

A single infusion of infliximab increases the serum endostatin level in patients with rheumatoid arthritis

Sirs,

The role of angiogenesis in pannus formation in patients with rheumatoid arthritis (RA) has been well established (1). Angiogenesis, defined as the growth of new capillaries from pre-existing vessels, is a complex phenomenon regulated by a number of factors acting as growth factors or inhibitors (2). Angiogenic growth factors and angiogenic inhibitors have been identified in the synovial tissue of patients with RA (3, 4). Endostatin is a newly characterized natural angiogenesis inhibitor (5) consisting of a proteolytically cleaved fragment of the carboxyl domain of type XVIII collagen (6). Nigashima *et al.* (4) determined the endostatin level in serum and synovial fluid from patients with RA and reported enhanced levels of angiogenic growth factors (basic fibroblast growth factor and vascular endothelial growth factor), but did not find differences in the endostatin level between RA patients and those with other joint diseases (inflammatory arthritis or osteoarthritis). In conclusion they suggested that increased angiogenesis resulted from an imbalance in production between proangiogenic factors and angiogenesis inhibitors. Endostatin levels were significantly increased after the patients were started on DMARD therapy (4).

The aim of the presented study was to determine the serum endostatin level in patients with rheumatoid arthritis before and after a single infusion of the TNF- α antagonist infliximab. Fifteen women with RA aged 47.6 ± 6.08 yrs (mean $\pm$ SEM; range 26–56 yrs) and 10 age-matched healthy female controls (age: 48.0 ± 7.6 yrs, range: 29–57 yrs) were investigated. All patients were rheumatoid factor positive. Blood samples were obtained between 7:30–8:00 AM after overnight fasting before and one day after the first therapeutic infusion of infliximab (Remicade, Schering-Plough). The drug was given intravenously at a dose of 3 mg/kg body mass. All patients were receiving methotrexate (mean $\pm$ SEM 10.25 ± 1.9 mg/week). The characteristics of the patients and controls (including ESR and C-reactive protein level) are shown in Table I.

The serum endostatin level was determined with a competitive enzyme immunoassay using a commercially available kit (Cytimmune Sciences Inc., Maryland, USA). There was no cross-reactivity with heat inactivated endostatin or human angiostatin.

The results are presented as means ($\pm$ SEM). Differences were tested for signifi-

Table I. Characteristics of the patients with rheumatoid arthritis and healthy controls (mean $\pm$ SEM).

Investigated group	Age [yr]	Serum CRP level [mg/l]	ERS [mm/hr]	Symptom duration (yrs.)
A. Controls (n=10)	48.0 ± 7.6	4.6 ± 1.5	4.6 ± 1.2	-
B. Rheumatoid arthritis (n=15)	47.6 ± 6.1	$30.26 \pm 5.88^*$	$32 \pm 10^*$	4.1 ± 0.6

Statistical significance of the difference from the corresponding controls. * $p < 0.0001$.

Table II. Serum endostatin level in the patients with rheumatoid arthritis before and after the infusion of infliximab and healthy controls (mean $\pm$ SEM).

Investigated group	Serum endostatin [ng/ml]
A. Controls	8.98 ± 2.31
B. Rheumatoid arthritis before the infliximab infusion	18.29 ± 1.55
C. Rheumatoid arthritis after the infliximab infusion	22.63 ± 2.68

Statistical significance of the differences: A-B $p < 0.0001$; A-C $p < 0.0001$; B-C $p < 0.05$.

cance using the Student's t-test or the Mann-Whitney U test where appropriate. Regression analysis was used to calculate the correlation.

Enhanced levels of endostatin were seen in the sera of patients with RA (Table II). Infliximab therapy resulted in an increase in serum endostatin. The increase was found in all the investigated patients although it ranged from 1%–36%. The mean relative change was $16.4 \pm 11.7\%$ (level before the infusion 100%). Correlations were found between the serum endostatin level before the infusion and the ESR ($r = 0.411191$), and between the endostatin level after the infusion and the ESR ($r = 0.578206$) as well as CRP ($r = 0.48624$). There was no correlation between the serum endostatin level and other indices.

The results obtained are akin to those reported by Nigashima *et al.* (4) who found enhanced serum levels of endostatin in patients with RA (they did not compare their results with healthy individuals). The values reported were relatively high and similar to our findings. It is interesting that they did not find a difference in serum endostatin level between RA patients and those with other joint involvements. It may suggest that enhanced endostatin production is a phenomenon occurring in injured joints irrespectively to the cause of the damage. Our results are concomitant with the finding of Nigashima *et al.* (4) indicating that medication with DMARD is associated with increase in endostatin level in serum. It may be speculated that tendency to normalize the imbalance between production of angiogenic growth factors and inhibitors is a common mechanism associated with effective management of the disease. Our results show that this alteration can be seen in a short time after a single infusion

of infliximab and are not limited to long term effects of DMARD only.

The role of TNF- α in angiogenesis remains unclear. TNF- α is considered an indirect angiogenesis inducer and TNF- α receptors are expressed on the endothelial cells. On the other hand, TNF- α is not considered as endothelial mitogen or stimulator of migration of these cells (endothelial motogen) (7). Application of infliximab results in a decrease in active TNF- α and this is a key mechanism for its therapeutic action. The present letter reports a novel beneficial effect of administration of infliximab, enhancement of serum endostatin. This effect may result in inhibition of new vessel formation and reduced pannus formation. Further studies to elucidate the role of infliximab in angiogenesis inhibition in RA patients are needed.

E.J. KUCHARZ* R. PIECZYRAK
J. GOZDZIK M. WIDUCHOWSKA
M. KOPEC H. ZAKLICZYNSKA
A. KOTULSKA M. SZARZYNSKA-RUDA
M. LEWICKI E. ZYCINSKA-DEBSKA

Department of Internal Medicine and Rheumatology, Medical University of Silesia, ul. Ziolowa 45/47, PL 40-635 Katowice, Poland. *Contact for all correspondence.

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Inflammatory polyenthesopathy in a patient with X-linked osteomalacia

Sirs,

X-linked hypophosphatemia (XLH) due to a renal tubular defect in phosphate transport and a mutation of the phosphate regulating gene, is the most common inherited form of rickets in developed countries (1-3). While the main radiographic feature in childhood is rickets, in adult life one predominantly sees generalised calcific enthesopathy (1, 4-6). Polisson *et al.* (1) suggested that the enthesopathy of XLH cannot be attributed to the other two common causes of generalised enthesopathy, i.e. diffuse idiopathic skeletal hyperostosis (DISH) or spondyloarthritis and, on the basis of histologic evaluation performed in selected patients, excluded the possibility of an inflammatory form. However, we would like to report the case of a patient with XLH who presented many aspects suggesting an inflammatory enthe-

sopathy.

A 42-year-old male patient presented with a 5-month history of diffuse joint pain and stiffness, mainly involving the shoulders and knees, which started abruptly after a long bicycle ride. The patient had been affected since the age of 18 months by an XLH for which, from the age of 5, he underwent several osteotomies in the legs. Treatment with phosphate and low dose vitamin D was introduced at the age of 15 and continued for 5 years. He was then well until the present episode. The family history was negative.

Physical examination revealed short stature (163.2 cm), bow legs, and loss of range of motion in the hips, knee and shoulders without significant swelling, but with tenderness and warmth over the shoulders and knees. Laboratory investigations revealed only a slight elevation of the ESR (35 mm/h) and a CRP of 1.7 mg/dl (normal < 0.6 mg/dl). X-rays detected extensive calcified enthesopathies on the supero-lateral patella, femoral metaphysis and pelvis, and multiple areas of hyperostosis in the tibia and fibula. In the left femur the diaphyseal bowing was associated with a mid-diaphyseal lateral active Looser-Milkman zone. Bone scintigraphy (Fig. 1) revealed intense up-take mainly in the left knee, right coxo-femoral area, and a small area in the diaphysis corresponding to the Looser-Milkman zone.

X-rays clearly demonstrated features of an enthesopathy that, due to the abrupt onset of symptoms, the elevated ESR and CRP, and the intense activity of the clinically affected areas shown on bone scintigraphy, seemed to be of the inflammatory type. However, no sacroiliac involvement or HLA-B27 were found. The patient was treated with 150 mg/day of sodium diclofenac for 3 months, and experienced a satisfactory recovery from his symptoms. Two years later he experienced some severe flares, mainly in the shoulder and knees, which occurred every 2-3 months and lasted approximately one week.

In 1985 Polisson *et al.* (1) reported that 69% of 26 patients with XLH were affected by generalised enthesopathy. These aspects should be considered as an integral part of XLH and are very frequent, being found in 33% of patients under the age of 30 with XLH, and in all those over this age (7).

The pathogenesis of the enthesopathy associated with XLH is unclear and seems to be unrelated to therapy. Unlike DISH, it is relatively symmetric and does not show significant spinal ligament hyperostosis. The differential diagnosis from spondyloarthritis was made based on the absence of sacroiliac involvement, subchondral bone erosions or syndesmophytes. Thus, the inflammatory features found in our patient are unusual

and difficult to explain. A possible cause could be the disruption in tissues surrounding the microcrystals contained in calcified deposits of the enthesopathies, probably induced by traumatic or metabolic changes, in our patient provoked by an unusually long bicycle ride.

L. PUNZI¹, MD, PhD, Associate Professor
P. ZUCCHETTA², MD, Assistant Professor
R. STRAMARE³, MD, Assistant Professor
M. PODSWIADEK¹, MD, Fellow
R. RAMONDA¹, MD, PhD, Rheumatologist
S. TODESCO¹, MD, Prof., Division Chief

¹Division of Rheumatology, Department of Medical and Surgical Sciences; ²Division of Nuclear Medicine; ³Department of Radiology, University of Padova, Italy

Address correspondence to: Dr. Leonardo Punzi, Division of Rheumatology, University of Padova, via Giustiniani no. 2, 35128 Padova, Italy. E-mail: punzireu@unipd.it

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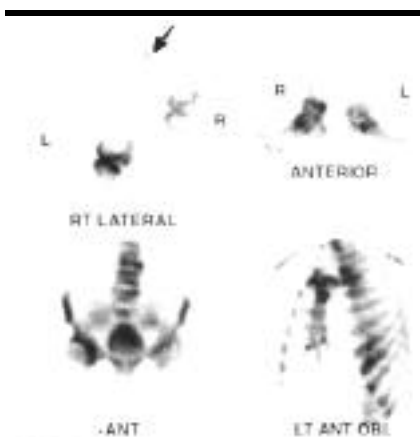


Fig. 1. ^{99m}Tc-MDP bone scan demonstrates intense uptake in the left knee and right coxo-femoral region. Note the deformity in the right femur and the small hyperactive area in the distal portion of the diaphysis. Milder hyperactivity can also be seen in the chondral part of numerous ribs and in the right tarsal region.