Apoptosis of peripheral blood lymphocytes in patients with juvenile idiopathic arthritis

E Smolewska, H Brozik, P Smolewski, M Biernacka-Zielinska, Z Darzynkiewicz, J Stanczyk

Background: Recent data suggested that abnormalities in mechanisms regulating apoptosis may have a role in the development of the rheumatoid process.

Objective: To evaluate different aspects of apoptosis in children with juvenile idiopathic arthritis (JIA).

Methods: The frequency of TUNEL positive peripheral blood (PB) lymphocytes (apoptotic index [AI]), as well as serum CD95 (APO1/Fas) antigen expression and serum levels of sFas and interleukin 15 (IL15), were examined in 44 cases of JIA. Results were correlated with type of onset, activity of JIA, and acute phase indicators.

Results: The AI of lymphocytes was significantly higher in patients with JIA than in controls (p=0.020). The mean AI of lymphocytes was increased in JIA with systemic type of onset and high activity (p=0.001). Moreover, IL15 levels in systemic disease were higher than in controls (p=0.012). An increased AI correlated with raised IL15 (p=0.046), erythrocyte sedimentation rate (p=0.005) and C reactive protein (CRP; p=0.017). Additionally, correlation was found between IL15 and CRP levels (p=0.039). CD95 and sFas levels were unchanged compared with controls.

Conclusion: PB lymphocytes of children with JIA have an increased tendency to undergo apoptosis. The degree of apoptosis depends on the type of onset and activity of JIA and correlates with serum levels of IL15. Further studies are needed to explain whether this is an epiphenomenon of the disease activity or is related to the pathogenesis of JIA.

Recent studies suggest that abnormalities in regulation of apoptosis may have a role in the pathogenesis of autoimmune disorders, including rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). Inhibition of apoptosis, with higher expression of anti-apoptotic Bcl-2 protein in synoviocytes, was found in patients with RA and JIA. Additionally, raised serum levels of the soluble form of Fas, anti-apoptotic sFas, were detected in RA. Proinflammatory cytokines such as tumour necrosis factor α, interleukin (IL)1, IL6, IL12, or IL15 are also believed to have a role in the development of the rheumatoid process.

The clinical course of JIA differs from that of RA, with more frequent systemic manifestations in children. A differentiation defect of T lymphocytes and their local activation in synovial tissue is considered to be the key event in both types of rheumatoid process. Less is known about homoeostasis maintained by equilibrium between cell proliferation and death among circulating immune cells. This study aimed at evaluating the incidence of apoptosis of lymphocytes in peripheral blood (PB) of children with JIA and correlating it with CD95 (APO1/Fas) antigen expression or serum levels of sFas and IL15, in different types of onset and activity of the disease.

Results:

**Patients**

Forty four children with JIA, aged 3–17 years (mean (SD) 11.6 (4.2)) were examined. A diagnosis of JIA was established according to Durban’s criteria. None of the children was given second line treatment before the study. Only non-steroidal anti-inflammatory drugs were used as a basic treatment, and these were stopped at least three days before collection of blood samples. The mean (SD) time interval from the first symptoms of JIA and enrolment in the study was 1.2 (1.8) years, reflecting a delayed diagnosis. Based on the type of onset, three subgroups were established: polyarthritis, oligoarthritis and systemic disease (fig 1). Furthermore, based on clinical and laboratory criteria three stages of JIA activity were stated: low, moderate, and high activity of the disease (fig 1). The mean (SD) values of routine laboratory tests in the children with JIA were: leucocytes 9.4 (3.8)×10⁹/l, lymphocytes 2.7 (2.8)×10⁹/l, haemoglobin 125 (1.6) g/l, platelets 445.2 (158.6)×10⁹/l, C reactive protein (CRP) 56 (16.3) mg/l, erythrocyte sedimentation rate (ESR) 46.7 (35.4) mm/1st h.

The controls comprised 30 healthy children, 22 girls and 8 boys, aged 4–17 years (mean (SD) 12.2 (2.9)).

**Abbreviations:** AI, apoptotic index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; JIA, juvenile idiopathic arthritis; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; RA, rheumatoid arthritis; TUNEL, TdT mediated dUTP-biotin nick end labelling

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Figure 1 Clinical characteristics of children with JIA. Three subgroups with different onset of disease were established: polyarthritis, oligoarthritis and systemic disease. Three stages of JIA activity were also distinguished, based on clinical and laboratory criteria: low activity (joint movement limited, without pain or swelling, no extra-articular symptoms, ESR <20 mm/1st h, CRP <10 mg/l); moderate activity (moderate intensity of arthritis, and/or slight temperature, ESR 20–60 mm/1st h, CRP 10–30 mg/l); and high activity (morning stiffness, pain and/or swelling of joints, and/or hepatosplenomegaly, fever, rash, and raised values for laboratory tests ESR >60 mm/1st h, CRP >30 mg/l).
Cell isolation
Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by centrifugation on Histopaque 1077 (Sigma, St Louis, MO, USA). Cells were then washed twice in phosphate buffered saline (PBS) buffer (Sigma) and resuspended in 0.5 ml of mixture of PBS/human serum albumin (1:1). Fifty microlitres of cell suspension, at a concentration of 5x10^5 cells/ml, was cytocentrifuged. Slides were fixed in 1% methanol-free formaldehyde (15 min/0°C), then in 70% ethanol (30 min/0°C). Simultaneously, serum was collected and stored at −80°C.

Apoptosis evaluation
Apoptosis was assessed by an in situ TdT mediated dUTP-biotin nick end labelling (TUNEL) of DNA strand breaks assay. Slides with fixed PBMC were washed in PBS subjected to the TUNEL assay using the APO-DIRECT kit provided by Phoenix Flow Systems, Inc (San Diego, CA, USA) and counterstained with propidium iodide in the presence of RNase. The intensity of cell fluorescence was measured by a laser scanning cytometer (CompuCyte, Cambridge, MA, USA); about 10 000 cells were measured for each sample. Taking advantage of the morphometric capabilities of laser scanning cytometry, we identified lymphocytes from other PBMC, based on the differences in area/red integral and red maximal pixel of DNA fluorescence. The percentage of TUNEL positive cells thus represents the apoptotic index (AI) of lymphocytes. The relocation feature of laser scanning cytometry allowed us to confirm the apoptotic mode of cell death by morphological criteria, as well as by the “sub-G1” DNA content based on cell staining with propidium iodide.

CD95 antigen
Expression of CD95 on cytospin preparations was examined immunohistochemically in an ABC system using anti-CD95 antibody (DAKO, Denmark), and assessed by light microscope (×40 and ×100 magnification) as the frequency of CD95 positive lymphocytes.

Serum sFas and IL15 levels
Serum concentrations of sFas and IL15 (both R&D Systems, UK) were measured by an enzyme linked immunosorbent assay (ELISA).

Other laboratory parameters
In all cases, in parallel with the evaluation of apoptosis, the PB, ESR, and CRP concentrations were estimated by standard methods.

Statistical analysis
Mean and standard deviations were calculated and compared using the Mann-Whitney U test. Correlations between variables were evaluated by the Spearman r test. Results were considered significant at p values <0.05.

RESULTS
Table 1 shows a detailed comparison of results.

### Table 1: The rate of apoptosis (TUNEL positive) of lymphocytes, expression of CD95, and serum levels of sFas and IL15 in children with JIA according to the type of onset and activity of the disease. Correlation between examined parameters and acute phase indicators

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>No</th>
<th>TUNEL (% cells) Mean (SD)</th>
<th>CD95 (% cells) Mean (SD)</th>
<th>sFAS (pg/ml) Mean (SD)</th>
<th>IL15 (pg/ml) Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>30</td>
<td>2.3 (2.9)</td>
<td>9.8 (5.5)</td>
<td>5024.5 (2184.5)</td>
<td>1.7 (0.6)</td>
</tr>
<tr>
<td>JIA - whole group</td>
<td>44</td>
<td>6.5 (7.4)</td>
<td>12.1 (7.3)</td>
<td>5761.3 (2856.1)</td>
<td>1.9 (1.3)</td>
</tr>
<tr>
<td>Type of onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligoarthritis</td>
<td>10</td>
<td>3.7 (3.9)</td>
<td>11.2 (9.4)</td>
<td>6308.0 (3512.1)</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>Polyarthritis</td>
<td>24</td>
<td>4.8 (7.1)</td>
<td>13.1 (7.2)</td>
<td>6561.0 (2427.2)</td>
<td>1.1 (0.4)</td>
</tr>
<tr>
<td>Systemic</td>
<td>10</td>
<td>13.5 (7.1)</td>
<td>9.7 (3.8)</td>
<td>5550.0 (3557.6)</td>
<td>3.6 (2.1)</td>
</tr>
<tr>
<td>Activity of the disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>11</td>
<td>2.7 (2.5)</td>
<td>13.2 (13.2)</td>
<td>6894.0 (2736.1)</td>
<td>1.6 (0.1)</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>4.9 (8.1)</td>
<td>10.9 (6.9)</td>
<td>5492.0 (2345.1)</td>
<td>1.8 (1.4)</td>
</tr>
<tr>
<td>High</td>
<td>18</td>
<td>9.2 (8.0)</td>
<td>12.6 (5.2)</td>
<td>5455.0 (3194.0)</td>
<td>2.3 (1.6)</td>
</tr>
<tr>
<td>Statistics (p values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control v JIA</td>
<td>0.020*</td>
<td>0.331 (0.424) (0.825)</td>
<td>0.031 (0.351) (0.046*)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Control v oligoarthritits</td>
<td>0.381</td>
<td>0.859 (0.318) (0.067)</td>
<td>0.026 (0.411) (0.050)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Control v polyarthritis</td>
<td>0.123</td>
<td>0.206 (0.411) (0.050)</td>
<td>0.026 (0.411) (0.050)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Control v systemic disease</td>
<td>0.001*</td>
<td>0.780 (0.867) (0.012)</td>
<td>0.026 (0.411) (0.050)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Control v low activity</td>
<td>0.437</td>
<td>0.938 (0.071) (0.612)</td>
<td>0.026 (0.411) (0.050)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Control v moderate activity</td>
<td>0.517</td>
<td>0.596 (0.496) (0.517)</td>
<td>0.026 (0.411) (0.050)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Control v high activity</td>
<td>0.001*</td>
<td>0.195 (0.878) (0.203)</td>
<td>0.026 (0.411) (0.050)</td>
<td>0.021 (0.411) (0.050)</td>
<td></td>
</tr>
<tr>
<td>Acute phase indicators</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v TdT</td>
<td>0.098</td>
<td>0.0313 (0.038) (0.867) (0.089)</td>
<td>0.0196 (0.038) (0.867) (0.089)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>v CD95</td>
<td>0.495</td>
<td>0.0737 (0.495) (0.0737)</td>
<td>0.0268 (0.495) (0.0737)</td>
<td>0.0268 (0.495) (0.0737)</td>
<td></td>
</tr>
<tr>
<td>v sFAS</td>
<td>0.017*</td>
<td>0.0460 (0.0460) (0.0460)</td>
<td>0.0469 (0.0469) (0.0469)</td>
<td>0.0469 (0.0469) (0.0469)</td>
<td></td>
</tr>
<tr>
<td>v IL15</td>
<td>0.003*</td>
<td>0.0502 (0.114) (0.089)</td>
<td>0.0036 (0.114) (0.089)</td>
<td>0.0036 (0.114) (0.089)</td>
<td></td>
</tr>
</tbody>
</table>

* Significant.

CRP, C reactive protein; ESR, erythrocyte sedimentation rate; r, Spearman rank coefficient.
Activity of JIA

There were significant differences between the AI in high activity JIA and low (p=0.040) or moderate (p=0.025) activity of the disease, as well as in healthy controls (p=0.001).

Acute phase indicators

The AI of lymphocytes correlated with the CRP concentration and the ESR (p=0.017 and p=0.005, respectively). Additionally, there was significant correlation between serum levels of IL15 and CRP (p=0.039). Serum sFas correlated inversely with lymphocyte count (p=0.038).

DISCUSSION

The results of our study provide evidence for enhanced apoptosis of PB lymphocytes in children with JIA who were not treated with immunosuppressive drugs. The highest AI rate was seen in systemic and in highly active disease.

A search of published reports produced only one related to apoptosis of PB lymphocytes in JIA. In that in vitro study Pig- natti et al evaluated anti-Fas monoclonal antibodies induced apoptosis, in CD3 and IL2 stimulated PBMC from patients with systemic and pauciarticular JIA. The authors found no significant differences in the frequency of apoptosis triggered by the Fas dependent pathway between children with JIA and controls, whereas distinctly increased cell death of PBMC induced by treatment was seen in children with JIA. Our study was the first attempt to evaluate the frequency, not induced by treatment, of in vivo apoptosis of PB lymphocytes in this disease; therefore it is difficult to compare these data directly. Our findings may reflect mechanisms which play a part in the development of the rheumatoid process. In an experimental animal model of RA, enhancement of apoptosis in synovial lining cells was seen at an early stage of the arthritis progression, followed by its inhibition in the prolonged phase of inflammation. A similar course of events may take place in PB lymphocytes.

For RA, the data are also fragmentary. Decreased catechol-amine induced cell death of RA B lymphocytes was reported by Wahle et al. They found raised IL15 in synovial fluid of patients with rheumatoid arthritis. Using flow cytometry they found that in vitro apoptosis of PBMC induced by treatment was seen in children with JIA. Our study was the first attempt to evaluate the frequency, not induced by treatment, of in vivo apoptosis of PB lymphocytes in this disease; therefore it is difficult to compare these data directly. Our findings may reflect mechanisms which play a part in the development of the rheumatoid process. In an experimental animal model of RA, enhancement of apoptosis in synovial lining cells was seen at an early stage of the arthritis progression, followed by its inhibition in the prolonged phase of inflammation. A similar course of events may take place in PB lymphocytes.

Scola et al showed different Fas/Fas in PBMC, serum, and synovial fluid in oligoarthritis JIA, suggesting a role for Fas expression in dysregulation of apoptosis in this disease, which may take place not only in affected joints but also in circulating immune system cells. In our study, expression of CD95 antigen on PB lymphocytes, as well as serum sFas levels, was not significantly higher in JIA than in healthy children. Scola et al are the only authors who reported IL15 in children with JIA. They found raised IL15 levels in synovial fluid, with a population of IL15 positive cells located predominately within perivascular aggregates. In our patients we found increased serum IL15 levels in children with systemic disease, correlating with the CRP concentration, which may reflect an association between IL15 and joint tissue alterations. Harada et al found raised levels of IL15 mRNA in the synovium of patients with RA, probably responsible for local activation of T lymphocytes. IL15 may cause the proliferation of fibroblast-like synoviocytes and inhibit their apoptosis, leading to uncontrolled hyperplasia of the synovium.

In conclusion, we found an increased tendency for PB lymphocytes to undergo apoptosis in children with JIA. The intensity of this phenomenon depended on the type of onset and activity of the disease and correlated with raised serum levels of IL15, but not with CD95 expression or serum concentration of sFas. Both the intensity of apoptosis and serum IL15 correlated with the acute phase indicator CRP levels. It is unclear, at present, whether the observed changes represent an epiphenomenon of disease activity or are related to the pathogenesis of JIA. To answer this question, further studies, including a detailed analysis of the mechanisms of apoptosis modulation during the rheumatoid process, are needed.

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REFERENCES


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Cyclosporin and methotrexate therapy

We read with interest the report by Gerard et al on the efficacy of cyclosporin monotherapy compared with methotrexate and cyclosporin combination therapy in patients with early rheumatoid arthritis. It is pleasing to see the increasing trend of publications looking at appropriate management strategies in early disease. We have previously reported a study comparing combination methotrexate, cyclosporin A, and intrarticular corticosteroids with sulfasalazine in a similar patient group.1

In our 48 week study there was no difference in American College of Rheumatology response, remission rates, or radiographic progression between the two groups at 48 weeks. The current cohort is similar in age though with shorter disease duration and a higher proportion of rheumatoid factor positive patients. Our study did show significantly fewer withdrawals due to lack of efficacy in the combination group than in the sulfasalazine monotherapy group (1/4 0.4 10/42), adding weight to the suggestion of the current study which demonstrated more effective retardation of radiographic progression in the combination treated group. These data suggest that the combination may be more effective in a larger study group.

However, combinations involving cyclosporin must be considered in the light of its significant toxicity. Both the current study and our own had significant periods of modestly raised serum creatinine and episodes of hypertension.

The difference in radiographic progression in the Gerards’ study compared with our own is interesting. The mean doses of cyclosporin and methotrexate in the combination therapy group at 48 weeks were similar in both studies, and it tempting to speculate that the difference in outcomes between the two studies reflects the difference in the comparator treatment—namely, sulfasalazine versus cyclosporin monotherapy. It appears that monotherapy with sulfasalazine is more effective than cyclosporin at retarding disease progression measured by radiographic erosion progression rate. We note that the corticosteroid dose in the Gerards’ trial is not reported, although it was presumably low judged by the number of injections given. Thus it would appear reasonable to conclude that although cyclosporin (as suggested by its mode of action) is effective in early disease, the benefits are insufficient compared with its toxicity to warrant routine use as first line treatment, either as monotherapy or in combination.

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References

Authors’ reply

With interest we read the remarks of Conaghan and Emery concerning the differences between our report and the study of Proudmund et al.

The Proudmund study compared the combination of methotrexate, cyclosporin, and intraarticular injections with sulfasalazine monotherapy in rheumatoid arthritis (RA). Like in our study, Proudmund et al noticed fewer withdrawals due to inefficacy in the combination therapy group, which underlines the importance of testing combination therapy in early disease.

Although tempting, it is difficult to compare outcome measures in Proudmund’s study and our study because of the differences in the study group and the lack of randomisation. We think that erosion scores in the two studies should not be compared when the interobserver differences are not known. We do not know if sulfasalazine or cyclosporin is better at retarding radiological progression, on the basis of the information from these two studies.

Conaghan and Emery conclude that cyclosporin cannot be used as a first line treatment in early RA, either as monotherapy or in combination therapy. We do not share that view. Cyclosporin toxicity was well controlled in a earlier study in early RA.2 The issue of nephrotoxicity with any treatment including cyclosporin is not resolved, although the guidelines state that toxicity is acceptable when dosage rules are closely guarded.3 We did not advocate the combination of methotrexate and cyclosporin as first line treatment in early RA because the data on efficacy were not sufficient. On the other hand, there is no evidence that the combination cannot be used because of toxicity.

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Cyclosporin A in rheumatoid arthritis

We read the paper by Gerard et al with interest. The authors are to be commended for the modest claims they make about the results of their study. They show that a combination of methotrexate and cyclosporin better retard radiographically visible progression than cyclosporin alone after one year in patients with early rheumatoid arthritis. (RA). It raises the question whether cyclosporin still has a place in the early treatment of this disease. One shortcoming of this study as stated in the paper is the lack of a methotrexate only arm. Furthermore, the study did not use optimal doses of methotrexate in the combined arm. Therefore, the possibility that the additional beneficial effects achieved in the combined arm at least in part might have been seen with methotrexate given in monotherapy cannot be excluded. The authors cite a number of studies supporting a retarding effect of cyclosporin, but fail to cite evidence that cyclosporin is not better

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than sodium aurothiomalate (Myocin) in this respect. This study stratified for the use of corticosteroids, in contrast with another observational study which claims that cyclosporin is better than a number of comparative disease modifying antirheumatic drugs, including chloroquine. The three year follow up of the stratified study still showed no difference in radiographic progression between the arms. Despite strict adherence to safety rules about dosing of cyclosporin, adverse renal effects were seen, which were not completely reversible.

This risk is, however, unsettled, and the main purpose of our comment. Cyclosporin is an indispensable drug in transplantation medicine and of unquestionable value in the treatment of unresponsive patients with conditions such as vasculitis and uveitis. A prospective biopsy study in patients with psoriasis and psoriatic arthropathies showed that all of around 30 patients developed interstitial fibrosis and arteriolar wall thickening characteristic of cyclosporin nephropathy. A similar study in patients with RA has not been published. A study published in 1996 stated: “Long term continuous treatment of RA with low dose cyclosporin does not result in more structural nephropathy than the disease process itself, in spite of substantial and persistent deterioration of the renal function.” This study compared renal biopsy results from 11 patients with RA treated for 24 months with 22 necropsy specimens. Although no morphological differences were apparent, creatinine clearance had diminished by 26% in the patients. The accompanying editorial pointed out the weaknesses of the study, based on small size, lack of pretreatment biopsies, and uncertainty about the control group.

A registry based study was published in 1996, consisting of 60 patients in all. It was not stated how the patients were selected for biopsy. The authors concluded that the low doses that had been given to 22 of the patients had not caused any renal damage. A more recent analysis performed in 1998 of cyclosporin related nephrotoxicity in autoimmune diseases concluded, however, that the treatment even with doses of 5 mg/kg day or lower was not without risks, and that renal biopsies should be seriously considered in patients treated with cyclosporin. This view is based on the slowly progressive interstitial fibrosis and arteriolar wall thickening characteristic of cyclosporin toxicity. A review published in 1991 examines the subject of renal toxicity and long term treatment with cyclosporin of autoimmune disease. It concludes that even strict adherence to recommended rules carries a substantial risk for irreversible changes after two years of treatment, and emphasises the need for rigorous risk-benefit analysis in each patient. In view of the lack of long term safety data based inter alia on systematic prospective biopsy results we feel that one should not use cyclosporin in patients with RA until other possible treatments have failed.

After the initial submission of this letter Fox et al published a report showing that cyclosporin given to patients with RA who were also treated with methotrexate, inhibits the oxidation of methotrexate to an inactive metabolite and thereby potentiates the effect of methotrexate. This will thus lead to a potentiation of the methotrexate effect and increased risks of adverse reactions when the drugs are combined.

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References

Authors’ reply
We thank Saxne and Wollheim for their kind remarks. Indeed, we were interested in whether the beneficial effects in the combination therapy group should be ascribed to the concerted action of the combining drugs rather than to the action of methotrexate alone. To test this hypothesis we selected a sample of 41 patients out of a cohort of 411 patients who had responded to cyclosporin plus methotrexate combination therapy in our study. These results give an indication that the effects seen in the combination therapy arm cannot be ascribed to methotrexate alone. Recently, Marchesoni et al published the results of a study showing that the combination of cyclosporin and methotrexate is more effective in retarding radiological progression than methotrexate alone.

The subject of nephrotoxicity of cyclosporin remains highly controversial. We agree with Saxne and Wollheim that structural damage to the kidney is not clearly demonstrated in patients with RA treated with cyclosporin. Reports in other autoimmune diseases cannot be extrapolated to RA but warrant a careful approach. Most reports on cyclosporin in RA state that impairment of the renal function is reversible if dosage guidelines are strictly followed. The study of Boers et al showed that nephrotoxicity is reversible. The study of Krien et al is an extension of the study of Zeidler et al. In the study of Zeidler dose reduction of cyclosporin was required if serum creatinine rose to >50% above the baseline, while guidelines recommend 30%. In the study of Krien it is clear that it was mainly patients who had a rise in creatinine >50% during cyclosporin treatment who were at risk of creatinine remaining high after discontinuation of cyclosporin. This again underlines the importance of the guidelines. We advocate the use of creatinine clearance measurement or calculation before starting cyclosporin treatment, to select patients at risk.

Data on renal function should be viewed from the point of view that renal function loss is common in patients with RA. It is not clear whether the patients in the study of Zeidler and Krien who were treated on the basis of the cyclosporin guidelines (a rise in creatinine no more than 30% is acceptable) were subjected to a greater risk of function loss than other patients with RA. Unfortunately, studies from Zachariae (on psoriasis and with higher cyclosporin dosages) and Vercauteren (not concerning patients with RA) do not shed light on this topic. Our concern is that on the basis of current knowledge on toxicity there is no reason to withhold cyclosporin from all patients with RA. However, questions about efficacy still have to be answered.

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References
1 van Ede AE, Loan RF, Rooil MJ, Huizinga TW, van de Laar MA, van Denderen CJ, et al. Effect of folate or folic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid
PostScript


Is methotrexate osteopathy a form of bone idiiosyncrasy?

I read the letter about low dose methotrexate (MTX) osteopathy with mixed feelings. On the one hand, it is not unusual for a woman to develop pelvic spontaneous fracture after 25 years of prednisone treatment. Longstanding inflammatory joint disease also affects bone. The patient had an active disease that is associated with osteoclast activation mediated by tumour necrosis factor-osteprotein. However, the authors underestimated other possible factors which might have had an influence on bone density. Menstrual cycle status was not discussed. Results of bone densitometry examination were not described despite long term steroid treatment. Risk factors such as family history, smoking, diet, and physical activity were not analysed.

Of note, besides pelvic fracture, increased technetium-99m uptake was seen in joint areas with normal standard radiographs. This may be due to active arthritis and enthopathy. We can draw no conclusions about the duration of the bone scan findings. Data about previous scans are absent. MTX in vitro does not affect the proliferation and further maturation of osteoblasts.' No adverse effect of low dose MTX (<30 mg/week) on bone formation in RA has been found. Studies have shown that low dose MTX treatment did not cause a decrease of bone density and was similar to that of the control groups. Summarising previous studies we can state that most patients have no increased risk of MTX osteopathy. Osteopathy resulting from high dose MTX treatment in children with malignancy occurs only in 9% of patients.

On the other hand, however, this young woman developed pelvic spontaneous fracture three months after the onset of MTX treatment. Severe leg pains increased by weight bearing and relieved by rest followed after four months of treatment. Such a rapid occurrence suggests hypersensitivity of the delayed type with targeting to bones. Bone targeted drug idiiosyncrasy may also be considered. Very delayed drug induced hyper-sensitivity affecting fat tissue of the abdomen has been reported previously. Other tissues may also be affected. Drug sensitivity tests may be helpful.

High and low dose MTX osteopathy have similar signs and symptoms, including a triad of severe low extremity pain (distal tibia), osteoporosis, and compression bone fractures occurring spontaneously or after minimal trauma. Both may develop even over a short period of time after the onset of MTX treatment. In both osteoporosis dosage groups scurvy-like lines may be seen on x ray examination, which may be normal at the start. Because the multiple controls receiving the same treatment in both groups do not have signs of such severe osteoporosis, it is assumed that an as yet unknown cause may be responsible. We propose hypersensitivity reaction or idiiosyncrasy, rapidly affecting bone tissue, may be such causes. There have been comparable reported rates of high and low dose (different by 70–100 fold) MTX osteopathy, independent of cumulative doses, pointing to the possible role of idiopathic or hypersensitivity aetiologies (table 1). Bone pain diminished within one month after stopping MTX treatment in both groups, and x ray findings returned to normal 5–7 months later. Proposed bone hypersensitiv-ity in MTX osteopathy may be compared with hypersensitivity lung or liver disease due to MTX treatment. These serious complications of MTX treatment may follow any cumulative dose of the drug. Recognising the phenomenon of MTX bone idiiosyncrasy or hypersensitivity may prevent the unnecessary or harmful proposal that MTX in vitro is a risk factor for osteoporosis and should be relatively contraindicated in patients with multiple risk factors for osteoporosis.

References


Low dose methotrexate osteopathy in a patient with polyarticular juvenile idiopathic arthritis

We read with some surprise the article by Rudler and colleagues proposing a case of a 36 year old woman with methotrexate (MTX) osteopathy. The authors report insufficiency fractures after low dose MTX treatment for

Table 1 Publications on high dose and low dose MTX osteopathy since the first report in 1970

<table>
<thead>
<tr>
<th>High dose</th>
<th>Low dose</th>
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<td>Cumulative dose: 7.5–144 g/m²</td>
<td>Cumulative dose: 97.5 mg–3.5 g/m²</td>
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<tr>
<td>Onset: 4–11 months</td>
<td>Onset: 3 months–8.5 years</td>
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<td>Warner et al, 1999</td>
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three months and further fractures two months later. They suggest that MTX osteopathy may be more common than expected in patients treated with low dose methotrexate, all the evidence suggests the opposite. MTX is now the most commonly prescribed disease modifying antirheumatic drug for rheumatoid arthritis in America and parts of Europe.\(^1\) We conservatively estimate that 120,000 patients receive low dose MTX in the UK alone, with historically a greater proportion of patients in America receiving the drug. Yet cases of proposed MTX osteopathy with low dose treatment are vanishingly rare (six reported cases in adults). Moreover, recent data suggest that low dose MTX has no effect on bone turnover at all.

In this case only a low dose of MTX was used and is the suggested cause of the fractures. Data from paediatric cases suggest that extremely high doses of MTX (20 g/m\(^2\), 80 g/m\(^2\), and 135 g/m\(^2\)) are associated with no significant change in markers of bone maturation of cells of the osteoblast lineage.\(^4\) Smaller cumulative doses have been implicated in adults, but in the only other published case with short duration (nine months) the patient received almost fivefold more MTX.\(^6\) It is surprising that the authors do not comment on the role of the high doses of prednisolone treatment (estimated cumulative dose of 92 g) or the presence of inflammatory disease of up to 27 years, both important risk factors for insufficiency fractures. There is a growing body of evidence to refute the fact that MTX has any clinically significant effect on bone mineral density (BMD) or a significant impact on the osteoblast lineage. Patel et al. carried out a prospective study of patients with psoriasis and low dose MTX treatment, and reported no significant change in markers of bone turnover or BMD after 21 months.\(^6\) Minaur et al. found that the proliferation and maturation of cells of the osteoblast lineage were not affected by MTX.\(^7\) In a study of 116 patients, no direct association of MTX with BMD or low bone turnover markers was found, and in a small subset, no impact on bone formation was shown by biopsy.\(^5\) There appears to be sufficient evidence to doubt the pathogenic role of MTX in this case. Further information about the treatment received by the patient in the study of Rudler et al., her BMD, parathyroid hormone levels, and long term outcome are necessary. Did she receive any treatment at all after her initial fractures? In the last paragraph the authors refer to other fractures. Are they implying that undue stress or activity contributed to the clinical picture? We believe they should be described as insufficiency fractures. The former are fractures occurring in otherwise normal bones by an abnormally applied mechanical load and the latter are due to abnormal bone.

Currently, it is thought that the possibility of a detrimental impact of MTX on the skeleton, even with concomitant corticosteroids, is low. It is important to emphasise that MTX has had a major impact in improving the health and bones (through corticosteroid sparing) of patients with inflammatory arthritis as well as other inflammatory conditions, which greatly outweighs any possible detrimental effects.

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**References**


**Authors’ reply to Rozin and Quinn et al**

We read with interest the comments by Rozin and by Quinn and colleagues about our recent publication on low dose methotrexate osteopathy in a patient with polyarticular juvenile idiopathic arthritis. Our report was not intended to suggest that MTX osteopathy may be more common than expected, and we agree that reported cases of low dose MTX osteopathy are exceedingly rare compared with the number of patients treated with MTX. Certainly, at first glance it might not be very surprising that this patient developed serial insufficiency bone fractures after 25 years of prednisone treatment. However, the temporal association with the introduction of MTX and the multiplicity of fractures was striking. We acknowledge that we did not provide further information about other possible factors that might have influenced the risk fracture in this patient. This 35 year old woman was not menopausal, did not smoke, and had a normal diet, and her physical activity was markedly restricted as her polyarticular joint involvement was severe. Unfortunately, family history of osteoporosis and bone mineral density were not assessed. We disagree with Rozin about his interpretation of the technetium-99m diphosphonate bone survey. The multiple areas of increased uptake are asymmetric, which would be unlikely for a flare of polyarticular juvenile idiopathic arthritis. Moreover, the enhanced uptake which was localised to the femoral condyles and right calcaneum is not compatible with joint involvement. The increased uptake is certainly too marked and too diffuse to be related to multiple enthesopathies, which would also be very unusual clinical features in this type of inflammatory rheumatism. In a scintigraphic study of the cruciate deficiency model of knee arthritis in dog, the uptake ratio (unstable cruciate/normal contralateral knee) did not exceed 2.0 (controls value: 1.0 to 1.0).\(^6\) Conversely, in a semiquantitative (‘‘scintimetric’’) \(^99m\)Tc diphosphonate scintigraph follow up study of patients with peripheral fractures, the uptake ratio (fracture/normal reference site) was much higher (5.0 to 8.0).\(^9\) In our patient the uptake ratio was 5.5 and 3.7 for the left knee/right knee and right calcaneum/left calcaneum, respectively, which is further evidence for the diagnosis of multiple fractures.

Data for the in vivo effect of MTX on osteoblasts are conflicting, but we agree with Rozin and Quinn and colleagues that the in vivo effect assessed on bone mineral density is reassuring in most studies.\(^6\) Moreover, better control of the inflammatory arthritides should allow an increase of physical activity, which in turn may improve osteoporosis. The hypothesis of bone hypersensitivity or idiosyncrasy to MTX that is discussed by Rozin is only speculative, but appealing. Finally, we obviously concur with both comments and agree that such an exceptional observation of MTX osteopathy should certainly be reported from the use of MTX in idiopathic juvenile arthritis or other inflammatory arthritides when it is indicated.

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**References**


**Clinical comparisons of RA between different populations:** are they feasible?

Rheumatoid arthritis (RA) is the most common chronic inflammatory disease,
In a published paper previously in the Annals, Dadoniene et al described and compared two cohorts of patients with RA from Vilnius (Lithuania) and Oslo (Norway). There were no significant differences in sex, age, extra-articular manifestations, location, or family history of RA between the groups. None the less, there were important differences in disease activity, disability, pain, economic status and medical care, drugs used and, to a lesser extent, genetic differences.

During the past years the HLA system has been gaining an increasingly important role in the pathogenesis of autoimmune diseases. HLA polymorphism has multiple effects on the immune system. HLA-DRB1 alleles have been associated with RA in a number of populations. In the third hypervariable region of their DR β1 chain, they share a sequence of amino acids named “the shared epitope” (SE). In a mestizo Colombian population we found that the SE **QKRA74** in DRB1*04 alleles had the strongest association with RA. However, we did not find any significant association between HLA and RA in African Colombians, emphasising the importance of genetic differences even among populations living within the same country.

There have been different findings from one area to another. In Latin America, the differences are important. In Chilean patients the HLA-DRB1*0401 was related to RA severity. In the Peruvian population an association between RA and the SE was not found. There was a lack of uniformity in the development of these trials, but they all shared a lack of association between DRB1*0401 and RA in the Latin American population. These findings suggest that SE inheritance and genetic influence may vary depending on the genetic background of the studied populations and even in apparently closely located countries. The previous study comparing the Norwegian and Lithuanian populations without inclusion of genetic typing may be misleading. Furthermore, not only may the HLA system play a part in the disease outcome and disease progression of these patients but pharmacogenetics may also be at least as important. The efficacy of methotrexate, salazopyrine, and other DMARDs in reducing the radiological progression of RA erosions has been proved; however, their efficacy and tolerability may be influenced by mutations in their metabolic pathways or in their cellular targets.

Epidemiology of autoimmune diseases is becoming more complex as our knowledge of HLA and genetics becomes more complete. The time is coming when diseases will be defined not only by their symptomatology but also by the genetic background of their hosts.

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References
5 Anaya JM, Carroza PA, Mantilla RD, Arcos-Burgos M. Rheumatoid arthritis association in Colombian population is restricted to HLA-DRB1*04 GQRAA alleles. Genes Immun 2002;3:56–8.

Authors’ reply
We thank Drs Cadena and Anaya for their important and interesting comments on our paper reporting differences in disease activity and health status between matched patients in Norway and Lithuania.
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International Congress on SLE and Related Conditions  
9–13 May 2004, New York, New York, USA  
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27–31 October 2004; Antalya, Turkey  
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Website: www.behcet2004.org

4th International Congress on Autoimmunity  
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VIIth European Lupus Meeting  
3–5 March 2005; Royal College of Physicians, London, UK

Corrections printed in the journal also appear on the Annals website www.annrheumdis.com and are linked to the original publication.

In fig 1 of this article the numbers of patients were corrected but the size of the boxes was not corrected at the same time. The correct figure is shown below.

One of the authors names was supplied incorrectly. The correct authors are as follows: Gerards A H, Landewe R B M, Prins A P A, Bruyn G A W, Goei Thé H S, Laan R F J M, Dijkmans B A C.