CORRELATIONS BETWEEN CLINICAL FORMS AND BIOMARKERS IN JUVENILE IDIOPATHIC ARTHRITIS

Andrea Somogyi Militaru, Ioan Sabau

REZUMAT

Introducere: Artrita juvenilă idiopatică este cea mai importantă boală reumatologică a copilăriei. **Obiective:** Studiul corelațiilor dintre semnele clinice și markerii inflamatori în AJI. **Material și metode:** 58 de copii diagnosticați și clasificați conform criteriilor ILAR, au fost evaluați clinic și biologic. **Rezultate:** Distribuția pacienților a fost următoarea: 17 poliartrite, 18 oligoartrite, 1 AJI sistemică, 22 spondiloartropatii (SpA). În lotul studiat, am observat o corelație bună între valorile VSH, CRP și scorurile funcționale. Anticorpii anti-CCP (ACPA) au fost pozitivi în doar 6 cazuri, dar în titru scăzut, toate cazurile asociind sindrom inflamator important la debutul bolii, dar nu am găsit corelație cu indicii funcționali. Nivelul plasmatic al interleukinelor pro-inflamtorii IL-1alfa, IL-beta, IL-6 a fost determinat în 8 cazuri. Ambele fracțiuni de IL-1 au avut nivele crescute în 3 cazuri de SpA (cu nivele normale de IL-6). Creșterea concentrației plasmatice a IL-6 a fost înregistrată în 3 poliartrite (valori normale ale IL-1). **Concluzii:** În lotul studiat nu am găsit corelație între ACPA și forma clinică de AJI. IL ar putea fi corelate cu forma clinică de AJI (Il-1 cu spondilopatii, IL-6 cu poliartrite). Studii ulterioare trebuie să confirme observațiile acestui studiu.

Cuvinte cheie: artrită juvenilă idiopatică, anticorpi anti-CCP, interleukine proinflamatorii

ABSTRACT

Introduction: Juvenile idiopathic arthritis (JIA) is the most important rheumatic disease of childhood. **Aim:** To study the correlations between clinical signs and inflammatory biomarkers in JIA. **Material and methods:** In 58 children, diagnosed and classified according to ILAR (International League of Associations for Rheumatology), evaluation consisted in clinical and laboratory examination (ESR, CRP, RF-rheumatoid factor, anticyclic citrullinated peptide antibody- ACPA, interleukins - ILs). **Results:** The distribution of patients was: 1 systemic JIA, 17 polyarthritis, 18 oligoarthritis, 22 spondyloarthropathies. There was a good correlation between the ESR, CRP values and the functional scores. ACPA was found positive in 6 cases, but borderline levels, all associating important inflammation at the onset, but no correlations with functional indexes. Plasma levels of IL-1alpha, IL-1betha, IL-6 pro-inflammatory interleukins was determined in 8 cases. Both fractions of IL-1 were increased in two cases of reactive arthritis and one juvenile spondylitis (with normal IL-6 levels). Enhancement of IL-6 (and normal IL-1 values) was observed in 3 children with polyarthritis. **Conclusions:** In the studied group, we found no correlations between clinical form of JIA and ACPA titre. Interleukins could be correlated with clinical form of JIA (IL-1 with spondyloarthropaty, IL-6 with polyarthritis). Further studies need to sustain these observations.

Key Words: juvenile idiopathic arthritis, ACPA, pro-inflammatory interleukins

INTRODUCTION

Juvenile idiopathic arthritis (JIA), previously called juvenile chronic arthritis or juvenile rheumatoid arthritis, is the most common chronic autoimmune disease of childhood. It is defined as an inflammatory arthritis of unknown origin in at least one joint persisting for more than 6 weeks in children younger than 16 years of age, and it affects 1 in 1000 children worldwide. This autoimmune disorder is a major cause of chronic disability in children.

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Juvenile idiopathic arthritis consists of a heterogeneous group of disorders with unknown etiology, and a lack of reliable biomarkers complicates diagnosis. The disease course is unpredictable, with no single marker able to monitor disease.

Biomarkers with the potential to differentiate those patients with aggressive JIA early in their disease have recently included anticyclic citrullinated peptide antibodies (ACPA). Although ACPA have been studied extensively in rheumatoid arthritis (RA), their significance in JIA has been evaluated only recently. Anti-CCP antibodies have a specificity of 98% and a sensitivity of 48% for RA, providing a useful diagnostic tool in RA.[1] They seem to play an important role in the pathogenesis of RA inflammation, because RA patients with ACPA have a more aggressive disease course with joint erosion and damage.[2,3] Citrullinated proteins may be targets of the local immune response in patients with RA and perpetuate a persistent state of synovitis leading to joint destruction. The role of ACPA in JIA remain controversial. Several

studies have generated varying results regarding their significance in the disease process. There was studies in which no statistically significant correlation between ACPA positivity and ESR or radiographic damage was detected.[4] Other study suggested that anti-CCP antibodies in JIA were not as prevalent as in adult RA, but could be useful in predicting joint damage.[5,6]

Although onset and disease course may differ, the subtypes of JIA share the occurrence of chronic inflammation of the joints. Monocytes, macrophages, fibroblasts and T cells within the inflamed microenvironment secrete many mediators that interact directly with the surrounding tissue and tend to have a pro-inflammatory character.[7-9] The produced interleukins (IL) regulate the production of inflammatory mediators from the surrounding tissue, whereas secreted chemotactic cytokines (chemokines) function as regulatory molecules that attract and direct the differentiation of new potent inflammatory cells to the site of inflammation.[4,9,10]

Evidence of an imbalance of pro-inflammatory cytokines in patients with inflammatory diseases includes the positive correlation of serum and synovial cytokine concentrations with JIA disease activity, an increase in antagonists or soluble receptors with a flare of arthritis and the effectiveness of JIA therapies that involve cytokine modulation.[9,11] The pro-inflammatory cytokines that have been reported to play a major role in JIA include interleukin 1-beta (IL-1β), tumor necrosis factor alpha and interleukin 6.[12,13] (Fig. 1)

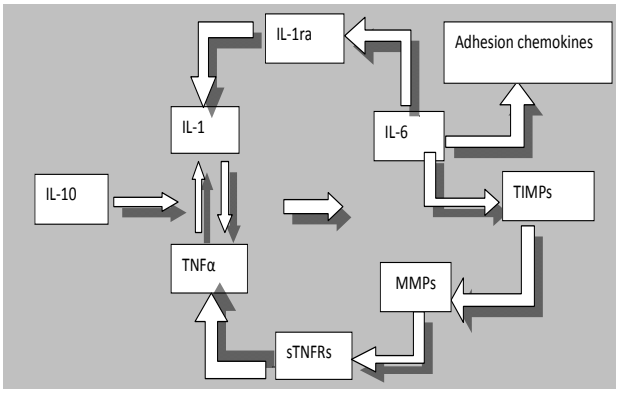

Figure 1. Positive and negative cytokine feedback. IL- interleukin; MMP – matrix metaloproteinase; sTNFR – soluble tumor necrosis factor receptor; TIMPs – tissue inhibitor of matrix metalloproteinase; TNF-alpha – tumor necrosis factor-alpha.

Circulating cytokines correspond to the activation status of immune-competent cells, and could be instrumental to monitor changes in this profile during treatment. Evaluating cytokines in plasma might help in identifying surrogate parameters for disease activity, disease severity, risk of side effects and treatment outcome.[11,12]

OBJECTIVES

The main goals of this study are to analyze certain biomarkers in a pediatric cohort with chronic arthritis, to investigate the interrelation between the inflammatory and immunologic biomarkers, and their correlations with the clinical form and the functional indexes of JIA.

MATERIAL AND METHODS

This study was initially retrospective, and then became prospective. We enrolled 58 children with chronic arthritis, assessed in the First Pediatric Clinic from "Louis Turcanu" Emergency Hospital for Children, Timisoara during the period of May 2005 – May 2011. All the patients were diagnosed and classified according to ILAR (International League of Associations for Rheumatology) criteria.

The study had full ethical approval of the Department. Informed consent was obtained either from parents or from the individuals directly if they were older than 12 years.

Evaluation of the patients consisted in medical history data collection, complete clinical assessment, functional evaluation and lab tests. Demographic and clinical characteristics of the patients included: age at the onset of JIA, sex, body weight, duration of JIA prior diagnosis.

Clinical parameters involved: number of swollen joints, of tender joints and of joints with limitation on passive motion. Presence of non-osteo-articular symptoms and signs (ophthalmological, gastrointestinal, dermatological, cardiovascular etc.) was assessed.

Functional assessment included: physician's and parent's global assessment of disease activity (with a 100-mm visual analogue scale -VAS, in which higher scores indicated more active disease); Disability Index score in Childhood Health Assessment Questionnaire –CHAQ, in which scores range from 0 (best) to 3 (worst) and parent's or patient's assessment of pain (through a 100-mm visual analogue scale in which higher scores indicated more severe pain).

All patients were assessed through laboratory exams, consisting in evaluation of: 1) inflammatory syndrome (blood cells count, ESR, CRP, plasma alpha2- and gamma-globulin levels, IgG levels); and 2) immunologic assessment (RF, and, just in a part of the cohort, was evaluated presence of anti-CCP antibody, plasma levels of certain pro-inflammatory interleukins: IL-1alpha, IL-1beta, IL-6).

Routine assay, consisting in latex agglutination,

measured 19S IgM RFs, but enzyme-linked immunosorbent assays (ELISAs) have been provided significantly more positive results than the routine ones. This is the reason why in our study we performed the measurement of IgM RF by ELISA. IL-1alpha, IL-1beta and IL-6 plasma levels were determined by ELISA also.

Imagistic evaluation was performed in every long lasting form of JIA and included x-ray and, in some cases, magnetic resonance investigation. Genetic assessment included HLA-B27 gene testing in the spondyloarthropathy group.

Active disease was defined by the presence of joint swelling or limitation of movement with either pain on movement or tenderness. Non-active disease (remission) was defined by the absence of joint swelling or limitation of movement with no pain on movement or tenderness.

Analysis of the results was performed with SPSS16 statistics program. The correlations were estimated using linear regression models.

RESULTS

Characteristics of the cohort

Clinical data permitted the division of the cohort into four major subgroups: (persistent) oligoarthritis, polyarthritis (including three extended oligoarticular JIA), systemic JIA and spondyloarthropaty (or enthesitis-related arthritis- ERA). Distribution of the patients is summarized in table I. Spondyloarthropathy group included: 4 cases of arthropathies associated with Crohn disease, 4 juvenile ankylosing spondylitis, 5 reactive arthritis and 9 patients with undifferentiated arthritis.

Table 1 summarizes the characteristics and descriptive statistics of our cohort.

Female patients were predominant both in oligoarticular and polyarticular JIA, but in the spondyloarthropathy group were more boys than girls. In our studied group, the highest medium age was found in spondyloarthropathy (12.4 years).

The ESR and CRP values presented an excellent correlation ($p<0.005$) with the disability index score, obtaining a R Sq Linear of 0.853 in ESR and CHAQ correlation, respectively 0.741 in CRP and CHAQ interrelation. (Fig. 2) VAS score had an even better statistic correlation both with ESR (R Sq Linear = 0.893) and CRP (R Sq Linear= 0.805) with $p<0.005$. (Fig. 3)

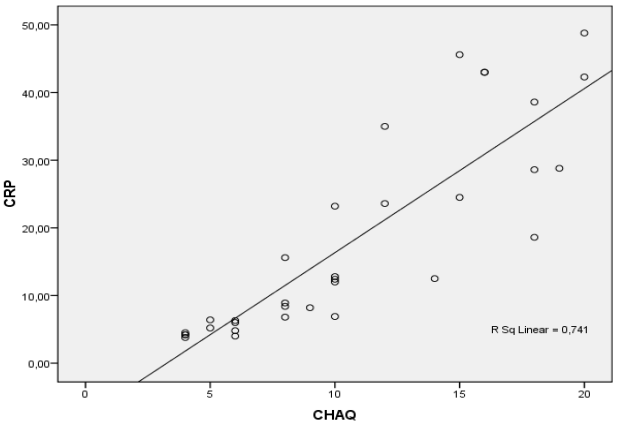

Figure 2. Correlation between CRP and CHAQ.

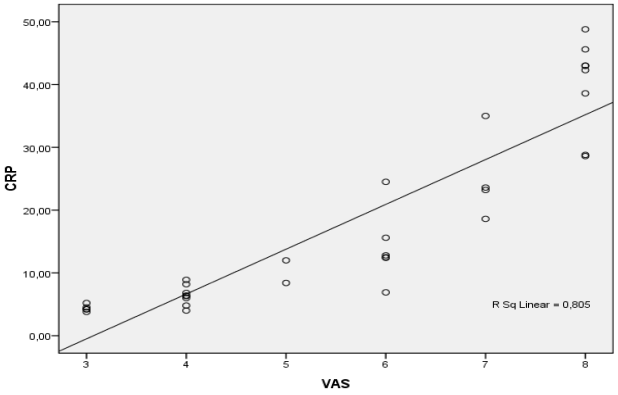

Figure 3. Correlation between CRP and VAS.

Table 1. Characteristics of the cohort and subgroups.

Characteristics	Cohort	Oligo JIA	Poly JIA	Systemic JIA	SpA
Patients number	58	18	17	1	22
Gender ratio F:M	32:26	12:6	10:7	1:0	10:12
Mean Age (years)	8.9	6.8	8.6	5.8	12.4
Mean ESR (mm/1h)	54.64±30.6	33.4±18.6	68.3±21	110	28.6±24.3
Mean CRP (mg/dl)	18.1±14.74	12.8±8.7	24.8±12.6	48	18.3±10.5
Mean VAS score	5.61±1.85	4.43±1.56	6.8±2.2	7	5.3±2.7
Mean CHAQ score	10.73±5.23	6.56±2.87	14.86±4.6	16	8.2±4.64
IgM RF positive (no)	4	0	4	0	0
Anti-CCP ab (no)	6	1	3	0	2

IgM RF detection

IgM RF has been evaluated in all 58 cases. In 4 patients, meaning 6.9% of the entire cohort, elevated IgM RF levels are determined by ELISA. All these cases belonged to polyarticular JIA and had important inflammation with severe functional disability at the onset of the disease. Two of them reached clinical remission on treatment, with excellent improvement of CHAQ, VAS scores, and the decreasing of ESR, CRP values, but without any change in IgM RF titre. This explains the absence of correlation between IgM RF titre and CHAQ, respectively VAS indexes ($p > 0.1$ in both). All three patients had radiographic evidence of joint damage at the onset of the disease, including joint space narrowing and joint erosion.

Anti-CCP antibody detection

Thirty-three cases were checked for anti-CCP antibodies. Positive, but very low levels were found, in just 6 children, with the following distribution: 3 polyarticular JIA (1 RF-positive and 2 RF-negative polyarthritis), 1 oligoarticular JIA, 1 case with ankylosing spondylitis and 1 patient with Crohn disease associating arthritis. We found no correlation of ACPA positivity and the clinical form of JIA.

We found no correlation between ACPA titre and functional indexes (CHAQ, VAS) and inflammatory biomarkers ($p>0.05$). 100% of anti-CCP positive cases had important inflammatory syndrome (more than five times normal levels of CRP, ESR) at the onset of JIA. The cases that reached clinical and biological remission on treatment (with the improvement of CHAQ, VAS, ESR, CRP) presented no decreasing in ACPA titer. Furthermore, the only one patient who had significantly high levels of anti-CCP (200UI/ml) was at the time of determination in clinical remission. All other children had ACPA titre≤0.8UI/ml, regardless of clinical, functional or inflammatory parameters.

In five cases from the six patients with borderline positive ACPA, radiographic evidence of joint damage was found on disease onset. The exception was the patient with arthropathy associated to Crohn disease, with an aggressive evolution of the intestinal inflammation, but no joint damage.

Interleukin plasma level evaluation

Plasma levels of IL-1alpha, IL-1betha, IL-6 pro-inflammatory interleukins was determined by ELISA in 8 cases. We studied the correlation of interleukin levels to inflammatory syndrome, immunologic biomarkers and functional indexes. We found no statistically significant correlations between IL plasma levels and ESR, CRP levels, IgM RF, ACPA titer, VAS and CHAQ scores ($p>0.05$; R Sq Linear < 0.1). This lack of correlation between pro-inflammatory interleukins and functional indexes could be explained by the different disease activity status of the patients. (Fig. 4)

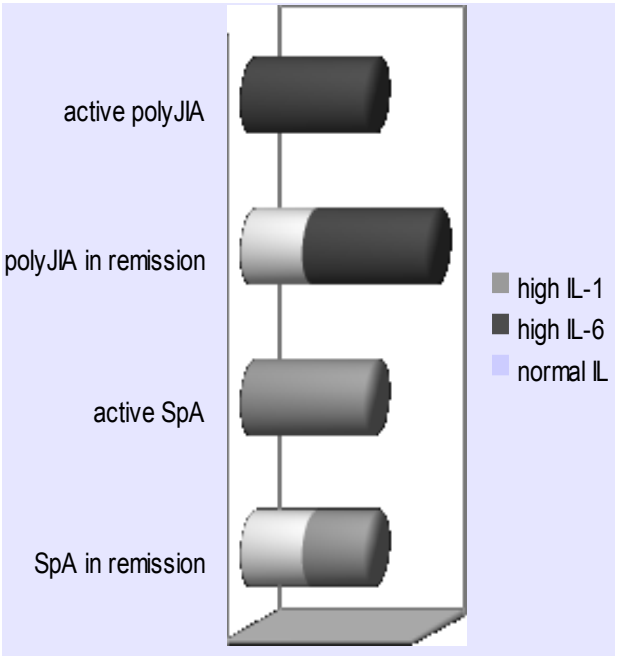

Figure 4. Interrelation between disease activity and ILs.

We observed a possible interrelation between IL type elevation and clinical form of JIA. Both fractions of IL-1, with normal IL-6 levels, were increased in spondyloarthropathies: two cases with reactive arthritis and one juvenile ankylosing spondylitis. Enhancement of IL-6 (and normal IL-1 values) was observed in 3 children with polyarthritis: 2 with clinical active disease, one in clinical remission on treatment, but high ACPA level and positive RF.

DISCUSSIONS

In diagnostic of rheumatoid arthritis, ACPA is an important biomarker, with higher specificity than RF and may better predict erosive disease.4,14 Because of this diagnostic and prognostic value of ACPA, recently, RA is reported to be sub-classified into two subsets by ACPA positivity, sub-division which could be useful in JIA as well.[14-18] Though, in JIA significance of ACPA remains to be determined. The low prevalence of ACPA in our cohort of JIA is in concordance with the result of other studies.[7,10,18,19] This point could support the supposition of the similarity between JIA and the ACPA negative RA, assumption with therapeutic and predictive implications in the management of JIA.

Numerous studies have shown that IgM RF-positive polyarthritis patients have a higher prevalence of anti-CCP antibodies, observation which was not

confirmed in our cohort. Limitation of the present study consists in the relative low number of cases.[5,6,10]

Another issue linked to the low prevalence of ACPA in JIA is the necessity of expanding the diagnostic tools in JIA with other biomarkers from the ACPAs family (anti-mutated-citrullinated-vimentin, anti-Sa antibody), similar to the tendency from rheumatoid arthritis.[20,21]

The pathogenic pathways of different subtypes of JIA are still up to debate. The correlation between IL and the form of JIA in our cohort is in concordance with the results of some studies, but in discordance with the outcome of others.[22-26] Once again, the low number of assessed cases represents an important limitation of our study.

The elevated level of pro-inflammatory interleukins in clinical remission JIA cases could support the concept of a sub-clinical, immunological disease activity.[27-31] This observation could have a practical importance in answering the question: "When to stop biotherapy in clinical remission JIA". Normal values of plasma interleukins could be markers for the treatment cut-off, in contrary high levels of IL could suggest the continuation of the anti-TNF therapy. Though, high levels of pro-inflammatory interleukins could be the result of other inflammatory condition out of articular area.

CONCLUSIONS

ESR and CRP are important biomarkers in assessment of disease activity and response to treatment, with good correlation with functional indexes.

IgM RF and anti-CCP antibody are not reliable markers in appreciation of disease activity or treatment response. However, the measurement of the mentioned biomarkers early in the course of JIA may be beneficial to distinguish aggressive disease and possibly initiate more aggressive treatment earlier in those patients.

Interleukins could be correlated with clinical form of JIA (IL-1 with spondyloarthropaty, IL-6 with polyarthritis). The interleukins could be more sensitive markers of the disease activity than the routine inflammatory markers or the functional indexes.

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