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► **To cite this version:**

Piotr Adrian Klimiuk, Stanislaw Sierakowski, Izabela Domyslawska, Justyna Chwiecko. Serum chemokines in patients with rheumatoid arthritis treated with etanercept. *Rheumatology International*, 2009, 31 (4), pp.457-461. 10.1007/s00296-009-1299-3 . hal-00583535

**HAL Id: hal-00583535**

**<https://hal.science/hal-00583535>**

Submitted on 6 Apr 2011

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## **Serum chemokines in patients with rheumatoid arthritis treated with etanercept**

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**Running title:** Etanercept and chemokines in RA

**Abstract**

**Objective.** Chemokines promote leukocyte traffic into the synovium, leading to the initiation and progression of the rheumatoid arthritis (RA). The aim of the study was to determine the effects of etanercept, a soluble tumour necrosis factor receptor (sTNFr), on the serum chemokines levels in patients with active RA.

**Methods.** Patients were treated with 50mg of subcutaneous injection of etanercept per week and methotrexate (10-25mg/week). Serum levels of interleukin-8 (IL-8), RANTES (regulated upon activation, normal T cell expressed and secreted) and monocyte chemoattractant protein-1 (MCP-1) were assessed by ELISA at months 0, 3, 6, 9 and at month 12, prior to injection.

**Results.** Three months treatment with etanercept diminished serum concentrations of IL-8, RANTES and MCP-1 ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively). Subsequent etanercept administrations prolonged decrease in serum chemokines levels and in the case of IL-8 even intensified the reduction of its concentration in serum. These changes were accompanied by significant decrease of disease activity score (DAS28) (in all cases  $p < 0.001$ ). Prior to the first etanercept administration serum concentrations of studied chemokines correlated with markers of RA activity such as the erythrocyte sedimentation rate (ESR) and DAS28. Following next drug injection such associations were less or not significant.

**Conclusion.** Therapy with etanercept and MTX, not only caused a clinical improvement, but also diminished serum chemokines levels in RA patients. Further treatment with etanercept sustained chemokines suppression.

**Key words:** chemokines, IL-8, RANTES, MCP-1, rheumatoid arthritis, etanercept.

## Introduction

Rheumatoid arthritis (RA) is characterized by infiltration of mononuclear cells into synovium. Enhanced angiogenesis and the proliferation of the synovium-lining layer may also be noticed. Macrophages, fibroblasts, lymphocytes and endothelial cells in rheumatoid synovium are supposed to contribute to the pathogenesis of the disease by several mechanisms including chemokines production [1, 2]. Chemokines have an important role in the migration of mononuclear cells into synovium, leading to the initiation and progression of the disease. These small proteins enhance the inflammation by recruiting and activating leukocyte subpopulations. Furthermore, chemokines like interleukin-8 (IL-8) or monocyte chemoattractant protein-1 (MCP-1) intensify infiltration of synovial tissue by inflammatory cells through the stimulation of neovascularisation of rheumatoid synovium [3, 4]. Chemokine production is stimulated by inflammatory cytokines like interleukin-1 (IL-1) and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) [5, 6]. In addition, chemokines promote synthesis of pro-inflammatory cytokines like IL-1, IL-6, TNF- $\alpha$  or matrix metalloproteinases engaged in the joint destruction in RA [3, 7]. Abundant presence of such chemokines like IL-8, RANTES (regulated upon activation, normal T cell expressed and secreted) or MCP-1 in rheumatoid synovium even in early stage of the disease, support the role of chemokines in RA [8, 9, 10].

Several studies have shown that anti-TNF- $\alpha$  treatment with infliximab, a chimeric anti-tumour necrosis factor  $\alpha$  antibody [11, 12] and etanercept, a soluble tumour necrosis factor receptor (sTNFr) [13, 14, 15] diminish clinical activity of RA. In our previous report, we demonstrated that infusions of infliximab reduce serum chemokines concentrations in RA patients [16]. In the present study, we investigated the effect of the one-year therapy with etanercept on serum IL-8, RANTES and MCP-1 serum levels in patients with active rheumatoid arthritis.

## Materials and methods

### Patients and samples

Eligible patients (3 men and 14 women) met the American College of Rheumatology 1987 revised diagnostic criteria for RA [17]. At the time of enrolment, patients had active RA, as manifested by at least 6 tender and 6 swollen joints, and two of the following: morning stiffness for more than 45 minutes, C-reactive protein (CRP) level of more than 20 mg/l, erythrocyte sedimentation rate (ESR) of more than 28 mm/h. None of the patients had previous history of tuberculosis or symptoms of infectious diseases in the previous 3 months. Chest x-rays performed prior to the first etanercept administration were normal in all patients. Mean patients age was 41.6 years (range 19-71 years), and mean disease duration was  $5.1 \pm 4.4$  years. All subjects were receiving methotrexate (MTX) (median 17.5 mg/week, range 10-25 mg/week) in a stable dose for at least 2 months and nonsteroidal anti-inflammatory drugs (NSAIDs) in a stable dose for at least 4 weeks before enrolment into the study. Fourteen patients were receiving corticosteroids (median 7.5 mg/day of prednisone or equivalent, range 5-10mg/day) in a stable dose for at least 4 weeks before beginning of the study. Such treatment regimen with MTX, NSAIDs and corticosteroids was maintained throughout the study.

Patients were receiving etanercept, a soluble tumour necrosis factor receptor (sTNFr), 50mg subcutaneously weekly during 12 months. Clinical and laboratory assessment were conducted prior to injection at baseline and at months 3, 6, 9, and 12. Blood samples were clotted for 30 minutes and next centrifuged for 15 minutes at 1000 x g. Serum aliquots were stored at  $-80^{\circ}\text{C}$  until assayed.

The local ethical committee approved the study protocol and all patients provided written informed consent.

### Clinical and laboratory measures

The evaluation included the number of tender joints (of 28 joints assessed), the number of swollen joints (of 28 assessed), three variables disease activity score that include 28-joint counts (DAS28) [18], the duration of morning stiffness, blood cell counts, including differential white blood counts, erythrocyte sedimentation rate (ESR), rheumatoid factor level, transaminases activity and creatinine concentration in serum. Measurements were performed at baseline and at months 3, 6, 9 and 12 (prior to etanercept injection).

### Enzyme-linked immunosorbent assays (ELISA)

The assessments of serum levels of studied chemokines (IL-8, RANTES, MCP-1) were determined by ELISA kits from R&D Systems, Wiesbaden-Nordenstadt, Germany, strictly according to the manufacturer's instructions. The sensitivity of the assays was 3.5 pg/ml (IL-8), 2 pg/ml (RANTES) and 5 pg/ml (MCP-1).

### Statistical analysis

The normally distributed data were analyzed by paired Student t-test. Wilcoxon signed rank test was used to compare the differences between non-normally distributed data. Correlations between variables were assessed by Spearman rank order test. P values lower than 0.05 were considered statistically significant.

## **Results**

### Serum concentrations of chemokines

Etanercept decreased serum concentrations of IL-8 after three months of treatment as compared to baseline values ( $p < 0.05$ ) (Figure 1). Continuation of therapy even more significantly diminished serum IL-8 levels as assessed at months 6, 9 and 12 (in all cases  $p < 0.001$ ). Down regulation of IL-8 was accompanied by the fall of serum concentrations of

RANTES as evaluated at months 3, 6, 9 and 12 ( $p < 0.01$ ,  $p < 0.001$ ,  $p < 0.001$  and  $p < 0.01$  respectively) (Figure 2). Three months treatment with etanercept suppressed also MCP-1 serum level ( $p < 0.001$ ) (Figure 3). Subsequent drug administrations prolonged MCP-1 reduction, although less significantly ( $p < 0.01$ ).

Correlations between serum levels of chemokines and clinical data

Down regulation of circulating chemokines was accompanied by significant decrease of three variables disease activity score that include 28-joint counts (DAS28) as assessed at months 3, 6, 9 and 12 (in all cases  $p < 0.001$ ) (data not shown). At baseline we demonstrated significant correlations between IL-8 and RANTES serum concentrations, and such markers of RA activity as the erythrocyte sedimentation rate (ESR) or DAS28 (in all cases  $p < 0.01$ ) (Table 1). Furthermore we observed significant correlations between MCP-1 serum levels and ESR or DAS28 ( $p < 0.01$  and  $p < 0.05$  respectively). During therapy with etanercept such associations were less or not significant. No associations between patient sex, age or disease duration with studied circulating chemokines were observed.

## Discussion

Chemokines are proteins, which work as mediators of inflammation process by recruiting and activating particular leukocyte populations. Along with adhesion molecules, they control the migration of mononuclear cells into synovium, leading to the initiation and progression of the rheumatoid arthritis. Chemokine synthesis is regulated by inflammatory cytokines like interleukin-1 (IL-1) and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) [5, 6]. Previously we revealed that another blocker of TNF- $\alpha$ , a chimeric anti-TNF- $\alpha$  monoclonal antibody (infliximab), not only suppress clinical activity of RA, but also down regulate serum levels of IL-8, RANTES and MCP-1 [16]. In current study, we analysed serum concentrations of IL-8, RANTES and

MCP-1 in patients with active RA treated with etanercept, a soluble tumour necrosis factor receptor (sTNFr).

IL-8 is a member of CXC chemokine family and is produced by different cells like macrophages, fibroblasts, T cells, neutrophils, endothelial cells and chondrocytes [3, 6]. Elevated IL-8 levels were found not only in serum [19, 20] but also in synovial fluid [20] of RA patients compared to patients with OA or in healthy controls. In our present study, we demonstrated that treatment with etanercept decreased serum levels of IL-8 in RA patients. Especially serum concentrations of that chemokine were reduced after 6 months of therapy and afterwards. Prior to etanercept administration serum IL-8 was correlated with clinical markers of disease activity like erythrocyte sedimentation rate (ESR) and disease activity score (DAS28). Circulating IL-8 was demonstrated by other investigators to correlate with serum MCP-1 [5]. We also observed the association between serum levels of IL-8 and MCP-1. Moreover, we noted the correlation between serum IL-8 and RANTES concentrations prior to the beginning of etanercept treatment. However, after subsequent drug injections such associations were less or not significant.

RANTES (regulated upon activation, normal T cell expressed and secreted) belongs to CC chemokine family, produced by T cells and synovial fibroblasts [21, 22], and involved in monocyte and T lymphocyte migration [3, 23]. Enhanced concentrations of RANTES were found in RA serum [19, 24] and synovial fluid [25] compared to OA patients and healthy individuals. In rheumatoid arthritis this chemokine was demonstrated to be predictive of radiological erosions [26]. In our study we showed that therapy with etanercept reduce serum levels of RANTES in RA patients. Further drug administrations prolonged circulating RANTES reduction in studied patients. Prior to the etanercept therapy serum RANTES concentrations were demonstrated by us to correlate with ESR and DAS28. Furthermore, serum RANTES was associated also with IL-8 and MCP-1 levels. As in the case of IL-8, after further etanercept administrations such correlations were less or not significant.



MCP-1 (monocyte chemoattractant protein-1) is produced by leucocytes, fibroblasts, endothelial cells and chondrocytes and is member of CC chemokine family [5, 6]. It attracts not only monocytes, but also T cells, natural killer cells and basophils, and plays a role in T cell differentiation and angiogenesis [3]. Elevated MCP-1 levels were revealed in serum and synovial fluid of RA patients [19, 27, 28]. In the current study, serum MCP-1 levels were down regulated following 3 months therapy with etanercept. Further drug injections maintained MCP-1 suppression, however to a lesser extent. Also others demonstrated serum MCP-1 levels reduction in RA patients during 6 months treatment with etanercept [29]. Other investigators correlated serum levels of MCP-1 with IL-8 concentrations [5], swollen joint count and Ritchie articular index [30] in RA. In our analysis prior to etanercept administrations circulating MCP-1 was correlated with clinical markers of disease activity like ESR and disease activity score (DAS28). Furthermore, we also noted the associations between serum concentrations of MCP-1 and IL-8 or RANTES concentrations. Following later on etanercept administrations these associations were less or not significant.

We found no correlations between patient sex, age or disease duration with serum concentrations of studied chemokines. As in other studies [13, 14, 15] parameters of disease activity such as ESR and disease activity score (DAS28) decreased after three months therapy with etanercept. Subsequent drug administrations caused further decrease of these parameters of disease activity (data not shown).

In conclusion, three months etanercept treatment combined with MTX, beside a rapid clinical improvement, down regulated serum IL-8, RANTES and MCP-1 levels in RA patients. Continued administrations of etanercept prolonged reduction of studied chemokines during one-year study period. Moreover, we revealed that serum concentrations of IL-8, RANTES and MCP-1 correlated with markers of disease activity such as ESR and the disease activity score (DAS28) prior to the first etanercept administration and to a lesser extent

following further drug injections. Therefore, these chemokines might be useful markers of the RA activity in patients with the anti-TNF- $\alpha$  therapy.

### **Key messages**

Therapy with etanercept and MTX cause a clinical improvement and diminish serum chemokines levels in rheumatoid arthritis (RA) patients during one-year observation. Serum concentrations of IL-8, RANTES and MCP-1 might be useful markers of the RA activity in patients with the anti-TNF- $\alpha$  therapy.

**Conflict of interest statement: None**

### **Acknowledgements**

Study was support by the grant (3-60766L) from Medical University of Bialystok, Poland.

## References

1. Tarrant TK, Patel DD. Chemokines and leukocyte trafficking in rheumatoid arthritis. *Pathophysiology*. 2006; 13: 1-14.
2. Vergunst CE, van de Sande MG, Lebre MC, Tak PP. The role of chemokines in rheumatoid arthritis and osteoarthritis. *Scand J Rheumatol*. 2005; 34: 415-425.
3. Haringman JJ, Ludikhuizen J, Tak PP. Chemokines in joint disease: the key to inflammation? *Ann Rheum Dis* 2004;63:1186-94.
4. Szekanecz Z, Pakozdi A, Szentpetery A, Besenyei T, Koch AE. Chemokines and angiogenesis in rheumatoid arthritis. *Front Biosci (Elite Ed)*. 2009; 1: 44-51.
5. Koch AE, Kunkel SL, Harlow LA, Johnson B, Evanoff HL, Haines GK, Burdick MD, Pope RM, Strieter RM. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. *J Clin Invest* 1992;90:772-9.
6. Pulsatelli L, Dolzani P, Piacentini A, Silvestri T, Ruggeri R, Gualtieri G, Meliconi R, Facchini A. Chemokine production by human chondrocytes. *J Rheumatol* 1999;26:1992-2001.
7. Klimiuk PA, Sierakowski S, Latosiewicz R, Cylwik B, Skowronski J, Chwiecko J. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in different histological variants of rheumatoid synovitis. *Rheumatology (Oxford)* 2002;41:78-87.
8. Katrib A, Tak PP, Bertouch JV, Cuello C, McNeil HP, Smeets TJ, Kraan MC, Youssef PP. Expression of chemokines and matrix metalloproteinases in early rheumatoid arthritis. *Rheumatology (Oxford)*. 2001; 40: 988-94.
9. Takahashi Y, Kasahara T, Sawai T, Rikimaru A, Mukaida N, Matsushima K, Sasaki T. The participation of IL-8 in the synovial lesions at an early stage of rheumatoid arthritis. *Tohoku J Exp Med*. 1999; 188: 75-87.
10. Haringman JJ, Smeets TJ, Reinders-Blankert P, Tak PP. Chemokine and chemokine receptor expression in paired peripheral blood mononuclear cells and synovial tissue of

- patients with rheumatoid arthritis, osteoarthritis, and reactive arthritis. *Ann Rheum Dis*. 2006; 65: 294-300.
11. Lipsky PE, van der Heijde DMFM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. *N Engl J Med* 2000;343:1594-602.
  12. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor  $\alpha$  monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet* 1999;354:1932-9.
  13. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999; 340: 253-259.
  14. van der Heijde D, Klareskog L, Rodriguez-Valverde V, Codreanu C, Bolosiu H, Melo-Gomes J, Tornero-Molina J, Wajdula J, Pedersen R, Fatenejad S; TEMPO Study Investigators. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. *Arthritis Rheum*. 2006; 54: 1063-74.
  15. van Riel PL, Taggart AJ, Sany J, Gaubitz M, Nab HW, Pedersen R, Freundlich B, MacPeck D. Add Enbrel or Replace Methotrexate Study Investigators. Efficacy and safety of combination etanercept and methotrexate versus etanercept alone in patients with rheumatoid arthritis with an inadequate response to methotrexate: the ADORE study. *Ann Rheum Dis* 2006;65:1478-1483.
  16. Klimiuk PA, Sierakowski S, Domyslawska I, Chwiecko J. Regulation of serum chemokines following infliximab therapy in patients with rheumatoid arthritis. *Clin Exp Rheumatol*. 2006; 24: 529-33.

17. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
18. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44-48.
19. Klimiuk PA, Sierakowski S, Latosiewicz R, Skowronski J, Cylwik JP, Cylwik B, Chwiecko J. Histological patterns of synovitis and serum chemokines in patients with rheumatoid arthritis. *J Rheumatol.* 2005; 32: 1666-1672.
20. Kaneko S, Satoh T, Chiba J, Ju C, Inoue K, Kagawa J. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines Cell Mol Ther* 2000;6:71-9.
21. Robinson E, Keystone EC, Schall TJ, Gillett N, Fish EN. Chemokine expression in rheumatoid arthritis (RA): evidence of RANTES and macrophage inflammatory protein (MIP)-1 beta production by synovial T cells. *Clin Exp Immunol* 1995;101:398-407.
22. Volin MV, Shah MR, Tokuhira M, Haines GK, Woods JM, Koch AE. RANTES expression and contribution to monocyte chemotaxis in arthritis. *Clin Immunol Immunopathol* 1998;89:44-53.
23. Ellingsen T, Buus A, Moller BK, Stengaard-Pedersen K. In vitro migration of mononuclear cells towards synovial fluid and plasma from rheumatoid arthritis patients correlates to RANTES synovial fluid levels and to clinical pain parameters. *Scand J Rheumatol* 2000;29:216-21.
24. Boiardi L, Macchioni P, Meliconi R, Pulsatelli L, Facchini A, Salvarani C. Relationship between serum RANTES levels and radiological progression in rheumatoid arthritis patients treated with methotrexate. *Clin Exp Rheumatol* 1999;17:419-25.

25. Volin MV, Shah MR, Tokuhira M, Haines GK, Woods JM, Koch AE. RANTES expression and contribution to monocyte chemotaxis in arthritis. *Clin Immunol Immunopathol* 1998;89:44-53.
26. Boiardi L, Macchioni P, Meliconi R, Pulsatelli L, Facchini A, Salvarani C. Relationship between serum RANTES levels and radiological progression in rheumatoid arthritis patients treated with methotrexate. *Clin Exp Rheumatol* 1999;17:419-25.
27. Koch AE, Kunkel SL, Harlow LA, Johnson B, Evanoff HL, Haines GK, Burdick MD, Pope RM, Strieter RM. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. *J Clin Invest* 1992;90:772-9.
28. Katrib A, Tak PP, Bertouch JV, Cuello C, McNeil HP, Smeets TJ, Kraan MC, Youssef PP. Expression of chemokines and matrix metalloproteinases in early rheumatoid arthritis. *Rheumatology (Oxford)* 2001;40:988-94.
29. Kageyama Y, Kobayashi H, Kato N, Shimazu M. Etanercept reduces the serum levels of macrophage chemotactic protein-1 in patients with rheumatoid arthritis. *Mod Rheumatol.* 2009;19:372-8.
30. Ellingsen T, Buus A, Stengaard-Pedersen K. Plasma monocyte chemoattractant protein 1 is a marker for joint inflammation in rheumatoid arthritis. *J Rheumatol* 2001;28:41-6.

## Legends for illustrations

Figure 1. Serum concentrations of interleukin-8 (IL-8) in rheumatoid arthritis (RA) patients, assessed by ELISA technique. Patients were treated with 50mg of subcutaneous injection of etanercept per week. Blood samples were obtained at months 0, 3, 6, 9 and 12 prior to injection. Box plots represent median (line), 25th and 75th percentiles (box), and 10th and 90th percentiles (whiskers). Significance of differences between pre-injection IL-8 values at month 0 and following months were expressed as: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Significance of differences between pre-injection values at month 3 and following months were expressed as: # $p < 0.05$ .

Figure 2. Serum concentrations of RANTES (regulated upon activation normal T cell expressed and secreted) in RA patients, determined and presented as described in the legend to Figure 1.

Figure 3. Serum concentrations of monocyte chemoattractant protein-1 (MCP-1) in RA patients, determined and presented as described in the legend to Figure 1.

**Table 1.** Correlations between serum concentrations of chemokines and DAS28 in RA patients studied.

	RANTES	MCP-1	ESR	DAS28
IL-8	0.699**	0.564*	0.703**	0.681**
RANTES		0.551*	0.647**	0.691**
MCP-1			0.681**	0.559*

RA: rheumatoid arthritis, IL-8, interleukin-8; RANTES, regulated upon activation normal T cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1; ESR: erythrocyte sedimentation rate; DAS28: three variables disease activity score that include 28-joint count.

Data expressed as r values (correlation coefficient) according to Spearman rank correlation: \*

$p < 0.05$ , \*\*  $p < 0.01$

DAS28 evaluations were performed, and blood samples were obtained on month 0, prior to first etanercept injection.







