Pediatric rheumatology

Circulating survivin indicates severe course of juvenile idiopathic arthritis

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Abstract

Background

Survivin is an anti-apoptotic protein that has been recently suggested as a predictive marker of joint destruction in adult rheumatoid arthritis. We assessed the presence of extracellular survivin in patients with juvenile idiopathic arthritis (JIA).

Methods

Survivin levels were assessed in the circulation of 46 patients with JIA and in the age- and gender-matched controls (n=46) having no inflammatory disease, by ELISA. Survivin levels were analyzed with respect to the onset type and the activity of the joint disease. The intensity of inflammation and cartilage turnover was measured as levels of IL-6, serum amyloid A protein (SAA), and cartilage oligomeric matrix protein (COMP), respectively.

Results

The levels of extracellular survivin were significantly higher in JIA compared to the controls (p=0.0002). High levels of survivin (above mean+2SD of the controls) were detected in 8/46 (17%) JIA patients. High survivin expression was associated with polyarticular onset, active phase of arthritis. In contrast, survivin was neither related to the levels of IL-6, SAA, nor to COMP.

Conclusion

Circulating survivin is expressed in a significant group of patients with JIA being associated to a severe course of the disease. It may be potentially used to select children with unfavorable prognosis of JIA who are in need of active pharmacologic treatment.

Key words

Juvenile idiopathic arthritis, survivin, inflammation, prognosis.

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Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood with a prevalence of 1 to 1000 in the population below the age of 16 years (1). JIA is characterized by hyperplasia of synovial tissue and pannus formation growing invasively into the cartilage, followed by cartilage and bone destruction. Despite the self-limiting nature of JIA, long-term outcome studies showed that a significant group of patients develop functional limitations related to generalized growth failure, leg-length discrepancy and joint destructions leading to replacement surgery (2, 3).

Identifying the patients at risk of developing progressive joint destruction and predicting outcome of JIA is the basis of optimal clinical management of these patients. At present, clinical signs at onset are widely used for prognosis of JIA. It is generally accepted that patients with polyarticular onset of arthritis are less likely to enter remission and are at higher risk to have erosive radiological damage at follow-up (4, 5). Persisting inflammation with concomitantly increased erythrocyte sedimentation rate, high levels of circulating C-reactive protein or high intra-articular levels of inflammatory cytokines as TNF-α, IL-1 and IL-6, are shown to be detrimental to the cartilage and bone development in patients with JIA (6-8). In adult patients with RA the degree of joint damage is traditionally assessed radiologically. In pediatric population the assessment of joint space narrowing and erosions is difficult because of variations of joint space with age and incomplete ossification (9, 10). In contrast to adult RA, the prognostic value of autoantibody production in JIA is less well established. Indeed, the presence of rheumatoid factor (RF) was associated with worse functional outcome and the prevalence of bone erosions in some studies (11), but was not predictive with respect to erosions in the others (3). Antibodies to cyclic citrullinated peptides, a newly recognized prognostic factor for adult RA, did not add prognostic specificity for the patients with JIA being expressed selectively in the RF-positive population (12). Thus, the heterogeneity of clinical manifestations of JIA, the absence of reliable laboratory or radiological marker predicting disease progression explains objective difficulties to distinguish patients with a high likelihood of poorer outcome of JIA and supports a necessity of search for better predictive modalities in JIA.

Synovial inflammation and extracellular matrix degradation are the critical hallmarks in the pathogenesis of JIA. Apoptosis is a physiological process that mediates the programmed cell death controlling the regeneration of the tissues. Chronic inflammation in the joints is associated with an impaired apoptotic elimination of activated and autoreactive cells resulting in hypertrophy of the inflamed synovia and the accumulation of inflammatory cells (13, 14). The role of apoptosis in the development of JIA is less well established. Donn *et al.* has reported high levels of soluble Fas, having anti-apoptotic properties in synovial fluids of patients with JIA (15). In contrast, increased apoptosis was reported in the peripheral blood lymphocytes of patients with JIA (16, 17). We have recently identified an anti-apoptotic protein, survivin as an important prognostic marker of the destructive joint disease in adults. Indeed, serum survivin levels were dramatically increased in about one-third of patients with adult RA being almost entirely associated with a destructive course of joint disease. In contrast, the presence of antibodies against survivin in the same cohort of patients with RA seemed to alleviate destructive process (18).

Survivin is a multifunctional protein transcribed as a wild-type 16.5 kD protein consisting of 142 amino acids and being found in cytoplasm, nucleus, and in mitochondria (19). Expression of survivin is present in the healthy tissues with high self-renewal rate such as gastrointestinal mucosa, hepatocytes, and vascular endothelium. Survivin is abanduntly expressed in most solid tumors and in hematologic malignancies (19, 20). It is believed that survivin has a causative role in cancer formation. The growing evidence suggests that survivin expression in tumor tissues is associated with clinical and morphological

characteristics of the malignancy being frequently used to predict efficiency of treatment and overall survival (21).

In the present study we assessed the frequency of extracellular survivin in a group of 46 patients with JIA and in 46 matched controls in relation to clinical type of arthritis, intensity of inflammation and cartilage turnover. The analysis indicates that high levels of serum survivin are associated with polyarticular type and active phase of the disease, the clinical features characteristic for JIA patients with poor long-term prognosis. These findings indicate that extracellular survivin may be a helpful tool in distinguishing patients with JIA who are at risk of progressive arthritis thus requiring additional attention of a rheumatologist.

Materials and methods
Patients
Plasma samples were collected from 46 consecutive patients with JIA attending the Rheumatology and Immunology Service at the National Children's Hospital Dr. Carlos Sáenz Herrera of San José de Costa Rica between August 2002 and December 2003. All the patients fulfilled the diagnostic criteria of JIA according to the International League of Associations for Rheumatology (22). Clinical and demographic characteristics of the patients are presented in Table I. According to the onset of JIA the patients were devided to the oligoarticular type of JIA (12 patients), polyarticular JIA (23 patients), and systemic JIA (11 patients). The activity of joint disease at the time of blood sampling was defined as active (presence of synovitis in 5 or more joints and/or extra-articular manufestations, 9 patients), stable (stable number of active joints and ongoing anti-rheumatic medication, 31 patients), inactive (no evidence of active synovitis and/or extrarticular manufestations and without medication for less than 2 years, 5 patients), and remission (as above for the time period longer than 2 years, 1 patient) (23). Thirty-three patients received anti-rheumatic treatment, among them 22 patients were treated with methotrexate, 9 patients received oral prednisone and 11 patients were

treated with non-steroidal anti-inflammatory drugs (NSAIDs). Nine patients had more than one treatment modality. Thirteen patients did not have any treatment at the time of blood sampling.

Controls
Plasma samples were collected from 46 children who matched the patients with JIA by age and gender. The children of the control group attended the National Children's Hospital Dr. Carlos Sáenz Herrera of San José de Costa Rica for regular health assessment. Five of them had obesity, 3 Wilson's disease, 2 myelomeningocele, 2 had athopic dermatitis, 5 had psychiatric disorders, 4 had an infection of the urinary tract, 1 anaemia, 1 proteinuria, 1 thrombocytopenia, 1 low blood pressure, 3 endocrine diseases, 1 epistaxis. The remaining seventeen children in the control group were healthy. Three children in the control group with Wilson's disease received D-penicilamine, 1 received imipramine because of depression and 1 had famotidin because of gastritis. None of the controls had known malignancy.

The study was approved by ethical committees of the Sahlgrenska University Hospital and the National Children's Hospital, Dr. Carlos Sáenz Herrera of San José de Costa Rica. The informed consent was obtained from all the patients and controls prior to blood sampling.

Sample preparation
Blood samples were obtained by vein punction and directly transferred into EDTA tubes, which were then centrifuged at 800xg for 15 minutes, stored and frozen at -20°C until use.

Determination of survivin levels
Survivin levels were determined by a sandwich ELISA using a pair of matched antibodies (rabbit anti-human survivin, R&D Systems, Abington, U.K.) as previously described (18). The matched pairs of plasma from patients with JIA and the controls were introduced into the parallel strips, in dilution 1 in 10 in PBS-BSA. The obtained absorbance values were compared to serial dilution of recombinant survivin

and presented as pg/mL. An arbitrary level of survivin corresponding to the mean plus two standard deviations of the control group (450 pg/ml) was defined as "high".

Determination of IL-6 levels
The level of IL-6 in the samples of patients with JIA and the controls was determined by the proliferation of the IL-6 dependent cell line, B13.29. Samples were diluted 1 in 200 in the Iscove's medium containing 10% heat inactivated FCS, 50 µg/ml gentamicyn, 1% L-glutamine, mercaptoethanol 5×10^5 M and put into the wells containing B13.29 cells, 5×10^4 cell/ml. Cells were allowed to grow for 72 hrs at 37°C, 5% CO_2. The effect of the test samples on the proliferation of IL-6-dependent cell line was assessed by incorporation of [^{3}H]thymidine (Amershan, Radiochemical Centre, U.K) added during the last 4 h of incubation. The amount of IL-6 was calculated using standard dilutions of recombinant IL-6 (Genzyme, Cambridge, MA). The levels of IL-6 were expressed in pg/ml

Determination of serum amyloid A protein (SAA) levels
SAA levels were determined by ELISA (Biosource, Camarillo, CA) following the manufacturer's recommendations. Samples were tested in the dilution 1 in 2000. The matched samples from the patients and controls were introduced into the parallel strips. The amount of SAA in the tested samples was recalculated using the serial dilution of the standard provided in the kit. The amount of SAA was expressed in µg/ml.

Determination of cartilage oligomeric matrix protein (COMP)
The levels of COMP were assessed by a quantitative ELISA produced by AnaMar Medical (Uppsala, Sweden). The manufacture's instructions were followed. Samples from JIA patients and controls were tested in parallel in the dilution 1 in 10. The amount of COMP in the samples was calculated using standards with known concentration of COMP provided by the manufacture. The levels of COMP were expressed in U/ml.

Determination of rheumatoid factor (RF)

Total RF, antibodies to immunoglobulin classes G, A, and M, was measure by an ELISA (Hycor Biomedical LTd, Penicuit, UK) following manufacture's instructions. Samples of patients and controls were diluted 1 in 100. The level of total RF was calculated using serial dilutions of the standards provided in the kit and expressed in U/ml. The sample was considered "positive" for RF if its level was above the mean+2SD of the control group (43 U/ml).

Determination of anti-nuclear antibodies (ANA)

ANA were determinate using Hep-2 ANA test system (Immuno Concepts, Sacramento, CA). Briefly, plasma samples diluted 1 in 40 in PBS were incubated on a glass slide with Hep-2 cells and incubated 30 minutes at room temperature. Following intensive washing and incubation with a FITC-labelled anti-IgG conjugate, Hep-2 cells were analysed in immunofluorescent light. The tested samples were compared to a serial dilution of a known positive standard. Those samples that gave immunofluorescent staining in the initial dilution were subjected to further analysis using serial dilutions of the sample. The sample was considered "positive" for anti-nuclear antibodies if the immunofluorescent staining was present in the dilutions above 1 in 80.

Statistical analysis

The levels of continuous variables (survivin, IL-6, SAA, COMP, and RF) are presented as the IQR (range). The comparison of levels in groups was performed using Mann-Whitney U test. Correlation between the variables was assessed using Z-correlation coefficient. For all statistical evaluation of the results, $p<0.05$ was considered significant All the statistical evaluations were performed using a StatView Power PC-Software.

Results

Clinical and demographic characteristics of the patient population and the control group are presented in Table I. In order to minimize the influence of demographic characteristics on the results, the pairs of patients and controls were composed to match by age and gender.

Levels of extracellular survivin are increased in patients with JIA

The determination of survivin in plasma samples of patients with JIA (n=46) and in the matched controls (n=46) showed that survivin levels were significantly higher in the group of JIA as compared with the controls (180[10-4270] versus 20[10-376] pg/ml, $p=0.0002$). Importantly, survivin levels were significantly higher in patients with JIA with systemic onset of arthritis (n=11, 453[10-4270] pg/ml) compared to oligoarticular (n=12, 153[10-1540] pg/ml, $p=0.0001$) type of the disease. In contrast, patients with polyarticular (n=22, 260[10-3470] pg/ml, ns) type of the disease did not differ significantly (Fig. 1a). Survivin levels were also significantly increased in patients having active arthritis (n=9, 332 [30-3470] pg/ml) compared to those with a stable (n=31, 180[10-4270] pg/ml, $p=0.023$) or inactive (n=5, 30[10-153] pg/ml, $p=0.0004$) phase of joint disease. The levels of survivin showed no correlation to the age of patient or to the duration of JIA. The patients treated with methotrexate had lower levels of survivin (n=22, 180 [10-490] pg/ml) compared to the remaining patients (n=24, 360[10-4270] pg/ml, not significant) indicating a more severe course of disease in this population. Survivin levels were not different in the patients treated with oral corticosteroids or with NSAIDs.

An arbitrary level of survivin corresponding to the mean plus two standard deviations of the control group (450 pg/ml) was defined as "high". The JIA patients were further dichotomized as having high (>450 pg/ml) or low (<450 pg/ml) levels of survivin. Eight (17%) of 46 patients with JIA had high levels of survivin compared to only one (2%) of 46 children in the control group ($p=0.0001$). Patients with high levels of survivin were found predominantly in the group with polyarticular onset of JIA (5/22, 23%) compared to only 1 of 12 (8%) patients with oligoarticular onset of the disease. High levels of survivin were also associated with the presence of autoantibodies. Indeed, high levels of survivin was registered in 5 of 12 patients with positive RF (42%) and in 2 of 3 positive for ANA. The JIA patients having positive RF had also higher levels of survivin (455[10-4270] pg/ml) compared to RF-negative (170[10-1540] pg/ml, $p=0.002$).

Extracellular levels of survivin are not related to the intensity of inflammation

The intensity of inflammation in the patients with JIA and in the matched controls was measured by circulating levels of IL-6 and SAA.

As expected, the levels of SAA were significantly higher in the samples of JIA patients as compared to the controls (72[3-1951] μg/ml versus 16[3-404] μg/ml, $p=0.04$). The levels of SAA were significantly higher in the samples of patients with systemic onset (114[4-1951] μg/ml) of JIA as compared to oligoarticular (15[4-868] μg/ml, $p=0.004$) and polyarticular (168[3-523] μg/ml,

Table I. Clinical and demographic characteristics of patients with juvenile idiopathic arthritis (JIA) and the controls.

	Patients with JIA n=46	Controls n=46
Age, years	12 ± 5	12 ± 5
Gender, female/male	35/11	35/11
Duration of JIA, years	5 ± 3	
RF positive (%)	12 (26%)	4 (9%)*
ANA positive (%)	3 (6%)	0 (0%)

RF: rheumatoid factor; ANA: antinuclear antibodies; MTX: methotrexate; NSAID: non-steroidal anti-inflammatory drugs.
*Patients with JIA were significantly more often positive for RF compared to the controls ($p=0.03$)
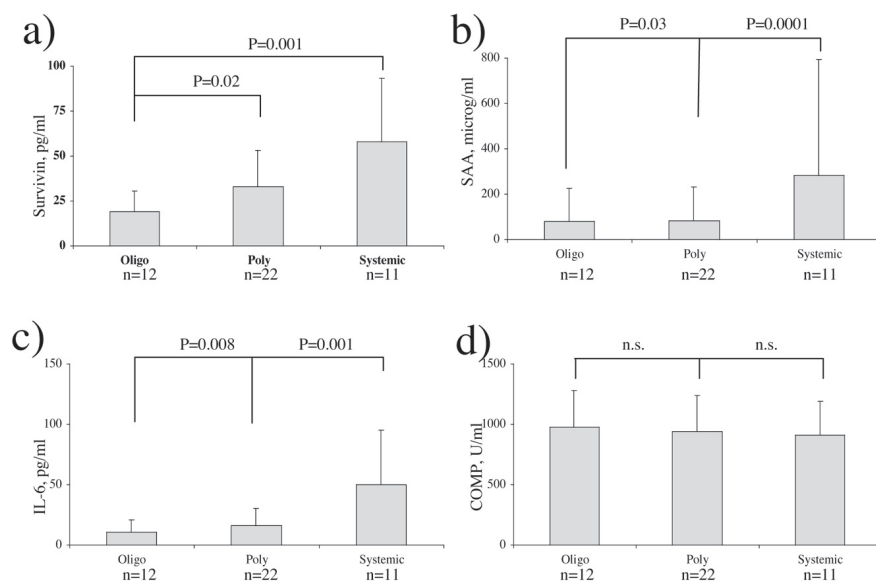
Fig. 1. Levels of circulating survivin, degree of inflammation and cartilage degradation in patients with different types of juvenile idiopathic arthritis (JIA).

Types of JIA by onset. Oligo: oligoarticular JIA (n=12); Poly: polyarticular JIA (n=22); Systemic: systemic JIA (n=11).

a) Levels of extracellular survivin (pg/ml); **b)** Levels of IL-6 (pg/ml); **c)** Levels of serum amyloid A protein (SAA, microg/ml); **d)** Levels of cartilage oligometric matrix protein (COMP, U/ml).

p=0.001) type of JIA (Fig. 1b). Just as in the case of circulating survivin, SAA levels were higher in the samples of JIA patients in the active phase of the disease compared to those in the stable and inactive phases (134[60-1951] μg/ml versus 43[3-523] μg/ml, p=0.05). However, there was no direct correlation between the levels of survivin and SAA indicating that the expression of these two proteins is controlled by different mechanisms. The levels of IL-6 followed the same tendency being higher in the samples of patients with JIA as compared to the controls (16[3-216] pg/ml versus 14[3-153] pg/ml). Levels of IL-6 were also significantly higher in the JIA patients with systemic onset of the disease (38[3-216] pg/ml, Fig. 1c). Levels of IL-6 showed a strong correlation to SAA levels (r=0.64, p=0.002), but not to the level of survivin (r=0.13).

Extracellular survivin levels and markers of cartilage turnover
The levels of COMP were tested in order to measure the ongoing cartilage destruction and to ascertain if survivin levels were related to this degradation product in patients with JIA.
The serum levels of COMP showed no difference between the JIA patients

and the controls (460[400-2020] U/ml versus 420[360-1926] U/ml, not significant). Consequently, no difference in COMP levels was found between the group of patients with high survivin levels and low survivin levels (480[400-2020] U/ml versus 420[400-2005] U/ml, not significant). The levels of COMP were similar independently of arthritis type or activity of the disease (Fig. 1d). No correlation was found between the levels of COMP and SAA or IL-6. With respect to treatment modality, we found that COMP levels were significantly lower in JIA patients treated with methotrexate (n=22, 790[400-1460] U/ml) compared to the remaining cohort of patients (n=24, 1050[440-2020] U/ml, p=0.02). The comparison of COMP levels with respect to gender showed that boys had significantly higher levels of circulating COMP compared to the girls (1120[600-1640] U/ml versus 800[400-2020] U/ml, p=0.036). This difference in the COMP levels was not dependent on the age of individuals and was consistently present in the JIA patients and in the controls. The levels of survivin were opposite to COMP with respect to gender being higher in girls (boys, n=11, 260[10-4270] pg/ml versus girls,

n=35, 127[10-570] pg/ml, not significant) and showed no correlation to the age of individuals.

Discussion
The assessment of patients with JIA showed the presence of high levels of extracellular survivin in 17% of cases but in only 2% of sex and age matched controls. This frequency of survivin is similar to the one reported in the cross-sectional study of patients with adult RA (18). High levels of survivin were associated with polyarticular and systemic onset of JIA as well as with the active phase of the joint disease (see Figure 1). This is of importance since the polyarticular/systemic type of arthritis and the inflammatory activity of JIA are important unfavourable prognostic parameters (1, 24). Also the other prognostic parameter, the presence of RF, was associated with higher levels of survivin in comparison to the JIA patients lacking RF. Taken together these observations place survivin among the possible indicators of unfavourable outcome in severe JIA. However, a prospective study should be undertaken to definitely set this item.
The patients with active JIA were also characterized by the increased intesity of inflammation, assessed in our study by the levels of IL-6 and SAA. Despite the fact that patients having high survivin were found within the group with active JIA, the levels of survivin were neither correlated to the levels of IL-6 nor to the levels of SAA. This lack of correlation between survivin and inflammation may be due to different physiological mechanisms regulating their expression or due to the different tissue origin of these proteins. The main source of IL-6 and SAA in circulation is hepatocytes, while the source of extracellular survivin is presently not identified. Survivin is not accumulated in the inflamed joints being found in similar amounts in circulation and in synovial fluid (18) making synovial tissue a less probable source of survivin during arthritis. Bone marrow may serve as another potential source of survivin taking into consideration the fact that most of mononuclear cells of the peripheral blood continuously express survivin

(25). We conclude that high levels of survivin are characteristic for a subgroup of patients with JIA and should not be considered as a simple acute phase protein.

Extracellular survivin has been suggested as a marker of necrosis and ongoing tissue destruction. To assess this possibility the levels of survivin were analysed in relation to COMP. COMP, a matrix protein produced by chondrocytes, is an established marker of cartilage turnover (26). Increased levels of COMP have been recently reported in relation to cartilage degradation in adults with RA and in osteoarthritis (27, 28) and being used for the evaluation of the efficacy of treatment of RA (27, 29). Survivin showed a lack of correlation to the COMP levels in the patients with JIA studied. Interestingly, patients treated with methotrexate had lower expression of both survivin as well as COMP compared to those without methotrexate. The true reason for this observation is unknown. We suggest that survivin might directly or indirectly participate in cartilage degradation, a mechanism being at least partly inhibited by methotrexate.

In conclusion, our findings indicate survivin as a biological indicator associated with the active joint disease and potentially helping to identify those patients with poor outcome of JIA being in need of additional attention of rheumatologist.

Acknowledgements

References

1. ADIB N, SILMAN A, THOMSON W: Outcome following onset of juvenile idiopathic inflammatory arthritis: I. frequency of different outcomes. *Rheumatology* (Oxford) 2005; 44: 995-1001.

2. FLATO B, SMERDEL A, JOHNSTON V *et al.*: The influence of patient characteristics, disease variables, and HLA alleles on the development of radiographically evident sacroiliitis in juvenile idiopathic arthritis. *Arthritis Rheum* 2002; 46: 986-94.

3. ARGUEDAS O, FASTH A, ANDERSSON-GARE B: A prospective population based study on outcome of juvenile chronic arthritis in Costa Rica. *J Rheumatol* 2002; 29: 174-83.

4. ADIB N, SILMAN A, THOMSON W: Outcome following onset of juvenile idiopathic inflammatory arthritis: II. predictors of outcome in juvenile arthritis. *Rheumatology* (Oxford) 2005; 44: 1002-7.

5. OEN K, MALLESON PN, CABRAL DA *et al.*: Early predictors of longterm outcome in patients with juvenile rheumatoid arthritis: subset-specific correlations. *J Rheumatol* 2003; 30: 585-93.

6. SAXENA N, AGGARWAL A, MISRA R: Elevated concentrations of monocyte derived cytokines in synovial fluid of children with enthesitis related arthritis and polyarticular types of juvenile idiopathic arthritis. *J Rheumatol* 2005; 32: 1349-53.

7. YOKOTA S, MIYAMAE T, IMAGAWA T *et al.*: Therapeutic efficacy of humanized recombinant anti-interleukin-6 receptor antibody in children with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum* 2005; 52: 818-25.

8. DE BEER FC, MALLYA RK, FAGAN EA, LANHAM JG, HUGHES GR, PEPYS MB: Serum amyloid-A protein concentration in inflammatory diseases and its relationship to the incidence of reactive systemic amyloidosis. *Lancet* 1982; 2: 231-4.

9. POZNANSKI AK, CONWAY JJ, SHKOLNIK A, PACHMAN LM: Radiological approaches in the evaluation of joint disease in children. *Rheum Dis Clin North Am* 1987; 13: 57-73.

10. VAN ROSSUM MA, ZWINDERMAN AH, BOERS M *et al.*: Radiologic features in juvenile idiopathic arthritis: a first step in the development of a standardized assessment method. *Arthritis Rheum* 2003; 48: 507-15.

11. FLATO B, LIEN G, SMERDEL A *et al.*: Prognostic factors in juvenile rheumatoid arthritis: a case-control study revealing early predictors and outcome after 14.9 years. *J Rheumatol* 2003; 30: 386-93.

12. VAN ROSSUM M, VAN SOESBERGEN R, DE KORT S *et al.*: Anti-cyclic citrullinated peptide (anti-CCP) antibodies in children with juvenile idiopathic arthritis. *J Rheumatol* 2003; 30: 825-8.

13. CHOU CT, YANG JS, LEE MR: Apoptosis in rheumatoid arthritis--expression of Fas, Fas-L, p53, and Bcl-2 in rheumatoid synovial tissues. *J Pathol* 2001; 193: 110-6.

14. BAIER A, MEINECKEL I, GAY S, PAP T: Apoptosis in rheumatoid arthritis. Cu*rr Opin Rheumatol* 2003; 15: 274-9.

15. DONN R, ZEGGINI E, SHELLEY E, OLLIER W, THOMSON W: Lack of association between juvenile idiopathic arthritis and fas gene polymorphism. *J Rheumatol* 2002; 29: 166-8.

16. HAAS JP, FRANK C, HAEFNER R, HERRMANN M, SPATH H, RUDER H: Inverted ratio of m-fas/s-fas expression in early onset pauciarticular juvenile chronic arthritis. *Eur J Immunogenet* 1999; 26: 325-9.

17. SMOLEWSKA E, BROZIK H, SMOLEWSKI P, BIERNACKA-ZIELINSKA M, DARZYNKIEWICZ Z, STANCZYK J: Apoptosis of peripheral blood lymphocytes in patients with juvenile idiopathic arthritis. *Ann Rheum Dis* 2003; 62: 761-3.

18. BOKAREWA M, LINDBLAD S, BOKAREW D, TARKOWSKI A: Balance between survivin, a key member of the apoptosis inhibitor family, and its specific antibodies determines erosivity in rheumatoid arthritis. *Arthritis Res Ther* 2005; 7: R349-58.

19. DUFFY MJ, O'DONOVAN N, BRENNAN DJ, GALLAGHER WM, RYAN BM: Survivin: A promising tumor biomarker. *Cancer Lett* 2007; 249: 49-60.

20. ALTIERI DC: Validating survivin as a cancer therapeutic target. *Nat Rev Cancer* 2003; 3: 46-54.

21. PENNATI M, FOLINI M, ZAFFARONI N: Targeting survivin in cancer therapy: fulfilled promises and open questions. *Carcinogenesis* 2007; 28: 1133-9.

22. KRUMREY-LANGKAMMERER M, HAFNER R: Evaluation of the ILAR criteria for juvenile idiopathic arthritis. *J Rheumatol* 2001; 28: 2544-7.

23. GARE BA, FASTH A: The natural history of juvenile chronic arthritis: a population based cohort study. II. Outcome. *J Rheumatol* 1995; 22: 308-19.

24. PACKHAM JC, HALL MA: Long-term follow-up of 246 adults with juvenile idiopathic arthritis: education and employment. *Rheumatology* (Oxford) 2002; 41: 1436-9.

25. ALTZNAUER F, CONUS S, CAVALLI A, FOLKERS G, SIMON HU: Calpain-1 regulates Bax and subsequent Smac-dependent caspase-3 activation in neutrophil apoptosis. *J Biol Chem* 2004; 279: 5947-57.

26. SAXNE T, HEINEGARD D: Involvement of nonarticular cartilage, as demonstrated by release of a cartilage-specific protein, in rheumatoid arthritis. *Arthritis Rheum* 1989; 32: 1080-6.

27. DEN BROEDER AA, JOOSTEN LA, SAXNE T *et al.*: Long term anti-tumour necrosis factor alpha monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. *Ann Rheum Dis* 2002; 61: 311-8.

28. DRAGOMIR AD, KRAUS VB, RENNER JB *et al.*: Serum cartilage oligomeric matrix protein and clinical signs and symptoms of potential pre-radiographic hip and knee pathology. *Osteoarthritis Cartilage* 2002; 10: 687-91.

29. LARSSON E, ERLANDSSON HARRIS H, LARSSON A, MANSSON B, SAXNE T, KLARESKOG L: Corticosteroid treatment of experimental arthritis retards cartilage destruction as determined by histology and serum COMP. *Rheumatology* (Oxford) 2004; 43: 428-34.