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Review

Biochemical marker research in hemophilic arthropathy: A systematic review

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ABSTRACT

Hemophilic arthropathy (HA) causes major morbidity. Breakthrough therapies reduce the bleeding frequency tremendously, but well-defined joint outcome assessments with a focus on early changes and subclinical damage are lacking. Biomarkers reflecting joint tissue turnover/inflammation might be useful to predict invalidating arthropathy. This systematic review summarized and categorized publications on blood/urinary biomarkers in HA to provide leads for implementation. A PubMed/EMBASE search was performed on September 9, 2019. All publications were assessed and allocated to one or several BIPED-categories, based on the utility of biomarkers. Of the initial 1307 publications found, 27 were eligible for inclusion. The majority (81%, $n = 32/42$) was cross-sectional in design, including relatively small numbers of patients (median 44, interquartile range 35–78). Fourteen percent ($n = 6/42$) investigated dynamic changes around a bleeding or treatment. Only two studies investigated the prognostic value of biomarkers. Most promising biomarkers were serum Coll2-1, COL-18N, COMP, C1,2C, C2M, CS846, MIF, plasma sVCAM-1 and urinary CTX-II. Comparing performances and pooling data was not possible due to heterogeneity. Currently, biomarker research in HA is still in an explorative stage and not yet sufficient for translation into daily practice. Clearly, larger homogeneous longitudinal studies in well-defined populations should be performed for further development.

1. Introduction

Hemophilia is an inherited coagulation disorder characterized by spontaneous and trauma-related bleeding, with musculoskeletal bleeding counting for 70–80% of all bleeding events. The main goal in the treatment of hemophilia is preventing these hemarthroses and subsequent arthropathy [1,2]. The introduction of prophylactic clotting factor replacement therapy significantly diminished the bleeding frequency, but patients with severe hemophilia on intermediate dose prophylaxis still experience 0.8–2.7 joint bleeds per year [3]. Joint bleedings, even the limited numbers, lead to the accumulation of iron in the joint cavity and have devastating effects on all joint components. Iron-laden synoviocytes induce an inflammatory response by producing cytokines and tissue destructive proteases like matrix-metalloproteinases (MMPs) [4]. This leads to hyperplasia and an increase in oxygen demand followed by a release of growth factors such as vascular-derived endothelial growth factor (VEGF). The newly formed fragile blood vessels cause vulnerability to repeat bleeds. Moreover, cartilage and bone are affected by the released cytokines and destructive proteases and together with the direct effects of blood, this results in tissue degradation [5]. Over time, this leads to irreversible joint damage, so called hemophilic arthropathy (HA). The chronic pain and limited daily functioning due to HA have a huge impact on quality of life in hemophilia patients and treatment options are limited.

At present, the development and progression of HA is monitored by clinical symptoms, motion analysis and the use of imaging techniques. Gait analysis may be useful to facilitate early diagnosis, but the relatively young patient population and ongoing musculoskeletal development in children may compensate for structural joint damage [6,7]. This young population is not likely to adopt a different motion pattern in other joints and moderations in functional activities like walking may remain subclinical.

Ultrasonography is relatively cheap and easy-accessible and can identify joint effusion, soft tissue changes and acute hemarthroses.

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Unfortunately, it has limitations regarding inter-observer variability, the need for a specially qualified observer and the difficulty to detect changes in deeper structures. Alternatively, Magnetic Resonance Imaging (MRI) can provide detailed information about small soft and hard tissue alterations, but the use is restricted due to high costs, relatively low accessibility and the need for sedation in young children. As a result, conventional radiography is also still used to monitor joint status. X-rays can visualize late osteochondral changes but have poor sensitivity in demonstrating early soft tissue changes. Imaging techniques provide only a cumulative result of past dynamic changes and in order to detect minor changes in an early stage of arthropathy, predict progression and eventually adapt clotting factor substitution therapy, a tool reflecting dynamical changes in the joint is favorable. [8,9] Biochemical markers reflecting the pathological processes due to a joint bleed, can potentially provide this significant information. Ideally, these biochemical markers monitor the ongoing rate of joint destruction and may even predict development and/or progression of arthropathy before it results in clinical symptoms or abnormalities on imaging. In clinical practice, they could be used to change the prophylaxis regime, switch to novel agents like emicizumab or to determine the right time for the start of additional treatments (e.g. anti-inflammatory drugs).

Moreover, biochemical markers may contribute to effectively evaluate joint protection in clinical trials. Recent studies monitored the short-term effects on joints by determining the annual bleeding rate (ABR). However, with this outcome measure, the effect on subclinical bleeding and subsequent joint tissue damage and inflammation is missed. Good and very good prophylaxis may both prevent overt bleedings, but only the very good prophylaxis may truly protect against long-term joint arthropathy and biomarkers may help to discriminate between these different types of prophylaxis. Also, the introduction of emicizumab, the first commercially available non-factor replacement product, in the treatment landscape of hemophilia is revolutionary and investigating a potential benefit over regular prophylaxis can only be achieved by very long follow-up or sensitive joint outcome measures.

HA has characteristics of both osteoarthritis (OA) and rheumatoid arthritis (RA) and biochemical markers have extensively been investigated in these joint diseases. Investigating biomarkers in these diseases is a complex challenge, as the involvement of multiple (small) joints, aggressive treatments, systemic inflammation in RA and comorbidities like liver or kidney dysfunction may influence biomarker metabolism [10]. Thus far, none of the markers had sufficient diagnostic or prognostic value at the level of an individual patient [11–13]. HA is attractive for biomarker research as it is mainly restricted to three pairs of large joints, has no systemic inflammatory component and is a fast progressing disease in relatively young and healthy patients with an unambiguous trigger for joint damage (bleeding). While the number of publications reporting about biochemical markers in HA is growing, a systematic overview of the performances of these biomarkers is lacking.

In order to give an overview about these performances and create a solid basis for future research, harmonization and organization of biochemical marker research by proper classification systems and effective, unambiguous communication is essential. The U.S. Food and Drug Administration and National Institutes of Health appointed the harmonization of terms used in medical product development and the translation of science as a priority need and focused on terms related to biomarkers. They developed the BEST (Biomarkers, EndpointS, and other Tools) Resource and give definitions of different kind of biomarkers (e.g. monitoring biomarker, diagnostic biomarker, pharmacodynamics biomarker) [14]. In this review, publications are categorized according to the BIPED-classification. The BIPED-classification is based on the utility of biomarkers (see *Methods* and Table 1) and most biochemical marker research has been performed in the OA field using this classification.

The present review aims to summarize and categorize publications on blood and urinary biochemical markers in HA and as such provide a basis for future research focusing on the potential of implementing biomarkers as well-defined joint outcome assessments [13].

Table 1
Analyses of biomarker performance within the BIPED-categories.

Abbreviation	Category	Description
B	Burden of disease	Biochemical markers associated with the severity of hemophilia or the severity of HA
I	Investigative	Biochemical markers in animals or the dynamical changes in markers upon a joint bleeding
P	Prognostic	Baseline biochemical markers predicting future outcomes, such as the risk for joint damage development or progression in a particular patient
E	Efficacy of intervention	Biochemical markers predicting whether an intervention will be efficacious and used to monitor the effect of an intervention or to determine which patients are eligible for the intervention
D	Diagnostic	Biochemical markers with the capacity to identify HA in the general population (comparison of markers between hemophilia patients and control populations) or biochemical markers with the capacity to diagnose a joint bleeding

2. Methods

A systematic search in PubMed and EMBASE was performed on September 9, 2019. The following search terms were used: 'biomarkers' AND 'hemophilia' OR 'hemophilic arthropathy'. Supplementary File 1 shows the detailed search strategy for PubMed and EMBASE. All articles were included if published in English. Abstracts were included if they were published in 2018 or 2019. Additional records from the reference list were identified manually. After a systematic deduplication using EndNote, two researchers independently screened all publications by title and abstract. The selected publications were assessed for eligibility by full-text screening. If full-text articles were unavailable, authors were contacted.

Studies were included when they reported about biochemical markers in blood and/or urine in case of HA. Articles evaluating bone turnover markers and the correlation with bone mineral density or osteoporosis only, without correlating the markers to HA, fell outside the scope of this review and were therefore excluded. Studies reporting on biochemical marker research in tissues, for example synovium biopsies, were not included because these are considered not relevant for implementation in daily practice. Disagreements were resolved by consensus with a third researcher.

Identified publications were analyzed and, based on the biochemical marker application, allocated to one or several BIPED-categories [13]. See Table 1.

Some publications contained more than one analysis for several BIPED-categories and were therefore allocated to more than one category. Data extraction and tabulation were performed by one author and verified by a second author. Due to the heterogenic study designs and outcome parameters comparison of biochemical marker performance and pooling of data was not possible.

3. Results

3.1. Search results and selection

A total of 1307 records were identified by searching PubMed (780) and EMBASE (527). After systematic deduplication, 1007 records of potentially eligible studies were screened on title and abstract. Most of the excluded publications reported about markers for inflammatory diseases, malignancies or genetic diagnoses and did not report about arthropathy. After full text screening, 27 publications were eligible for

inclusion (Fig. 1). Most of the included publications had relatively small patients populations (median 44, interquartile range 35–78). All studies were allocated to one or more BIPED-categories and summarized in Table 2. This resulted in 42 items. In total, 81% (n = 34/42) was cross-sectional in design, 5% (n = 2/42) longitudinal and 14% (n = 6/42) investigated the dynamical change of biomarkers in response to a joint bleed or treatment. In total 72 different biochemical markers were studied, all reflecting different processes in the development and/or progression of arthropathy, see Fig. 2. Biochemical marker abbreviations are listed in Supplementary File 2 with a description on their nature.

3.2. Burden of disease

Eighteen articles could be classified in the burden of disease category. These studies investigated the correlation of biochemical markers with the degree of HA. Five studies compared biochemical marker levels in patients with severe, moderate or mild hemophilia [15–20]. Patients with severe hemophilia had statistically significant lower levels of serum sclerostin and higher levels of serum COMP compared to patients with moderate or mild hemophilia [15,17]. Levels of plasma soluble E-/P-selectin and VCAM-1 did not significantly differ between these groups [18]. Differences in levels of serum b-ALP and vitamin D3 were contradictory or unclear [16,20].

Comparing the correlations of biomarkers with the severity of HA was hampered by the very heterogeneous assessments for the degree of HA, using functional scores, history and different imaging techniques with varying scoring methods (Supplementary File 3).

Considering cartilage turnover markers, five studies investigated the relationship of serum COMP with arthropathy with inconclusive results [17,21–24]. Two studies reported a significant positive association of COMP with the joint space narrowing (JSN) on X-ray.

JSN is an important and widely accepted indicator of cartilage loss and frequently reported in evaluations of relationships between radiographic arthropathy and biochemical markers [21,25,26].

Three other studies reported weak negative or no correlations with the severity of HA on imaging. CTX-II was investigated in urine and serum. Urinary CTX-II was only studied once and showed a significant correlation with JSN (rs = 0.35) and total radiographic Pettersson score (PS; rs = 0.39) [21]. The correlation of serum CTX-II with total PS was investigated in two studies with contradictory results. However, reported correlations were weak [21,22]. Jansen et al. also found significant correlations for serum CS846 and serum C1,2C with JSN (rs 0.42 and rs = 0.29) and PS (rs = 0.31 both). In order to increase correlations of single biomarkers, combined indexes were used. In this study, the combined index of urinary CTX-II and serum C1,2C and CS846 increased the correlation with JSN (rs = 0.70) and PS (rs = 0.67) [21]. Comparison between studies is difficult, as correlations for biochemical markers can change depending on the assessment of HA. For example, the correlations for serum CS846 and X-ray parameters could not be confirmed by Oldenburg et al. [23] They only reported a significant correlation of serum CS846 with MRI score in a small subpopulation (n = 22) of patients treated on demand. Besides heterogeneous assessments for HA, different populations might also explain the differences between correlations. The contribution of soft tissue and osteochondral changes to the total MRI score in joints of subjects with hemophilia is shown to be age-dependent. In patients <16 years old, the soft tissue component was 81%, while in patients between 16 and 26, this component was 49% [27]. Oldenburg included patients between 12 and 35 years old, while Jansen had a cohort of patients with a mean age of 35. As CS846 is an

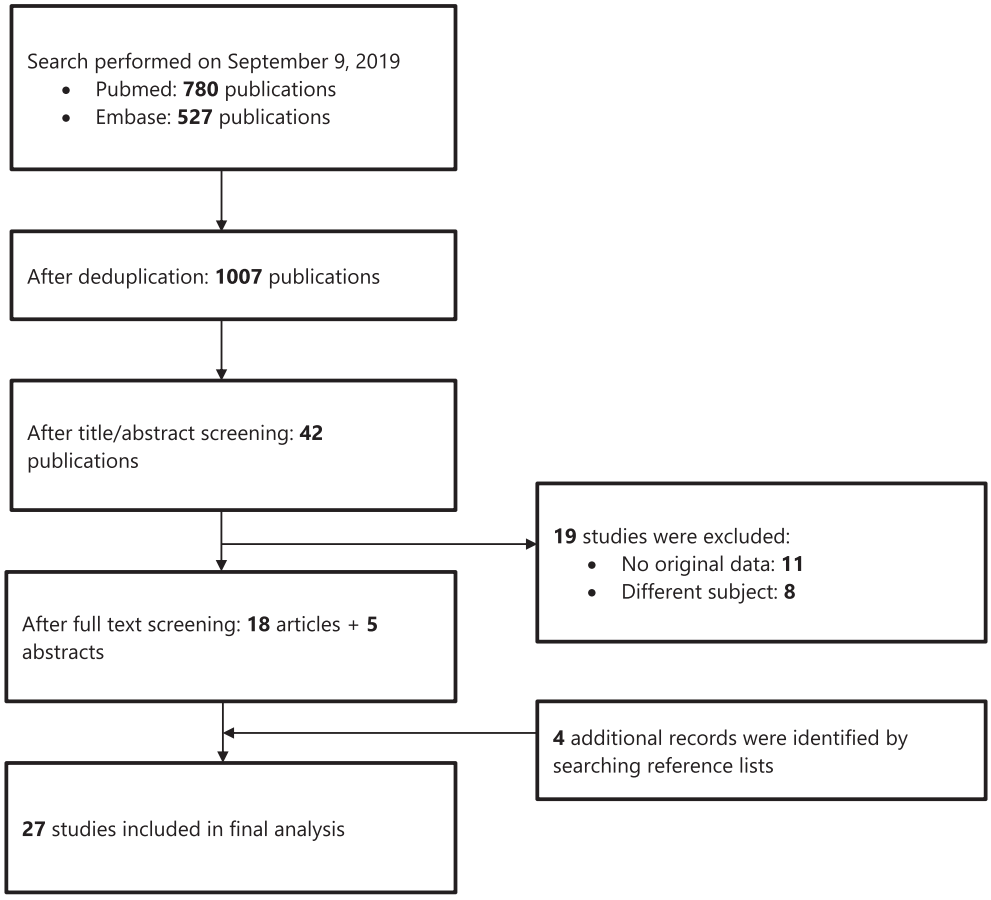

Fig. 1. Flow diagram.

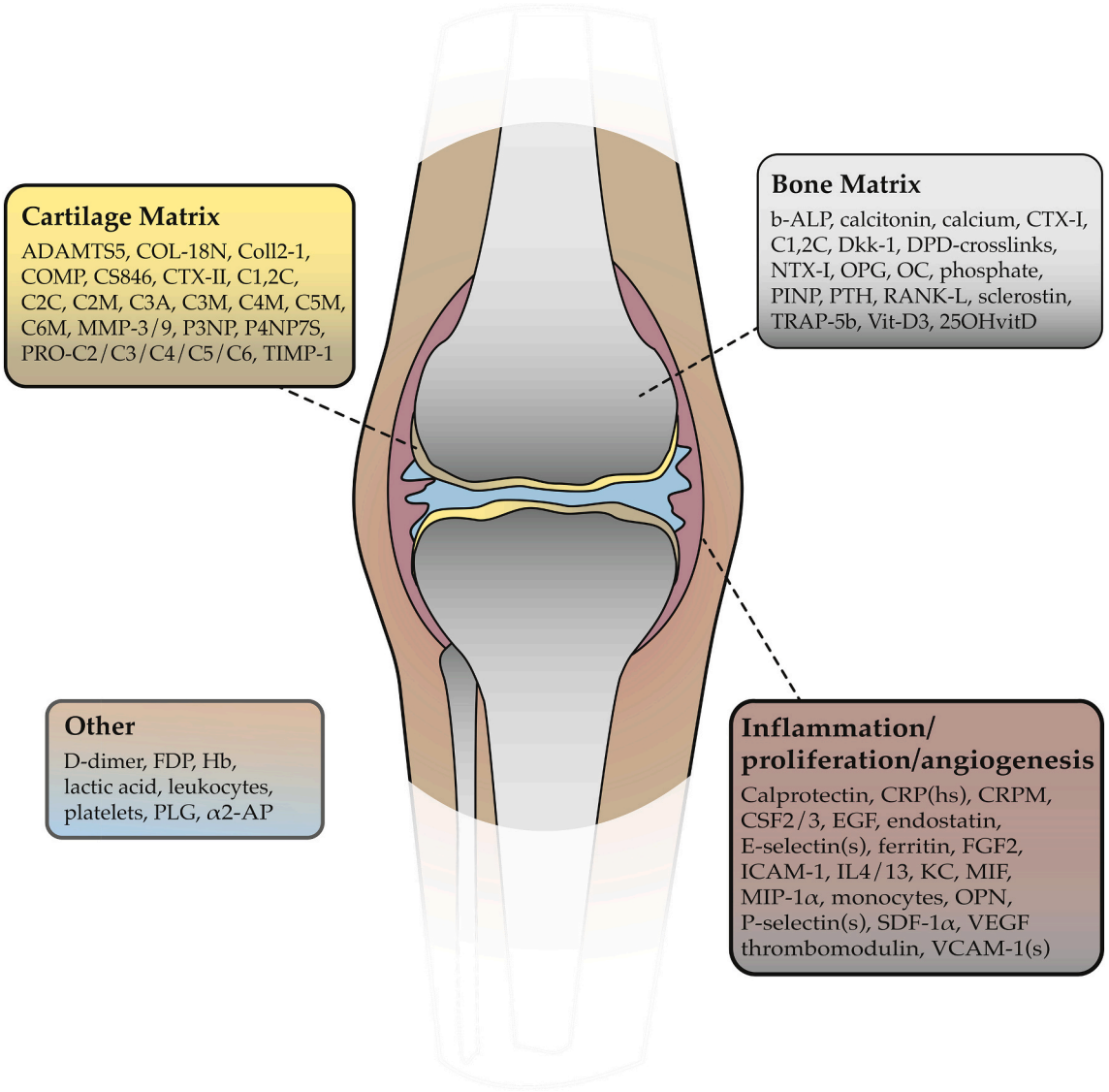

Fig. 2. *Joint with all studied biochemical markers.*

osteochondral marker and not a soft tissue marker, it might be higher in older patients, explaining why the study by Oldenburg, with a relatively young population, only found a significant correlation of CS846 with the MRI score in a small subpopulation of patients treated on demand. This phenomenon was also shown for osteopontin, a potential marker for synovitis. Osteopontin had a significant correlation with the MRI score in immature but not mature subjects [27].

Another parameter for the degree of arthropathy is the annual bleeding rate (ABR), which is directly associated with the degree of arthropathy on X-rays assessed by the PS [28]. Serum COL-18N, although studied only once, was significantly associated with the ABR and might be promising [29]. Data on serum PRO-C2 are also very limited, but showed a significant correlation with the PS [22].

Looking at bone turnover markers, CTX-I gained most attention and was studied in six different studies. However, only one study showed a significant (but weak) association with the degree of arthropathy on X-ray, while the other studies reported non-significant relations [16,21–23,30].

Another frequently studied ($n = 4$) bone marker but with very heterogeneous results is serum osteocalcin. One study reported a significant negative associations of serum osteocalcin with the Colorado Hemophilia Pediatric Joint Physical Examination Scale (CHPJPES), while three other studies did not report significant associations between serum osteocalcin and Hemophilia Joint Health Score (HJHS) or PS [16,30–32]. Again, this might be explained by the heterogeneous assessments of HA. However, urinary osteocalcin did show a negative correlation with the Orthopedic Joint Score (OJS) [33]. Other bone turnover markers were studied less frequently. Serum TRAP-5b and sclerostin were investigated in one and two studies, respectively, and had a significant correlation with the severity of HA [15,16,30]. Correlations of serum osteoprotegerin and RANK-L were contradictory [30,32]. As we already mentioned for CS846 and osteocalcin, the correlation of some bone markers with the severity of HA also depended on the assessment of HA. Serum Dkk-1, vitamin D and b-ALP were significantly correlated with physical examination scores but not with imaging scores/number of affected joints [15,16,30,32].

In regard to inflammation including angiogenesis, serum and plasma VEGF, plasma soluble VCAM-1 and high sensitive serum/plasma CRP were investigated in four studies. Plasma soluble VCAM-1 was significant higher in patients with more severe arthropathy on X-ray or more joints involved [18]. Patients with early joint disease showed a 10-fold increase in plasma VEGF-A compared to patients with advanced joint disease in one study. In contrast, two other studies reported no significant differences for serum or plasma VEGF and different stages of HA

Table 2
All studies summarized and allocated to one or more 'BIPED'-categorie. The items in italics are abstracts only.

Rf	Biochemical markers	Investigation groups	Conclusions
Burden of disease			
A	P: VEGF-A	PWH + early HA (n = 10) vs advanced HA (n = 13) based on joint bleeding history + imaging	**SS**: early HA: higher VEGF-A (mean ± SEM: 227.7 ± 33.4 vs 22.4 ± 1.2 pg/ml)
B	S: Dkk-1, sclerostin	PWH + HA (n = 89) PWH, severe (n = 16) vs mild/moderate (n = 74)	**SS**: PS + sclerostin/Dkk-1: rs = −0.254; rs = −0.319; PWH, severe: lower sclerostin levels (mean ± SD: 36.1 ± 24.7 vs 50.6 ± 26.7 pmol/l)
C	S: b-ALP, CTX-I, NTX-I, OC, TRAP-5b	PWH + HA (n = 70)	**SS**: PS knees/ankles and CTX-I: rs = 0.311/0.288; and TRAP-5b: rs = 0.367/0.365; Arnold-Hilgartner knees/ankles and CTX-I: rs = 0.349/0.313 and TRAP = 5b: rs = 0.81/0.254. Other significant correlations: nr of affected joints with CTX-I + b-ALP: rs = −0.273 + −0.314; and severity of hemophilia with b-ALP: H = -7.178; **NSS**: Other correlations
D	S: COMP	PWH (children) + target joint; severe hemophilia (n = 15) vs moderate (n = 7) or mild (n = 8)	**SS**: COMP + JSN(rs = 0.64)/ total PS(rs = 0.42)/ FISH(rs = −0.44)/ nr of joints affected(rs = 0.49)/ nr of joint bleeds last yr(rs = 0.82); **SS**: COMP higher in PWH severe (mean ± SD 757 ± 211.3 ng/ml) vs moderate (403.6 ± 86.5 ng/ml)/ mild (211.3 ± 74.3 ng/ml)
E	S: ADAMTS5, COMP, CRPM, CTX-I/II, C2M, hsCRP PINP, PRO-C2	Severe PWH + HA (n = 35)	**SS**: PS + PRO-C2/CTX-II (rs = 0.34/0.37); Gilbert + CTX-II (rs = 0.36); **NSS**: other markers
F	S: COMP, CS846, CTX-I/II, C1,2C, C2C; U: CTX-I/II	PWH + HA (n = 36)	**SS**: PS + uCTX-II/C1,2C/CS846: rs = 0.387/0.314/0.312; JSN + uCTX-II/C1,2C/CS846/COMP: rs = 0.348/0.291/0.424/0.284; Increased correlation: combined index uCTX-II, COMP, CS846 (+JSN, rs = 0.703; +PS, rs = 0.665)
G	P: CRP, Hb, leukocyte; S: endostatin, ferritin, ICAM-1, lactic acid, MIF, thrombomodulin, VEGF	Severe PWH + early HA (n = 6) vs advanced HA (n = 13) vs without HA (n = 16) (based on physical examination, X-ray, MRI)	Advanced HA vs early HA; **SS**: higher Hb in advanced; **NSS**: leukocyte, ferritin, lactic acid, VEGF, trombomodulin, endostatin; HA vs no HA; **SS**: higher CRP and lower lactic acid
H	S: COL-18 N	PWH (n = 35)	**SS**: COL-18 N and ABR: rs = 0.45
I	P: MMP-3/9, VEGF; S: COMP, CS846, CTX-I, TIMP-1	Severe PWH (n = 117); subpopulation (n = 22) treated on demand	**SS**: CS846 + MRI scores in subpopulation: rs = 0.436; **NSS**: other markers, CS846 in whole cohort
J			

Table 2 (*continued*)

Rf	Biochemical markers	Investigation groups	Conclusions
	P: soluble *E*-selectin, soluble P-selectin, solubleVCAM-1	PWH, mild, moderate, severe PWH + HA (n = 35) Group 1 = PS 0–4 every joint; Group 2 = PS ≥ 5 any joint; Group 3 = PS ≥ 5 more than 2 joints	**NSS:** VCAM-1, E-selectin, P-selectin in PWH mild vs moderate vs severe **SS**: VCAM-1 higher in group 2 and 3
K	*S: MMP-3*	*PWH + subjective symptoms of HA (n = 56)*	***NSS:** MMP-3 + joint scores*
L	*S: Coll2-1, COMP*	*Severe PWH + severe HA (n = 30)*	***SS:** US score + Coll2-1/COMP: rs = −0.437/−0.431; **NSS:** biomarkers + ABR/HJHS*
M	*S: b-ALP, vitamin D3*	*PWH, severe (n = 99), moderate (n = 96) and mild (n = 10)*	***SS:** number of joints involved + b-ALP(r = −0.14); higher b-ALP in severe PWH (r = 0.08) with higher number of joints involve. Vitamin D3: More deficiencies in severe hemophilia. No correlation with number of joints involved (unknown significance).*
N	S: calcitonin, OC, PTH, 25OHvitD	Severe PWH (children) (n = 44)	**SS**: Colorado Hemophilia Paediatric Joint Physical Examination Scale +25OHvitD/OC: rs = −0.323/−0.313
O	S: ALP, OC, OPG, RANK-L	PWH (children) (n = 26)	**NSS**: HJHS + RANK-L/OPG/OC/ALP
P	S: ALP, calcium, CTX-I, Dkk-1, OC, OPG, phosphate, PTH, RANK-L, sclerostin, 25OHvitD	Severe PWH (children) (n = 44)	**SS**: HJHS + sclerostin (after age-adjustment)/PTH/25OHvitD/OPG/RANK-L: rs = 0.222/−0.11/−0.296/−0.184/0.194
Q	*U: bone ALP, DPD crosslinks, OC*	*PWH (n = 75)*	***SS:** Orthopedic Joint Score + uOC (r = −0.272);* **NSS**: bone AP/DPD crosslinks
Investigative			
R	P: C4M, PRO-C4	F8−/− mice + induced hemarthrosis treated with two doses of rhFVIII or saline-only (n = 7 to 10 per group)	Saline-treated mice; 2 weeks after the bleeding vs baseline: elevation **SS**: C4M; 1.3-fold; **NSS**: PRO-C4 1.2-fold. (implying increased Col4 turnover; increase prevented by 200 IU/kg recombinant human FVIII prophylaxis)
S	S: calprotectin	F8−/− mice + induced hemarthrosis (n = 10) vs unpunctured F8−/− mice (n = 3)	Punctured mice: calprotectin higher at 2 (mean ± SE 58 ± 23 pg/ml) and 12 (21 ± 7 pg/ml) weeks vs no calprotectin.
T	S: C2M, C3M, C4M, C6M, PRO-C5, P3NP, P4NP7S	F8−/− rats (n = 24) vs wild-type rats (n = 18), all with induced hemarthroses on day 0 and 14	**SS**: F8−/− rats: increase C4M (day 15), P4NP7S (day 15 + 21), P3NP (day 7), PRO-C5 (day 7 + 21), C2M (day 15 + 28) and decrease C3M (day 14) after knee bleed. **NSS**: decrease C6M. **SS**: F8−/− rats: C2M (day 15) + degree of cartilage degradation histologically/degree of overall arthropathy histologically rp = 0.85/rp = 0.75. C2M:

(*continued on next page*)

Table 2 (*continued*)

Rf	Biochemical markers	Investigation groups	Conclusions
			response to bleeding similar in WT and F8−/−, C3M and PRO-C5 more pronounced in F8−/− (more hemophilia specific)
U	S: COMP, CS846, C1,2C; U: CTX-II	PWH + joint bleed (*n* = 10) Dogs + induced hemarthroses (n = 7)	**SS**: PWH: increased uCTX-II (+52%) and CS846 (+14%) (day 5) after joint bleed. Dogs: increased uCTX-II (from 75% to 155%) (day 2–7); COMP (+46%) (baseline-day 2) **NSS**: other markers
G	P: CRP, Hb, leukocyte; S: endostatin, ferritin, ICAM-1, lactic acid, MIF, thrombomodulin, VEGF	Severe PWH with acute joint bleeding (n = 10)	**SS**: (Intraindividual); during bleeding vs 1 month after bleeding: MIF (mean 74,801 vs 23,692 pg/ml), CRP (16.3 vs 3.1 mg/dl), ICAM (359.5 vs 399.8 pg/ml) **NSS**: endostatin, ferritin, Hb, lactic acid, leukocyte, thrombomodulin, VEGF.
Prognostic			
V	S: CS846; U: CTX-II	PWH + HA (*n* = 31) Outcome: joint damage progression (PS) after 6.5 yr	Individual markers: no prediction **SS**: combined index uCTX-II + CS846: OR 8.8, 95%CI 1.1–70.6. Differed between slow and fast progressors (median − 0.095 vs 0.33)
L	*S: Coll2-1, COMP*	*Severe PWH + severe HA (n = 30) Outcome: change in US score after 3 m*	***SS**:sampled again after 3 months; ▲US score + ▲Coll2-1/COMP: rs = −0.797/−0.768; **NSS**: ▲biomarkers + ▲ABR/▲HJHS*
Efficacy of intervention			
L	*S: Coll2-1, COMP*	*Severe PWH + severe HA (n = 30). 11 changed on-demand to prophylactic treatment*	***NSS**:lower in on-demand vs prophylactic treatment*
Diagnostic			
A	P: MMP-9, SDF-1α, VEGF-A	PWH + HA (*n* = 25–76) vs patients + bleeding disorder without arthropathy (*n* = 17–41) vs healthy persons (n = 16–26) > *n differs for different markers*	**SS**: PWH + HA: 4-fold elevation in MMP-9, SDF-1α, VEGF-A
D	S: COMP	PWH (children) + target joint (n = 30) vs healthy boys (*n* = 20)	**SS**: PWH: higher COMP (mean ± SD 529 ± 288.1 vs 285 ± 63.2 ng/ml)
E	S: ADAMTS5, COMP, CRPM, CTX-I/II, C2M, hsCRP PINP, PRO-C2	Severe PWH + HA (n = 35) vs matched controls (*n* = 43)	**SS:** PWH increased C2M, CTX-II, COMP (+25%), CTX-I (+30%), hsCRP (+50%) decreased ADAMTS5 (−10%), PINP (−25%), CRPM (−25%); AUC: C2M (0.70), COMP (0.65), ADAMTS5 (0.67), PINP (0.63), CTX-I (0.74), hsCRP (0.67), CRPM (0.76), combination C2M, CRPM, ADAMTS5 (0.85) **NSS**: PRO-C2 (0.56); CTX-II (0.61)
G	P: CRP, Hb, leukocyte, monocyte, platelet; S:	Severe PWH with (n = 10) and without (n	Acute joint bleed group vs healthy control: **SS**:

Table 2 (*continued*)

Rf	Biochemical markers	Investigation groups	Conclusions
	endostatin, ferritin, ICAM-1, lactic acid, MIF, thrombomodulin, VEGF	= 25) acute joint bleeding vs healthy children (n = 22)	higher CRP, ferritin, lactid acid. No difference: VEGF, MIF, trombomodulin, endostatin, ICAM-1; PWH (with and without acute joint bleed) vs healthy control; **NSS**: Hb, leukocyte, monocyte, platelet PWH acute joint bleed vs no acute joint bleed: no clear differences inter-individually.
J	P: soluble E-selectin, soluble P-selectin, solubleVCAM-1	PWH + HA (n = 35) vs healthy persons (n = 20)	**SS**: PWH: higher VCAM-1 (mean ± SD 683.0 ± 392.3 vs 475.6 ± 85.75 ng/mL), only in PWH, severe, not in mild/moderate. **NSS**: E-selectin and P-selectin
W	P: VEGF	PWH (n = 24) without recent bleeding (within 3 months) vs non-hemophilic undergoing diagnostic arthroscopy (n = 9)	Low levels in both groups and below the ELISA detection level (<9 pg/mL)
S	S: calprotectin	PWH (n = 40) vs bleeding disorder controls without hemarthrosis (n = 36) vs healthy controls (n = 23)	**SS**: PWH: increased calprotectin (mean ± SE 57 ± 4 vs 38 ± 2 vs 32 ± 3 pg/mL)
L	*S: Coll2-1, COMP*	*Severe PWH + severe HA (n = 30)* vs *healthy controls (n = 19)*	***SS**: PWH: lower Coll2-1 (212,612.07 ± 91,921.67* vs *349,563.06 ± 83,985.03 pg/ml) and COMP (1295.83 ± 542.04* vs *1995.34 ± 364.16 ng/ml)*
X	S: C3A, C3M, C4M, C5M, C6M, PRO-C3/C5/C6, P4NP7S, PRO-C3/C5/C6	PWH + HA + high ABR (n = 35) vs matched controls (n = 43)	**SS**: PWH: upregulated C4M (11%); downregulated C3M (13%), C3A (13%), PRO-C5 (25%), C5M (11%); **NSS**: C6M, PRO-C3, P4NP7S
N	S: calcitonin, OC, PTH, 25OHvitD	Severe PWH (children) (n = 44) vs healthy controls (n = 40)	**SS**: PWH higher PTH (mean ± SD 48.2 ± 23.1 vs 28.3 ± 15.8 pg/ml), lower 25OHvitD (9.1 ± 4.9 vs 42.2 ± 6.8 ng/ml) and OC (2.76 ± 2.08 vs 7.22 ± 1.66 ng/ml) **NSS**: calcitonin
O	S: ALP, OC, OPG, RANK-L	PWH (children) (n = 26) vs matched controls (n = 13)	**SS**: PWH lower OPG (mean ± SD 15.78 ± 2.53 vs 23.79 ± 4.39 pg/ml) and higher RANK-L (21.04 ± 4.78 vs 18.58 ± 2.28 ng/ml) and OC (5.35 ± 2.29 vs 3.09 ± 0.61 ng/ml) ALP not measured in controls.
P	S: ALP, calcium, CTX-I, Dkk-1, OC, OPG, phosphate, PTH, RANK-L, sclerostin, 25OHvitD	Severe PWH (children) (n = 44) vs matched controls (n = 40)	**SS**: PWH higher intact PTH (mean ± SD 31.10 ± 16.09 vs 19.5 ± 3.89 ng/l), OC (10.09 ± 5.34 vs 6.54 ± 3.28 nmol/l), sclerostin (1845 ± 671 vs 1521 ± 285 pg/ml) lower 25OHvitD (69.15 ± 36.50 vs 96.60 ±

(*continued on next page*)

Table 2 (*continued*)

Rf	Biochemical markers	Investigation groups	Conclusions
			36.75 nmol/l) **NSS**: other markers
Y	S: CRP, D-D, endostatin, FDP, ferritin, Hb, ICAM-1, leukocyte, MIF, monocyte, platelet, PLG, thrombomodulin, VEGF, α2-AP	Severe PWH (n = 144) vs healthy controls (n = 90) PWH without joint bleeding (n = 78) vs acute joint bleeding (n = 66)	PWH vs healthy controls (and also for joint bleed vs no acute joint bleed): **SS**: higher CRP, D-D, ferritin, FDP, leukocyte, MIF, PLG, VEGF; **NSS**: endostatin, Hb, ICAM-1, lactic acid, monocyte, platelet, thrombomodulin, α2-AP; **SS**: AUC in diagnosis of acute joint bleed: CRP: 0.829 (sensitivity 88.4%, specificity 67.9%); VEGF: 0.758 (sensitivity 82.8%, specificity 68.3%)
Z	*P: CSF2, CSF3, EGF, FGF2, IL4, IL13, MIP-1α, KC*	*Severe PWH (n = 37) Group 1 = No bleeding/target joints/ synovitis; Group 2 = Target joints and/or synovitis, no bleeding; Group 3 = Bleeding in the joint*	***SS**: Group 3 vs 1: CSF2, EGF, FGF2, IL4, IL13, MIP-1α decreased (ES, Cohen 1.25–2.18); SS unknown: Group 3 vs 1: KC, CSF3 elevated (ES = 0.80–1.54); Group 3 vs 2 and 2 vs 1: CSF2, CSF3, EGF, IL4 different (group 3 vs 2: ES = 0.53–1.17) and (group 2 vs 1: ES = 0.20–0.5)*
B	S: Dkk-1, RANK-L, RANK-L/OPG ratio, sclerostin	PWH (n = 89) vs matched controls (n = 30)	**SS**: PWH lower Dkk-1 (median ± IQR 21.24 ± 17.18 vs 26.16 ± 15.32 pg/ml), sclerostin (47.4 ± 26.93 vs 250 ± 250 pmol/l), (21.24 ± 17.18 vs 26.16 ± 15.32 pg/ml), higher RANK-L (0.23 ± 0.03 vs 0.04 ± 0.03 pmol/l), RANK-L/OPG ratio (0.063 ± 0.25 vs 0.005 ± 0.11)

References (*number in reference list manuscript*): **A.** Acharya 2011 (*34*); **B.** Anagnostis 2018 (*15*); **C.** Anagnostis 2014 (*16*); **D.** Hassab 2016 (*17*); **E.** Hua 2017 (*22*); **F.** Jansen 2009 (*21*); **G.** Karapinar 2014 (*35*); **H.** Kjeld 2018 (*29*); **I.** Oldenburg 2016 (*23*); **J.** Tseng 2016 (*18*); **K.** Ogata 2011 [53]; **L.** Sun 2019 (*24*); **M.** Mandal 2019 (*20*); **N.** Alioglu 2012 (*31*); **O.** Christoforidis 2010 (*32*); **P.** Giordano 2016 (*30*); **Q.** Holstein 2019 (*33*); **R.** Cooke 2019 (*37*); **S.** Haxaire 2018 (*39*); **T.** Manon-Jensen 2016 (*36*); **U.** van Vulpen 2015 (*38*); **V.** Pulles 2018 (*40*); **W.** Zetterberg 2014 (*42*); **X.** Manon-Jensen 2017 (*41*); **Y.** Xu 2020 (*19*); **Z.** Song 2018 (*43*)

Abbreviations: biochemical markers, see list abbreviations. ABR = annual bleeding rate, AUC = area under the curve, ELISA = enzyme-linked immune sorbent assay, ES = effect size, H = Kruskal-Wallis test, HA = hemophilic arthropathy, HJHS = hemophilia joint health score, IQR = interquartile range, IU = international units, MRI = magnetic resonance imaging, NSS = non-statistically significant, OR = odds ratio, P = plasma, PS=Pettersson score, PWH = patients with hemophilia, Rf = reference, rp = Pearson correlation coefficient, rs = Spearman's rank correlation coefficient, S = serum, SD = standard deviation, SE(M) = standard error of the mean, SS = statistically significant, U = urinary, US = ultrasound, vs = versus, ▲ = change.

[34] [23,35]. Comparing biochemical markers in different body fluids (plasma versus serum) provided inconsistent results for plasma CRP and serum high-sensitive CRP [22,35].

3.3. Investigative

Within this domain only a limited number of studies (n = 5) could be retrieved of which most studies were using an experimental in vivo model.

3.3.1. Animal studies

Type II collagen degradation, measured as C2M, may be a predictive marker for cartilage degradation and arthropathy development. An induced joint bleeding (on day 0) in hemophilic rats resulted in an unaffected serum C2M level after the first joint bleed. However, significantly increased levels after the second joint bleeding (on day 14) could be demonstrated. The serum levels of C2M after the second joint bleed significantly correlated with the degree of arthropathy on histology. Other collagen markers like serum C4M and P4NP7S increased significantly one day after the second joint bleeding, whereas serum C3M significantly decreased directly after the joint bleeding. Serum PRO-C5 and P3NP increased significantly one week after the first and second joint bleeding [36]. In hemophilic mice, plasma levels of C4M and PRO-C4 were also significantly increased two weeks after an induced hemarthrosis [37].

The change of urinary CTX-II and serum COMP, CS846 and C1,2C in response to a joint bleed, was investigated upon experimentally induced hemarthrosis in dogs, resulting in a significant increase in urinary CTX-II from day two to seven (from 75% to 155%) and serum COMP from baseline to day two (+46%) [38].

The concept that joint bleedings can promote a systemic pro-inflammatory condition and that inflammatory markers could be a suitable biomarker for the detection of hemarthrosis was investigated in hemophilic mice with and without induced joint bleeding. It turned out that serum calprotectin, a marker for residual inflammation, was higher in the hemarthrosis induced mice at two and twelve weeks compared with control hemophilic mice, where calprotectin was not detectable [39].

3.3.2. Studies in hemophilia patients

The animal data of the dog study were confirmed in a prospective study in patients with hemophilia. Urinary CTX-II (+52%) and serum CS846 (+14%) increased significantly five days after a joint bleeding compared with baseline in patients with hemophilia. Serum COMP and C1,2C levels did not significantly change [38].

Change in inflammatory markers upon joint bleeding was studied by Karapinar et al. Intra-individual serum ICAM-1, MIF and plasma CRP levels changed significantly in response to a joint bleeding (mean ICAM-1 359.5 pg/ml versus 399.8 pg/ml; mean MIF 74801 pg/ml versus 23,692 pg/ml; mean CRP 16.3 mg/dl versus 3.1 mg/dl at time of the bleeding versus one month after the bleeding) [35].

3.4. Prognostic

This category is considered to be the most important for implementation in clinical trials (and clinical practice), yet only two studies have been published so far. Pulles et al. included 31 hemophilia patients and followed them for a mean of 6.5 years. None of the individual markers (urinary CTX-II, serum C1,2C, CS846, COMP) measured at baseline predicted joint damage progression. However, the combined index of urinary CTX-II and serum CS846 was significantly associated with radiographic joint damage progression (odds ratio (OR) 8.8, 95% confidence interval (CI) 1.1–70.6). The discriminative ability of the prediction model of the combined index was 'acceptable' with an area under the curve (AUC) of 0.77 (95% CI: 0.60–0.95) [40]. A conference abstract describing 30 patients with severe hemophilia and severe arthropathy showed a correlation with the change of serum Coll2-1 and COMP and the change of an ultrasound score after three months follow-up [24].

3.5. Efficacy of intervention

So far there is only one study published investigating the efficacy of treatment on biochemical markers. Sun et al. reported via a conference abstract on concentrations of cartilage turnover biomarkers reflecting cartilage breakdown (Coll2-1 and COMP) in serum from 30 patients with

severe hemophilia A with severe arthropathy. Their preliminary data suggested that patients treated on-demand had lower cartilage turnover markers compared with patients treated on prophylactic basis though no statistically significance could be demonstrated [24].

3.6. Diagnostic

Sixteen studies compared biochemical markers between hemophilia patients and control patients. The most frequent studied biomarkers were serum COMP, VEGF and bone markers RANK-L and osteocalcin.

Serum COMP was studied in three different publications with contradictory results. Two studies reported a significantly higher level of COMP in patients with HA compared to healthy controls, whereas one study reported significantly lower levels of COMP [17,22,24]. While some cartilage and collagen markers were significantly increased in hemophilia patients compared to controls (C2M, CTX-II, C4M), other markers, also reflecting cartilage and collagen degradation, were significantly decreased (ADAMTS5, Coll2-1, C3A, C3M, C5M) in hemophilia patients. Cartilage formation markers did not differ between patients with HA and healthy controls, with the exception for the marker serum PRO—C5, which was significantly downregulated in hemophilia patients [22,24,41]. When the serum biomarkers C2M, CRPM and ADAMTS5 were combined, hemophilia patients could be distinguished from control subjects with an 85% accuracy [22].-

Regarding markers for bone metabolism, hemophilia patients revealed significant higher levels of serum parathormone and significant lower levels of 25-OH vitD in two out of two studies [30,31]. Levels of serum osteocalcin were reported as significantly higher in two studies, while contrasted by another study reporting significant lower levels of osteocalcin for hemophilia patients [30–32]. These conflicting results were also reported for serum sclerostin. [15,30] There were no obvious explanations for the discrepancy between the studies, other than the age of the participants (children versus adults) and the use of different assays. Some markers (RANK-L, CTX-I, Dkk-1, OPG) were reported as significantly different between hemophilia patients and controls, but other studies did not show this significance [15,22,30,32].

Acharya et al. investigated levels of plasma VEGF-A, SDF-1α and MMP-9 in hemophilia patients with joint disease versus patients with a bleeding disorder without joint disease and found a significant 4-fold elevation in hemophilia patients. Comparison of plasma and serum VEGF-levels in hemophilia patients and healthy controls was reported in four different studies with contradictory results [19,34,35,42]. Levels of plasma MMP-9, SDF-1α, soluble VCAM-1 and serum calprotectin, (high sensitive) CRP, plasminogen, FDP, D-dimer, ferritin and MIF were significantly increased in hemophilia patients compared to controls, whereas results for plasma leukocyte and serum lactic acid were unclear or contradictory [18,19,35,39]. Plasma hemoglobin, monocyte, platelet, soluble *E*-selectin and P-selectin and serum α2-AP, endostatin, thrombomodulin and ICAM-1 did not differ between subjects.

The studies mentioned above investigated the capacity of biochemical markers to *diagnose HA in the general population* and were therefore allocated to the diagnostic category. However, this approach is not relevant to the condition hemophilia as HA is a long-term complication of an already diagnosed disease. Studies investigating the value of biochemical markers in differentiating a bleed from a flare of HA are more useful and were allocated to the diagnostic category as well. Three studies investigated the differences between hemophilia patients with and without an acute joint bleeding [19,35,43]. Statistically significantly increased levels for serum D-dimer, ferritin, FDP, leukocyte, plasminogen and VEGF were reported in patients with acute joint bleeding. The other way, statistically significant decreases were seen for plasma EGF, CSF2, IL4/13, FGF2, MIP-1α in patients with a joint bleeding compared to patients without a bleeding or synovitis. Results for serum/plasma CRP and serum MIF were contradictory. No clear differences were found in patients with severe hemophilia with and without acute joint bleeding for the serum markers endostatin, ferritin, ICAM-1, lactic acid, thrombomodulin, VEGF and hemoglobin. Another important diagnostic tool is detecting active synovitis, for example by measuring osteopontin. Osteopontin is described in one conference abstract, showing that in hemophilia patients (≤16 years) with clinical synovitis, osteopontin was significantly higher compared to hemophilia patients without synovitis (and also compared to healthy controls) [27].

4. Discussion

After conducting this systematic review providing an overview of the current state of biochemical marker research in hemophilic arthropathy, we have to conclude that although promising in theory, none of the investigated markers in hemophilic arthropathy is currently sufficient for implementation in daily clinical practice. Clearly, larger homogenous longitudinal studies in well-defined populations should be performed to study the prognostic value of the most promising markers.

A further quest for robust biochemical markers that provide information on dynamic changes in tissue turnover and inflammation is needed because many new therapeutic options for hemophilia are being developed and with that well-defined and sensitive joint outcome assessments are needed to demonstrate their efficacy in joint protection. As such, biochemical marker research in HA is gaining more attention. Recently, a narrative review was published by Rodriguez-Merchan giving a general overview of biochemical marker studies in hemophilia [44]. We conducted a systematic review providing a comprehensive and complete overview of the current state of biomarker research in HA and categorized the biochemical markers to the different BIPED-criteria.

For clinical use in hemophilia, biomarkers in the prognostic category are by far the most important. These biomarkers are a baseline characteristic who predict future outcomes, such as the risk for joint damage development or progression in a particular patient.

Till date, only one full article and one conference abstract reported on the prognostic properties of biochemical markers. In this respect, the urinary marker CTX-II and serum markers CS846, COMP and Coll2-1 might be promising [24,40].

Likewise, only one study investigated markers reflecting changes upon an intervention (efficacy of intervention category) [24]. These markers may predict whether the intervention (started after the first baseline sampling) will be efficacious and can be used to monitor the effect of an intervention or to determine which patients are eligible for the intervention. Research in this category is very scarce and the existing limited data is pointing towards the serum markers Coll2-1 and COMP.

The diagnostic distinction between a joint bleed and a flare of HA is also clinically relevant, in contrast to the relevance of discriminating between HA patients and healthy control individuals. Unfortunately, the majority of the studies in the diagnostic category investigated biomarkers enabling *identification of HA in the general population* and only three studies in this category investigated the capacity to diagnose synovitis or acute joint bleeding[19,35,43]. In immature subjects, osteopontin was significantly higher in hemophilia patients with synovitis compared to hemophilia patients without synovitis. Regarding acute joint bleeding, three studies hinted towards some inflammatory markers that might be useful, but high inter-individual differences exist. This variability can potentially be diminished by a combined index of biomarkers capturing all pathogenic processes as it is known that patients with a similar bleeding history show a marked variability in joint damage with some patients developing osteochondral degeneration and other patients suffering from chronic synovitis [45].

Studies allocated to the investigative category are not limited by these inter-individual differences, as they investigate the change of biomarkers upon a joint bleeding within one patient. Karapinar et al. showed that serum ICAM-1, MIF and plasma CRP did not have the capacity to differentiate between patients with an without an acute joint bleeding, but intra-individual measurement of these markers showed a significant change in response to a joint bleeding. This emphasized the fact that biomarkers differ inter-individually suggesting that biomarkers

should be developed and used as a follow-up method within a patient, rather than using the same cut-off values for all patients [35]. However, for some of these markers it takes a few days before breakdown products of joint tissue can be measured in blood or urine, making them clinically less feasible as a tool to diagnose acute joint bleeding. Still, these studies can be useful to understand the changes in joint homeostasis after a joint bleeding and can be helpful in a first selection of interesting biomarkers. In this respect, urinary CTX-II, serum CS846, MIF, ICAM and plasma CRP in patients with hemophilia may be promising [35,38].

A next step in selecting promising markers to detect early changes even before imaging can visualize these changes or predict joint damage development/progression, is correlating biochemical markers with the severity of hemophilia (as a proxy for the severity of joint involvement) or the severity of arthropathy. This approach is used in publications allocated to the burden of disease category. At present, implemented markers for the severity of HA are imaging and symptoms recorded by health care providers or patients. In this category, more frequently studied biomarkers showed conflicting results. Significant correlations were reported for some cartilage and inflammation markers (serum: PRO—C2, COL-18N; plasma: soluble VCAM-1), but these studies were very limited [18,22,29]. Fig. 3 shows a summary of the most important results in the different BIPED-criteria.

As it turns out, biochemical marker research in HA is very heterogeneous and the practical use of biomarkers defines their requirements. Biomarkers used as a point-of-care tool in decision making in an individual patient are subjected to strict requirements regarding sensitivity, specificity and inter-individual differences. The use of biomarkers on population level (e.g. to monitor treatment effects) is much more feasible. The majority of the studies investigated biomarkers that have been discovered and validated for other purposes. In order to discover new biomarkers specific for HA, studying the multi-factorial pathobiology of HA will become paramount. Unbiased omics studies (e.g. proteomics), approaching biological systems as a whole and investigating multiple molecules simultaneously, will pave the way for innovative biomarker research and may reveal completely novel biomarkers that are specific for HA.

Biomarker research has some important limitations. First, the lack of a reference standard to assess the severity of HA is a major challenge. Physical function scores can reflect the functional joint status, but do not necessarily reflect damage to specific joint structures, complicating the association with biomarkers. In patients with HA, discrepancies between clinical function and imaging are well known. In both children and adults, joints without abnormalities in the HJHS showed osteochondral changes during routine ultrasound examination [46,47]. Stephensen et al. systematically reviewed the measurement properties of outcome measures used to evaluate physical function in children with hemophilia and concluded that evidence for the ability of outcome measurements to detect changes in physical function is limited and test-retest repeatability is lacking (apart from the HJHS) [48].

Radiologically, the degree of arthropathy is mainly assessed by the use of X-ray, which is especially suited for visualization of bone structures. Changes in cartilage and to a greater extent in synovial tissue are difficult to determine. Ultrasonography and MRI are a good alternative in patients with absent or limited arthropathy, as synovial changes are a strong predictor for 5-year bleeding and progression of arthropathy. Therefore, assessment of joint status by MRI or ultrasonography will lead to a more representative association, but these techniques also have their limitations as mentioned in the *Introduction* [8,49,50].

Moreover, some markers are highly influenced by other factors. Biomarker research in HA is attractive as it has no systemic inflammatory response. However, the risk of co-infection with human immunodeficiency virus (HIV)- or hepatitis C virus (HCV)-infection in hemophilia patients is increased due to contaminated blood products in the past and this can influence inflammatory marker levels. For example, HIV mono-infection is associated with a 88% higher CRP level in men, whereas HCV co-infection leads to substantially lower CRP levels [51]. Most articles lack adjustment for these contributing factors and therefore it is unclear whether CRP levels can be associated with arthropathy. Also, bone markers do not necessarily reflect bone status as they are often age-dependent and might be different in children compared to adults and also between children. Moreover, bone mineral density is significantly lower in severe hemophilia patients than in controls, both in adults and children, and also HCV- and HIV-infection affects bone mass as well as physical activity [52]. Furthermore, tissue specificity should be taken into account when interpreting biomarker levels in blood or urine. Most connective tissues are widespread throughout the body. The release of biochemical markers by joint tissue must overwhelm the release from other connective tissues. Finally, different assays, different batches and material from different suppliers contribute to heterogeneous assessments and may explain the observed contradictory results.

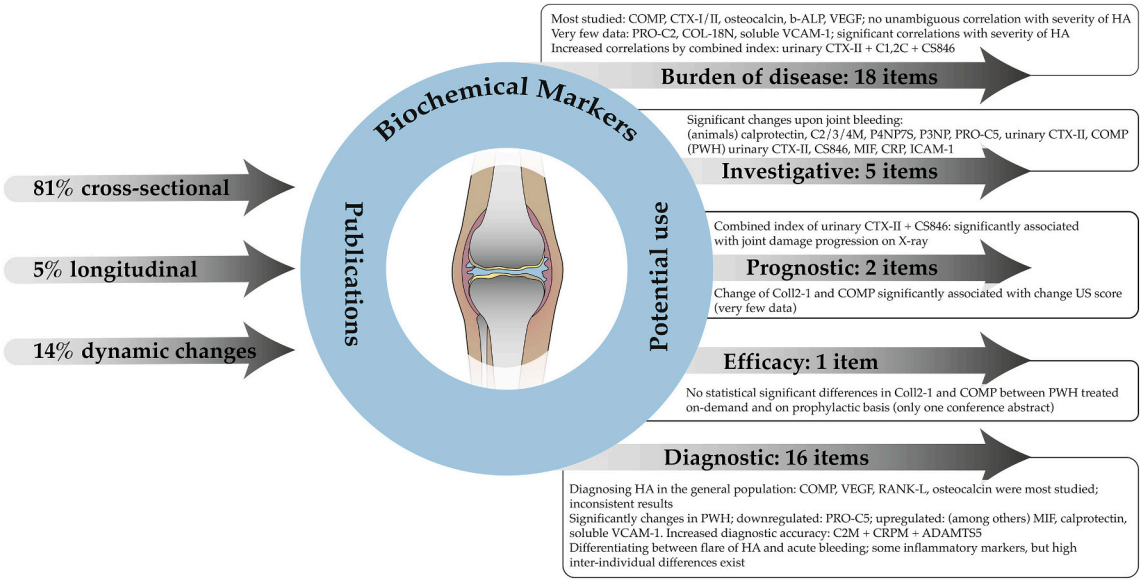

Fig. 3. Summary of the most important results in the different BIPED-categories.

5. Summary and future considerations

In conclusion, most biochemical markers for HA are still in an explorative stage and research in this area comes with challenges. The current rapid changes in the treatment landscape embarrasses longitudinal biomarker measurements under the same circumstances (e.g. patients may switch from regular prophylactic therapy to emicizumab). Also, translation to other treatment centers or countries can be difficult as there may be differences in laboratory techniques and procedures. Preferably, biomarkers should be stable during collection and storage and the assessment should be accurate and not too expensive.

We advise larger longitudinal studies with multiple measurements within one patient to eliminate inter-individual variability and study the prospective value of these markers. These studies should ideally be performed by consortia and the pharma industry when investigating new products and should include well-defined populations and preferably assess joint status by a method sensitive to little changes in all joint structures, e.g. MRI. In order to enlarge the quality and efficacy of biochemical marker research, the homogeneity of study designs should be increased and publication bias should be avoided. We should not only focus on the performance of single biochemical markers, but also consider combined indexes to increase correlations and diagnostic accuracy. Interventions where clear joint improvements are expected are an opportunity to study the change of biochemical markers. Ultimately, this may lead to translation into daily practice and more personalized medicine in hemophilia patients and gives potential for further development and utilization of biomarkers in HA.

Practice points

- Recurrent joint bleeds are the hallmark of hemophilia and can lead to invalidating arthropathy.
- Currently, progression of arthropathy is monitored by clinical symptoms, motion analysis and imaging techniques which provide a cumulative result of past dynamic processes.
- Biomarkers may monitor joint status more closely than the present diagnostic methods, by the reflection of subclinical damage and minor alterations in an early stage.
- Biomarker research in hemophilia is very heterogeneous, with different patient populations, relatively small sample sizes and the lack of a reference standard to assess arthropathy.

Research agenda

- More accurate evaluation of joint protection in clinical trials is urgently needed, especially with the numerous recent breakthroughs, accompanied by high costs.
- Categorizing biomarkers based on their utility (BIPED-classification) is of utmost importance to provide a solid basis for future research.
- To achieve translation into daily practice, the homogeneity of study designs should be increased, inter-individual variability should be eliminated as much as possible and combined indexes of biomarkers to increase correlations should be considered.

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Competing interest

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References

[1] Fischer K, Collins P, Björkman S, Blanchette V, Oh M, Fritsch S, et al. Trends in bleeding patterns during prophylaxis for severe haemophilia: observations from a series of prospective clinical trials. Haemophilia 2011;17:433–8. https://doi.org/10.1111/j.1365-2516.2010.02450.x.
[2] Stephensen D, Tait R, Brodie N, Collins P, Cheal R, Keeling D, et al. Changing patterns of bleeding in patients with severe haemophilia a. Haemophilia 2009;15:1210–4. https://doi.org/10.1111/j.1365-2516.2008.01876.x.
[3] Fischer K, Steen Carlsson K, Petrini P, HolmströmHolmstr M, Ljung R, Marijke van den Berg H, et al. Intermediate-dose versus high-dose prophylaxis for severe hemophilia: comparing outcome and costs since the 1970s. Blood 2013;122:1129–36. https://doi.org/10.1182/blood-2012-12.
[4] van Vulpen LFD, Thomas S, Keny SA, Mohanty SS. Synovitis and synovectomy in haemophilia. Haemophilia 2020:1–7. https://doi.org/10.1111/hae.14025.
[5] Pulles AE, Mastbergen SC, Schutgens REG, Lafeber FPJG, van Vulpen LFD. Pathophysiology of hemophilic arthropathy and potential targets for therapy. Pharmacol Res 2017;115:192–9. https://doi.org/10.1016/j.phrs.2016.11.032.
[6] Forneris E, Andreacchio A, Pollio B, Mannucci C, Franchini M, Mengoli C, et al. Gait analysis in children with haemophilia: first Italian experience at the Turin Haemophilia Centre. Haemophilia 2016;22:e184–91. https://doi.org/10.1111/hae.12920.
[7] Tijskens D, Lobet S, Eerdekens M, Peerlinck K, Hermans C, Van Damme A, et al. Paediatric patients with blood-induced ankle joint arthritis demonstrate physiological foot joint mechanics and energetics during walking. Haemophilia 2020:1–9. https://doi.org/10.1111/hae.14128.
[8] Doria AS. State-of-the-art imaging techniques for the evaluation of haemophilic arthropathy: present and future. Haemophilia 2010;16:107–14. https://doi.org/10.1111/j.1365-2516.2010.02307.x.
[9] Seuser A, Khayat CD, Negrier C, Sabbour A, Heijnen L. Evaluation of early musculoskeletal disease in patients with haemophilia: results from an expert consensus. Blood Coagul Fibrinolysis 2018;29:509–20. https://doi.org/10.1097/MBC.0000000000000767.
[10] Lafeber FPJG, van Spil WE. Osteoarthritis year 2013 in review: biomarkers; reflecting before moving forward, one step at a time. Osteoarthr Cartil 2013;21:1452–64. https://doi.org/10.1016/j.joca.2013.08.012.
[11] van Spil WE, Szilagyi IA. Osteoarthritis year in review 2019: biomarkers (biochemical markers). Osteoarthr Cartil 2020;28:296–315. https://doi.org/10.1016/j.joca.2019.11.007.
[12] Gavrilă BI, Ciofu C, Stoica V. Biomarkers in rheumatoid arthritis, what is new? J Med Life 2016;9:144–8.
[13] Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, et al. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthr Cartil 2006;14:723–7. https://doi.org/10.1016/j.joca.2006.04.001.
[14] FDA-NIH, BiomarkerWorkingGroup. BEST (biomarkers, EndpointS, and other tools) resource 2020.
[15] Anagnostis P, Vakalopoulou S, Christoulas D, Paschou SA, Papatheodorou A, Garipidou V, et al. The role of sclerostin/dickkopf-1 and receptor activator of nuclear factor kB ligand/osteoprotegerin signalling pathways in the development of osteoporosis in patients with haemophilia a and B: a cross-sectional study. Haemophilia 2018;24:316–22. https://doi.org/10.1111/hae.13384.
[16] Anagnostis P, Vakalopoulou S, Vyzantiadis TA, Charizopoulou M, Karras S, Goulis DG, et al. The clinical utility of bone turnover markers in the evaluation of bone disease in patients with haemophilia a and B. Haemophilia 2014;20:268–75. https://doi.org/10.1111/hae.12271.
[17] Hassab HMA, El-Gendy WM, El-Noueam KI, Abd El Ghany HM, Elwan MMA. Serum cartilage oligomeric matrix protein reflects radiological damage and functional status in hemophilic arthropathy patients. Egypt Rheumatol 2016;38:241–5. https://doi.org/10.1016/j.ejr.2015.09.005.
[18] Tseng YH, Chiou SS, Zeng YS, Tsai SP, Chen CS, Liao YM, et al. Soluble vascular cell adhesion molecular-1 is a potential biological indicator of hemophilic arthropathy. Med (United States) 2016:95. https://doi.org/10.1097/MD.0000000000005384.
[19] Xu H, Zhong R, Wang K, Li X, Zhao Y, Jiang J, et al. Diagnostic values of inflammatory and Angiogenic factors for acute joint bleeding in patients with severe hemophilia a. Clin Appl Thromb 2020;26. https://doi.org/10.1177/1076029619892683.
[20] P. Mandal, A. Jitani, A. Bhowmik, D. Gantait PC. Bone health in hemophilic Arthropathy - a systematic study from eastern India. Res Pract Thromb Haemost 2019;3:449.
[21] Jansen NWD, Roosendaal G, Lundin B, Heijnen L, Mauser-Bunschoten E, Bijlsma JWJ, et al. The combination of the biomarkers urinary C-terminal telopeptide of type II collagen, serum cartilage oligomeric matrix protein, and serum chondroitin sulfate 846 reflects cartilage damage in hemophilic arthropathy. Arthritis Rheum 2009;60:290–8. https://doi.org/10.1002/art.24184.
[22] Hua B, Olsen EHN, Sun S, Gudme CN, Wang L, Vandahl B, et al. Serological biomarkers detect active joint destruction and inflammation in patients with haemophilic arthropathy. Haemophilia 2017;23:e294–300. https://doi.org/10.1111/hae.13196.

[23] Oldenburg J, Zimmermann R, Katsarou O, Zanon E, Kellermann E, Lundin B, et al. Potential biomarkers of haemophilic arthropathy: correlations with compatible additive magnetic resonance imaging scores. Haemophilia 2016;22:760–4. https://doi.org/10.1111/hae.12936.
[24] Sun X, Zhuang J, Zhou X, Liu Z, Sun J. Relationship between serum cartilage turnover biomarkers and hemophilic arthropathy severity in adult patients with severe hemophilia a in China. Res Pract Thromb Haemost 2019;3:273–4.
[25] Conaghan PG. Osteoarthritis Assessment of Structural Change Working Group19; 2011. p. 606–10. https://doi.org/10.1016/j.joca.2011.02.018.Summary.
[26] Van Spil WE, Nair SC, Kinds MB, Emans PJ, Hilberdink WKHA, Welsing PMJ, et al. Systemic biochemical markers of joint metabolism and inflammation in relation to radiographic parameters and pain of the knee: data from CHECK, a cohort of early-osteoarthritis subjects. Osteoarthr Cartil 2015;23:48–56. https://doi.org/10.1016/j.joca.2014.09.003.
[27] Hakobyan N. Osteopontin as a biomarker for early stages of blood-induced joint disease in hemophilia patients. WFH Conf Abstr 2014;63:1.
[28] Fischer K, Van Hout BA, Van Der Bom JG, Grobbee DE, Van Den Berg HM. Association between joint bleeds and Pettersson scores in severe haemophilia. Acta Radiol 2002;43:528–32.
[29] Kjeld NG, Hua B, Karsdal MA, Sun S, Manon-Jensen T. The endothelial specific isoform of type XVIII collagen correlates to annual bleeding rate in haemophilia patients. PLoS One 2018;13. https://doi.org/10.1371/journal.pone.0190375.
[30] Giordano P, Brunetti G, Lassandro G, Notarangelo LD, Luciani M, Mura RM, et al. High serum sclerostin levels in children with haemophilia a. Br J Haematol 2016;172:293–5. https://doi.org/10.1111/bjh.13481.
[31] Alioglu B, Selver B, Ozsoy H, Koca G, Ozdemir M, Dallar Y. Evaluation of bone mineral density in Turkish children with severe haemophilia a: Ankara hospital experience. Haemophilia 2012;18:69–74. https://doi.org/10.1111/j.1365-2516.2011.02587.x.
[32] Christoforidis A, Economou M, Papadopoulou E, Kazantzidou E, Farmaki E, Tzimouli V, et al. Comparative study of dual energy X-ray absorptiometry and quantitative ultrasonography with the use of biochemical markers of bone turnover in boys with haemophilia. Haemophilia 2011;17. https://doi.org/10.1111/j.1365-2516.2010.02385.x.
[33] Holstein K, Witt L, Rolvien T, Schmidt T, Amling M, Barvencik F, et al. Bone mineral density and bone microstructure in patients with haemophilia in northern Germany: preliminary findings of a single Centre study. Haemophilia 2019;25:120–1.
[34] Acharya SS, Kaplan RN, Macdonald D, Fabiyi OT, DiMichele D, Lyden D. Neoangiogenesis contributes to the development of hemophilic synovitis. Blood 2011;117:2484–93. https://doi.org/10.1182/blood-2010-05-284653.
[35] Karapinar TH, Karadaş N, Özek G, Tüfekçi Ö, Atabay B, Türker M, et al. The investigation of relationship between joint findings and serum angiogenic and inflammatory factor levels in severe hemophilia a patients. Blood Coagul Fibrinolysis 2014;25:703–8. https://doi.org/10.1097/MBC.0000000000000131.
[36] Manon-Jensen T, Karsdal MA, Nielsen LN, Kjelgaard-Hansen M, Vandahl B, Olsen EHN, et al. Altered collagen turnover in factor VIII-deficient rats with hemophilic arthropathy identifies potential novel serological biomarkers in hemophilia. J Thromb Haemost 2016;14:2419–29. https://doi.org/10.1111/jth.13518.
[37] Cooke EJ, Wyseure T, Zhou JY, Gopal S, Nasamran CA, Fisch KM, et al. Mechanisms of vascular permeability and remodeling associated with hemarthrosis in factor VIII-deficient mice. J Thromb Haemost 2019;17:1815–26. https://doi.org/10.1111/jth.14567.
[38] van Vulpen LFD, van Meegeren MER, Roosendaal G, Jansen NWD, van Laar JM, Schutgens REG, et al. Biochemical markers of joint tissue damage increase shortly after a joint bleed; an explorative human and canine invivo study. Osteoarthr Cartil 2015;23:63–9. https://doi.org/10.1016/j.joca.2014.09.008.
[39] Haxaire C, Hakobyan N, Pannellini T, Carballo C, McIlwain D, Mak TW, et al. Blood-induced bone loss in murine hemophilic arthropathy is prevented by blocking the iRhom2/ADAM17/TNF-a pathway. Blood 2018;132:1064–74. https://doi.org/10.1182/blood-2017-12-820571.
[40] Pulles AE, Mastbergen SC, Foppen W, Schutgens REG, Lafeber FPJG, van Vulpen LFD. The combination of urinary CTX-II and serum CS-846: promising biochemical markers to predict radiographic progression of haemophilic arthropathy—an exploratory study. Haemophilia 2018;24:e278–80. https://doi.org/10.1111/hae.13554.
[41] Manon-Jensen T, Hua BL, Olsen EHN, Sun S, Gudme CN, Li J, et al. Increased basement membrane turnover and strongly attenuated interstitial matrix turnover is a key pathological feature of haemophilia. Haemophilia 2017;23:e515–8. https://doi.org/10.1111/hae.13329.
[42] Zetterberg E, Palmblad J, Wallensten R, Morfini M, Melchiorre D, Holmström M. Angiogenesis is increased in advanced haemophilic joint disease and characterised by normal pericyte coverage. Eur J Haematol 2014;92:256–62. https://doi.org/10.1111/ejh.12227.
[43] Song X, Enockson C, Fogg L, Boggio L, Simpson M, Hakobyan N. Circulating biochemical markers of early joint bleeding: validation study in humans. Res Pract Thromb Haemost 2018;2:69.
[44] Rodriguez-Merchan EC. Serological biomarkers in hemophilic arthropathy: can they be used to monitor bleeding and ongoing progression of blood-induced joint disease in patients with hemophilia? Blood Rev 2020;41:100642. https://doi.org/10.1016/j.blre.2019.100642.
[45] van Vulpen LFD, Mastbergen SC, Lafeber FPJG, Schutgens REG. Differential effects of bleeds on the development of arthropathy – basic and applied issues. Haemophilia 2017;23:521–7. https://doi.org/10.1111/hae.13236.
[46] Foppen W, van der Schaaf IC, Fischer K. Value of routine ultrasound in detecting early joint changes in children with haemophilia using the "Haemophilia early Arthropathy detection with UltraSound" protocol. Haemophilia 2016;22:121–5. https://doi.org/10.1111/hae.12769.
[47] Timmer MA, Foppen W, Schutgens REG, Pisters MF, Fischer K. Comparing findings of routine Haemophilia joint health score and Haemophlia early Arthropathy detection with UltraSound assessments in adults with haemophilia. Haemophilia 2017;23:e141–3. https://doi.org/10.1111/hae.13147.
[48] Stephensen D, Drechsler WI, Scott OM. Outcome measures monitoring physical function in children with haemophilia: a systematic review. Haemophilia 2014;20:306–21. https://doi.org/10.1111/hae.12299.
[49] Foppen W, Van Der Schaaf IC, Beek FJA, Mali WPTM, Fischer K. MRI predicts 5-year joint bleeding and development of arthropathy on radiographs in hemophilia. Blood Adv 2020;4:113–21. https://doi.org/10.1182/bloodadvances.2019001238.
[50] Foppen W, van der Schaaf IC, Beek FJA, Mali WPTM, Fischer K. Diagnostic accuracy of point-of-care ultrasound for evaluation of early blood-induced joint changes: comparison with MRI. Haemophilia 2018;24:971–9. https://doi.org/10.1111/hae.13524.
[51] Reingold JS, Wanke C, Kotler DP, Lewis CE, Tracy R, Heymsfield S, et al. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels the fat redistribution and metabolic change in HIV infection (FRAM) study. J Acquir Immune Defic Syndr 2008;48:142–8. https://doi.org/10.1097/QAI.0b013e3181685727.
[52] Iorio A, Fabbriciani G, Marcucci M, Brozzetti M, Filipponi P. Bone mineral density in haemophilia patients: a meta-analysis. Thromb Haemost 2010;103:596–603. https://doi.org/10.1160/TH09-09-0629.
[53] Ogata K. Matrix metalloproteinase (MMP)-3 is not effective for evaluating hemophilic arthropathy. Rinsho Byori 2011;59:37–41.