INFLAMMATORY AND IMMUNOLOGIC BIOMARKERS CORRELATED WITH THERAPEUTIC OUTCOME IN JUVENILE IDIOPATHIC ARTHRITIS

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Abstract

Introduction: Juvenile idiopathic arthritis (JIA) is the most important rheumatic disease of childhood. *Aim:* To study the correlations between biomarkers and the therapeutic response 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, alpha2-and gamma-globulins, total IgM and IgG, immunoglobulins, anticyclic citrullinated peptide antibody- ACPA, antinuclear antibodies-ANAs, interleukins – ILs). The outcome was assessed by ACR Pedi (American College of Rheumatology)score. *Results:* Patients distribution regarding diagnosis was: of patients was: 1 systemic JIA, 17 polyarthritis, 18 oligoarthritis, 22 spondyloarthropathies. There was a good, but inverted correlation between the ESR, CRP, serum immunoglobuline values and the ACR Pedi30 scores on NSAID treatment. ACPA was found positive in 6 cases, all associating important inflammation at the onset, but no correlations with ACR scores. ANAs was found positve in just 3 cases of extended oligoarthritis, all associating ocular complications. 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:* Important biologic inflammatory syndrome at the moment of JIA diagnosis suggests a highly active disease, indicating a more aggressive therapeutic approach. Positive ACPA is less prevalent than in rheumatoid arthritis, but its positivity denotes an erosive course of JIA. ANAs were correlated with extended oligoarthritis and ocular complications. Interleukins could be correlated with clinical form of JIA (IL-1 with spondyloarthropaty, IL-6 with polyarthritis) and could suggest the timing of biological therapy withdrawal. Further studies are needed to sustain these observations.

Keywords: juvenile idiopathic arthritis, biomarkers, ACPA, pro-inflammatory interleukins, ACR Pedi score

Introduction

Juvenile idiopathic arthritis (JIA), with a prevalence varying from 16 to 150 per 100,000 (1) is the most common chronic rheumatic condition in childhood and represents an important cause of short and long term disability. It is not a disease, but an exclusion diagnosis that gather together all forms of arthritis that begin before the age of 16 years, persist for more than 6 weeks, and are of unknown origin.

Laboratory examination in JIA

Although the laboratory may provide support for a diagnosis of chronic arthritis, no laboratory test or combination of studies can confirm the diagnosis. The laboratory can be used to provide evidence of inflammation, to support the clinical diagnosis and as a research tool to understand more completely the pathogenesis of the disease.

ESR and CRP.

The Westergren erythrocyte sedimentation rate (ESR) is a useful but not totally reliable measure of active disease at onset and during follow-up of children with arthritis. It is occasionally helpful in monitoring the therapeutic efficacy of treatment, although it does not correlate with the articular response to medication.(2) The C-reactive protein (CRP) level may be a more reliable monitor of the inflammatory response; at least it is less often increased in a child in whom no clinical inflammatory disease can be found.(3)

Rheumatoid factor.

The diagnostic importance of RF seropositivity in a child with possible chronic arthritis is mitigated by the frequent occurrence of abnormal titers in the other connective tissue disorders of childhood.(4) RFs are common in children with later onset age and polyarticular disease and is associated with articular erosions, or a poorer functional class.(5)

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*Note about financial support: The first author, Andrea Somogyi Militaru, benefitted by a grant from the PhD programme POSDRU/88/1.5/S/63117.
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Serum immunoglobulins.

Elevated levels of immunoglobulins are correlated with activity of the disease and reflect the acute-phase response. Extreme hypergammaglobulinemia is present in the sickest children and returns toward normal with clinical improvement. In general, persistent hypegammaglobulinemia is an important hallmark of deteriorating clinical course and poor therapeutic response. Significantly increased concentrations of IgG, IgA are present in children with active disease, whereas elevated IgM levels are characteristic of the disease itself.(6)

Antinuclear antibodies.

Tests for ANAs are more useful than those for RF in diagnostic and classification. Standardized serum dilution titers are usually low to moderate. The frequency of ANAs is highest in girls of younger age of onset, especially in those with oligoarticular disease, and lowest in older boys and whose with systemic -onset disease. ANAs reach their highest prevalence in children who have oligoarthritis and uveitis. Therefore, determination of ANA seropositivity is supportive of the diagnosis and is important in identifying children with the highest risk for chronic uveitis.(7)

Anti-cyclic citrullinated peptide.

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. (8) 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. (9,10) 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. Role of ACPA in JIA remains controversial. Several studies have generated varying results regarding their significance in the disease process. They showed no statistically significant correlation between ACPA positivity and ESR or radiographic damage was detected.(Another study suggested that anti-CCP antibodies in JIA were not as prevalent as in adult RA, but could be useful in predicting joint damage. (5,11,12)

Interleukins.

Although onset and disease course may differ, the subtypes of JIA share the occurrence of chronic inflammation of 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.(12-14) 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. (11,14,15) 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 (14) and the effectiveness of JIA therapies that involve cytokine modulation. 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. (16,17)

Circulating cytokines correspond to the activation status of immune-competent cells, and it 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.(16,18)

Therapy in JIA

Recently the ACR has issued recommendation for the treatment of JIA (19). Recommendations are based on a step up approach which requires the subsequent use of drugs with greater power once the previous treatment(s) failed. Recommendation are proposed for five functional categories of JIA and according to the level of disease activity (low, moderate, and high) and the presence of poor prognostic features specific for each JIA group. In addition the recommendations provide guidance for the safety monitoring of NSAID, methotrexate (MTX) and TNF-α inhibitors.

Assessment of therapeutic answer in JIA.

The validated criteria to evaluate response to therapy in JIA were adopted by the ACR and are now known as the ACR Pediatric 30. According to the ACR Pediatric 30, patients are considered responders to a given therapy if they demonstrate at least 30% improvement from baseline in at least 3 of any 6 JIA core set variables, with no more than 1 of the remaining variables worsening by more than 30%. Commonly, patients also are evaluated for higher level of improvement (ACR Pediatric 50, 70, 90, and 100). Variables included in the JIA core set variables include: (1) the number of joints with active arthritis, (2) the number of joints with limited range of motion; (3) the physician's global assessment of disease activity; (4) the parent assessment of child's overall well-being; (5) a validated measure of functional ability, usually measured by the disability index of the Childhood Health Assessment Questionnaire (CHAQ), (6) a laboratory measure of inflammation, either the ESR or CRP.

Objectives

The main goals of this study were to analyze inflammatory and immunologic biomarkers in a pediatric cohort with chronic arthritis, and to investigate the predictive role of these biomarkers by studying the interrelation between the lab tests at the disease's onset and the therapeutic response in different subtypes of JIA.

Material and methods

It was a retrospective study, followed by a prospective one. 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 –October 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 complete medical history, 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 to diagnosis.

Clinical parameters involved: number of swollen joints, of tender joints and of joints with limitation on passive motion. Presence of extra-articular symptoms and signs (ocular, gastrointestinal, dermatological, cardiovascular etc.) had been 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, serum IgM and IgG levels); and 2) immunologic assessment (RF, ANA, ACPA, and, IL-1alpha, IL-1betha, IL-6 in 13,6% of cases).

Routine assay, consisting in latex agglutination, measured 19S IgM RFs, but enzyme-linked immunosorbent assays (ELISAs) have 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. ACPA, ANAs and plasma levels of ILs 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.

The patients have been treated according to ACR Pediatric recommendations, and the therapeutic response has been assessed according to the ACR Pediatric criteria at one month (on treatment with NSAIDs), at 3 months (on DMARD therapy) and at 6 months (on biological treatment only or combined with DMARD, according to the form of JIA).

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

Results

Characteristics of the cohort

Clinical data permitted the division of 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. The 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).

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
ANA positive (no)	3	0	3 (extended oligo JIA)	0	0

IgM RF detection

IgM RF has been evaluated in all 58 cases by ELISA. In 4 patients, (6.9%), elevated levels of IgM RF were found, all belonging to polyarticular JIA. RF detection permitted the sub-classification of the polyarticular JIA (RF positive and RF negative polyarthritis). All these cases, had important inflammation with severe functional disability at the onset of the disease and had radiographic evidence of joint damage at the moment of diagnosis, including joint space narrowing and joint erosion.

Inflammatory biomarkers evaluation

The values of ESR and CRP levels at disease's onset were divided up into two groups: moderate (less than five times of normal values) and important biologic inflammation (more than five times the normal value: above 50 mm/1h for ESR, and more than 25mg/dl for CRP). Distribution of cases according to the biologic inflammatory syndrome and JIA forms are illustrated in figure no 1 and no 2 (revealing that the systemic JIA and the 70% of polyarticular cases presented an important inflammatory syndrome at disease onset). We studied the interrelations between the inflammatory biomarkers' values (ESR, CRP , fibrinogen, alpha2-, and gamma-globulins , IgM, IgG) at disease onset and therapeutic response.

We found a significant negative correlation between both ESR (r = - 0.70, p <0.001) and CRP (r = - 0,74, p <0,001) values and ACR score obtained on NSAID therapy (Figure no 3 and 4).

Very similar results were obtained in the study of correlations between fibrinogen (r = - 0,63, p <0,001), serum aplha2-globuline levels (r = - 0,59, p <0,001), gamma-globuline (r = - 0,67, p <0,001), IgM (r = - 0,41, p =0,002), IgG (r = - 0,59, p =0,002) and ACR Pedi30 score on NSAID therapy at one month.

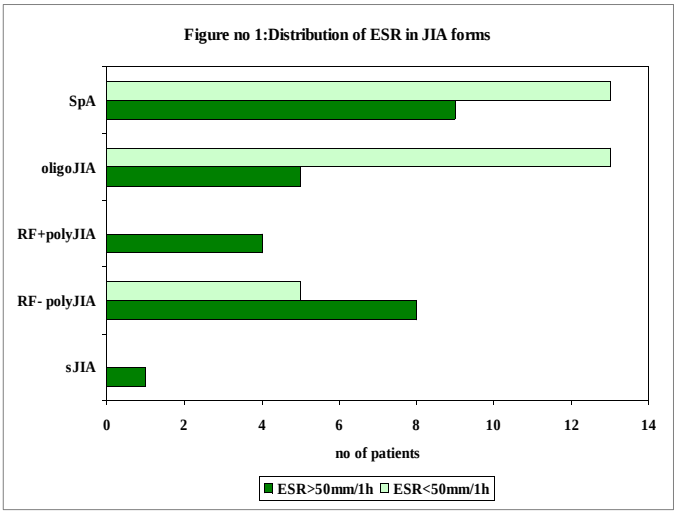

Figure no1. Distribution of ESR values in JIA forms.

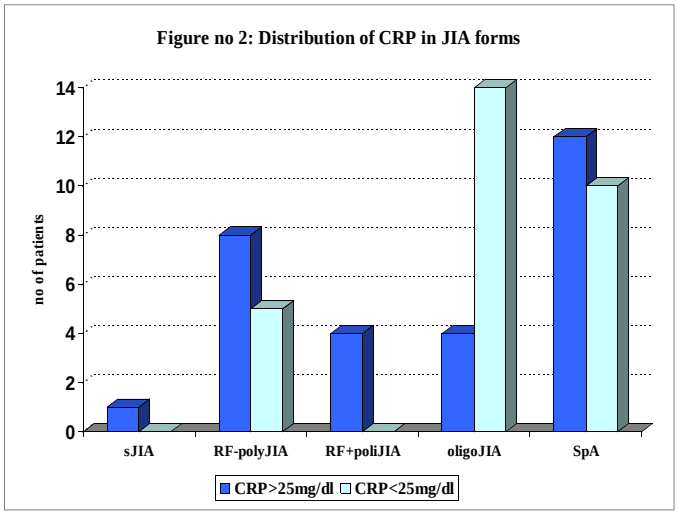

Figure no 2. Distribution of CRP values in JIA forms.

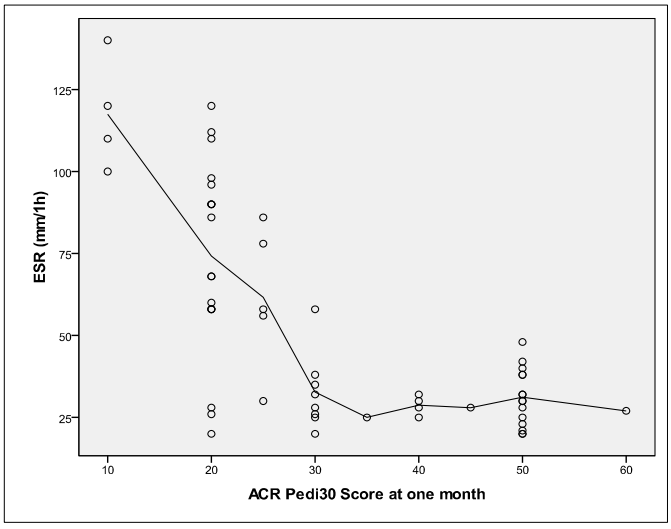

Figure no 3. Correlation between ESR values at the onset and ACR Pedi30 score.

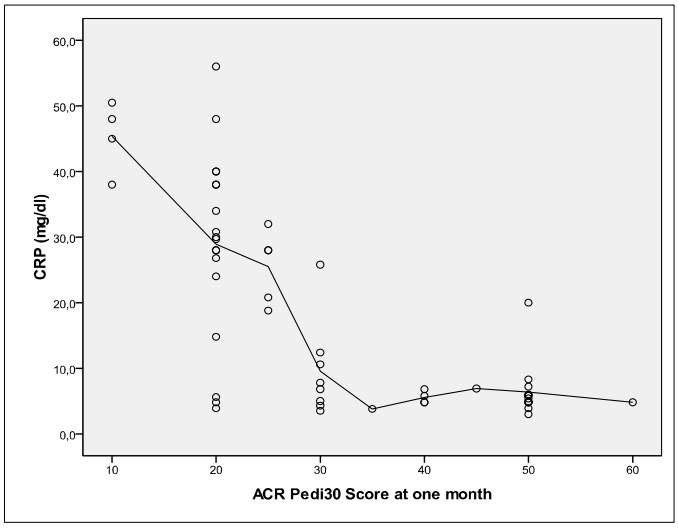

Figure no 4. Correlation between CRP values at disease's onset and ACR Pedi30 score.

IMMUNOLOGIC BIOMARKERS EVALUATION

Anti-CCP antibody detection

In 43 cases ACPAs presence was assessed. Positive, but very low levels were found, in just 9 children, with the following distribution: 6 polyarticular JIA (4 RF-positive and 2 RF-negative polyarthritis), 1 oligoarticular JIA, 2 cases of ERA (an ankylosing spondylitis and 1 patient with Crohn disease associating arthritis). We found no correlation of ACPA positivity and the clinical form of JIA.

100% of ACPA positive cases had important inflammatory syndrome at onset of JIA, had no improvement on NSAIDs at 1 month (under ACR Pedi30), poor evolution on DMARD treatment (average ACR Pedi35 score) and all cases needed aggressive treatment (DMARD and biological association) in evolution. We found no correlation between ACPA values and ACR Pedi score at one, three or six months ($p>0.05$). The cases that reached clinical and biological remission on treatment (with the improvement of CHAQ, VAS, ESR, CRP) presented no decreasing in ACPA titer.

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.

ANAs detection

Antinuclear antibody detection was found positive in just three cases (5,1% of total cases), but in all children disease's history revealed an oligoarticular onset, extended to polyarticular JIA. Ocular complications were checked, and we found in all cases uveitis (in two cases asymptomatic iridocyclitis).

Interleukin plasma level evaluation

Plasma levels of IL-1alpha, IL-1betha, IL-6 pro-inflammatory interleukins were determined by ELISA in 9 cases, but in different disease activity status of the patients.(Figure no 5)

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. All three ILs plasma levels were found elevated in the active systemic JIA case.

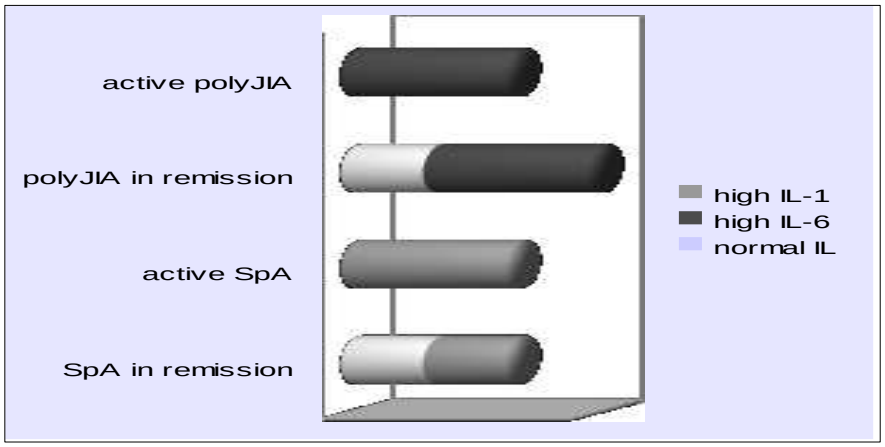

Figure no 5. Interrelation between JIA forms, disease activity and ILs.

Discussions

ESR and CRP are important biomarkers in assessment of disease activity and response to treatment, with good correlation with functional indexes and have an important role in prognosis of the each individual case. (13,20-22) One of the questions raised in consequence of our study's results is if a high disease activity at onset of JIA, reflected by an important increase of inflammatory biomarkers, could or could not be a predictive factor for an aggressive course? For answering, we observed that in our cohort 44% of cases with highly elevated values of inflammatory biomarkers belonged to polyarticular JIA. This issue is highlighted by the newest ACR recommendations, which proposed concerning the polyarticular JIA treatment to over leap NSAIDs, and to star treatment with the DMARDs .(19)

The other inflammatory biomarkers (serum globulins and immunoglobulins) presented less sensitivity in comparison with CRP in appreciation of disease activity in our cohort. The significantly elevated serum levels of both IgG and IgM at the moment of diagnosis reflected a highly active disease, though IgM plasma concentration remained increased even in cases with improving outcome.(6)

In diagnostic of rheumatoid arthritis, ACPA is an important biomarker, with higher specificity than RF and may better predict erosive disease. (11,23) 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. (23-27) 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. (13,15,27,28) 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 ACPA, observation which was confirmed in our cohort. (5,12,15)

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. (29,31-33)

Studies (34) raise the issue that all the available scores (ACR Pedi 30, the 3 versions of the Juvenile Arthritis Disease Activity Score based upon 10-, 27-, and 71-joint counts JADAS,) used in the assessment of disease activity in JIA can lead to a miss-classification of active versus inactive disease. These scores apply ESR or CRP as inflammatory biomarkers, but the researchers continue to detect more sensitive biomarkers. A possible solution could be the interleukins. Elevated level of proinflammatory interleukins in clinical remission JIA cases could support the concept of a sub-clinical, immunological disease activity.(31,34-37) This observation could have a practical importance as well, in answering the question: "When to stop biological therapy in clinical remission JIA". Normal interleukins values could be markers for withdrawal of biological therapy, while, in contrary, high levels of IL could suggest the continuation of the anti-TNF treatment. Nevertheless, high levels of pro-inflammatory interleukins could also be the result of other inflammatory condition out of articular area.

Limitation of the present study consists in the relatively low number of cases.

Conclusions

ESR and CRP correlate closely, show similar test characteristics and are feasible and valid tools for assessing disease activity in JIA. Higher values of ESR and CRP at the first study visit significantly predicted physical disability, damage, no remission on NSAID medication, and use of DMARDs during the disease course.

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.

Prevalence of ANAs was low in our cohort of JIA, but determination of ANA seropositivity is important in identification of children with the higher risk for chronic uveitis.

ACPA, even if is much less prevalent than in RA, represents a reliable biomarker for an aggressive form of JIA, underlying the necessity for a complex therapeutic approach.

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.

Acknowledgments

This study was possible thanks to POSDRU/88/1.5/S/63117 scholarship, and is included in the grant with the title: "Immunogenetical studies and therapeutic correlations in juvenile idiopathic arthritis".

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