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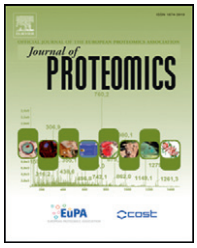

Vitamin D binding protein isoforms as candidate predictors of disease extension in childhood arthritis

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ABSTRACT

Introduction. Juvenile idiopathic arthritis (JIA) comprises a poorly understood group of chronic autoimmune diseases with variable clinical outcomes. We investigated whether the synovial fluid (SF) proteome could distinguish a subset of patients in whom disease extends to affect a large number of joints.

Methods. SF samples from 57 patients were obtained around time of initial diagnosis of JIA, labeled with Cy dyes and separated by two-dimensional electrophoresis. Multivariate analyses were used to isolate a panel of proteins which distinguish patient subgroups. Proteins were identified using MALDI-TOF mass spectrometry with expression verified by immunochemical methods. Protein glycosylation status was confirmed by hydrophilic interaction liquid chromatography.

Results. A truncated isoform of vitamin D binding protein (VDBP) is present at significantly reduced levels in the SF of oligoarticular patients at risk of disease extension, relative to other subgroups ($p<0.05$). Furthermore, sialylated forms of immunopurified synovial VDBP were significantly reduced in extended oligoarticular patients ($p<0.005$).

Conclusion. Reduced conversion of VDBP to a macrophage activation factor may be used to stratify patients to determine risk of disease extension in JIA patients.

1. Introduction

About one in every thousand children in the UK suffers from juvenile idiopathic arthritis (JIA) [1]. JIA is a heterogeneous group of inflammatory disorders affecting the musculoskeletal system. Of the seven subsets of JIA identified according to ILAR classification [2], oligoarticular, extended oligoarticular, and polyarticular are the commonest. Adverse outcomes can present to varying degrees regardless of disease subtype [3]. In approximately 25% of children with oligoarticular JIA, over time the

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disease spreads to involve many joints, a condition known as extended oligoarticular disease [4]. Clinical, laboratory or radiologic parameters cannot accurately predict disease extension. Extended oligoarticular JIA is much more difficult to treat due to its characteristic resistance to second-line therapies [5]. It is therefore important to define more sensitive markers to determine the risk of inflammation spreading to previously unaffected joints. If it was possible to identify these children earlier, more effective therapies could be instigated to prevent joint and periarticular damage.

Previous studies have suggested that measurement of a selected set of synovial fluid or plasma proteins may be used to discriminate clinically and biologically relevant JIA subgroups [6–9]. A recent study reported a significant reduction in the ratio of CD4:CD8 positive T cells with a corresponding increase in the levels of CCL5 in the synovial fluid of extended oligoarticular patients [10].

Post-translational modifications of proteins are frequently overlooked as candidates are identified in biomarker discovery studies. However, covalent modifications of proteins by oxidation, phosphorylation or glycosylation can have profound effects on protein transport, function, stability and recognition. Growing evidence suggests a significant role for glycosylation in a range of arthritic and autoimmune disorders [11,12]. Specifically, protein glycosylation motifs affect a wide variety of innate and adaptive immunological processes including inflammation, cellular infiltration, cell communication and adhesion, and lymphocyte tolerance [13–17]. Changes in the glycosylation of proteins such as acute-phase proteins and antibodies have already been recorded in the synovial fluid and plasma of arthritis patients, but no relationship to clinical subtype or outcome has yet been established in JIA [18–22].

This study is focused on identifying protein isoforms in a de novo cohort of children with newly diagnosed JIA that will predict disease spread. The synovial fluid proteome of the persistent oligoarticular patient subgroup was compared to that of patients who show a spread after the first 6 months post diagnosis to involve five or more joints i.e., the extended-to-be oligoarticular subgroup. Novel mass spectrometry based analyses were employed to resolve protein post-translational modifications which are not apparent by conventional antibody based methods.

2. Materials and methods

2.1. Patients

Fifty-seven patients with newly diagnosed *untreated* JIA according to International League Against Rheumatism criteria entered this study and were followed for 1 year. At the time of initial sampling there were 34 children with oligoarticular arthritis, 18 with polyarticular arthritis (16 rheumatoid factor negative) and 5 with psoriatic or enthesitis related arthritis. Patient data shown in Table 1 refers to clinical findings at the time of joint aspiration and biopsy i.e. at initial presentation before disease extension. Disease extension was defined as 5 or more joints involved after 6 months from disease commencement. At 1 year, 8 oligoarticular cases had been reclassified as having extended oligoarticular JIA.

Patients were examined by a consultant rheumatologist (M.E.R.) who confirmed their diagnosis. For the purposes of this study, only initial synovial fluids from children with disease duration of less than 1 year and steroid and DMARD naive were included. Arthrocentesis and subsequent joint steroid injection were performed according to clinical need.

Clinical details recorded included subtype of JIA, age, sex, disease duration, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Treatments applied after samples were drawn are also listed. Local inflammation was defined as both joint swelling and pain on physical examination. All SFs

Table 1 – Patient demographics and laboratory characteristics.

Characteristic	Subtype				Total
	Oligoarticular	Extended-to-be oligoarticular	Polyarticular	Psoraitic/enthesitis	
	(n=26)	(n=8)	(n=18)	(n=5)	(n=57)
Sex, no. (females)	21	7	15	2	45
Age at time of biopsy (year)	5.6 (4.4)	6.1 (4.5)	9.9 (5.0)	12.8 (3.1)	7.7 (5.1)
Disease duration (months)	6.2 (4.3)	7.4 (7.5)	7.4 (7.2)	9.0 (7.9)	7.0 (6.0)
Swollen joint count	2.3 (1.2)	3.0 (0.9)	10.9 (9.4)	–	4.5 (5.5)
Neutrophils ($\times 10^8$ cells/L)	4.7 (2.4)	5.9 (1.7)	5.4 (1.8)	6.7 (1.4)	5.3 (2.1)
Lymphocytes ($\times 10^8$ cells/L)	3.7 (1.7)	4.5 (2.1)	3.0 (1.7)	2.4 (1.1)	3.5 (1.8)
Monocytes ($\times 10^8$ cells/L)	0.8 (0.3)	1.0 (0.3)	0.8 (0.4)	0.7 (0.3)	0.8 (0.4)
ESR (mm/h)	16.3 (15.0)	36.5 (32.7)	38.6 (35.0)	48.0 (22.8)	29.8 (28.4)
C reactive protein (mg/L)	8.5 (14.5)	11.4 (7.8)	30.9 (43.2)	44.0 (44.2)	20.1 (32.2)
Rheumatoid factor status					
+ve	0	0	2	0	2
−ve	26	8	16	5	55
Antinuclear antibody status					
+ve	12	5	9	1	27
−ve	14	3	9	4	30

Clinical and demographic characteristics of the study subjects at presentation. Values are the mean±standard deviation or the number of subjects. CRP = C-reactive protein and ESR = erythrocyte sedimentation rate.