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Biomarkers and immunological parameters in haemophilia and rheumatoid arthritis patients: a comparative multiplexing laboratory study

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Abstract

Introduction: Haemophilia (HA) and rheumatoid arthritis (RA) patients may develop joint damage caused by recurrent joint bleedings in HA or by chronic inflammation in RA. Only few data exist for biomarker studies in these patients.

Aim: The objective of the present study is to assess a large array of biomarkers in peripheral blood samples obtained from HA patients without or with arthropathy and to compare pattern to RA patients and healthy controls.

Methods: A panel of biomarkers was assessed in 129 men (40 HA patients without arthropathy, 23 HA patients with arthropathy, 23 RA patients and 43 control subjects). 37 different biomarkers (cytokines, angiogenesis-related proteins) were analysed using a multiple analyte profiling technology and supplemented by acute phase proteins, coagulation and immunological parameters.

Results: Evidence for systemic inflammation was obtained by increased acute phase reactants in all patient groups. 13 or 14 from 42 soluble parameters demonstrated significant differences ($p < .05$) between HA patients without arthropathy and healthy controls, or between HA patients with arthropathy and healthy controls, respectively. Largely overlapping patterns were obtained except for interleukin-7 being increased in HA patients without arthropathy and being decreased in HA in the presence of arthropathy.

Conclusions: In addition to data supporting systemic inflammation, we provide evidence for a common biomarker profile in HA patients and RA patients compared to healthy controls. A distinctive biomarker profile for HA patients with arthropathy did not appear except for interleukin-7 demonstrating specific changes depending on the absence or presence of arthropathy in HA patients.

KEYWORDS
haemophilic arthropathy, rheumatoid arthritis, biomarker study, joint bleeding

1 | INTRODUCTION

Joints are the most frequent bleeding site in patients with haemophilia A and B. Replacement therapy with coagulation factor VIII (FVIII) or IX (FIX) effectively reduces bleeding rates. However, even the best standard of care can not entirely prevent the occurrence of overt and subclinical joint bleedings which collectively lead to chronic structural and functional joint damage.[1] In about eighty per cent of haemophilia patients, recurrent joint bleeding episodes cause joint damage, predominantly affecting knees, elbows and ankles conceptualized as target joints.[2] The progression of the disease leads to chronic synovitis and the development of haemophilic arthropathy (HAP), a disabling condition characterized by chronic pain, joint deformities with loss of function and reduced quality of life.[3]

The current strategy to control HAP is prevention of recurrent bleeding episodes by prophylactic factor replacement.[4] However, despite identical haemophilia classification (mild, moderate, severe) and treatment standards, bleedings rates and HAP development vary underlining the clinical heterogeneity of haemophilia patients.[5] A multitude of factors modulates the clinical HAP phenotype. In addition, there is no linear progression in the development of HAP with individually defined delay between joint bleeds, radiographically detectable lesions and loss of function.[6]

Haemophilia may be considered the prototypical disease leading to blood-induced joint damage (BIJD). Like BIJD in arthritis, the pathogenesis of HAP remains incompletely understood. The joint space is physiologically free of blood. When blood enters the joint space, arthritis typically develops irrespective of the underlying cause (injury, chronic inflammation, surgery, coagulation defect). In the early stages of HAP, blood-derived iron deposits mediate oxidative stress and chronic inflammation (synovitis) extending to the articular cartilage surface and resulting in cartilage damage.[7] Synovitis is a highly complex, chronic inflammatory process involved in the development of HAP, characterized by synovial hyperplasia as the outcome of the interplay between synovial cell proliferation and apoptosis, by cartilage destruction triggered and sustained by inflammatory mediators and immigrating inflammatory cells and by neoangiogenesis. Anabolic growth factors (eg TGF, IGF, FGF) and catabolic cytokines (eg IL-1, TNF) variably initiate and propagate cellular processes,[8] which ultimately lead to the progression of the disease including subchondral bone damage and loss of bone mass. Later stages of HAP are mainly characterized by dysregulation of bone turnover and enhanced bone resorption.[9] In brief, the initial phase of HAP is mainly characterized by inflammation and demonstrates strong similarities with rheumatoid arthritis (RA).[10] In late-stage HAP, inflammatory aspects persist with degenerative processes taking over with similarities to osteoarthritis (OA).[11,12]

In destructive joint disease, biomarkers have emerged as a useful tool to monitor cartilage degradation and bone turnover.[13] Biomarker panels have been established as part of clinical practice in RA patients with the assumption that monitoring joint damage may result in individualized treatment regimens tailored for the stage and dynamic pattern of joint damage.[14] However, despite the striking similarities between HAP and arthropathies of other origin, only few biomarker studies in HAP have been conducted and recently summarized.[15] Therefore, we performed a cohort study exploring biomarkers (immunological, inflammatory, angiogenesis-related parameters and cytokines) in HA and RA patients with the aim to identify common and differing biomarker profiles related to the presence or absence of arthropathy. The chosen biomarkers have been shown to be relevant to angiogenesis, cell proliferation, cell adhesion, apoptosis and inflammation.[16]

2 | METHODS

2.1 | Study design

The cohort study was conducted at the Haemophilia Centre, 2nd Department of Medicine, University Hospital Frankfurt/Main, Germany. Male participants were recruited between May 2016 and December 2017. Written informed consent was obtained from all patients. The study protocol was approved by the Ethics Committee of the Medical Faculty, University Frankfurt/Main (vote number 439/13).Exclusion and inclusion criteria were defined a priori. All consecutive male patients admitted to our hospital for HA and RA were enrolled for this study. As participation in the study was part of a regular medical visit, the investigators were fully aware of the clinical status of the patients at all the time. However, due to the fact that all consecutive patients were asked to participate a selection bias could be excluded. Inclusion criteria for the HA group were severe, moderate or mild haemophilia A and B, with or without arthropathy and no history of inhibitors or chronical inflammatory disease. The RA group required a diagnosis according to ACR/EULAR criteria. Exclusion criteria were active bleeding within the last four weeks, chronic viral infection (hepatitis, HIV), malignancy, history of myocardial infarction or acute inflammation of any origin. Arthropathy was characterized by painful swelling, functional impairment, typical radiology images and/or orthopaedic interventions. Male healthy blood donors served as control group.

2.2 | Laboratory analyses

Venous blood samples were drawn from all participants at a single time point as part of their regular medical visit. Serum and plasma samples were obtained by centrifugation at 2600 g for 15 minutes and stored frozen at −80°C until all samples were analysed at the same time. 24 cytokines and 13 angiogenesis-related proteins (Table 1) were measured using multiplex kits (Bio-Plex Pro™ Human Cancer Biomarker Panel 1, Panel 2 and Bio-Plex Pro™ Human Th17 Cytokine Panel, Bio-Rad Laboratories). The fluorescent bead-based technology utilizes the Luminex® 200 (Luminex Corporation) for read-out and was carried out according the manufacturer's instructions including at least seven point dilution standard curves followed

TABLE 1 Parameters analysed in haemophilia patients without/with arthropathy, in rheumatoid arthritis patients and in healthy blood donors

Cytokines
Interleukin−1b (IL−1b)
IL−1ra
IL−2/4/5/6/7/8/9/10/12/13/15/17
Eotaxin
Fibroblast Growth Factor (FGF basic)
Granulocyte Colony Stimulating Factor (G-CSF)
Granulocyte Macrophage Colony Stimulating Factor (GM-CSF)
Interferon Gamma (IFN-γ)
Interferon Gamma-Induced Protein 10 (IP−10)
Monocyte Chemoattractant Protein−1 (MCP 1, MCAF)
Platelet-Derived Growth Factor Subunit B (PDGF-BB)
Macrophage Inflammatory Protein 1b (MIP−1b)
Regulated upon activation, normal T-cell expressed and secreted (RANTES)
Tumour Necrosis Factor-α (TNF-α)
Angiogenesis markers
Vascular Endothelial Growth Factor (VEGF)
Vascular Endothelial Growth Factor Receptor 1 (VEGFR−1)
Vascular Endothelial Growth Factor Receptor 2 (VEGFR−2)
Stem Cell Factor (SCF)
Platelet Endothelial Cell Adhesion Molecule (PECAM−1)
Epidermal Growth Factor Receptor (EGFR)
Epidermal Growth Factor Receptor 2 (HER2 neu)
Tyrosine Kinase Receptor TIE−2
Follistatin
Hepatocyte Growth Factor (HGF)
Osteopontin
Prolactin
Leptin
Acute phase proteins
C-reactive protein (CRP)
α−2-macroglobulin
Ferritin
Fibrinogen
Haptoglobin
Miscellaneous
Full blood count
Anti-CCP antibody

by calculations using Bio-Plex® software (Bio-Plex Manager®, Bio-Rad Laboratories). The sensitivity, specificity and limit of detection of the assays can be found in the manufacturer's tech note.[17,18] Coagulation parameters were obtained using the BCS® coagulation analyser (Siemens Medical Solutions Diagnostics). Full blood count, acute phase proteins and anti-CCP antibody titre were analysed using standard methods.

2.3 | Statistical analysis

All samples were taken at a single time point, and data were compared between groups (HA patients without or with arthropathy, RA patients and healthy blood donors) using Wilcoxon-Mann-Whitney test and BiAS software (Epsilon Verlag GmbH). *P* values <.05 for paired samples were considered statistically significant.

3 | RESULTS

3.1 | Study subjects

We enrolled for the study 129 male participants older than 30 years with the assumption that beyond the age of 25 years, growth-related bone and cartilage turnover would not interfere with the study [19]. The first group including 63 HA patients (haemophilia A, n = 55; haemophilia B, n = 8), including severe (n = 27), moderate (n = 10) and mild haemophilia (n = 26). HAP was established in 23 haemophilia A patients (severe, n = 16; moderate, n = 4; mild, n = 3). The second group of patients consisted of 23 male RA patients, and the control group of 43 male healthy blood donors. The median age of HA patients was 49 years, of RA patients 63 years and of the control group 47 years, respectively (patients characteristics are specified in Table 2). At the time of sampling, none of the study participants showed signs or symptoms of active bleeding or ongoing inflammation. As overweight has an impact on joint health and chronic inflammation, we excluded patients with a body mass index above 25 kg/m^2.

3.2 | Comparison between haemophilia patients without arthropathy, rheumatoid arthritis patients and healthy controls

13 from a total of 42 soluble parameters (31%) demonstrated significant differences ($p < .05$) between HA patients without arthropathy and healthy controls (Table 3). Among the acute phase reactants, ferritin and α-2-macroglobulin were significantly increased. Cytokines and angiogenesis markers were increased (IL-7, Leptin, PECAM-1, IL-10, IL-12, IP-10) or decreased (VEGFR-1, VEGFR-2, HGF, Follistatin, MIP-1b), respectively. When comparing HA patients without arthropathy to RA patients, only two parameters with significant differences could be identified with IL-7 increased ($p = .024$) and VEGFR-1 decreased ($p = .002$) in HA patients.

TABLE 2 Clinical characteristics of the patients (n = 129).

Patient subgroup	n (age [years])
Haemophilia	63 (49)
Haemophilia A	55
Haemophilia B	8
Without arthropathy	40
Severe	11
Moderate	6
Mild	23
With arthropathy	23
Severe	16
Moderate	4
Mild	3
Rheumatoid Arthritis	23 (63)
Control group	43 (47)

TABLE 3 Parameters significantly different in HA patients without arthropathy and RA patients compared to healthy controls.

Parameter	Patients	Mean (median)	Min-Max	*p*
Ferritin (ng/mL)	HA	213 (155)	15.0-1645	<.001 vs controls
	RA	219 (215)	16.0-649	.228 vs HA
	Control group	40.0 (29.0)	6.00-138	
MIP−1b (pg/mL)	HA	74.2 (70,2)	30.7-140	<.001 vs controls
	RA	86.7 (74.1)	37.1-185	.543 vs HA
	Control group	124 (117)	67.6-352	
Leptin (pg/mL)	HA	3309 (2794)	1020-12034	<.001 vs controls
	RA	3455 (2751)	1034-14088	.385 vs HA
	Control group	2127 (1625)	711-5321	
PECAM−1 (pg/mL)	HA	5391 (5207)	3735-9264	<.001 vs controls
	RA	5354 (5102)	3214-9330	.718 vs HA
	Control group	4546 (4509)	2833-6242	
IL−7 (pg/mL)	HA	44.1 (8.10)	2.10-1125	.002 vs controls
	RA	20.2 (13.5)	2.10-86.4	.024 vs HA
	Control group	18.5 (15.7)	1.80-81.8	
IL−10 (pg/mL)	HA	79.0 (24.5)	15.8-1989	.002 vs controls
	RA	102 (29.5)	7.20-1521	.369 vs HA
	Control group	22.8 (18.5)	6.30-115	
IL−12 (pg/mL)	HA	42.1 (22.0)	14.0-313	.010 vs controls
	RA	69.6 (26.8)	0.60-784	.401 vs HA
	Control group	23.5 (16.6)	1.40-106	
Alpha−2-Macroglobulin (mg/dl)	HA	237 (210)	114-480	.017 vs controls
	RA	208 (179)	115-328	.532 vs HA
	Control group	177 (164)	102-469	
VEGFR−1 (pg/mL)	HA	278 (248)	94.5-539	<.001 vs controls
	RA	435 (435)	129-804	.002 vs HA
	Control group	518 (459)	73.1-1243	
VEGFR−2 (pg/mL)	HA	3447 (3481)	1511-6092	<.001 vs controls
	RA	3373 (3135)	1457-6273	.478 vs HA
	Control group	4084 (3995)	2259-5238	
IP−10 (pg/mL)	HA	786 (586)	164-4140	<.001 vs controls
	RA	876 (652)	268-3522	.13 vs HA
	Control group	457 (379)	245-1941	
HGF (pg/mL)	HA	945 (873)	375-1783	<.001 vs controls
	RA	1041 (898)	626-1752	.185 vs HA
	Control group	1362 (1398)	797-1851	
Follistatin (pg/mL)	HA	935 (898)	370-1670	<.001 vs controls
	RA	1013 (949)	433-1789	.553 vs HA
	Control group	1249 (1240)	555-2391	

3.3 | Comparison between haemophilia patients with arthropathy, rheumatoid arthritis patients and healthy controls

14 from a total of 42 soluble parameters (33%) demonstrated significant differences ($p < .05$) between HA patients with arthropathy and healthy controls (Table 4). The increase in the acute phase reactants C reactive protein, ferritin and α-2-macroglobulin was accompanied by an increased white blood cell count. Cytokines and angiogenesis markers were increased (IP-10, PECAM-1, IL-10, IL-12, VEGF,) or decreased (MIP-1b, HGF, VEGFR-2, TIE-2, IL-7, FGF basic), respectively. When comparing HA patients with arthropathy to RA patients, the only significant difference obtained was a decreased ferritin ($p = .008$) in HA patients with arthropathy.

TABLE 4 Parameters significantly different in HA patients with arthropathy (HA +AP) and RA patients compared to healthy controls

Parameter	Patients	Mean (median)	Min-Max	*p*
MIP−1b (pg/mL)	HA +AP	85.6 (68.9)	34.0-292	<.001 vs controls
	RA	86.7 (74.1)	37.1-185	.711 vs HA +AP
	Control group	124 (117)	67.6-352	
HGF (pg/mL)	HA +AP	894 (861)	536-1543	<.001 vs controls
	RA	1041 (898)	626-1752	.136 vs HA +AP
	Control group	1362 (1398)	797-1851	
IP−10 (pg/mL)	HA +AP	1041 (612)	210-3722	<.001 vs controls
	RA	876 (652)	268-3522	.663 vs HA +AP
	Control group	457 (379)	245-1941	
VEGFR−2 (pg/mL)	HA +AP	3394 (3322)	1620-5257	<.001 vs controls
	RA	3373 (3135)	1457-6273	.571 vs HA +AP
	Control group	4084 (3995)	2259-5238	
TIE−2 (pg/mL)	HA +AP	8030 (8353)	2387-13177	<.001 vs controls
	RA	7456 (5710)	4679-18001	.148 vs HA +AP
	Control group	11153 (11152)	6400-17458	
Ferritin (ng/ml)	HA +AP	136 (128)	17.0-452	<.001 vs controls
	RA	219 (215)	16.0-649	.008 vs HA +AP
	Control group	40.0 (29.0)	6.00-138	
CRP (mg/dL)	HA +AP	0.60 (0.33)	0.01-2.42	.008 vs controls
	RA	0.58 (0.29)	0.08-3.25	.648 vs HA +AP
	Control group	0.12 (0.06)	0.01-0.54	
Alpha−2-Macroglobulin (mg/dL)	HA +AP	234 (214)	116-367	.001 vs controls
	RA	208 (179)	115-328	.202 vs HA +AP
	Control group	177 (164)	102-469	
Leucocytes (/nl)	HA +AP	6.89 (7.00)	2.50-12.9	.039 vs controls
	RA	7.48 (7.00)	3.40-13.2	.695 vs HA +AP
	Control group	5.66 (5.27)	2.80-10.4	
PECAM−1 (pg/mL)	HA +AP	5089 (4905)	3535-7068	.030 vs controls
	RA	5354 (5102)	3214-9330	.601 vs HA +AP
	Control group	4546 (4509)	2833-6242	
IL−7 (pg/mL)	HA +AP	13.3 (9.40)	2.10-58.3	.045 vs controls
	RA	20.2 (13.5)	2.10-86.4	.135 vs HA +AP
	Control group	18.5 (15.7)	1.80-81.8	
IL−10 (pg/mL)	HA +AP	28.3 (25.5)	16.2-64.1	.006 vs controls
	RA	102 (29.5)	7.20-1521	.420 vs HA +AP
	Control group	22.8 (18.5)	6.30-115	
IL−12 (pg/mL)	HA +AP	33.1 (22.0)	16.0-118	.014 vs controls
	RA	69.6 (26.8)	0.60-784	.557 vs HA +AP
	Control group	23.5 (16.6)	1.40-106	
FGF basic (pg/mL)	HA +AP	64.7 (68.9)	24.5-131	.003 vs controls
	RA	75.8 (72.9)	49.4-111	.264 vs HA +AP
	Control group	85.7 (81.0)	23.2-159	
VEGF (pg/mL)	HA +AP	20.7 (17.4)	8.10-58.7	.020 vs controls
	RA	30.1 (20.7)	1.00-120	.679 vs HA +AP
	Control group	16.5 (12.4)	1.00-81.4	

4 | DISCUSSION

The pathophysiology of haemophilic arthropathy including the complex interplay of recurrent bleeding episodes and immunopathological mechanisms has not been fully elucidated. It is generally accepted that blood extravasated into the joint triggers acute and/or chronic intra-articular inflammation.[9] However, the participating mediators including cytokines and angiogenesis modulators have only partially been characterized.[20] In a few studies, synovial fluid samples obtained from HA patients or from factor VIII knock-out mice with needle-induced knee joint hemarthrosis, or synovial tissue samples from HA patients have been used to quantify cytokines including IL-1ß, TNF-α, and IL-6.[11,21–23] In addition, cartilage co-culturing studies with whole blood and IL-4 and/or IL-10 suggested their involvement in the synthesis rate of the proinflammatory cytokines IL-1ß, TNF-α and IL-6 as well as proteoglycan turnover and chondrocyte apoptosis rates.[23–26]

Except for their collection during orthopaedic interventions, synovial fluid samples from haemophilia patients are not routinely available for diagnostic purposes. Therefore, it is tempting to speculate if local findings on soluble mediators can be reproduced using blood samples. However, only a few biomarker studies of haemophilic arthropathy have been published involving cartilage and bone degradation markers, inflammation and angiogenesis-related mediators.[27–29] However, to the best of our knowledge, the present study is the first to systematically investigate blood levels of a large array of mediators involved in the pathogenesis of haemophilic arthropathy in haemophilia patients without or with arthropathy and to compare their patterns to a cohort with rheumatoid arthritis and a control group. Using peripheral blood samples, we quantified 42 different immunological, inflammation, coagulation, angiogenesis-related parameters and cytokines in 129 men (40 HA patients without arthropathy, 23 HA patients with arthropathy, 23 patients with RA and 43 control subjects). Following statistical analyses of the data, we could identify similarities and differences among the immunological parameters and biomarkers between the populations.

RA is a systemic inflammatory disease primarily involving the synovium of multiple joints. In addition to the chronic, symmetric polyarthritis, signs and symptoms of systemic disease are present in almost all patients. In contrast, in HA patients, haemophilic arthropathy typically affects a single joint conceptualized as target joint with no clinically overt systemic inflammatory disease. These generally accepted differences notwithstanding, it was surprising to obtain laboratory evidence of ongoing inflammation in HA patients as demonstrated by increased acute phase reactants irrespective of the presence of arthropathy. Although clinically going largely unnoticed, a chronic inflammatory disease should therefore be proposed in HA patients. RA patients were shown to have more significantly more pronounced ferritin concentrations compared to HA patients with arthropathy probably reflecting the systemic character of the disease process.

In the present study, HA patients with arthropathy demonstrated significantly increased peripheral blood vascular endothelial growth factor (VEGF) concentrations suggesting ongoing proangiogenic activity similar to RA patients with increased VEGF values. In contrast, HA patients without arthropathy did not demonstrate increased VEGF concentrations. Data on VEGF in haemophilia patients are contradictory. In a recently published study, serum VEGF was highest in bleeding HA patients followed by nonbleeding HA patients, and controls.[29] However, in another study of HA patients, VEGF values were within the normal range and did not show a significant correlation with joint status as documented by magnetic resonance imaging.[27] It is generally accepted that neoangiogenesis contributes to the development of haemophilic arthropathy.[8] Angiogenesis is a highly complex process involving a multitude of cellular and soluble factors displaying proangiogenic or antiangiogenic activity. Unfortunately, there is no single laboratory parameter to reflect angiogenesis in its spatiotemporal dimension. Nevertheless, the association of increased VEGF with joint bleeding or arthropathy reported in the present and other studies makes it a candidate for validation and follow-up studies.

Among the cytokines, a largely overlapping pattern was obtained when comparing in HA patients without or with arthropathy, RA patients, and controls. A notable exception was interleukin-7 (IL-7) being increased in HA patients without arthropathy, and being decreased in HA in the presence of arthropathy. IL-7 belongs to the group of proinflammatory cytokines and triggers the synthesis of additional cytokines including TNF alpha. Increased levels have been described in synovial fluid samples from RA patients whereas data on circulation levels remain a point of debate.[30] To the best of our knowledge, IL-7 has not been previously investigated in HA patients. Increased IL-7 in HA patients in the absence of arthropathy versus RA patients and controls, and decreased IL-7 in HA patients in the presence of arthropathy versus controls may point to different pathophysiological mechanisms. Increased IL-7 may initially perpetuate synovitis in HA patients. At a later stage with clinically overt arthropathy, reduced IL-7 may contribute to joint destruction by modulating circulating T cells. Circulating IL7 should be investigated in more detail in HA patients including follow-up studies and correlations with radiological and clinical scores.

The present study did not focus on a single or a few candidate biomarkers in haemophilia patients. Rather, it employed a multiplexing laboratory strategy to include a large array of cytokines and angiogenesis-related proteins. Previous candidate biomarker studies compared haemophilia patients to controls,[28,31] whereas the present study included RA patients to actively seek similarities and differences in the biomarker patterns. Except for interleukin-7, no distinctive biomarker pattern could be demonstrated confirming the current view that peripheral blood biomarkers are unlikely to add value to the diagnostic approach or to have an impact on patient management from a practical point of view.[15] However, the scientific value of this and related findings should be explored in further clinical studies including outcome assessment criteria as there continues to a need of sensitive imaging techniques and biomarkers alike.[32]

4.1 | Strengths and limitations

The present study is the first to compare blood levels of a large array of biomarkers and immunological parameters involved in the pathophysiology of joint damage in HA and RA using a multiplexing laboratory approach. We enrolled for the study 129 men (40 HA patients without arthropathy, 23 HA patients with arthropathy, 23 patients with RA, and 43 control subjects) and analysed 42 parameters with the aim to compare pattern between RA and HAP, and eventually to identify potential biomarkers for the development of HAP. However, there are several limitations to this study. The analysed populations showed some differences in age reflecting clinical reality with RA patients being older (median age 63 years) compared to HA patients (median age 49 year). Haemophilic children were not included and may be subject to a similar study to focus on newly beginning and/or developing arthropathy. Since HAP resembles RA in the early disease stages and osteoarthritis in the more advanced stages, OA patient would have been also an interesting control group. All laboratory measurements were performed at a single time point. The study was designed to identify candidate biomarkers rather to perform a follow-up study to consider their fluctuations and dynamic patterns. Furthermore, scores for radiological evaluation (ie Pettersson score) and physical examination (ie Gilbert score) were not considered in the present study. Their application would have generated subgroups in number and size precluding meaningful statistical considerations and interpretation. Further prospective studies are needed to evaluate the clinical potential of selected biomarkers. They should be performed as follow-up studies including clinical and radiological scoring of arthropathy.

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CONFLICT OF INTEREST

R.T. received a research grant and scientific congress travel expenses from Bayer and serves as a consultant for Bayer. A.W. declares no conflict of interest. WM has received fees from Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, Takeda/Shire, and uniQure.

AUTHOR CONTRIBUTIONS

R.T. led project and study design, performed the data analyses and wrote the paper, A.W. contributed to the study management, data collection and analyses, W.M. contributed to the study design, project management and manuscript writing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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