factor replacement therapy was 3. Furthermore, changes in follow-up (28/77; 36%, CI 26-48%) and mobilisation advice (25/77; 32%, CI 22-44) were the most common. Ultrasound findings led to more restrictive mobilisation advice in 15 episodes and more liberal mobilisation advice in 10 episodes. Based on the ultrasound findings, prescription of ant-inflammatory medication changed in 5 episodes: 1 refrain from prescription and 4 extra prescriptions. Referral to a primary care physiotherapist was thought to be unnecessary in 3 episodes. On the contrary, additional referrals to a primary care physiotherapist were made in 2 episodes. In 8 episodes, patients were referred to the radiology department for an x-ray to rule out fracture (n=3) or arthropathy (n=1); an additional diagnostic ultrasound (n=3); both an additional diagnostic ultrasound and an x-ray to rule out fracture (n=1). Changes in treatment plans occurred with a similar prevalence in healthy joints (38%; CI 24-54%) versus arthropathic joints (41%; CI 18-67%, p=1) and children (47%; CI 30-65%) versus adults (33%; CI 19-49%, p=0.24). Patients with multiple episodes had little effect on the results, as the proportion of changes in treatment (37%; CI 26-50%) and factor replacement therapy (33%; CI 22-45%) after ultrasound was similar when only the first episode of each patient was analysed.

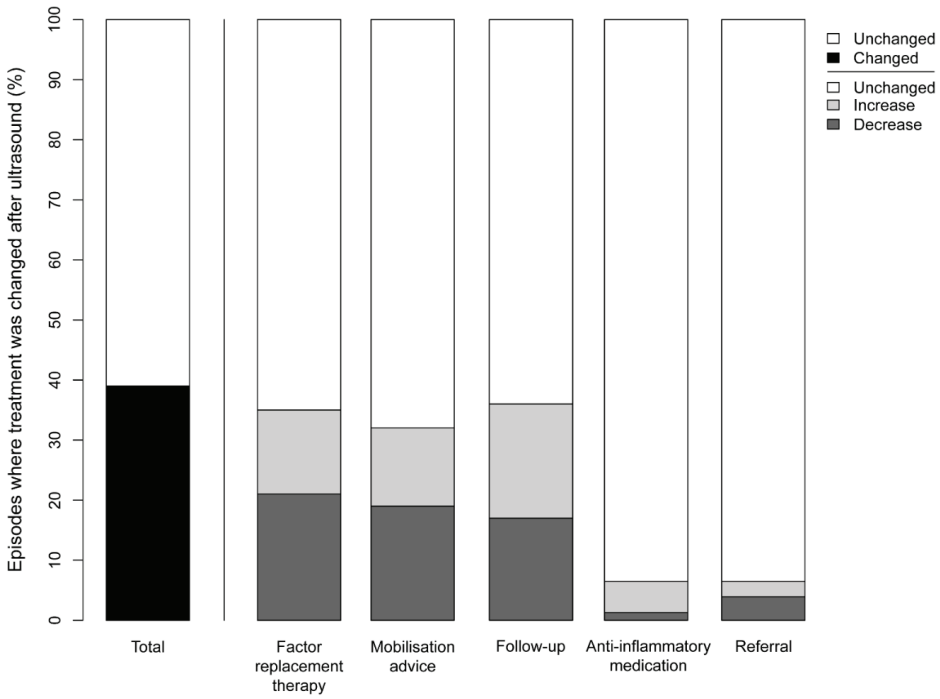

Figure 4. Stacked bar chart visualizing changes in treatment plans after ultrasound assessment.

Clinical symptoms for identifying joint bleeding

The prevalence of clinical symptoms in joint episodes (n=61) according to the presence or absence of ultrasound confirmed joint bleeding is summarized in Table 3. The sample size of our study did not allow detailed analyses of the predictive value of clinical symptoms for joint bleeding. However, pre-existing haemophilic arthropathy seemed to be associated with joint bleeding. Besides pre-existing haemophilic arthropathy, increasing pain intensity and diffuse pain seemed to have the highest PPV for joint bleeding. The NPV was low for all three clinical signs. Traditional symptoms associated with joint bleeding, such as the presence of pain, limitation of AROM, and improvement of symptoms after factor concentrate treatment, were not associated with joint bleeding.

Table 3. Clinical symptoms in joint episodes with and without confirmed intra-articular bleeding.

	Joint bleed (n=33)	No joint bleed (28)	PPV (CI)	NPV (CI)
Haemophilic arthropathy*	13 (39%)	4 (14%)	0.76 (0.56-97)	0.48 (0.33-0.63)
Cause				
Trauma	12 (36%)	17 (61%)		
Overexertion	8 (24%)	3 (11%)		
Unknown	13 (39%)	8 (29%)		
Pain	33 (100%)	27 (96%)		
Pain localisation				
Local	*13 (39%)*	*21 (75%)*		
Diffuse	*18 (55%)*	*4 (14%)*	*0.82 (0.66-0.98)*	*0.38 (0.22-0.55)*
n.a.	*2 (6%)*	*2 (7%)*		
Type of pain				
Stabbing	*8 (24%)*	*10 (36%)*		
Pressing	*15 (46%)*	*5 (18%)*		
Other	*3 (9%)*	*2 (7%)*		
n.a.	*7 (21%)*	*10 (36%)*		
Pain in rest	17 (52%)	10 (36%)		
Sleep disrupted by pain§	12 (41%)	3 (13%)		
Painful weightbearing¶	23 (100%)	24 (89%)		
Painful AROM	30 (91%)	23 (82%)		
Pain decreased after FVIII treatment†	14 (61%)	9 (53%)		
Course of pain				
Constant	*5 (15%)*	*9 (32%)*		
Increasing	*26 (79%)*	*11 (39%)*	*0.70 (0.56-0.85)*	*0.29 (0.11-0.47)*
Increasing with motion	*0 (0%)*	*4 (14%)*		
Start pain (decreasing with motion)	*2 (6%)*	*4 (14%)*		
AROM limitation	27 (82%)	20 (71%)		
Warmth	22 (67%)	12 (43%)		
Swelling	31 (94%)	19 (68%)		
Local	10 (30%)	11 (39%)		
Diffuse	21 (68%)	8 (42%)		
Discoloration	4 (12%)	4 (14%)		
Red	0 (0%)	2 (7%)		
Blue	4 (12%)	2 (7%)		

Table 3. Clinical symptoms in joint episodes with and without confirmed intra-articular bleeding. (continued)

	Joint bleed (n=33)	No joint bleed (28)	PPV (CI)	NPV (CI)
Gait[¶]				
No weightbearing	6 (26%)	5 (19%)		
Asymmetric	12 (52%)	15 (56%)		
Limited stability	1 (4%)	2 (7%)		
No abnormalities	4 (17%)	5 (19%)		

*% might not add up to 100% due to rounding, *HJHS/HEAD-US cartilage and bone sum score/Pettersson score>0, § Applicable to 52/61 joint episodes as in 9 episodes patients were assessed on the day of symptom onset, ¶ Only applicable to knee and ankle episodes (n=50), † Applicable to 40/61 joint episodes as patients received factor replacement therapy prior to assessment in only 40/61 episodes, PPV: positive predictive value, NPV: negative predictive value, CI: 95% confidence interval, AROM: active range of motion*

DISCUSSION

In this prospective cross-sectional study, we evaluated the impact of ultrasound in addition to clinical assessment on the diagnosis and treatment of acute musculoskeletal episodes in children and adults with haemophilia and VWD, including joints with (29%) and without (67%) pre-existing haemophilic arthropathy. Ultrasound findings impacted the diagnosis in 28/77 acute musculoskeletal episodes (36%, CI 26-48%) and impacted treatment in 30/77 (39%; CI 28-51%). This means that 3 patients need to be scanned by ultrasound in order to change either the diagnosis or the treatment of one patient (NNS= 3). Clinical symptoms evaluated in the current study were not able to identify joint bleeding.

Strengths and limitations

A strength of the current study is the relatively large heterogeneous study population. The mix of age, disease type and severity, and joint status provides a good representation of the daily variance in patients presenting with acute musculoskeletal complaints. Therefore, the results of our study provide a good impression of the impact of ultrasound in the daily practice of a haemophilia treatment center. Another strength of the study is the structured prospective data collection through the use of a CRF. This ensured data quality and reduced missing values. In addition, the structured collection of clinical symptoms on the CRF provided insight into the clinical decision-making process and ensured that clinicians made a well-grounded clinical diagnosis. Lastly, the ultrasound assessments were performed by two trained and experienced physiotherapists, which increased the accuracy of the ultrasound findings and reduced interobserver variability.

A limitation of the study is that only patients who visited the outpatient clinic with an acute musculoskeletal complaint were included. Therefore, patients without home treatment or home-treated patients who were unsure whether or not a bleeding event had occurred may be overrepresented in our study. The impact we observed is therefore representative of outpatient clinics in Western Europe, where self-infusion is common. Our results may not be fully generalisable

to the home setting or to countries where self-infusion is not common. The study design has another limitation. The clinical assessment was always followed by an ultrasound, after which the final diagnosis and treatment were determined. This allowed the ultrasound assessment to act as a 'safety net'. In cases of doubt based on clinical assessment, clinicians may have been more confident in their clinical diagnosis and treatment plan, because they were aware that the final diagnosis and treatment was made after ultrasound. Our results may therefore underestimate the impact of ultrasound as clinicians when unsure in their clinical diagnoses are more likely to treat the episode as a bleed.

Comparison to previous literature

Three previous studies have investigated the value of ultrasound in diagnosis (and treatment) of acute painful musculoskeletal episodes in adults with haemophilia[14,24,25]. These reported changes in diagnosis after ultrasound in 16% (CI 6-32%) to 65% (CI 48-79%) of episodes, which is similar to the proportion of changed diagnoses in our study (36%; CI 26-48%). Two of the previous studies reported changes in treatment after ultrasound in 16% (CI 6-32%) and 73% (CI 56-85%) [14,24]. The proportion of treatment changes in our study (39%; CI 28-51%) was similar or lower.

Differences between our estimates and those of previous studies cannot be explained by the type of patients included. Previous studies investigated only musculoskeletal episodes in adults with haemophilia, including a large number of joints with pre-existing haemophilic arthropathy. Our study included a large number of children (44% of episodes), and most joints were without haemophilic arthropathy. However, the prevalence of changes in diagnosis and treatment in the subgroup of adults and/or joints with haemophilia arthropathy was similar to our overall population.

Still, differences between our estimates and the previous results may be due to the smaller sample sizes of the previous studies (37-42 episodes) compare to our 77 episodes. Furthermore, two studies included only patient-perceived haemarthroses[24,25], while our study and the study by Ceponis et al.[14] included all types of painful acute musculoskeletal episodes. Other differences were the time intervals between symptom onset and inclusion (previous studies <72h; current study <7 days) and the diagnostic outcomes used. The previous studies differentiated between bleeding and non-bleeding episodes. In our study, we divided the non-bleeding episodes into different diagnoses, which allowed for changes in diagnosis within the non-bleeding group. However, if we had only differentiated between bleeding and non-bleeding episodes, our results would not have been significantly different (diagnosis change in 24/77 episodes; 31% CI 21-43%).

Clinical relevance and future research

Our results showed that discrepancies between clinical diagnosis and ultrasound findings were common in a heterogeneous group of patients presenting with acute musculoskeletal complaints. These discrepancies demonstrate that ultrasound impacts diagnosis and treatment in patients with and without pre-existing arthropathy. Ultrasound can improve the differentiation between joint bleeding and arthropathy-related complaints, and between joint bleeding and non-haemophilia-related musculoskeletal complaints.

In addition to the previously reported good accuracy compared to magnetic resonance imaging and reproducibility of ultrasound for musculoskeletal assessment in haemophilia[18–21], we now show an impact of ultrasound on the management of acute musculoskeletal episodes. Ultrasound is non-invasive, relatively inexpensive and can be performed quickly by trained clinicians. We therefore encourage the use of ultrasound in addition to clinical assessment for the management of acute musculoskeletal complaints in people with bleeding disorders. Ultrasound may easily be incorporated into the standard diagnostic work-up for patients presenting to the outpatient clinic with an acute musculoskeletal complaint. In addition, patients could be encouraged to attend the clinic in case of a musculoskeletal complaint. Interestingly, there are several initiatives investigating the possibility of remote (artificial intelligence-assisted) ultrasound by patients at home or by non-haemophilia health professionals close to home[24,35–39]. In addition to increasing the accessibility of ultrasound, future research should focus on the (long-term) impact and cost-effectiveness of ultrasound-guided management of acute musculoskeletal complaints in people with bleeding disorders. To our knowledge, there is only limited research on the short-term effects of ultrasound-guided treatment[14,24].

CONCLUSION

Frequent discrepancies between clinical diagnosis and ultrasound findings were observed in acute musculoskeletal episodes in a heterogeneous cohort of children and adults with haemophilia and VWD with and without haemophilic arthropathy. Ultrasound findings in addition to clinical assessment impacted diagnosis in 36% and treatment plans in 39% of episodes, which corresponds to a number needed to scan of 3 for a change in diagnosis and/or treatment. Our results show that it is difficult to correctly diagnose an acute musculoskeletal episode based on clinical assessment in both joints with and without haemophilic arthropathy. We therefore encourage implementation of ultrasound in the management of acute musculoskeletal complaints in haemophilia and VWD patients.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Clinical symptoms (n=77)

	n	%
Pain	76	99%
Pain localisation		
Local	*45*	*58%*
Diffuse	*27*	*35%*
n.a.	*4*	*5%*
Type of pain		
Stabbing	*23*	*30%*
Pressing	*29*	*38%*
Other	*5*	*6%*
n.a.	*19*	*25%*
Pain at rest	38	49%
Sleep disrupted by pain§	21	27%
Painful weightbearing¶	59	95%
Painful AROM	69	90%
Pain decreased after factor replacement therapy†	25	32%
Course of pain		
Constant	*16*	*21%*
Increasing	*51*	*66%*
Increasing with motion	*4*	*5%*
Start pain (decreasing with motion)	*6*	*8%*
AROM limitation	63	82%
Warmth	44	57%
Swelling	63	82%
Local	*29*	*38%*
Diffuse	*34*	*44%*
Discoloration	15	19%
Red	*2*	*3%*
Blue	*13*	*17%*
Gait¶		
No weightbearing	*13*	*21%*
Asymmetric	*37*	*60%*
Limited stability	*3*	*5%*
No abnormalities	*9*	*15%*

% might not add up to 100% due to rounding, § Applicable to 68/77 episodes as in 9 episodes patients were assessed on the day of symptom onset, ¶ Only applicable to lower extremity episodes (n=62), †Applicable to 49/77 episodes as patients received factor replacement therapy prior to assessment in only 49/77 episodes, AROM: active range of motion

Supplementary Table 2. Ultrasound findings (n=77)

	n	%
Effusion/fluid collection (n=77)	44	57%
Minimal/moderate	*10*	*13%*
Large	*34*	*44%*
Synovial hypertrophy (n=70)¶	9	13%
Mild/moderate	*8*	*11%*
Severe	*1*	*1%*
Synovial hyperaemia (n=68)¶†	6	9%
Small spots	*5*	*7%*
Confluent vessel in <50% tissue of interest	*1*	*1%*

% might not add up to 100% due to rounding; ¶ Available for joint ultrasound only (n=70/77); †missing for 2 joints.

Supplementary Table 3. Treatment before and after ultrasound assessment (n=77)

	Before ultrasound Median/n (IQR/%)	After ultrasound Median/n (IQR/%)	Change n (%)
Factor replacement therapy	58 (75%)	50 (65%)	27 (35%)
Total dose (IU)	4000 (500-10000)	3000 (0-8000)	
Duration (days)	3 (1-4)	2 (0-4)	
Anti-inflammatory treatment	6 (8%)	8 (10%)	5 (6%)
Mobilisation			25 (32%)
Unload	*33 (43%)*	*26 (34%)*	
Limited loading	*18 (23%)*	*19 (25%)*	
limit intensive loading	*5 (6%)*	*10 (13%)*	
Mobilisation guided by pain	*19 (25%)*	*17 (22%)*	
No mobilisation restriction	*2 (3%)*	*5 (6%)*	
Referral			5 (6%)
Physiotherapist p.c.	*12 (16%)*	*10 (13%)*	
Physiotherapist p.c. + orthopaedic surgeon	*0 (0%)*	*1 (1%)*	
Follow-up	49 (64%)	46 (60%)	28 (36%)
Follow-up interval (days)	7 (7-7)	*7 (4-10)*	

% might not add up to 100% due to rounding; Physiotherapist p.c.: referral to a primary care physiotherapist

CHAPTER 8

Monitoring recovery of joints after bleeding: physical examination and ultrasound are complementary

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ABSTRACT

Aim

Traditionally, recovery after a joint bleed in people with bleeding disorders is evaluated by clinical symptoms. Following a bleed, however, asymptomatic joints may still show synovial hypertrophy and effusion on ultrasound. We evaluated the duration of full recovery from a joint bleed. Additionally, we determined how recovery differed when assessed by physical examination and ultrasound.

Methods

In this retrospective cohort study, we investigated joint bleeds in elbows, knees and ankles of people with haemophilia or Von Willebrand disease who attended the Van Creveldkliniek between 2016 and 2021. Physical examination (warmth, swelling, range of motion and gait) and ultrasound (effusion and synovial hypertrophy) were performed within 7 days after the onset of the bleed, 1 week after the first examination and monthly thereafter until patients had recovered fully. Joint bleeds were treated in line with the current international treatment guidelines.

Results

We evaluated 30 joint bleeds in 26 patients. The median recovery time was 1 month (range 0.3-5 months). In 47% of the joint bleeds, the recovery took longer than 1 month. The moment of recovery based on physical examination and ultrasound differed in 27% of bleeds. Both persistent abnormalities at physical examination in joints with normalized ultrasounds and persistent ultrasound findings in clinically recovered joints occurred.

Conclusion

Joint bleed recovery can take long and recovery times differed per bleed. Recovery differed when assessed by physical examination or ultrasound. Therefore, both should be used to closely monitor recovery of joint bleeds and offer personalized care.

INTRODUCTION

People with haemophilia have a (functional) deficiency in clotting factor VIII or IX resulting in an increased bleeding tendency. Joint bleeds account for up to 80% of all bleeds[1]. People with severe haemophilia still have approximately one joint bleed per year despite prophylactic clotting factor replacement therapy[2]. In people with von Willebrand disease (VWD), the (functional) deficiency in von Willebrand factor also results in joint bleeds, albeit less frequently[3]. Joint bleeds lead to both acute and long-term pain and disability through subsequent joint damage[1, 4, 5]. In the acute phase, the intra-articular blood induces chondrocyte apoptosis and triggers synovial inflammation. Concomitant synovial hypertrophy and neo-angiogenesis increases susceptibility to re-bleeding. In the long-term, synovial inflammation damages the cartilage and eventually the underlying bone, resulting in haemophilic arthropathy[4, 5].

The treatment of joint bleeds aims to stop the bleed, to prevent re-bleeding and development of synovitis, and to regain physical functioning[6, 7]. Treatment consists of clotting factor replacement therapy and (partial) immobilization of the joint, followed by functional rehabilitation. Inadequate treatment may result in persistent synovial hypertrophy, which is a risk for re-bleeding and chronic synovitis[6, 8].

Traditionally, the treatment effect and start of the physical rehabilitation are based on clinical symptoms, including pain, swelling, warmth and functioning of the joint. Treatment with clotting factor is advised until the bleed has stopped and clinical symptoms decline. However, current international treatment guidelines do not advise on specific follow-up intervals after a joint bleed[6, 7]. Joint bleed recovery is often not routinely monitored, since most joint bleeds are home-treated. Furthermore, clinical symptoms do not always adequately represent the current status of the joint[9, 10]. Clinical evaluation can be complemented with ultrasound assessment. Ultrasound can accurately assess the synovium, joint effusion, cartilage and joint bleeds[11-15]. In addition, ultrasound can detect synovial hypertrophy in joints without clinical symptoms[16]. Ultrasound is therefore recommended as an additional tool for diagnosing early joint bleeds and monitoring synovitis[6].

The role of ultrasound in monitoring joint bleed recovery is not well established yet. Two studies followed-up joint bleeds with physical examination (PE) and ultrasound. The first study reported a mean of 13 days for range of motion to recover, while ultrasound findings resolved after a mean of 20 days[17]. The second study reported that painless joints still showed synovial hypertrophy and effusion on ultrasound a week after onset of the bleed[18]. Still, it remains unknown how often subclinical findings on ultrasound occur after a joint bleed and how long it takes for joint bleeds to recover fully.

The aim of this cohort study was twofold. First, we evaluated how long it took for joint bleeds to recover fully. Second, we compared PE and ultrasound findings after a joint bleed to estimate the added value of ultrasound for monitoring joint bleed recovery.

METHODS

Study design and study population

In this retrospective cohort study, we followed-up joint bleeds in people with haemophilia or Von Willebrand disease (VWD) who attended the Van Creveldkliniek between April 2016 and April 2021. Patients were included if they had a joint bleed in an ankle, knee or elbow, confirmed by ultrasound. A joint bleed was confirmed by ultrasound when complex intra-articular joint effusion was observed. We only included joint bleeds if patients were examined within 7 days of onset of complaints, and if follow-up was available until full recovery. Patients could be included multiple times with distinct bleeds.

According to the local clinic's protocol, joint bleed recovery was followed-up with both PE and ultrasound examination. Visits were scheduled within 7 days after the onset of the bleed, 1 week after the first examination and monthly thereafter until patients had recovered fully. The study was approved by the institutional Medical Research Ethics Committee (19-665/C).

Treatment of joint bleeds

Joint bleeds were treated in line with the World Federation of Haemophilia (WFH) guidelines for management of haemophilia[6, 19]. Patients received factor replacement therapy and/or Desmopressin to achieve target peak factor VIII or IX levels of 60% for at least two consecutive days. Subsequently, treatment was adjusted based on clinical signs and ultrasound findings. At the start of treatment, patients were advised to (partially) immobilise the affected joint, then they followed rehabilitation to regain pre-bleed functionality. According to standard care, anti-inflammatory medication was administered in case of persistent synovial hypertrophy[6].

Assessment of joint bleed recovery

Joint bleed recovery was assessed by PE followed by ultrasound examination. Swelling, active range of motion and gait were reported according to the Haemophilia Joint Health Score (HJHS) version 2.1[20]. Warmth of the joint was reported as 'present' or 'absent'. Effusion and synovial hypertrophy were assessed and reported according to the Haemophilia Early Arthropathy Detection with UltraSound (HEAD-US) protocol[21]. All examinations were performed using a single ultrasound scanner (Esaote, MyLab 25 Gold, Genova, Italy) with a 7.5–12 MHz linear transducer. PE and ultrasound were performed by a physiotherapist (MT) or paediatric haematologist (KF), both trained and experienced in using the HJHS and HEAD-US protocol.

Outcomes

The primary outcome measure was time to full recovery of the joint bleed. Full recovery was defined as normalisation of all clinical findings (joint swelling, joint warmth, active range of motion, gait) and ultrasound findings (joint effusion and synovial hypertrophy). In joints with pre-existing abnormalities established during previous routine clinical and ultrasound assessment, normalisation to the pre-bleed joint status was considered as full recovery. The secondary outcome was the difference in time to recovery between PE and ultrasound. Full recovery as determined by

PE was defined as return to the pre-bleed status of all clinical findings. Full recovery as determined by ultrasound was defined as return to the pre-bleed status of all ultrasound findings.

Data extraction

Extracted patient characteristics from the electronic patient records included bleeding disorder and severity, age, inhibitor status and treatment regimen (prophylaxis or on demand). Joint status prior to the bleed was established by the number of lifetime joint bleeds in the affected joint and the clinical and radiological joint status based on the last reported joint specific HJHS (range 0–20), HEAD-US score (range 0–8) and/or Pettersson score (range 0–13)[22]. Joint status was defined as normal if all scores were 0, as minimal-mild haemophilic arthropathy if the scores were <1/3 of the maximum joint score (HJHS <7, HEAD-US <3 and/or Pettersson score <5), or as moderate-severe haemophilic arthropathy if the scores were ≥1/3 of the maximum joint score (HJHS≥7, HEAD-US≥3 and/or Pettersson score was ≥5). When multiple scores were available, the worst score prevailed for grading the severity of haemophilic arthropathy. For each joint bleed, location, cause of bleeding (trauma or unknown), period of bleed-related (altered) clotting factor replacement therapy in days, duration of anti-inflammatory treatment in weeks and physical therapy interventions used (immobilisation, exercise therapy and/or coaching regarding physical activities) were documented. Initial clotting factor replacement therapy was defined as the period in days in which treatment was altered compared to the baseline treatment regimen. Treatment alterations after initial bleed treatment were defined as temporary intensified prophylaxis (intensified dose or frequency of prophylaxis for weeks-months), permanent intensified prophylaxis and switches of regimen or product. During follow-up clinical and ultrasound outcomes were collected at each visit.

Analysis

Patient and joint bleed characteristics were reported as medians with ranges, or frequencies with percentages. Time to full recovery of clinical and ultrasound abnormalities was assessed for all bleeding episodes, and were reported as median duration in months with ranges. time to full recovery was summarized in a cumulative incidence curve. The Wilcoxon signed rank test was used to compare recovery as assessed by PE or ultrasound. To investigate differences in recovery as assessed by PE or ultrasound according to recovery time, joint bleeds were divided into three groups based on time to full recovery: recovery <2 weeks, 2 weeks–1 month and >1month. To investigate the influence of disease severity, bleeding cause and joint type, recovery times were compared between people with severe and non-severe haemophilia, between traumatic bleeds and bleeds with unknown cause, and between elbows, knees and ankles, using the Mann-Whitney U test or Kruskal Wallis test. p-values of < .05 were considered significant. We created heat maps to search for patterns in the recovery of individual parameters of the PE and ultrasound. All analyses were performed using RStudio (version 1.3.1093).

RESULTS

Patient and joint characteristics

Patient and joint characteristics are summarized in Table 1. We identified 30 bleeding episodes in 28 joints of 26 patients who were followed according to our local protocol. Two patients were included twice with two distinct bleeding episodes in one ankle, one patient was included with one bleeding episode in each knee, and one patient was included with an ankle bleed and an elbow bleed. The cohort included 7 adults and the median age at the time of bleeding was 13.8 years (range 2.6–43.1). Half of the patients had mild haemophilia (n = 13, 50%), 3 had moderate haemophilia (12%) and 10 had severe haemophilia (38%). All patients with severe haemophilia and one with moderate haemophilia were on prophylaxis.

Overall, joint health status prior to the bleed was good: 14 joints (50%) were healthy without abnormalities prior to the bleed and 3 (11%) had minimal to mild haemophilic arthropathy. For 11 joints (39%), no information regarding the joint status prior to the bleed was available. However, pre-existent joint damage was not expected because these joints recovered without residual abnormalities.

Table 1. Patient and joint characteristics

	Median or n	Range or %
A) Patient characteristics (n= 26)		
Age (years)	13.8	2.6-43.1
Disease		
Haemophilia A	22	85%
Haemophilia B	3	12%
Von Willebrand Disease	1	4%
Haemophilia severity		
Severe	10	38%
Moderate	2	8%
Mild	13	50%
Prophylactic treatment	11	42%
Positive inhibitor status	1	4%
B) Joint characteristics of affected joints (n= 28)		
Baseline joint status[$]		
Normal	14	50%
Minimal – mild HA	3	11%
Moderate – severe HA	0	0%
Not available[¶]	11	39%
Lifetime joint bleeds[$]	0	0-4

% might not add up to 100% due to rounding, HA: Haemophilic arthropathy;
Normal: joint-specific score HJHS=0/HEAD-US=0/Pettersson score=0; Minimal-mild HA: joint-specific score HJHS<7/HEAD-US<3/Pettersson score<5; Moderate-severe HA: joint-specific score HJHS≥7/HEAD-US≥3/Pettersson score≥5; [$]*Characteristics on joint level of the joints affected by the joint bleeds (n=28)* [¶]*All mild haemophilia patients.*

Joint bleed and treatment characteristics

Characteristics of the joint bleeds and treatment are available in Table 2. Most joint bleeds occurred in ankles (n = 18, 60%), followed by knees (n = 9, 30%) and elbows (n = 3, 10%). Most bleeds had a traumatic origin (n = 22, 73%). In 8 joint bleeds, the bleed occurred spontaneously (27%).

Bleeds treated with clotting factor replacement therapy (n = 29/30) were treated over a median period of 6 days (range 2–29). The bleed in the patient with VWD was treated with Desmopressin for 2 days. In six bleeds, the initial bleed treatment was followed by temporary intensified prophylaxis (range 1 week to 5 months). After four bleeds, the treatment regimen was permanently altered: 1 patient switched from on demand treatment to prophylaxis, 1 patient switched from recombinant factor VIII to emicizumab prophylaxis, and for 2 patients their prophylaxis was intensified permanently. In nine bleeds, patients received anti-inflammatory treatment with Celecoxib for a median duration of 3.5 weeks (range 1–12). All patients (partly) immobilised the affected joint

(n = 30, 100%). The immobilisation was followed by coaching regarding physical activities in 28 bleeds (93%) and/or exercise therapy in 18 bleeds (60%).

Table 2. Characteristics of joint bleeds and treatment (n=30)

	Median or n	Range or %
A) Joint bleeds (n=30)		
Cause		
Trauma	22	73%
Unknown	8	27%
Joint		
Ankle	18	60%
Knee	9	30%
Elbow	3	10%
B) Treatment		
Period of initial clotting factor replacement therapy (days)[¶]	6	2-29
Anti-inflammatory treatment	9	30%
Anti-inflammatory treatment duration (weeks)	3.5	1-12
Physical therapy		
(Partial) immobilization	30	100%
Coaching on activities	28	93%
Exercise therapy	18	60%

[¶] *Period in days in which treatment was altered compared to the baseline treatment regimen, does not correspond to the number of days with administered factor concentrate.*

Joint bleed recovery

Duration of joint bleed recovery

The cumulative incidence of fully recovered joint bleeds over time is shown in Figure 1. Joint bleeds recovered in a median of 1 month (range 0.3–5 months). The recovery rate was 10% within 2 weeks, 53% after 1 month and 100% after 5 months. Recovery times were comparable (p = .48) when assessed by PE (median 1 month, range 0–5) or ultrasound (median 1 month, range 0.3-5). Duration of full recovery was similar (p = .37) in people with severe (median 2 months, range 1–5) and non-severe haemophilia (median 1 month, range 0.3–5). Within the current cohort, we found no difference in the duration of full recovery (p = .23) between traumatic bleeds (median 1.5 months, range 0.3–5) and bleeds of unknown cause (median 1 month, range 0.3–3), nor a difference in the duration of full recovery (p = .20) between elbows (median 1 month, range 1–1), knees (median 2 months, range 1–5) and ankles (median 1 month, range 0.3–5).

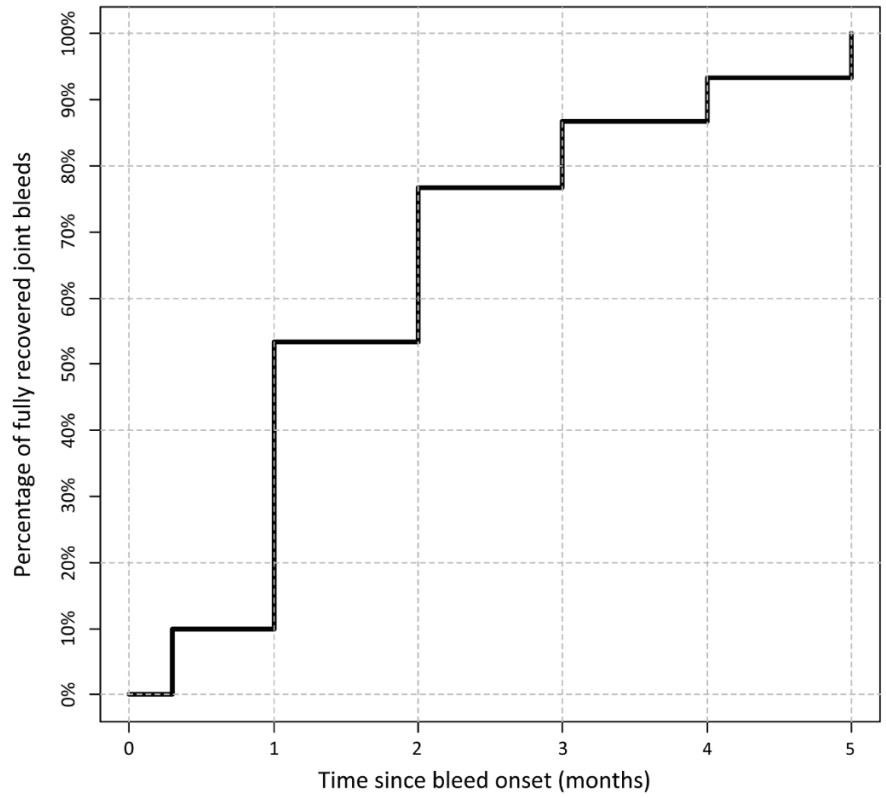
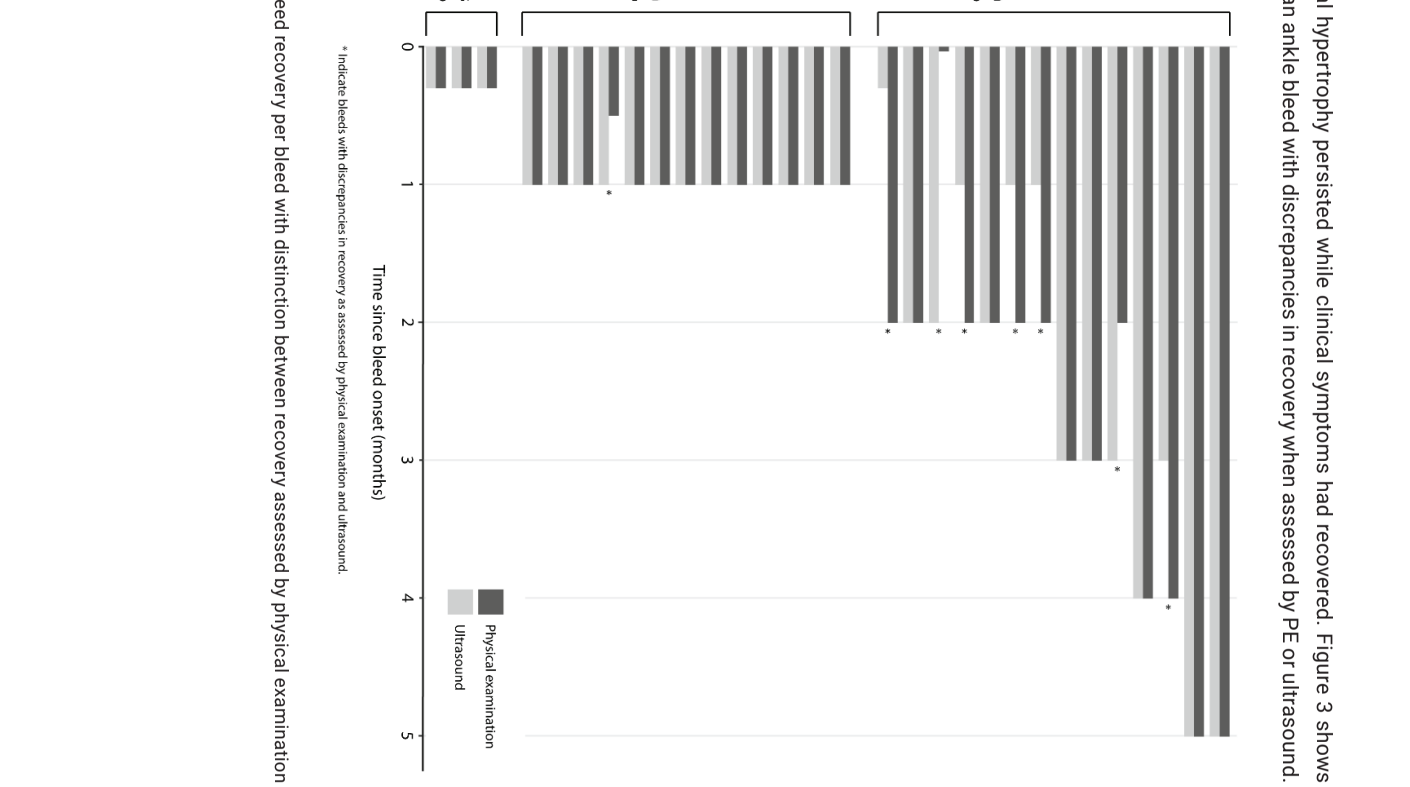


Figure 1. The cumulative incidence of full recovery of joint bleeds over time.

Discrepancies between PE and ultrasound

Recovery of all joint bleeds with distinction between recovery assessed by PE and ultrasound is shown in Figure 2. In the majority of joint bleeds (n = 22, 73%), the last clinical symptoms and the last abnormalities on ultrasound recovered simultaneously. In eight joint bleeds however, the last clinical symptoms and abnormalities on ultrasound recovered at different timepoints. An overview of the joint bleeds with discrepancies in recovery according to PE and ultrasound is available in Table 3. In the three bleeds with recovery within 2 weeks clinical symptoms and abnormalities on ultrasound recovered simultaneously. In 1/13 bleeds with recovery between 2 weeks and 1 month synovial hypertrophy on ultrasound persisted while clinical symptoms had already recovered. In 7/14 bleeds with recovery > 1 month, recovery differed when assessed by PE and ultrasound. Abnormalities on ultrasound recovered before clinical symptoms in five bleeds. In these bleeds, active range of motion and gait abnormalities persisted while abnormalities on ultrasound had recovered. Clinical symptoms recovered before abnormalities on ultrasound in two bleeds. In both

bleeds, synovial hypertrophy persisted while clinical symptoms had recovered. Figure 3 shows an example of an ankle bleed with discrepancies in recovery when assessed by PE or ultrasound.



* Indicate bleeds with discrepancies in recovery as assessed by physical examination and ultrasound.

Figure 2. Joint bleed recovery per bleed with distinction between recovery assessed by physical examination and ultrasound.

Table 3. Joint bleed episodes with discrepancies in clinical recovery and normalization of ultrasound findings.

Patient characteristics		Joint bleed characteristics							
		Joint		Bleeding episode			Recovery		
Age (years)	Haemophilia severity	Joint	Baseline	Cause	Initial clotting factor replacement therapy (days)[1]	Anti-inflammatory treatment (weeks)	Physical examination	Ultrasound	Parameters with delayed recovery
10	Severe	Ankle	HJHS 0 HEAD-US 0	Trauma	3[2]	-	No abnormalities	2 months	Effusion, Synovial hypertrophy
11	Severe	Ankle	HJHS 0	Trauma	2[4]	4	2 months	3 months	Synovial hypertrophy
2	Moderate	Elbow	n.a.	Unknown	5[3]	-	2 weeks[5]	1 month	Synovial hypertrophy
13	Mild	Ankle	n.a.	Unknown	6	3	2 months	1 month	Swelling, warmth, gait
16	Mild	Knee	n.a.	Trauma	10	1	2 months	1 week	AROM, gait
16	Mild	Knee	n.a.	Trauma	22	-	2 months[5]	1 month	AROM, gait[6]
6	Severe	Ankle	HJHS 0	Trauma	11	-	4 months	3 months	Gait
9	Severe	Ankle	HJHS 1	Trauma	6	-	2 months	1 month	Gait

HJHS = Haemophilia Joint Health Score; HEAD-US = Haemophilia Early Arthropathy Detection with Ultrasound score; n.a. = not available; AROM = Active range of motion; 1 = Total duration of the initial treatment, does not correspond with daily treatment; 2 = Patient switched to emicizumab prophylaxis after initial bleed treatment; 3 = Patient started prophylactic treatment after initial bleed treatment; 4 = Prophylaxis was intensified after one month based on findings during follow-up; 5 = Complete gait follow-up unavailable; 6 = Recovery of gait unavailable because of joint bleed in the other knee during follow-up.

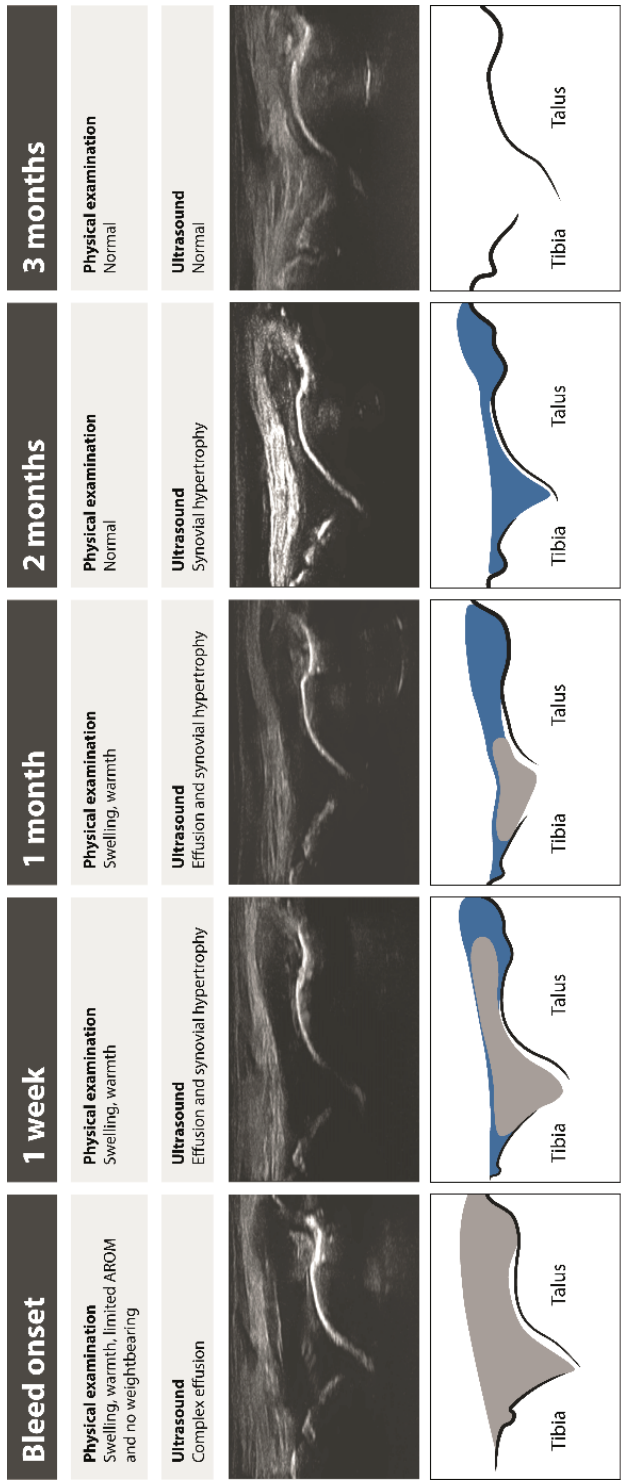


Figure 7. Recovery of an ankle bleed in a 11-year-old patient with severe haemophilia A.

Recovery of individual parameters

Median recovery times of individual parameters of the PE and ultrasound examination are available in Table S1. We did not find a consistent pattern in the order in which the individual parameters of the PE and ultrasound examination recovered. A heat map illustrating the recovery of the individual clinical and ultrasound parameters is available in Figure S1.

DISCUSSION

In this retrospective study, we evaluated the duration of recovery from a joint bleed in people with haemophilia or Von Willebrand disease. Furthermore, we determined the discrepancies between PE and ultrasound when assessing joint bleed recovery. In 47% of the 30 joint bleeds, recovery took longer than one month. In 10% of the bleeds synovial hypertrophy was detected by ultrasound examination in clinically recovered joints, and in 17% of the bleeds clinical abnormalities were detected when ultrasound abnormalities were resolved.

Compared to two previous studies[17, 18] we observed longer recovery times after joint bleeds and less discrepancies between PE and ultrasound. These differences may be explained by the use of different definitions for recovery. We defined clinical recovery as absence of warmth, swelling, active range of motion limitations and gait abnormalities, while Aznar et al.[18] defined clinical recovery as absence of pain. Due to our more detailed definition, clinical recovery usually took more than 1 week, which explains why we observed less subclinical synovial hypertrophy and/or effusion. However, the prevalence of synovial hypertrophy and/or effusion 1 week after bleed onset in our study (87%, 95% Confidence interval (CI) 69–96) was comparable to or slightly higher than the prevalence of subclinical synovitis and/or effusion in the study by Aznar et al. (60%, CI 26–88). We observed a longer median recovery time for ultrasound findings (median 1 month) compared to the study by De la Corte-Rodriguez et al.[17](mean 20 days), which may be due to different definitions as well. We used absence of effusion and synovial hypertrophy to mark ultrasound recovery, while they used absence of bloody effusion. Furthermore, differences in recovery times for range of motion and ultrasound findings between our study and the study by De la Corte-Rodriguez may be due to their weekly follow-up schedule compared to our monthly follow-up.

Strengths and limitations

A strength of our study is the standardised follow-up using validated assessment tools, despite the retrospective study design. The follow-up visits were planned at set timepoints, which makes comparison between different bleeds possible and kept missing values to a minimum. The assessments were performed by one haematologist and one physiotherapist. While interobserver variability was minimized by the use of the validated and standardised HJHS score and HEAD-US protocol[20, 23, 24].

A limitation of our study might be selection bias. Our study population included predominantly young patients and patients with mild haemophilia. In addition, most patients had a good baseline joint health status. Our selective population can be explained by our retrospective study design and

the organisation of haemophilia care in the Netherlands: young people with haemophilia and people with mild haemophilia are usually treated and monitored at the haemophilia care centre when they have a joint bleed. People with severe haemophilia receive home treatment for their bleeds and do not visit the clinic for each bleed[25]. We did not find significant differences in recovery time between people with severe and non-severe haemophilia. Furthermore, clinical symptoms and pathophysiology of joint bleeds can be considered similar between age groups. Hence, we expect that the results can still be generalized to populations with predominantly adults and people with severe haemophilia. However, clinical symptoms and ultrasound findings may differ between arthropathic joints and healthy joints[9]. Therefore, our results might not be completely generalizable to the recovery process in arthropathic joints.

Excluding patients with incomplete follow-ups might have induced selection bias as well. Lost to follow-up might not have occurred randomly yet be related to fast(er) recovery: patients that seemed recovered might have cancelled their follow-up. Therefore, including only patients that were monitored until full recovery might have led to an overestimation of the average recovery time.

Another limitation may be the assumption that the 39% of joints without baseline status were healthy. In the case of unobserved baseline abnormalities, this may have introduced information bias into the recovery times. However, bias from unobserved baseline abnormalities seems negligible as all joints without baseline status recovered without residual abnormalities.

Clinical relevance

The current treatment guidelines do not propose an explicit follow-up period after a joint bleed[6, 7]. In our study, joint bleeds had long and variable recoveries (range 0.3-5 months). Both PE and ultrasound abnormalities could still be present days to months after onset of the bleed. We therefore recommend to incorporate monitoring the recovery of joint bleeds into regular care. We propose routine follow-up with PE and ultrasound 1 month after bleed onset, since that seems the most effective moment to identify prolonged recovery based on our data. It would give more insight into the course of the recovery process, which could lead to timely detection and treatment of ongoing synovitis or functional abnormalities. Treatment could be individualised based on the findings during follow-up: clotting factor replacement therapy may be intensified until full recovery, additional anti-inflammatory medication may limit synovial inflammation, (partial) immobilisation may be continued, and/or coaching on activities and exercise therapy may be initiated to restore joint function. However, the effectiveness of these treatment alterations still needs to be established.

Discrepancies between PE and ultrasound in the longer recovery processes show the added value of using both because they focus on different aspects of the joint. PE focuses on impairment and functionality[23], while ultrasound focuses on detection of synovitis[21]. Both examinations provide valuable information to guide treatment decisions and must be seen as complementary.

Future research

In our relatively small cohort, we did not find significant differences in recovery time between different bleeding causes, different joints and different haemophilia severities. Future research should focus on larger cohort studies, to enable determining risk factors for prolonged recovery.

In addition, we were unable to investigate the effect of treatment compliance on the recovery time. Treatment compliance is therefore a potential risk factor that remains to be investigated. These risk factors could indicate patients who would benefit from treatment adjustments and/or intensive monitoring. Second, the effectiveness of the proposed routine follow-up visit one month after bleed onset should be established in a prospective study. Third, the optimal treatment adjustments to change the course of the recovery process remain to be established.

CONCLUSIONS

Joint bleed recovery can take long and recovery times differed from bleed to bleed. In 47% of the joint bleeds, the recovery took longer than one month. PE and ultrasound have a different focus and provide complementary information. Therefore, both should be used to monitor joint bleed recovery. Monitoring joint bleeds with PE and ultrasound, for example 1 month after bleed onset, will provide more insight in recovery of the individual joint and enable personalised care.

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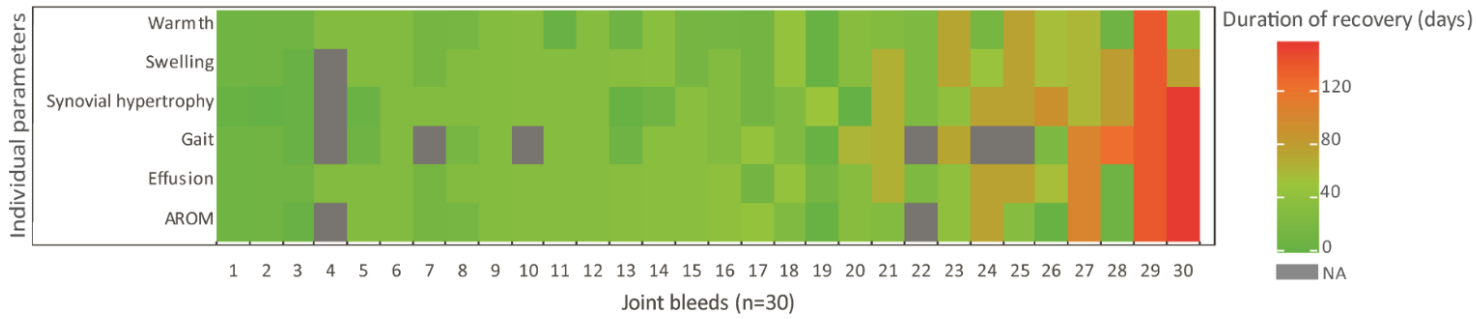
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. Heatmap showing the recovery of individual parameters of the physical examination and ultrasound.
Interpretation: *The columns represent the 30 individual bleeds. The rows correspond to the recovery time of individual parameters measured during physical examination and ultrasound. The colour of the cells indicates the relative speed of recovery to all parameters in all joint bleeds. The colour ranges from green for fast recovery, to red for long recovery. Joints with short recovery times are presented on the left in the figure, joints with long recovery times on the right. No patterns were evident in the order in which the parameters normalised.*

Supplementary Table 1. Recovery times of individual parameters.

	Median (months)	Range
Physical examination	**1**	**0-5**
Warmth	1	0-5
Swelling	1	0-5
Range of motion	1	0-5
Gait	1	0-5
Ultrasound examination	**1**	**0.3-5**
Effusion	1	0.3-5
Synovial hypertrophy	1	0-5

CHAPTER 9

Summary and Discussion

SUMMARY

Haemophilia is a X-linked inherited coagulation disorder that results in an increased bleeding tendency. The increased bleeding tendency is caused by a deficiency of (functional) clotting factor VIII (haemophilia A) or IX (haemophilia B). Most bleeding episodes occur in the large synovial joints (elbows, knees, ankles). Recurrent joint bleeding eventually leads to irreversible haemophilic arthropathy, which causes pain, reduced functionality and thus reduced quality of life. Prophylactic treatment prevents most bleeding episodes. However, (subclinical) joint bleeding and inflammation still occur. Surprisingly, even in the absence of clinically overt joint bleeding, long-term progression to arthropathy is observed. Subclinical bleeding and inflammation are therefore thought to contribute to the development of arthropathy. Early detection of these subclinical processes is becoming increasingly important in the prevention of arthropathy as overt joint bleeding becomes rare with new replacement therapies. This thesis focused on the detection of subclinical bleeding, the screening for subclinical joint inflammation, and the use of ultrasound in the management of acute joint episodes.

Detection of subclinical bleeding

Chapter 2 demonstrated that MRI T1 and T2 relaxometry could quantitatively distinguish synovial fluid from haemorrhagic joint effusion *in vitro*. The lowest detectable blood concentrations were 5% using T2 mapping at 3 Tesla and 10% using T1 mapping at 1.5 Tesla. **Chapter 3** demonstrated good feasibility and reproducibility of the T2-relaxometry method at 3 Tesla *in vivo*. **Chapter 4** described evidence for subclinical joint bleeding in people with severe haemophilia on long-term prophylaxis. Joints without a history of bleeding in adolescents and adults with severe haemophilia A on prophylaxis were assessed by conventional 3 Tesla MRI. MRI-detected synovial hemosiderin depositions, observed in in 16% of the 43 joints, provided evidence of previous subclinical joint bleeding in patients with severe haemophilia A.

Screening for subclinical joint inflammation

Chapters 5 and 6 are dedicated to screening for (subclinical) synovial proliferation as a proxy for joint inflammation. **Chapter 5** reviewed literature on the occurrence of joint swelling at physical examination and synovial hypertrophy on ultrasound in routine examinations of people with haemophilia. The overall low sensitivity of joint swelling for ultrasound-detected synovial hypertrophy illustrated an underestimation of synovial hypertrophy by physical examination alone. Therefore, ultrasound appears to have added value in screening for subclinical synovial hypertrophy. The results of our own cross-sectional study in **Chapter 6** provide further support for the value of ultrasound screening for subclinical synovial proliferation. Subclinical synovial proliferation was observed in at least one joint in 43% of the 79 screened Dutch severe haemophilia A patients without recent joint bleeding. Both 'active' inflammatory synovial proliferation (22%) and 'inactive' fibrotic synovial proliferation (22%) were prevalent in the cohort. The role of ultrasound is further emphasized by the finding that biochemical markers of (synovial) inflammation and osteochondral damage could not distinguish patients without synovial proliferation from those with active or inactive synovial proliferation.

Ultrasound in management of acute joint episodes

Although subclinical joint disease can be seen on imaging it remained unclear whether it would have an impact on patient management. The cross-sectional study in **Chapter 7** evaluated the impact of ultrasound, in addition to clinical assessment, on the diagnosis and treatment of acute musculoskeletal episodes in people with haemophilia and von Willebrand disease (VWD). The 77 acute musculoskeletal episodes occurred in a heterogeneous population including children (44%) and adults (56%) with (29%) and without (67%) pre-existing haemophilic arthropathy. Ultrasound findings often impacted diagnosis (36%) and treatment (39%). The number of patients that needed to be examined with ultrasound to change the diagnosis and/or treatment of one patient was 3. The results showed that it is difficult to correctly diagnose an acute musculoskeletal episode based on clinical assessment in both joints with and without haemophilic arthropathy. In **Chapter 8**, the recovery of 30 joint bleeds in people with haemophilia and VWD was monitored using ultrasound and physical examination. Joint bleeds could take a long time to recover (47% > 1 month) and recovery times varied between bleeds. Recovery differed between ultrasound and physical examination in 27% of the bleeds. Both persistent abnormalities at physical examination in joints with normalized ultrasound and persistent ultrasound findings in clinically recovered joints were observed.

DISCUSSION

This thesis focused on the imaging of (sub)clinical joint changes in people with haemophilia and the role of imaging in clinical decision making in haemophilia care. Three main topics were discussed: detection of subclinical joint bleeding, screening for subclinical joint inflammation, and the role of ultrasound in the management of acute joint episodes.

Key findings

Detection of subclinical joint bleeding

- Quantitative MRI T1 and T2 relaxometry can differentiate between haemorrhagic joint effusion with low blood concentrations and synovial fluid *in vitro*.
- T2 relaxometry of synovial fluid in haemophilia patients is feasible at 3 Tesla with good interobserver and intraobserver reproducibility.
- Conventional MRI of joints without a history of bleeding showed evidence of previous subclinical bleeding in 16% of people with severe haemophilia A on prophylaxis.

Screening for subclinical joint inflammation

- Physical examination underestimates the prevalence of ultrasound-detected synovial proliferation.
- Biochemical markers failed to identify ultrasound-detected subclinical synovial proliferation.

Ultrasound in management of acute joint episodes

- Ultrasound, when added to clinical assessment, often changes the diagnosis and treatment of acute musculoskeletal complaints in people with haemophilia and von Willebrand disease.
- Ultrasound and physical examination provide complementary information in monitoring joint bleed recovery.

Subclinical bleeding

Subclinical joint bleeding may include both clinically missed and asymptomatic joint bleeding and asymptomatic leakage of microscopic amounts of blood into a joint[1]. Manco-Johnson et al. first proposed the subclinical bleeding theory after observing MRI abnormalities in joints without a history of prior life-time bleeding in a prospective trial. They hypothesized that chronic microbleeding into the joints caused the joint deterioration visible on MRI. They also hypothesized that prophylaxis could prevent the subclinical bleeding and joint deterioration[2]. Findings supporting subclinical bleeding have since been described[3–7]. However, the microbleeding hypothesis is not universally accepted[1]. The hypothesis that prophylaxis can prevent subclinical bleeding has not been confirmed either. Erythrocyte-derived iron that accumulates as haemosiderin

deposits in joints is a sign of previous joint bleeding. We observed haemosiderin deposits on MRI in 16% of the joints without a history of bleeding in people with severe haemophilia A with lifelong access to prophylaxis **(Chapter 4)**. These haemosiderin deposits suggest that Dutch intermediate dose[8] prophylaxis cannot fully prevent subclinical bleeding. In the joints with haemosiderin deposits, concomitant synovial hypertrophy (29%) or osteochondral changes (14%) were also observed.

It remains unknown whether the observed haemosiderin deposits in **Chapter 4** are remnants of missed joint bleeds or microbleeding. To verify the existence of microbleeding, we need techniques that can detect small percentages of blood in the synovial fluid of an asymptomatic joint. If T1/T2 relaxometry described in **Chapter 2 and 3** is able to distinguish haemorrhagic effusion from synovial fluid in patients, this non-invasive MRI technique can be used in the aetiological research to detect microbleeding. However, the relaxation time shortening observed *in vitro* in haemorrhagic effusion first needs to be validated in patients at clinically available field strengths (1.5 and 3 Tesla). This requires a cross-sectional study in patients with (suspected) joint bleeding, a design that would be challenging in clinical practice. There is preliminary evidence that ultrasound can also detect small amounts of blood in synovial fluid[13,14]. Although ultrasound is a more accessible and rapid method, it has disadvantages. The assessment of synovial fluid is qualitative/subjective and the imaging quality is dependent on the experience of the operator. Irrespective of the measurement method, an aetiological study to detect microbleeding should ideally be a longitudinal study in which asymptomatic joints are frequently assessed to identify microbleeding. In addition, overt joint bleeding and joint complaints should be recorded in detail by the participants. The practical feasibility of such a study is limited by the burden on participants and the length of follow-up required.

The (longer-term) clinical consequences of previous subclinical bleeding are a topic of interest. MRI-detected synovial hypertrophy and haemosiderin are associated with an increased bleeding risk and later osteochondral changes[9,10]. In addition, osteochondral changes on MRI are predictive for development of arthropathy visible on x-ray[9]. From previous studies it is known that arthropathy on x-rays is negatively associated with physical functioning[11]. In **Chapter 4**, osteochondral changes were also observed in 11% of joints without MRI-signs of previous subclinical bleeding. This is similar to the prevalence of knee cartilage defects in the same age category in the general population[12]. This suggests that osteochondral abnormalities may not be entirely haemophilia-related and may represent 'normal' asymptomatic osteoarthritis. Hence, aiming for preventing all osteochondral abnormalities in the treatment of haemophilia is probably overtreatment.

Since subclinical signs of previous bleeding are associated with progression to functionally limiting arthropathy, monitoring of joint status in people with haemophilia using imaging is important and may be used to guide treatment. MRI is currently considered the reference standard for detecting (early) joint changes in haemophilia[15–17]. However, routine joint health screening using MRI is currently not feasible due to limited availability, high costs, relatively long duration of

the examination, and need for sedation in young children. MRI is best used as a troubleshooter in the case of difficult clinical cases where physical examination and ultrasound are inconclusive. Furthermore, MRI should be used as a reference standard in research on accuracy of diagnostic tests and could be used as sensitive outcome measure of joint health in clinical trials of drug efficacy. However, frequent full diagnostic MRI of 6 index joints seems too time-consuming and burdensome for study participants. MRI examinations should therefore be tailored to the research question with a trade-off between duration and detail of imaging. Examples of such trade-offs are the limited MRI protocol used by Zwagemaker et al.[5], MRI assessment of 1/6 index joints combined with ultrasound assessment of all 6 joints **(Chapter 4)**, or limiting MRI assessment to baseline and end-of-study visits.

'Active' versus 'inactive' subclinical synovial proliferation

The results in **Chapter 5** indicate that subclinical synovial proliferation can be detected by ultrasound while being missed by physical examination. Therefore, we recommend that ultrasound should be used in screening for subclinical synovial proliferation. The results of routine ultrasound screening in joints without recent bleeding **(Chapter 6)** show that subclinical synovial proliferation is common (43%). We hypothesized that subclinical synovial proliferation could be 'active inflammatory' or 'inactive fibrotic'[18,19]. In particular, active synovial proliferation may be reversible after early detection and treatment with anti-inflammatory medication. Early detection and treatment of such reversible synovial proliferation could break the vicious circle and prevent bleeding and progression to arthropathy. A diagnostic method to distinguish active from inactive synovial proliferation is not yet available. Ultrasound Doppler assessments have been used for this purpose. However, Doppler may lack sensitivity, as a Doppler signal in synovial proliferation is rarely observed in joints of people with haemophilia[20]. The biomarkers investigated in **Chapter 6** were non-discriminative and are therefore, as of yet, unsuitable for distinguishing between active and inactive synovial proliferation. A future prospective study[21] at our center will investigate whether synovial proliferation can be altered over time with COX-2 inhibitors and explore whether baseline (imaging) characteristics can predict the reversibility of synovial proliferation. This study will provide insight into the clinical impact of joint inflammation findings on routine ultrasound screening.

The role of ultrasound in haemophilia care

Joint ultrasound is increasingly used in haemophilia care, both in acute situations and for the assessment of arthropathy[22–24]. The increased use of ultrasound is not surprising as it is a safe, non-invasive, relatively inexpensive method that can provide rapid, real-time information. Furthermore, the accuracy of ultrasound in detecting soft tissue joint changes is comparable to that of MRI[25,26] and the reproducibility of findings is high, even among non-radiologists[27].

Ultrasound is currently recommended by the World Federation of Haemophilia (WFH) as a sensitive tool for routine assessment of joint health[15]. This is consistent with the findings in **Chapter 5 and 6**.

The WFH also recommends the use of ultrasound in diagnosing early joint bleeding and monitoring of synovitis after joint bleeding[15]. However, these recommendations are limited and non-specific. The recommendation on the diagnosis of joint bleeding states the added value in differentiating bleeding from arthropathy flare-ups. The results in **Chapter 7** indicate that the impact of ultrasound can be broader than solely differentiating bleeding from arthropathy. Ultrasound findings also had diagnostic and therapeutic impact in differentiating joint bleeding from other musculoskeletal diagnoses in children and in joints without arthropathy. To strengthen the rationale for the use of ultrasound in the management of acute joint episodes, future studies should investigate the cost-effectiveness and impact of ultrasound-guided treatment changes on longer-term joint health.

The WFH recommendation on the use of ultrasound to monitor the development of synovitis after bleeding only advises frequent monitoring until the situation is controlled. No details are given regarding the frequency of monitoring and the definition of a controlled situation. An initial routine follow-up, including a combination of physical examination and ultrasound assessment 1 month after the onset of bleeding , as suggested in **Chapter 8,** may provide a starting point for more specific monitoring advice. Future studies should further establish the effectiveness of the proposed routine 1-month follow-up after joint bleeding. Future studies should also determine the optimal treatment adjustments to improve the course of the joint bleed recovery.

The results in **Chapters 7 and 8** show that physical examination alone is not sufficient for diagnosing acute joint complaints and monitoring recovery from joint bleeding. As ultrasound is a promising tool in the management of acute joint episodes, its implementation and accessibility should be increased. Ultrasound could be incorporated into the existing diagnostic workflow of the haemophilia treatment center (HTC) outpatient clinic[28]. Point-of-care ultrasound could be performed by clinicians at the HTC. In difficult cases, close collaboration with the radiology department is essential to discuss images and/or refer for additional imaging. Ideally, ultrasound should also be available for home-treated patients who do not attend the clinic every time they experience a joint bleed. There are several ways to improve access to ultrasound for these patients. Ultrasound could be performed by a trained healthcare professional close to the patient's home. For example, a radiologist at a local hospital. A 'home-delivered ultrasound programme', where a physiotherapist visits the patient's home to perform an ultrasound, has also been studied[29]. In addition, studies of (artificial intelligence-assisted) patient-performed home ultrasound are ongoing, although the feasibility and cost-effectiveness of such programs have not yet been established [30–34].

General conclusion

This thesis presented evidence that subclinical joint bleeding has occurred in patients with severe haemophilia despite prophylaxis and in the absence of any clinical joint bleeding during their lifetime. It remains unknown whether these were clinically missed joint bleeds or microbleeds. Prevention of joint bleeding remains an unmet need in haemophilia, as it is an independent cause of joint damage. The data in this thesis further showed that synovial proliferation can be missed by physical examination alone. Subclinical synovial proliferation was often detected by routine

ultrasound. Future research should determine if and how synovial proliferation can be differentiated into (so far irreversible) fibrotic remnants and reversible active inflammation, and whether anti-inflammatory treatment is beneficial. Finally, studies in this thesis showed that ultrasound has a real impact on clinical management of acute joint episodes. Ultrasound provides information complementary to clinical assessment in diagnosing acute joint episodes and monitoring recovery after joint bleeding. Ultrasound should be implemented in haemophilia care as a screening tool for (subclinical) joint damage, as a diagnostic tool in acute joint episodes, and as a monitoring tool for recovery from joint bleeding. MRI remains the reference standard for imaging early joint changes in haemophilia. MRI is best used as a troubleshooter in difficult clinical cases and as a sensitive outcome measure in research.

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APPENDICES

ONDER DE OPPERVLAKTE: BEELDVORMING VAN (SUB)KLINISCHE GEWRICHTSVERANDERINGEN BIJ HEMOFILIE

Hemofilie is een zeldzame erfelijke stollingsstoornis die bijna alleen bij mannen voorkomt. Bij hemofilie is er te weinig stollingsfactor in het bloed. Bij hemofilie A is dit factor VIII en bij hemofilie B factor IX. Door het tekort hebben mensen met hemofilie een hogere kans op bloedingen. Deze bloedingen komen vaak voor in de grote gewrichten: enkels, knieën en ellebogen. De gewrichtsbloedingen zorgen voor schade aan de gewrichten. Hoewel herstel van schade in eerste instantie nog gedeeltelijk mogelijk is, veroorzaken herhaalde bloedingen op termijn onherstelbare gewrichtsschade. De gewrichtsschade leidt tot pijn en verminderde functionaliteit van het gewricht, wat de kwaliteit van leven vermindert.

De behandeling van hemofilie is gericht op het voorkomen van bloedingen (profylactische behandeling) en zo snel mogelijk stoppen van bloedingen. Op deze manier probeert men te voorkomen dat gewrichtsschade ontstaat. Profylactische behandeling voorkomt de meeste bloedingen. Helaas kan de huidige profylaxe niet alle gewrichtsbloedingen voorkomen en komt gewrichtsschade nog steeds voor. Ook wanneer er geen zichtbare gewrichtsbloedingen optreden kan gewrichtsschade ontstaan of toenemen. Waarschijnlijk wordt die schade veroorzaakt door onopgemerkte (subklinische) bloedingen en ontsteking (inflammatie). Vroege opsporing van deze subklinische bloedingen en inflammatie is belangrijk om het proces van gewrichtsschade te stoppen of te voorkomen.

Dit proefschrift beschrijft de beeldvorming van (subklinische) gewrichtsveranderingen bij hemofilie. Het onderzoek richtte zich op het opsporen van subklinische gewrichtsbloedingen, het screenen op subklinische gewrichtsinflammatie en het gebruik van echografie bij het diagnosticeren en behandelen van acute gewrichtsklachten.

Aantonen van subklinische gewrichtsbloedingen

Het eerste deel van het proefschrift is gericht op het aantonen van subklinische gewrichtsbloedingen. In **hoofdstuk 2** wordt een experimentele kwantitatieve MRI-methode beschreven (MRI-relaxometrie) die in staat is om buiten het lichaam lage concentraties bloed te detecteren in buisjes met gewrichtsvloeistof. In **hoofdstuk 3** wordt beschreven dat deze MRI-relaxometrie ook uitgevoerd kan worden in het lichaam in de gewrichtsvloeistof van mensen met hemofilie. In **hoofdstuk 4** werd met standaard MRI aangetoond dat bij mensen met ernstige hemofilie A op profylaxe in 16% van de gewrichten zonder voorgeschiedenis van bloedingen toch tekenen waren van doorgemaakte subklinische bloedingen.

Screening op subklinische gewrichtsinflammatie

Het tweede deel van het proefschrift gaat over het screenen op subklinische gewrichtsinflammatie. Gewrichtsinflammatie uit zich onder andere in verdikking van de binnenbekleding van het gewricht (synoviale hypertrofie). De literatuurstudie in **hoofdstuk 5** laat zien dat met alleen routine lichamelijk

onderzoek het voorkomen van echografisch gedetecteerde subklinische synoviale hypertrofie wordt onderschat. **Hoofdstuk 6** onderschrijft het belang van echografische screening op subklinische synoviale hypertrofie. Bij echografische screening van gewrichten van patiënten met ernstige hemofilie A bleek 44% van de patiënten synoviale hypertrofie te hebben. Biomarkers in bloed en urine konden de echografisch aangetoonde subklinische synoviale hypertrofie niet identificeren.

Echografie bij de behandeling van acute gewrichtsklachten

Het derde deel van het proefschrift beschrijft de toegevoegde waarde van echografie bij het diagnosticeren en behandelen van acute gewrichtsklachten. De studie in **hoofdstuk 7** laat zien dat het toevoegen van echografie aan de klinische beoordeling van acute gewrichtsklachten de diagnose en behandeling vaak verandert. Echografie zorgde voor een verandering in diagnose of behandeling in 1 op de 3 patiënten. In **hoofdstuk 8** werd het herstel van gewrichtsbloedingen onderzocht. Het herstelproces werd met zowel lichamelijk onderzoek als echografie gevolgd. Het onderzoek liet zien dat echografie informatie geeft die de informatie van het lichamelijk onderzoek aanvult.

Conclusie

Concluderend kwamen subklinische gewrichtsbloedingen voor bij mensen met ernstige hemofilie die profylaxe gebruikten. Het blijft onbekend of dit klinisch gemiste bloedingen of microbloedingen waren. Daarnaast kwam subklinische synoviale hypertrofie vaak voor. Resten van subklinische bloedingen en synoviale hypertrofie worden in verband gebracht met onomkeerbare gewrichtsschade op de lange termijn. Het is daarom belangrijk om de gewrichten van mensen met hemofilie regelmatig te controleren. MRI is het meest gevoelig voor het aantonen van gewrichtsveranderingen bij hemofilie, maar MRI is niet overal beschikbaar, duurt langer en is duurder dan echografie. Het onderzoek in dit proefschrift laat zien dat het gebruik van echografie toegevoegde waarde heeft bij screening op (subklinische) gewrichtsveranderingen, bij het diagnosticeren en behandelen van acute gewrichtsklachten en bij het volgen van het herstelproces na gewrichtsbloedingen. MRI kan het beste worden gebruikt wanneer klinische beoordeling en echografie niet toereikend zijn en als gevoelige uitkomstmaat in onderzoek.

Toekomstig onderzoek moet uitwijzen of er onderscheid gemaakt kan worden tussen omkeerbare synoviale hypertrofie door actieve inflammatie en onomkeerbare synoviale hypertrofie als blijvende restafwijking. Wanneer dit onderscheid gemaakt kan worden, kan actieve synoviale inflammatie misschien behandeld worden met ontstekingsremmers.

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CURRICULUM VITAE

Floor (Flora Hendrica Pieternella) van Leeuwen was born on the 25th of February 1995 in Nijmegen, the Netherlands. After graduating cum laude from high school (Canisius College, Nijmegen) in 2013, she started her medical studies at Utrecht University. Early in her studies, she developed an interest in radiology, which led to several research internships and clerkships in radiology. During her elective clerkship in radiology in 2018 (Gelre Ziekenhuizen, Apeldoorn), Dr W. Foppen, introduced her to research in haemophilic arthropathy. In 2018-2019 Floor did a 5-month research internship at the Space Medicine Team of the European Astronaut Centre (ESA, Cologne, Germany), where she contributed to research on radiation protection in astronauts. After returning from Cologne, she started working on the first research projects on imaging of (sub)clinical joint changes in haemophilia: she helped to prepare the BEGIN study, followed by a research internship on joint bleed recovery in people with bleeding disorders supervised by Dr M.A. Timmer. She obtained her medical degree in 2020, after which she continued the started research projects as a PhD candidate at the UMC Utrecht under the supervision of Dr M.A. Timmer, Dr W. Foppen, Dr K. Fischer and Prof. Dr P.A. de Jong. During her PhD, she completed a postgraduate Masters in Epidemiology. The results of her PhD, which was a collaboration between the department of radiology and the Van Creveldkliniek, are described in this thesis. In January 2024, Floor will start her training as a radiologist at Meander MC in Amersfoort.

LIST OF PUBLICATIONS

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