Increased expression of macrophage colony-stimulating factor in ankylosing spondylitis and rheumatoid arthritis

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Increasing evidence suggest that macrophage colony-stimulating factor (M-CSF) plays a part in rheumatoid arthritis, but few studies have focused on its role in ankylosing spondylitis. This study aimed at investigating M-CSF expression and its clinical significance in active ankylosing spondylitis and rheumatoid arthritis.

Sandwich ELISA was used to measure serum M-CSF levels in 12 active patients with ankylosing spondylitis (Bath Ankylosing Spondylitis Disease Activity Index of ≥ 3)¹ and 12 active patients with rheumatoid arthritis.² Eleven serum samples of healthy volunteers were examined as controls. We also examined M-CSF mRNA expression in peripheral blood mononuclear cells (PBMCs) using real-time reverse transcription-polymerase chain reaction. The relative fold increase in M-CSF mRNA expression in patient groups was determined by $2^{-\Delta\Delta C_T}$.³ Considering that tumour necrosis factor (TNF) α and interleukin (IL) 1 α have the ability to induce M-CSF,⁴ we also investigated their levels and correlations with M-CSF. Sensitivities of ELISA kits are as follows: M-CSF, 9 pg/ml (Minneapolis, Minnesota, USA); TNF α , 5 pg/ml (Beckman, Immunotech, Marseille, France); and IL1 α , 1.5 pg/ml (Cayman Chemical Company, Michigan, USA).

The mean (standard deviation (SD)) concentrations of serum M-CSF were significantly increased in patients with ankylosing spondylitis (2234 (2154) pg/ml; $p < 0.001$) and in patients with rheumatoid arthritis (2148 (1803) pg/ml, $p < 0.001$) as compared with that in controls (232 (46) pg/ml). A total of 7 of the 12 patients with ankylosing spondylitis and 10 of the 12 patients with rheumatoid arthritis had increased C reactive protein concentrations (> 1 mg/dl). As table 1 shows, the serum level of M-CSF significantly correlated with Bath Ankylosing Spondylitis Disease Activity Index, erythrocyte sedimentation rate and serum immunoglobulin A (IgA) in the ankylosing spondylitis group, as well as with disease activity scores, C reactive protein and serum albumin in the rheumatoid arthritis group.

Table 1 The correlation coefficients between serum macrophage colony-stimulating factor levels and disease activity variables

	AS (n = 12)	RA (n = 12)
BASDAI	0.62*	—
Schober test	−0.37	—
Sacroiliitis score	0.44	—
Disease activity scores	—	0.63*
Morning stiffness	—	−0.11
Grip strength	—	0.06
Platelet count	0.11	0.08
ESR	0.61*	0.55
CRP	0.40	0.58*
Serum level of IgA	0.68*	0.22
Serum level of IgG	0.07	−0.02
Serum level of IgM	−0.21	0.02
Serum level of ALB	−0.26	−0.64*

ALB, albumin; AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; RA, rheumatoid arthritis.

* $p < 0.05$; —, not applicable.

M-CSF mRNA expression in PBMCs is 5.6-fold and 7.3-fold increased in patients with ankylosing spondylitis and rheumatoid arthritis, respectively, when compared with that in controls (fig 1).

The mean (SD) serum TNF α concentration was significantly increased in patients with rheumatoid arthritis (335 (388) pg/ml; $p < 0.01$) as compared with that in controls (12 (15) pg/ml), whereas no significant difference was found between patients with ankylosing spondylitis (34 (60) pg/ml) and controls. The mean (SD) levels of IL1 α were significantly increased in both patients with ankylosing spondylitis and rheumatoid arthritis (110 (92) and 120 (78) pg/ml, respectively; both $p < 0.01$) when compared with that in controls (3 (9) pg/ml). However, we failed to find any significant correlation between M-CSF and TNF α ($r = 0.48$) in patients with rheumatoid arthritis, or between M-CSF and IL1 α ($r = 0.39$ and 0.11 , respectively) in patients with ankylosing spondylitis and rheumatoid arthritis.

Our results showed an increased serum level of M-CSF in patients with ankylosing spondylitis, which is consistent with earlier studies suggesting that macrophages are crucial for inflammatory responses in patients with ankylosing spondylitis.⁵ Yang *et al*⁶ have studied M-CSF in patients with ankylosing spondylitis, but they were unable to find a significant difference in the serum M-CSF level between patients with ankylosing spondylitis and controls. Koth⁷ reported that M-CSF can exist in several forms and different target sites used in different ELISA kits should be considered to explain different findings. Notably, the assay buffers from R&D Systems (Minneapolis, Minnesota, USA) we used are designed to prevent any interactions that could potentially interfere. In our data, M-CSF mRNA expression in PBMCs was significantly increased in patients with ankylosing spondylitis and rheumatoid arthritis, suggesting that PBMCs may play a part in increased circulating M-CSF levels, although inflammatory sites, such as sacroiliac joints, and involved peripheral joints may also contribute to increased

Figure 1 Change (Δ) CT of each subject in ankylosing spondylitis (AS), rheumatoid arthritis (RA) and control (con) groups. The relative increase in macrophage colony-stimulating factor mRNA ($\Delta\Delta C_T$) was determined by this formula, $\Delta\Delta C_T = C_{T, M-CSF} - C_{T, \beta-actin}$, where C_T was the cycle of reverse transcriptase-polymerase chain reaction at which the amplification plot crossed the threshold during the exponential phase of amplification. The subtraction of mean ΔC_T of controls from that of patients with AS or RA was referred to as $\Delta\Delta C_T$. Fold increase in M-CSF in PBMC in patients with AS or RA was determined by $2^{-\Delta\Delta C_T}$.

serum M-CSF. Additionally, significant correlations between M-CSF and activity index in patients with ankylosing spondylitis and rheumatoid arthritis also highlight the potential role of M-CSF in evaluating the activity of the two diseases.

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Cyclic intravenous pamidronate treatment in children with nodulosis, arthropathy and osteolysis syndrome

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Nodulosis, arthropathy and osteolysis (NAO) syndrome is a rare autosomal recessive multicentric osteolysis.^{1,2} Few therapeutic studies have proved the efficacy of pamidronate in children with genetic osteoporotic syndrome such as osteogenesis imperfecta.^{3,4}

Therefore, we conducted an uncontrolled prospective study to assess the effect of cyclic intravenous pamidronate treatment on the clinical course of NAO syndrome in children and its effect on bone mineral density.

Seven children with NAO syndrome aged 5–14 years were treated with intravenous pamidronate between June 2003 and July 2004. The treatment was given after informed consent was obtained from the parents.

Pamidronate was diluted in 250 ml of isotonic saline and infused over a 4 h period on each of three consecutive days every 3 months for 1 year. The dose was 2 mg/kg/infusion. The patients were maintained on 800 IU/day vitamin D, and at least 800 mg/day of elemental calcium supplement. The patients had clinical and functional assessments using the Childhood Health Assessment Questionnaire and the Visual Analogue Scale.⁵

All patients had normal serum levels of calcium, magnesium, phosphorus, alkaline phosphatase, parathyroid hormone, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, serum osteocalcin, urine spot for cross-linked N-telopeptide type I collagen (NTX), calcium, phosphorus and creatinine. x Rays of hands and feet, and bone mineral density (BMD) of the lumbar spine and the whole body, were assessed before and at the end of the treatment course. z Scores and the number of standard deviations (SD) for BMD above or below the mean for age-matched controls were derived on the basis of the manufacturer's data. The possible side effects were monitored.

The treatment was well tolerated by all patients. They reported a decrease in limb and joint pain and improvement

in functional ability. However, it was not significant ($p < 0.094$; table 1). All patients had significant and progressive reduction in urinary NTX with treatment ($p < 0.01$), and low BMD in the lumbar spine ranging from 0.1 to 0.59 (mean (SD) 0.4 (0.18)) with z scores ranging from –2 to –5.5 (mean (SD) –3.25 (1.35)). At the end of treatment, the mean (SD) BMD increased significantly to 0.63 (0.04) ($p < 0.03$) and z scores to –1.08 (1.2) ($p < 0.03$). Increased mineralisation was observed on conventional x ray in one patient.

Idiopathic osteolysis is a rare skeletal disorder, with progressive bone destruction.^{6–8} Recently, the mutational inactivation of matrix metalloproteinase-2 has been identified in patients with NAO syndrome, which represents the first hereditary deficiency of a metalloproteinase described in man.² Several studies have reported beneficial effects of bisphosphonate treatment in children with different genetic osteoporotic syndromes.^{4,9,10} We treated seven patients with NAO using cyclic intravenous pamidronate, which resulted in

Table 1 Changes observed in patients with nodulosis, arthropathy and osteolysis during pamidronate treatment

	Before	After	p Value
Urinary NTX	550.57 (367)	252 (204)	0.01
Alkaline phosphatase	255.7 (163.1)	134.9 (36.6)	0.094
z Score lumbar BMD	–3.25 (1.35)	–1.08 (1.23)	0.03
z Score whole body BMD	–1.72 (1.57)	–1.18 (1.08)	0.08
VAS	1.3 (0.27)	0.77 (0.62)	0.06
CHAQ	1.73 (1.0)	1.29 (0.76)	0.094

BMD, bone mineral density; CHAQ, Childhood Health Assessment Questionnaire; NTX, cross-linked N-telopeptide type I collagen; VAS, Visual Analogue Scale. Values are mean (SD).