CONCISE REPORT

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 (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 synovocytes, 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 homeostasis 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.

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).

Patients and Methods

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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Cell isolation
Peripheral blood mononuclear cells (PBMC) were isolated from heparinised 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 5×10⁶ 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 cytometry (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.

Apoptotic index
The mean AI of lymphocytes was significantly higher in systemic JIA than in controls (p=0.020). The AI correlated positively with IL15 levels (p=0.046, r=0.402). No correlation between AI and CD95 expression, serum sFas, or IL15 levels was apparent.

Type of onset and apoptosis
The mean AI of lymphocytes was significantly higher in systemic JIA than in controls (p=0.001).

No significant correlation was found between either the frequency of CD95+ lymphocytes vs serum sFas levels or vs type of onset of JIA.

The mean serum IL15 level in systemic disease was increased (p=0.012), whereas in oligoarthritis it was decreased (p=0.046) compared with controls. IL15 levels in systemic JIA were higher than those in oligoarthritis (p=0.009) and polyarthritis (p=0.006).
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 Pignatti 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 catecholamine induced cell death of RA B lymphocytes was reported by Wahle et al. Courtyard et al. found an increased rate of apoptotic PB lymphocytes in RA patients compared with controls, without correlation with serum sFas levels, similar to our JIA data.

Haas et al. showed different Fas/sFas 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 predominantly 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 corresponded 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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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.1 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 intra-articular corticosteroids with sulfasalazine in a similar patient group.2

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 (1/40 v 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 moderately 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 Proudman et al.

The Proudman study compared the combination of methotrexate, cyclosporin, and intra-articular injections with sulfasalazine monotherapy in rheumatoid arthritis (RA). Like in our study, Proudman 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 Proudman’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.3 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.4 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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References


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 retards radiographically visible progression than cyclosporin alone after one year in patients with early rheumatoid arthritis (RA). It raises the question whether cyclosporin A still has a place in the early treatment of this disease. One short-coming 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...
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 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 arthritis showed that all of around 30 patients developed interstitial fibrosis and arteriolar wall thickening characteristic of cyclosporin-induced nephrotoxicity in autoimmune diseases. 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 cause 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 induced 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 who develop even slight renal function impairment. This view is based on the slowly progressive interstitial fibrosis and arteriolar wall thickening characteristic of cyclosporin toxicity. A review published in 1997 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 interim analysis 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 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 potential problem: the methotrexate effect 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 group of 41 patients out of a cohort of 411 patients who had participated in the methotrexate/folate supplementation study which was published recently. These 41 patients were matched for age, sex, disease duration, and clinical disease activity. All 41 patients had early rheumatoid arthritis (RA) and were treated with methotrexate as their first disease modifying antirheumatic drug (DMARD; median dose 15 mg/week). Of these 41 patients, 19 (47%) had an American College of Rheumatology (ACR)20 response after one year of treatment, 9 (22%) had an ACR50 response, and 3 (8%) had an ACR70 response. The patients who had responded to methotrexate monotherapy were in the same range as the proportions of patients who had responded to cyclosporin monotherapy, and substantially lower than the proportions 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 withholding 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.22 The study of Boers et al showed that nephrotoxicity is reversible.23 The study of Kviën et al is an extension of the study of Zeidler et al.24 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 Kvien 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 Kvien 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 conclusion 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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1 van Ede AE, Loan RF, Roed MJ, Huizinga TW, van de Laar MA, van Denderen CJ, et al. Effect of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid
Is methotrexate osteopathy a form of bone idiocrasy?
I read the letter about low dose methotrexate (MTX) osteopathy with mixed feelings.2,3 On the one hand, it is not unusual for a woman to develop pelvic spontaneous fractures 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-osteooprogerin. 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 densitometric measurement 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. This rapid effect on bone 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 fractures 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 idiocrasy may also be considered. Very delayed drug induced hypersensitivity 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 idiocrasy, rapidly affecting bone tissue, may be such cases. 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 hypersensitivity 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 idiocrasy or hypersensitivity may prevent the unnecessary or harmful proposal that MTX treatment 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.1 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

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<tr>
<th>High dose Cumulative dose: 7.5–144 g/m²</th>
<th>Low dose Cumulative dose: 97.5 mg–3.5 g/m²</th>
<th>Onset: 4–11 months</th>
<th>Onset: 3 months–8.5 years</th>
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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. 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/m², 80 g/m², and 135 g/m²) are associated with MTX osteopathy. 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. 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 over 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’ follow up. Minnaar et al. found that the proliferation and maturation of cells of the osteoblast lineage were not affected by MTX. In a study of 116 patients, no direct association of MTX with BMD or overall bone turnover markers was found, and in a small subset, no impact on bone formation was shown by biopsy. There appears to be sufficient evidence to doubt the pathogenic role of MTX in this case. Further information about the treatment of the patient in the study of Rudler et al. on bone mineral density and bone turnover. Ann Rheum Dis 2003;62:186–7.


Authors’ reply to Rozin and Quinn et al

We read with interest the comments by Rozin and Quinn and colleagues about our recent publication on low dose methotrexate (MTX) 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 polyartic 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 scintigraphic follow up study of patients with peripheral fractures, the uptake ratio (fracture/normal reference site) was much higher (5.0 to 8.0). 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. Moreover, better control of the inflammatory arthritis 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 reinforce fractures due to 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,
affecting about 1% of the white population, particularly female patients, and has considerable physical, psychological, and social repercussions.1

In a paper published previously in the Annals, Dadoniene et al described and compared two cohorts of patients with RA from Vilnius (Lithuania) and Oslo (Norway).2 There were no significant differences in sex, age, extra-articular manifestations, location, or family history of RA between the groups. None of the less, there were important differences in disease activity, disability, pain, emotional, mental and general health with patients in the Vilnius group having the worst scores. The number of patients who had never used a disease modifying anti-rheumatic drug (DMARD) was similar in both groups. Vilnius patients had more commonly used azathioprine, sulfasalazine, and antimarial drugs, whereas Oslo patients had used methotrexate, gold salts, cyclosporin, and n-penicillamine. Surgery was more common in the Oslo patients. That study was developed to compare the evolution and outcomes of two different populations with RA and was the first to include health related quality of life. The authors also introduced the differences between these groups to differences in economic status, 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.3 HLA-DRB1 alleles have been associated with RA in a number of populations. In the third hypervariable region of their DRβ1 chain, they share a sequence of amino acids named “the shared epitope” (SE).4

In a mestizo Colombian population we found that the SE 30QRRAA in DRB1*0404 and *0408 and the SE influenced the radiographic evolution of hands erosions.5 In the Argentinian population the DRB1*0404 was also important but only DRB1*0404 and *0401 was related to RA severity.6 In the Peruvian population an association between RA and the SE was not found.7 There was a lack of uniformity in the development of these trials, but they all showed 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 population 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, sulfasalazine, 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.8,9

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


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.1

Cadena and Anaya focus on the difference in the genetics of the HLA system or pharmacogenetic differences as a potential explanation for our findings. They refer to several studies, mainly from their own region of the world, where genetic markers have been associated with disease severity and progression. We agree that rheumatoid arthritis is associated with genes, mainly in the region encoding the major histocompatibility complex genes. However, the relative importance of genes is controversial also because low disease concordance has been found in monozygotic twins. Some of the genetic studies indicate only a limited influence of genetic factors on disease susceptibility and progression, and this may suggest a relatively stronger importance of environmental factors.1

However, we completely agree with the comments of the authors that genetic factors, ideally, should have been examined in both populations. However, blood samples were not available for such analyses, but our results would have been stronger if data on the genetic background of the populations had also been available.

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References


FORTHCOMING EVENTS

American Back Society: Advanced Diagnosis and Treatment for Neck and Back Pain 2004
13–15 November 2003; Las Vegas, Nevada
24 CME category 1 units
Tel: +1 510 536 9929
Fax: +1 510 536 1812
Email: info@americanbacksoc.org
Website: http://www.americanbacksoc.org

Fourth International Symposium on Clinical and Economic Aspects of Osteoporosis and Osteoarthritis
14–17 November 2003; Nice, France
Contact: Organisation Secretariat, YP Communication, 108 boulevard G Kleyer, 4000 Li ge, Belgium
Tel: +32 (4) 254 12 25
Fax: +32 (4) 254 12 90
Email: yoland@piettecommunication.com
Website: http://nice.piettecommunication.com

2nd International Forum on Geronto-Rheumatism
27–29 November 2003; Amsterdam, The Netherlands

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International Congress on SLE and Related Conditions
9–13 May 2004, New York, New York, USA
Contact: The Oakley Group, 2014 Broadway, Suite 250, Nashville, Tennessee 37203, USA
Tel: +1 615 322 2785
Fax: +1 615 322 2784
Email: Lupus2004@theoakleygroup.com
Website: http://www.lupus2004.org

IOF World Congress on Osteoporosis
14–18 May 2004, Rio de Janeiro, Brazil
Abstract deadline 14 November 2003
IOF awards are available for scientists:
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IOF Servier Young Investigator Fellowship: 40 000
Contact: Congress Secretariat at info@osteofound.org
Website: www.osteofound.org

International Society for the Study of the Lumbar Spine
31 May–5 June 2004; Porto, Portugal
Deadline for abstracts 15 November 2003
Contact: International Society for the Study of the Lumbar Spine, 2075 Bayview Avenue, Room MG 323, Toronto, Ontario, Canada M4N 3M5
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XIIth International Conference on Behçet’s Disease
27–31 October 2004; Antalya, Turkey
Contact: Congress Secretariat, FIGUR Congress and Organization Services Ltd. STI, Ayazmaderesi Cad, Kardat Sok. No: 7 80888 Dikilitas, Istanbul, Turkey
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CORRECTIONS

Apoptosis of peripheral blood lymphocytes in patients with juvenile idiopathic arthritis
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.

Cyclosporin A monotherapy versus cyclosporin A and methotrexate combination therapy in patients with early rheumatoid arthritis: a double blind randomised placebo controlled trial
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, Bruijn G A W, Goëi Thé H S, Laan R F J M, Dijkmans B A C.