Haemophilia
The Official Journal of the World Federation of Hemophilia,
European Association for Haemophilia and Allied Disorders and
the Hemostasis & Thrombosis Research Society

Haemophilia (2016), **22**, 760–764 DOI: 10.1111/hae.12936

ORIGINAL ARTICLE *Musculoskeletal*

Potential biomarkers of haemophilic arthropathy: correlations with compatible additive magnetic resonance imaging scores

J. OLDENBURG,* R. ZIMMERMANN,† O. KATSAROU,‡ E. ZANON,§ E. KELLERMANN,¶ B. LUNDIN** and P. ELLINGHAUS††

**University Clinic Bonn, Bonn; †Kurpfalz Hospital and Haemophilia Centre for Children and Adults, Heidelberg, Germany; ‡Laikon General Hospital, Athens, Greece; §University of Padua Medical School, Padua, Italy; ¶Bayer Vital GmbH, Leverkusen, Germany; **Lund University and Skåne University Hospital, Lund, Sweden; and ††Bayer Pharma AG, Wuppertal, Germany*

Introduction: Although biomarkers are useful diagnostic tools to assess joint damage in osteoarthritis and rheumatoid arthritis, few data exist for biomarkers of haemophilic arthropathy. **Aim:** To evaluate the association between biomarkers and compatible additive magnetic resonance imaging (MRI) scores in patients with severe haemophilia A. **Methods:** Patients aged 12–35 years with no history of factor VIII (FVIII) inhibitors were enrolled in a controlled, cross-sectional, multinational investigation. Patients received primary or secondary prophylaxis or on-demand treatment with FVIII and underwent MRI on four joints (two ankles, two knees). Soluble biomarkers of cartilage and bone degradation, inflammation, and angiogenesis were assessed (serum levels of C-terminal telopeptides of type I collagen [CTX-I], cartilage oligomeric matrix protein [COMP], chondroitin-sulphate aggrecan turnover 846 epitope [CS846], tissue inhibitor of metalloproteinase 1 [TIMP-1]; plasma levels of vascular endothelial growth factor [VEGF], matrix metalloproteinases 3 and 9 [MMP3, MMP9]). Relationships between biomarkers and MRI scores were evaluated using Spearman rank correlation. **Results:** Biomarkers were assessed in 117 of 118 per-protocol patients. Mean and median CTX-I, COMP, TIMP-1, MMP3, MMP9, and VEGF values were within normal ranges (reference range not available for CS846 in healthy volunteers). No correlations between biomarkers and MRI scores were found, with the exception of CS846, which showed significant correlation in a subgroup of 22 on-demand patients ($r = 0.436$; $P = 0.04$). **Conclusions:** Compatible additive MRI scores showed no clear correlations with any of the potential biomarkers for haemophilic arthropathy in the overall population. CS846 levels were significantly correlated with MRI scores in patients treated on demand.

Keywords: arthropathy, biological markers, haemophilia A, joints, magnetic resonance imaging

Introduction

Biochemical markers can provide clinically useful diagnostic tools for monitoring changes in cartilage and bone turnover in people with destructive joint disease. In the fields of osteoarthritis (OA) and rheumatoid arthritis (RA), urine and blood biomarkers for degradation of cartilage, bone and synovial tissue have been studied in the evaluation of severity and progression of joint damage and in the determination of the effects of treatment [1–5]. Haemophilia A is an inherited disease caused by deficient coagulation factor VIII (FVIII) that results in spontaneous and trauma-related bleeding. Over time, recurrent bleeding into joints leads to inflammation, synovitis, and subsequent destruction of cartilage and bone (i.e. haemophilic arthropathy, which is also characterized by soft-tissue contractures, muscular atrophy, and angular deformities [6]). Development of arthropathy in patients with haemophilia A can be prevented or delayed by regular prophylactic infusions of FVIII products, thus avoiding joint bleeds and their sequelae [7–9]. Haemophilic arthropathy has some characteristics similar to degen-

Correspondence: Johannes Oldenburg, Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Sigmund-Freud-Str 25, 53127 Bonn, Germany.
Tel.: +49 228 287 15176/15175; fax: +49 228 287 14783;
e-mail: johannes.oldenburg@ukb.uni-bonn.de

Accepted after revision 2 February 2016

erative joint disease (i.e. OA) and inflammatory joint disease (i.e. RA) [5,10], but few data are available on the use of biomarkers for assessing the severity of joint disease in patients with haemophilia A. The current study explored biomarkers that have been identified in patients with OA and RA as reflecting severity of joint damage and evaluated the applicability of these biomarkers to haemophilic arthropathy.

Materials and methods

We conducted an exploratory analysis to evaluate the association between potential biomarkers of haemophilic arthropathy and compatible additive magnetic resonance imaging (MRI) scores [11] in patients with severe haemophilia A. Data for this analysis were derived from a cross-sectional, multicohort, multinational, epidemiologic, interventional investigation in which 129 patients aged 12–35 years treated with FVIII primary or secondary prophylaxis or on-demand treatment underwent MRI of four joints (two ankles, two knees) [12]. A plasma and a serum sample was collected from each patient at the time of enrolment for measurement of the levels of soluble biomarkers of cartilage and bone degradation, inflammation, and angiogenesis.

We assessed levels of the following markers: cartilage oligomeric matrix protein (COMP), a non-collagenous extracellular matrix protein in the thrombospondin family; chondroitin-sulphate aggrecan turnover 846 epitope (CS846), a glycosaminoglycan epitope on the large proteoglycan aggrecan; C-terminal telopeptides of type I collagen (CTX-I), a fragment released during degradation of type I collagen; matrix metalloproteinase 3 (MMP3), a stromelysin; matrix metalloproteinase 9 (MMP9), a gelatinase that degrades collagen of the extracellular matrix; tissue inhibitor of metalloproteinase 1 (TIMP-1), an endogenous metalloproteinase inhibitor; and vascular endothelial growth factor (VEGF), a pro-angiogenic factor (Table 1). The levels of all markers were measured in serum, with the exception of VEGF, MMP3, and MMP9, which were measured in platelet-poor plasma samples [13]. To avoid a circadian impact on the levels of biomarkers [14], blood sampling should be performed in the morning.

Serum COMP levels were assessed using the Wieslab® hCOMP enzyme-linked immunosorbent assay (ELISA) kit (Osteomedical, Hiddenhausen, Germany), and CS846 levels were quantified using CS846 ELISA (IBEX Pharmaceuticals, Montreal, QC, Canada). Serum CTX-I levels were measured using CrossLaps ELISA (Immunodiagnostics, Frankfurt, Germany). Serum levels of TIMP-1 were assessed using the Human TIMP-1 Immunoassay, and plasma levels of MMP3, MMP9, and VEGF were assessed using the Quantikine Human Total MMP3 Immunoassay, the Human MMP9 Immunoassay, and the Human VEGF Immunoassay respectively (R&D Systems, Heidelberg, Germany). Relationships between biomarkers and compatible additive MRI scores were evaluated using Spearman rank correlation.

Results and discussion

Data on serum or plasma levels of potential biomarkers of haemophilic arthropathy were available for 117 of 118 patients in the per-protocol population (i.e. patients without major protocol violations). Results are summarized in Table 1. Despite some individually high or low values, mean and median levels of CTX-I, COMP, MMP9, TIMP-1, VEGF, and MMP3 were within normal ranges for healthy subjects provided by the supplier of the respective assays. No normal ranges were available for CS846.

Higher compatible additive MRI scores indicate worse haemophilic arthropathy [11]. In our analysis, with the exception of CTX-I levels (which showed a

Table 1. Biomarker levels* and correlation with compatible additive MRI score (per-protocol population).

Biomarker	Marker for	Normal range†	Biomarker levels (n = 117)		Correlation with MRI score‡
			Mean ± SD	Median (range)	
COMP, μg mL^{-1}	Cartilage degradation	0.99–2.54	1.5 ± 0.3	1.6 (0.0–2.0)	0.100
CS846, ng mL^{-1}	Cartilage formation	§	255.3 ± 213.9	200.3 (0.0–1722.2)	0.053
CTX-I, ng mL^{-1}	Bone degradation	0.115–0.748	0.7 ± 0.6	0.6 (0.0–3.5)	−0.203
MMP3, ng mL^{-1}	Joint cartilage destruction	2.1–64.0	17.4 ± 9.4	16.6 (0.0–74.4)	−0.121
MMP9, ng mL^{-1}	Joint cartilage destruction	169–705	244.5 ± 161.8	219.1 (0.0–939.7)	0.156
TIMP-1, ng mL^{-1}	Periarticular bone loss	87–524	121.3 ± 41.2	121.8 (0.0–242.9)	0.005
VEGF, pg mL^{-1}	Inflammation and angiogenesis	62–707	226.4 ± 177.0	191.3 (0.0–845.5)	−0.084

COMP, cartilage oligomeric matrix protein; CS846, chondroitin-sulphate aggrecan turnover 846 epitope; CTX-I, C-terminal telopeptides of type I collagen; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; TIMP-1, tissue inhibitor of metalloproteinase 1; VEGF, vascular endothelial growth factor.

*Serum levels were measured for all the biomarkers except VEGF, MMP3, and MMP9, which were measured in platelet-poor plasma samples.

†Normal ranges for healthy patients as provided by supplier of test kits.

‡Spearman rank correlation coefficient for the correlation of biomarker and compatible additive MRI score.

§No normal range for healthy patients was provided by supplier of test kits. Serum CS846 levels in patients with haemophilic arthropathy who had not experienced any joint bleeds in the previous 3 months have been reported [5].

borderline significant result [$r = -0.203$]), biomarker levels did not correlate with MRI scores in the total study population (Table 1; Spearman correlation coefficients, $0.2 > r > -0.2$; all $P > 0.05$). However, the analysis revealed a significant positive correlation between serum CS846 levels and MRI scores in the subpopulation of 22 patients who were treated on demand ($r = 0.436$; $P = 0.04$; scatter plot shown in Fig. 1).

It has been reported that MMP3 levels increase in patients with RA as the synovium becomes inflamed [2]. TIMP-1 was proposed as a serum marker of periarticular bone loss in a study of patients with RA [15]. In patients with severe haemophilia A, levels of biomarkers of inflammation (C-reactive protein and macrophage migration inhibitory factor) increase during acute bleeding episodes whether or not the patient has joint damage [16]. In patients with haemophilia who have joint disease, plasma concentrations of the proangiogenic biomarkers VEGF-A, stromal cell-derived factor-1, and MMP9 are increased compared with healthy controls and compared with patients with bleeding disorders without joint disease [17,18].

In the study from which our data were derived, patients treated with FVIII on demand showed worse compatible additive MRI scores, worse clinical joint scores, and higher annualized joint bleeding rates than those treated with primary or secondary prophylaxis [12]. In our analysis, significantly increased levels of the cartilage formation marker CS846 were observed in on-demand patients with worse MRI scores (i.e. positive Spearman correlation). The relatively short half-lives of the studied biomarkers combined with substantially more frequent bleeding in on-demand patients (range, 5.2–39.4 index joint bleeds year^{-1}) than in patients who received primary or secondary prophylaxis (range, 0–10.6 index joint bleeds year^{-1}) [12] could potentially explain the more readily detectable CS846 levels in on-demand patients.

Compatible additive MRI scores mainly reflect lifetime accumulated joint damage, whereas biomarker levels reflect ongoing tissue deterioration and/or regeneration (i.e. present disease activity). Because joint arthropathy is a degenerative condition that can develop over decades [6,17], metabolic changes may be moderate during this process. Because of the low frequency of joint bleeding in the prophylaxis group in this study, biomarkers related to direct consequences of bleeding or chronic synovitis would likely not be correlated with MRI scores in prophylaxis patients because biomarker half-life is short and the MRI analyses were unlikely to be performed within a sufficient time frame of a joint bleeding event. In the on-demand group, the higher frequency of joint bleeding could result in higher present disease activity at the time of the MRI analysis; thus, in patients treated on demand, biomarkers of synovitis can be expected to correlate with pathologic joint findings because these patients are more likely to show some signs of active synovitis. Consequently, CS846 levels may reflect the altered joint metabolism that exists after a bleeding event. Although the correlation between the time of last bleed and the expression of biomarkers was not specifically investigated in this study, in another study involving 10 haemophilia patients, biochemical markers of joint damage such as CS846 were shown to increase within a week after a single joint bleed [19].

Few published data exist on biomarker correlations with joint status in patients with haemophilia. In a

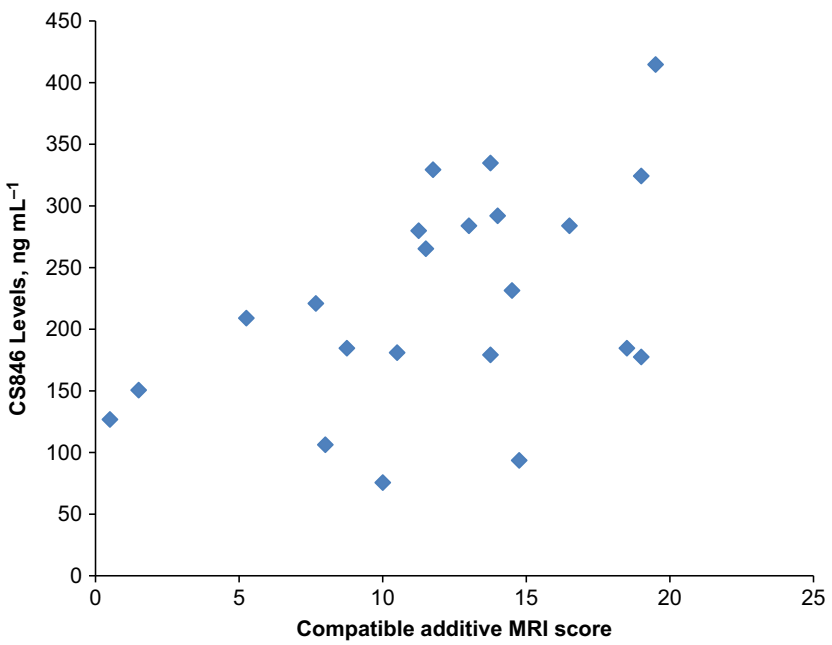

Fig. 1. Correlation between the compatible additive MRI score and CS846 levels in patients treated on demand ($n = 22$).

study of patients with haemophilia A or B with varying degrees of arthropathy, levels of biomarkers of cartilage destruction or synthesis (urinary C-terminal telopeptide of type II collagen [CTX-II], serum cartilage cleavage products [C1,2C], and serum CS846) significantly correlated with total Pettersson (radiographic) scores, but levels of CTX-I did not correlate with total or bone-specific items of the Pettersson score [5]. Furthermore, regression analyses showed that the combination of urinary CTX-II, serum COMP, and serum CS846 significantly correlated with radiographic scores [5]. Stronger correlations in this study than in our analysis can potentially be explained by use of radiography in the former study because radiography is less sensitive in detecting joint changes than MRI [7,20–23]. Therefore, positive radiographic scores would be expected to reflect only more severe joint changes relative to positive MRI scores. Groups of patients with positive radiographic scores may have more bleeds and higher present disease activity compared with groups of patients with positive MRI scores.

In haemophilia, joint arthropathy is not a systemic disease. Arthropathy in patients with haemophilia is restricted to certain joints, and the severity and activity of the arthropathy depends on the frequency and severity of bleeding events in individual joints [6]. Analysis of biomarkers of joint disease in patients with haemophilia may be more complex than in patients with RA or OA.

Conclusion

Our analysis is the first investigation of correlations between MRI findings and potential biomarkers of haemophilic arthropathy. We found that compatible additive MRI joint scores did not show any clear correlations with potential biomarkers of haemophilic arthropathy, except for CS846 in on-demand patients. The data from the on-demand subgroup suggest that biomarker levels may correlate with bleeding incidence, but we were unable to prove this relationship because most patients in our study population were treated with prophylaxis to prevent bleeding. Our results showed that biomarkers and MRI joint status reflect different disease properties, convey complementary information, and support the value of prophylaxis, which results in lower disease activity compared with on-demand therapy.

Acknowledgements

J. Oldenburg was the principal investigator of the study; contributed to the clinical study protocol, design and study report; and reviewed all manuscript drafts. B. Lundin was the lead radiologist of the study; supervised the design, implementation and interpretation of the MRI investigations; and reviewed all manuscript drafts. E. Kellermann contributed to the design and conduct of the study and the clinical study protocol and report, and reviewed all manuscript drafts. R. Zimmermann, O. Katsarou, and E. Zanon were coinvestigators for the study and reviewed all manuscript drafts. P. Ellinghaus contributed to the design and conduct of the biomarker measurements of the study and the clinical study protocol and reviewed each manuscript draft. All authors approved the final version of the manuscript.

This study was funded by Bayer Pharma AG, Leverkusen, Germany. Medical writing assistance was provided by Jane A. Phillips, PhD, from Complete Healthcare Communications, LLC, and was fully funded by Bayer.

The study investigators were as follows: *Germany:* D. Franke (Magdeburg), J. Oldenburg (Bonn), H. Pollmann (Münster), R. Zimmermann (Heidelberg); *Greece:* O. Katsarou (Athens), G. Theodossiades (Athens); *Italy:* G. Mazzucconi (Rome), E. Santagostino (Milan), A. Tagliaferri (Parma), E. Zanon (Padova); *Spain:* F. Lopez (La Coruna), F. Querol (Valencia); *Sweden:* E. Berntorp (Malmö), M. Homström (Stockholm); *United Kingdom:* P. Chowdary (London).

Disclosures

J. Oldenburg received reimbursement for attending symposia/congresses and/or honoraria for speaking or consulting, and/or funds for research from Bayer and other companies. O. Katsarou is a member of the European Haemophilia Standardization Board sponsored by Baxter. E. Zanon has acted as a paid consultant for Bayer, Baxter, Novo Nordisk, and Grifols. E. Kellermann is an employee of Bayer Vital GmbH. B. Lundin is employed by the Center for Medical Imaging and Physiology at Skåne University Hospital and has been under contract to Bayer for work performed for the cross-sectional study 12948 and is under contract for work performed for SPINART. P. Ellinghaus is an employee of Bayer Pharma AG. R. Zimmermann has no conflicts of interest to declare.

References

1 Lindstrom TM, Robinson WH. Biomarkers for rheumatoid arthritis: making it personal. *Scand J Clin Lab Invest Suppl* 2010; **242**: 79–84.

2 Karsdal MA, Woodworth T, Henriksen K *et al.* Biochemical markers of ongoing joint damage in rheumatoid arthritis–current and future applications, limitations and opportunities. *Arthritis Res Ther* 2011; **13**: 215.

3 Patra D, Sandell LJ. Recent advances in biomarkers in osteoarthritis. *Curr Opin Rheumatol* 2011; **23**: 465–70.

4 Garnero P, Delmas PD. Biomarkers in osteoarthritis. *Curr Opin Rheumatol* 2003; **15**: 641–6.

5 Jansen NW, Roosendaal G, Lundin B *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.

6 Srivastava A, Brewer AK, Mauser-Bunschoten EP *et al.* Guidelines for the management of hemophilia. *Haemophilia* 2013; **19**: e1–47.

7 Manco-Johnson MJ, Abshire TC, Shapiro AD *et al.* Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**: 535–44.

8 Brackmann HH, Eickhoff HJ, Oldenburg J, Hammerstein U. Long-term therapy and on-demand treatment of children and adolescents with severe haemophilia A: 12 years of experience. *Haemostasis* 1992; **22**: 251–8.

9 Manco-Johnson MJ, Kempton CL, Reding MT *et al.* Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose-formulated recombinant factor VIII in adults with severe hemophilia A (SPINART) [published correction appears in *J Thromb Haemost*. 2014;12:119-122.]. *J Thromb Haemost* 2013; **11**: 1119–27.

10 Valentino LA. Blood-induced joint disease: the pathophysiology of hemophilic

arthropathy. *J Thromb Haemost* 2010; **8**: 1895–902.
11 Lundin B, Babyn P, Doria AS *et al.* Compatible scales for progressive and additive MRI assessments of haemophilic arthropathy. *Haemophilia* 2005; **11**: 109–15.
12 Oldenburg J, Zimmermann R, Katsarou O *et al.* Controlled, cross-sectional MRI evaluation of joint status in severe haemophilia A patients treated with prophylaxis vs. on demand. *Haemophilia* 2015; **21**: 171–9.
13 Olson FJ, Schmidt C, Gummesson A *et al.* Circulating matrix metalloproteinase 9 levels in relation to sampling methods, femoral and carotid atherosclerosis. *J Intern Med* 2008; **263**: 626–35.
14 Kong SY, Stabler TV, Criscione LG, Elliott AL, Jordan JM, Kraus VB. Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. *Arthritis Rheum* 2006; **54**: 2496–504.
15 Murphy E, Roux-Lombard P, Rooney T, Fitzgerald O, Dayer JM, Bresnihan B. Serum levels of tissue inhibitor of metalloproteinase-1 and periarticular bone loss in early rheumatoid arthritis. *Clin Rheumatol* 2009; **28**: 285–91.
16 Karapnar TH, Karadas N, Ozek G *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.
17 Acharya SS. Hemophilic joint disease – current perspective and potential future strategies. *Transfus Apher Sci* 2008; **38**: 49–55.
18 Acharya SS, Kaplan RNM, Macdonald D, Fabiyi OT, Dimichele DM, Lyden DMP. Neoangiogenesis contributes to the development of hemophilic synovitis. *Blood* 2011; **117**: 2484–93.
19 van Vulpen LF, van Meegeren ME, Roosendaal G *et al.* Biochemical markers of joint tissue damage increase shortly after a joint bleed; an explorative human and canine in vivo study. *Osteoarthritis Cartilage* 2015; **23**: 63–9.
20 Pergantou H, Platokouki H, Matsinos G *et al.* Assessment of the progression of haemophilic arthropathy in children. *Haemophilia* 2010; **16**: 124–9.
21 Yu W, Lin Q, Guermazi A *et al.* Comparison of radiography, CT and MR imaging in detection of arthropathies in patients with haemophilia. *Haemophilia* 2009; **15**: 1090–6.
22 Nuss R, Kilcoyne RF, Rivard GE, Murphy J. Late clinical, plain X-ray and magnetic resonance imaging findings in haemophilic joints treated with radiosynoviorthesis. *Haemophilia* 2000; **6**: 658–63.
23 Hassan TH, Badr MA, El-Gerby KM. Correlation between musculoskeletal function and radiological joint scores in haemophilia A adolescents. *Haemophilia* 2011; **17**: 920–5.

