Research article

A pilot study of serum calprotectin ability to be a potential biomarker of erosive course juvenile arthritis

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Abstract

Background: Juvenile Idiopathic Arthritis (JIA) is the most common chronic arthritis in children. There is no reliable laboratory test for JIA. Some researchers have reported that the serum level of calprotectin may be correlated with arthritis activity.

Objective: The aim of this study was to investigate the potential role of serum calprotectin in predicting the erosive course of JIA.

Methods: Level of serum calprotectin (sCal) was evaluated in 70 children with JIA (50 active JIA / 20 inactive JIA) and 20 adolescents with non-rheumatic joint disease. In comparison the serum level of IL6, TNF-alpha, vimentin were assessed.

Results: sCal level was higher in patients with active erosive course JIA (R^2 = 0.4159, T = 4.336, OR erosive JIA = 3.3193, 95%CI 1,7006-6.4789, p=0.0079). Serum level of vimentin, IL6 and TNF-alpha were not always correlated with active stage JA and erosive joint damage (p>0,05). The ROC analysis of the sCalc showed that a cut-off point more of 2,9 µg/ml may be high prognostic factor for related erosive JIA (AUC 0,837±0,0553, 95%CI 0,711-0.923).

Conclusions: The serum levels of calprotectin are significantly associated with erosive course of JIA. These results suggest that calprotectin might be superior to serum IL6 and TNF-α for aggressive erosive course of JIA.

Key words: Juvenile arthritis, Activity, Serum calprotectin, Erosive course

Introduction

Juvenile Idiopathic Arthritis (JIA) is a chronic childhood inflammatory autoimmune joint disease[1]. Juvenile arthritis comprises a group of heterogeneous forms of arthritis characterized by unknown cause persistent joint inflammation lasting longer than 6 weeks. Typical classic forms of JIA (systemic, oligo, poly, psoriatic, enthesopathy) are well known[2]. Some laboratory tests neither rule in nor rule out disease. An elevated ANA titer is not a diagnostic criterion for JIA. Erythrocyte sedimentation rate can be normal despite marked involvement of arthritis[3,4]. Paediatric rheumatologists use clinical diagnostic ILAR criteria (ILAR 1997; 2001; the Edmonton revision 2004) to confirm juvenile arthritis[5]. Some researchers reported that the serum level of calprotectin (S100A8/A9 or MIF-Related Protein 8/14) were correlated with arthritis activity[6,7].

Materials and methods

This study evaluated the level of serum calprotectin in 70 children with JIA and 20 adolescents with non-rheumatic joint pain. The study group consisted of 50 children with active JIA. Twenty children with inactive JIA and 20 adolescents with non-rheumatic joint disease were from comparison groups. All 70 children with chronic inflammatory joint disease fulfilled the ILAR criteria for JIA. The active JIA subtypes were as follows: oligoarticular in 15 (oJIA), polyarticular RF- negative in 17 (pJIA), enthesitis-related arthritis in 18 (erJIA) patients (Table 1). No patients were classified as undifferentiated arthritis. No patients were with uveitis. Inactive JIA consisted of 6 children with oJIA, 7 – pJIA, 7 – erJIA. The study used standard treatment regimens for different subtypes JIA. Methotrexate was applied in all children with JIA. Biologic agents were used in patients who did not respond to 6 months of DMARDS. Laboratory tests of study group were performed at onset JIA. Levels of serum calprotectin (sCal), vimentin, interleukin-6 (sIL6) and Tumour Necrosis Factor (sTNF-α)

were collected before treatment was commenced. Other children were laboratory tested (sCal, vimentin, sIL6 and sTNF-α) after confirmed inactive JIA or non-rheumatic joint disease. Patients' age, onset and course of JIA, radiology data were collected for each patient. This study was carried out at the Rheumatology Department of H. Turner National Medical Research Center for Children's Orthopaedics and Trauma Surgery, from 2018 – 2021.

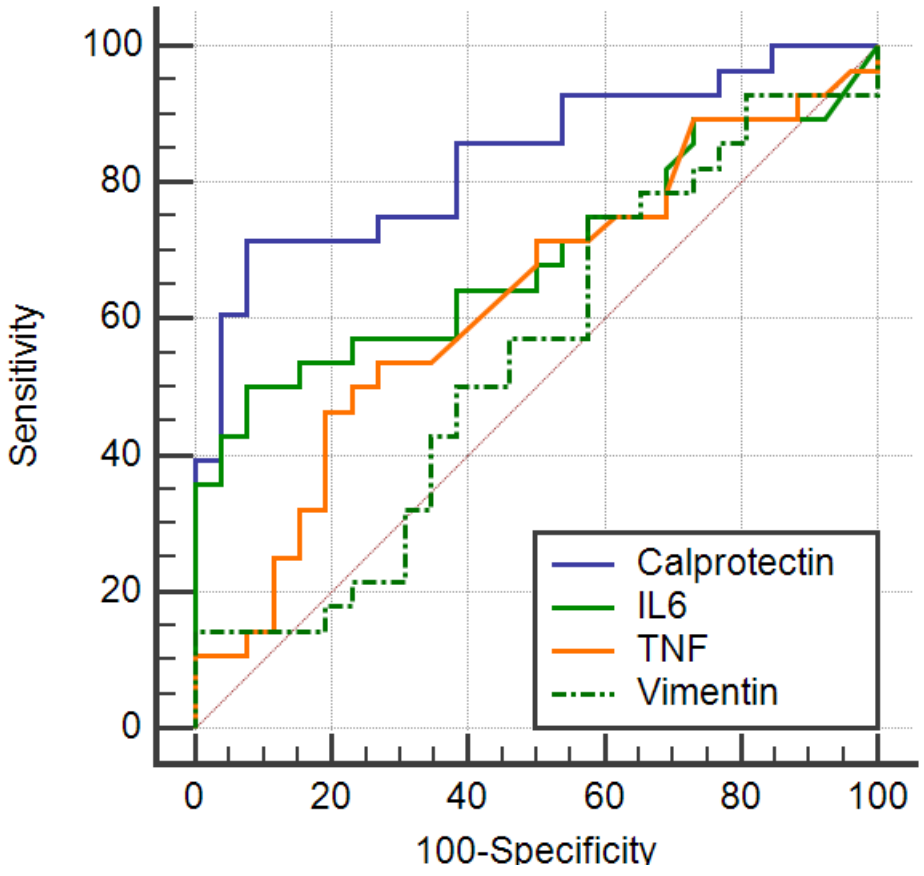
Achievement of inactive disease and clinical remission of JIA were evaluated according to Wallace criteria[8]. Assessment of X-ray changes in juvenile arthritis was performed using a modified scoring method by Steinbrocker[9]. Disease activity range was evaluated by Juvenile Arthritis Disease Activity Score (JADAS27-ESR)[10]. Digital data were statistically processed using a software package Microsoft Excel and Statistica 6.0. Quantitative indicators distribution is given as a median [5th; 95th percentile]. To determine the significance of differences between the groups we used nonparametric dispersion analysis (ANOVA) by Kruska–Wallis test (for independent groups) Wilcoxon criterion (for dependent groups). The correlation between the parameters under study was analyzed using Spearman rank correlation analysis (r). In all statistical analysis procedures p=0.05 was taken for a critical significance level of null statistical hypothesis.

Immunological research was carried out in the laboratory of H.Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery (Bogdanova S.L., Derkach E.A. chief). The serum concentration of tumour necrosis factor alpha / sTNF-α were determined using enzyme-linked immunosorbent assay (Elisa-TNF-alfa; Vector-Best, Russia). The serum concentration of interleukin 6 / sIL6 were determined by electrochemiluminescence immunoassay (ECLIA) method on Cobas E411 (Roche, Switzerland). The serum concentration of calprotectin / sCal were determined by enzyme-linked immunosorbent assay (MIF-Related Protein 8/14 ELISA; Buhlmann Laboratories AG/ Basel, Switzerland).

Results

Mean value of sCal in patients with active JIA at onset (n=50) and inactive disease (n=20) (Figure 1). Level of sCal in active oJA were 2,61 µg/ml [1,015; 3,935], inactive oJA - 1,258 µg/ml [0,772; 2,254], active pJA - 5,845 µg/ml [3,408; 8,005], inactive pJA - 1,36 µg/ml [0,678; 2,342], active erJA - 2,98 µg/ml [0,897; 6,876], inactive erJA - 0,94 µg/ml [0,429; 1,92]; sCal level in children with non-rheumatic joint pain - 1,288 µg/ml [0,513; 2,364].

Figure 1: Distribution of serum calprotectin levels in JIA patients with active and inactive disease



It was observed that, out of the total JIA patients studied, part of patients in active JIA were having high value sCalc compared with inactive disease. The association was found to be statistically significant only in children with active pJA (p<0,01). Then the clinical course of JIA was compared for the association with the serum levels of IL6, TNF-α, vimentin and calprotectin among patients. For this, all children with active JIA were divided into three groups based on their treatment and aggressive disease. The average follow-up was 26 [18; 42] months. 1st gr – consist of 14 children with mild form successful treated by methotrexate over 2 years (4 erJA /10 oJA, JADAS27 at onset 3.2 – 4.6), 2nd gr – 19 children with severe JA and delayed-onset erosion which was treated unsuccessfully by methotrexate over 1 year (14 erJA /5 oJA, JADAS27 at onset 4.8 – 8.4), 3rd gr - 17 children with early erosive JA which initial treatment were methotrexate and anti-TNF drugs (all pJA, JADAS27 at onset > 13). In the 1st group sCal level were 1,0175 µg/ml [0,45; 2,378], sIL6 - 2,94 pg/ml [1,549; 5,617], vimentin - 9,872 U/ml [3,87; 18,81], sTNF-α - 1,144 pg/ml [0,397; 3,757]. In the 2nd sCal level were 3,81 µg/ml [2,48; 5,992], sIL6 16,15 pg/ml [1,769; 48,85], vimentin - 13,632 U/ml [2,319; 44,492], sTNF-α - 1,18 pg/ml [0,204; 3,54]. In the 3rd sCal level were 5,845 µg/ml [3,408; 8,005], sIL6 - 11,048 pg/ml [1,5; 33,7], vimentin - 17,22 U/ml [4,212; 52,1], sTNF-α - 10,5 pg/ml [0,5; 50,43]. Statistic analysis revealed a correlation between sCalc and active erosive JA (R^2 = 0.4159, T = 4.336, OR erosive JA = 3.3193 (predictor factor), 95%CI 1,7006-6,4789, p=0.0079). Serum level of vimentin, IL6 and TNF-α were not correlated with risk of erosive course JA (Table 2). The ROC analysis of the sCalc showed that a cut-off point more of 2,9 µg/ml may be high prognostic factor for related erosive JA (AUC 0,837±0,0553, 95%CI 0,711-0.923, (Figure 2, Table 3).

Table 1: Clinical and laboratory features of patient study group (active JA) at onset disease

Groups of children	Value and frequency		
	oJA	pJA	erJA
Parameters, Me [25Q;75Q]	(n=15)	(n=17)	(n=18)
Girls, abs (%)	15 (100%)	13 (76,5%)	12 (66,7%)
Age of onset JA, years*1	3 [2; 5]	5 [2; 9]	11 [8; 15]
Number of active joints, abs*2	1 [1; 2]	8 [6; 14]	3 [2; 3]
JADAS27	5,2 [3,2; 7,4]	18,2 [14,4; 22,8]	7 [5,2; 8,4]
ESR, mm/h	14 [6; 28]	22 [16; 48]	12 [6; 22]
CRP, mg/l	3,0 [1,4; 6,2]	4,4 [3,2; 13,6]	2,8 [1,2; 5,6]
Hemoglobin, g/l	114 [110; 128]	112 [108; 124]	120 [114; 132]
Leucocytes, 10^9/l	8,0 [6,8; 10,6]	7,4 [6,2; 10,8]	6,4 [5,2; 8,8]
Trombocytes, 10^9/l	442 [408; 486]	480 [434; 526]	438 [412; 468]
Gamma globulin, %	21,6 [19,2; 22,0]	22,4 [20,4; 23,8]	21,0 [18,4; 22,4]
ANF ≥ 1/160, abs (%)	15 (100%)	15 (88,2%)	12 (66,7%)

Note: *1 – age of children at the onset JIA; *2 – number of active joints at the onset JIA; JADAS: Juvenile Arthritis Disease Activity Score; ESR: erythrocytes sedimentation rates; СРБ: C-reactive protein; ANF: antinuclear factor

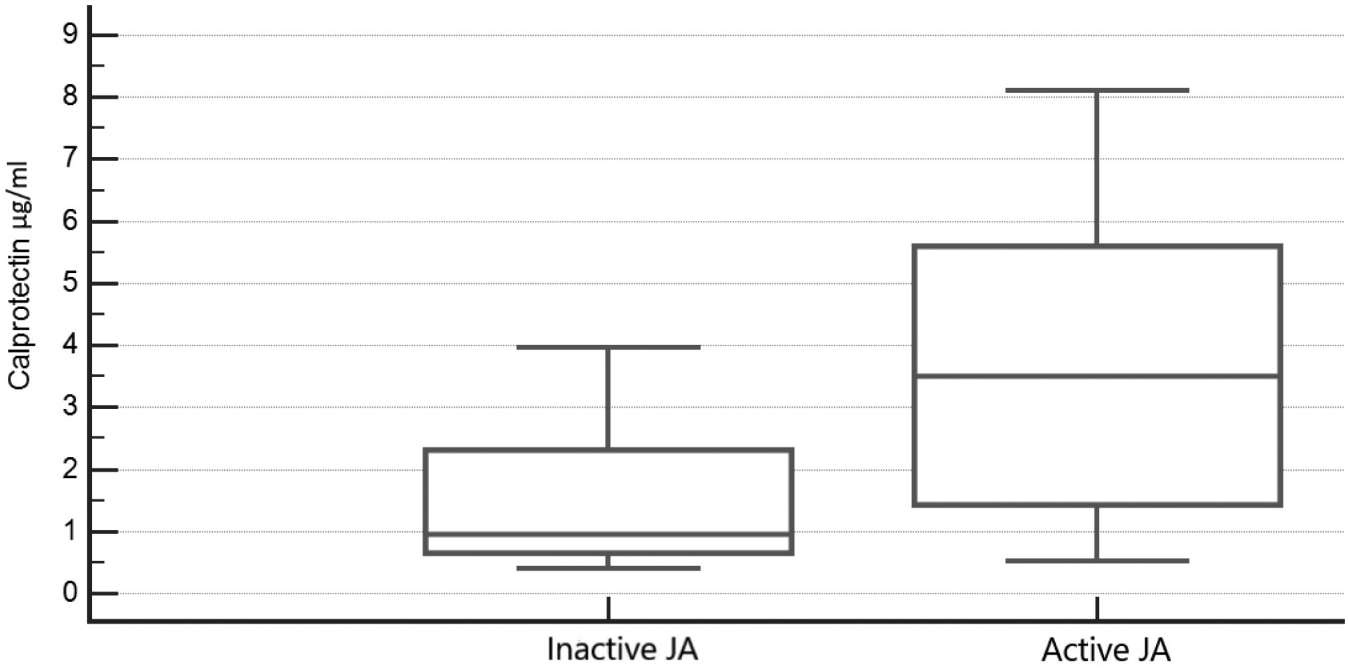
Table 2: Poisson regression modeling of aggressive erosive course of JA in children

Parameters	Odds Ratio	95%CI	Coefficient	Standard error	P-value
Calprotectin	3,3193	1,7006 to 6,4789	1,19975	0,34122	p=0,0004
Vimentin	1,0922	0,9979 to 1,1953	0,088151	0,046045	p=0,0556
Interleukin-6	1,0036	0,9371 to 1,0747	0,035678	0,034944	p=0,5104
Tumor necrosis factor alpha	1,0342	0,9356 to 1,1433	0,033664	0,051143	p=0,0726

Table 3: ROC analysis of laboratory predictors for aggressive erosive course of JA

Parameters	AUC	Standard error	95%CI
Calprotectin	0,837	0,0553	0,711 to 0,923
Vimentin	0,545	0,0806	0,404 to 0,681
Interleukin-6	0,689	0,0743	0,548 to 0,808
Tumor necrosis factor alpha	0,627	0,0773	0,485 to 0,755

Figure 2: ROC curves of serum calprotectin, IL6, TNF-alpha and vimentin for predicting aggressive erosive course of Juvenile Arthritis



Discussion

In this study we investigated the possibility of serum calprotectin as an indicator of early aggressive JIA. It's related to the absence of specific radiographic findings in early disease and low imaging ability of erosion changes in X-ray. To meet the objectives of the study, the approach to diagnosis and management of JIA in one medical center was used. According to the national (Russian) clinical guidelines the degree of laboratory activity is assessed by the level of CRP and ESR[11]. Only titer of ANF (Antinuclear factor) and vimentin may be used to indicate the aggressiveness JIA. Calprotectin doesn't appear in the guidelines for JIA[12].

Calprotectin (MPR 8/14 and S100A8/A9) is a calcium- and zinc-binding protein that belongs to the S100 family (total molecular weight of 36.5kDa). Calprotectin is present in large quantities of neutrophil granulocytes, less in activated monocytes and macrophages [13]. This protein plays a major role in inflammatory reactions and is considered to be a positive acute phase protein. Calprotectin is released following granulation of neutrophil granulocytes during interaction of cells with inflammatory activated endothelium. It is implicated in the innate immune response as a damage-associated molecular pattern protein[14].

Most multicenter studies have demonstrated correlated serum level of calprotectin with activity JIA[15]. The level of calprotectin can reflect the degree of inflammatory activity of JA[16]. Some studies revealed that serum calprotectin levels in patients with active systemic JIA were higher than in active non-systemic JIA. However, not all researchers agree that calprotectin may be a predicting factor of flares in JIA. Also correlation between ultrasound dates and concentration of sCalc needs clarification[17,18].

The study results showed substantially higher serum calprotectin levels in children with active JIA compared to data in inactive disease and non-inflammatory arthropathy. However, despite the lack of differences,

research revealed that serum calprotectin were higher in children with active erosive course of JIA. It is known, that early erosive changes in children with non-systemic JIA define marker of aggressive course. The present study aimed to find the correlation between serum level of calprotectin and aggressiveness of JIA. The study showed that calprotectin was a more sensitive laboratory marker of the aggressive course of non-systemic JIA.

Conclusion

One of the currently unresolved problems in paediatric rheumatology is identification of markers of aggressive course of JIA. Serum calprotectin can serve as one of these markers. Studies confirm that the serum levels of calprotectin are significantly associated with JIA disease activity. Our results suggest that calprotectin might be superior to serum IL6 and TNF-α as a marker for erosive course of JIA.

Conflict of interest: None to declare.

Financing source: Not specified.

Ethical approval: The study was approved by the local ethics committee at H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery (Protocol No. 1 dated 01.20.2014).

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