

Received: 2003.04.22
Accepted: 2003.08.20
Published: 2004.02.25

Reduction of soluble adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) and vascular endothelial growth factor levels in serum of rheumatoid arthritis patients following multiple intravenous infusions of infliximab

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Source of support: self financing

Summary

Introduction:

The purpose of this study was to determine the effect of repeated infusions of infliximab, a chimeric anti-tumor necrosis factor (anti-TNF)- α antibody, on the levels of soluble adhesion molecules and vascular endothelial growth factor (VEGF) in patients with active rheumatoid arthritis (RA).

Materials and Methods:

The treatment design consisted of 9 infusions of infliximab (3 mg/kg) at weeks 0, 2, 6, and every 8 weeks thereafter. All patients had been receiving methotrexate (MTX; 7.5–20 mg/week). Serum levels of soluble intercellular adhesion molecule (sICAM)-1, vascular cell adhesion molecule (sVCAM)-1, E-selectin (sE-selectin), and VEGF were measured by ELISA at weeks 0, 2, 6, 14, and 38 prior to infusion, and at week 62.

Results:

A remarkable decrease in serum sICAM-1 ($p < 0.001$), sVCAM-1 ($p < 0.01$), sE-selectin ($p < 0.01$) and VEGF ($p < 0.001$) levels was observed in RA patients after the initial dose of infliximab. The second administration of the drug was followed by an even more significant suppression of serum sICAM-1, sVCAM-1, sE-selectin, and VEGF ($p < 0.001$ in all cases). Further infliximab infusions also significantly reduced serum soluble adhesion molecules and VEGF concentrations, although these were less effective. Infliximab treatment induced a significant decrease in the number of monocytes observed until the end of the study.

Conclusions:

Our study, besides a rapid suppression of disease activity, showed that serum soluble adhesion molecules and VEGF concentrations are down-regulated following anti-TNF- α antibody therapy combined with MTX. Repeated doses of infliximab sustained the reductions in the soluble adhesion molecules and VEGF concentrations, although they were less effective than the first and second infusions of infliximab.

Key words:

sICAM-1 • sVCAM-1 • VEGF • rheumatoid arthritis • infliximab

Abbreviations:

CRP – C-reactive protein, **ESR** – erythrocyte sedimentation rate, **MTX** – methotrexate, **NSAIDs** – nonsteroidal anti-inflammatory drugs, **RA** – rheumatoid arthritis, **sICAM-1** – soluble intercellular adhesion molecule-1, **sVCAM-1** – soluble vascular cell adhesion molecule-1; **TNF- α** – tumor necrosis factor α , **VEGF** – vascular endothelial growth factor

Full-text PDF:

http://www.aite-online.org/pub/aite/vol_52/no_1/4459.pdf

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INTRODUCTION

The accumulation of mononuclear cells, increased angiogenesis, and the proliferation of the synovium-lining layer are the characteristic features of rheumatoid synovitis^{9, 11, 33}. Destruction of the synovial tissues in rheumatoid arthritis (RA) is mediated by cytokines and matrix metalloproteinases. Their production by macrophages and fibroblasts seems to be regulated by lymphocytes^{12, 14, 26}. Mononuclear-cell activation, circulation and migration to inflammatory sites are modulated by adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and E-selectin^{3, 24, 25, 37}. Stimulation of synovial tissue neovascularization by endothelial growth factors increases inflammatory cell migration^{4, 28}. Furthermore, also soluble forms of VCAM-1 (sVCAM-1) and of E-selectin (sE-selectin) were shown to possess angiogenic activity¹⁵. Therefore, cell adhesion molecules and endothelial growth factors are supposed to play an important role in the infiltrating of the rheumatoid synovium with mononuclear cells, leading to the initiation and progression of the disease¹³.

Single administration of the anti-tumor necrosis factor (anti-TNF)- α monoclonal antibody infliximab, besides decreasing RA activity^{7, 18, 21, 23}, was shown to reduce serum concentrations of soluble cell adhesion molecules^{20, 29} and endothelial growth factors³⁰. The present study was undertaken to evaluate the efficacy of repeated infusions of infliximab in decreasing serum soluble adhesion molecule and vascular endothelial growth factor (VEGF) levels in patients with active RA.

MATERIALS AND METHODS

Patients and samples

The open (non-placebo-controlled) study recruited 13 patients who fulfilled the American College of Rheumatology 1987 revised criteria for RA¹. None of the patients had a previous history of tuberculosis or symptoms of infectious diseases in the last 3 months. Chest x-rays done before the first infliximab infusion were normal in all patients. One patient discontinued the study regimen due to tuberculosis, which was diagnosed after the 3rd infliximab infusion. Another developed herpes zoster 4 weeks after the 6th drug infusion. Both patients discontinued infliximab infusions and received appropriate treatment. No other adverse events were observed among the remaining 11 patients, who completed 9 infusions of infliximab.

Due to incomplete sampling, data of the 11 patients were studied (1 man and 10 women). All patients had

active RA, as defined by 6 or more tender joints, 6 or more swollen joints, and two of the following: morning stiffness for more than 45 min, C-reactive protein (CRP) level of more than 20 mg/l, or erythrocyte sedimentation rate (ESR) of more than 28 mm/h. Mean age of the studied patients was 45.2 years (range 21–65 years), and mean $\pm$ SD disease duration was 10.6 ± 6.6 years. Seven patients were at the 3rd, and 4 patients were at the 4th radiological stage of disease according to Steinbrocker's criteria³¹. Nine patients were seropositive and 2 patients were seronegative. All patients had been receiving methotrexate (MTX) (median 12.5 mg/week, range 7.5–20 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 the beginning of the study. Five patients had been using corticosteroids (median 5 mg/day of prednisone, range 5–10 mg/day) in a stable dose for at least 4 weeks before the beginning of the trial. The treatment regimens with MTX, NSAIDs and prednisone were continued during the study period.

Patients received 9 infusions of infliximab, a chimeric anti-TNF- α antibody, at weeks 0, 2, 6, and every 8 weeks thereafter with the same dose (3 mg/kg). Each infusion lasted at least 2 h. We studied blood samples obtained at weeks 0, 2, 6, 14, and 38 prior to infusion, and at week 62 (8 weeks after the last drug administration). Blood samples were clotted for 30 min and then centrifuged for 10 min at 1000 g. Serum aliquots were stored at -80°C until assayed. The study protocol was approved by the local ethics committee and the patients' written consent was obtained.

Clinical and laboratory analysis

The assessment included the number of tender joints (of 68 joints assessed), the number of swollen joints (of 66 assessed), the duration of morning stiffness (in minutes), white blood cell counts, including differential blood counts, platelets counts, ESR, CRP concentration measured by a radial immunodiffusion kit (Nanorid, The Binding Site Ltd., Birmingham, UK) and rheumatoid factor level. Radiological assessment of joint destruction was performed according to Steinbrocker's criteria³¹. Table 1 presents the characteristics of the patient population.

Enzyme-linked immunoassays

The serum levels of soluble ICAM (sICAM)-1, sVCAM-1, and sE-selectin were determined by commercial ELISA kits (Bender MedSystems, Vienna, Austria). Levels of VEGF were measured with the ELISA kit from R&D Systems, Wiesbaden-Nordenstadt, Germany. The analyses were performed according to the manufacturers' instructions. The sensitivities were 3.3 ng/ml for

Table 1. Clinical and laboratory characterization of 11 RA patients treated with infliximab (3 mg/kg) at weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54

	Week 0	Week 2	Week 6	Week 14	Week 38	Week 62
ESR (mm/h)	69.1±21.7 65.0 (52.0–84.8)	41.4±16.5 42.0 (27.3–55.5)	37.1±11.3 36.0 (29.8–45.8)	39.6±25.9 35.0 (19.8–54.5)	37.5±16.1 38.0 (26.8–49.8)	42.1±20.3 42.0 (24.0–55.3)
No. of tender joints (0–68)	27.1±6.5 26.0 (21.5–31.3)	16.9±4.3 16.0 (14.3–20.5)	11.3±5.1 11.0 (7.8–13.5)	12.5±4.1 12.0 (9.3–14.5)	11.8±5.2 11.0 (8.5–15.3)	14.2±5.3 13.0 (9.5–17.3)
No. of swollen joints (0–66)	16.4±4.4 16.0 (13.3–19.8)	11.1±3.7 12.0 (9.0–12.8)	9.2±3.8 9.0 (6.3–12.5)	10.6±3.0 10.0 (8.5–13.3)	10.7±2.9 10.0 (9.0–12.8)	11.2±3.3 11.0 (9.3–12.8)
WBC (x 10 ⁹ /l)	7.6±2.4 6.9 (6.1–10.1)	8.1±2.1 7.5 (6.1–9.3)	7.7±2.4 7.5 (6.4–9.7)	8.2±3.1 7.0 (6.7–9.5)	8.3±3.6 7.4 (5.8–9.8)	7.8±2.9 7.9 (5.7–8.7)
Lymphocytes (x 10 ⁹ /l)	1.9±0.7 1.7 (1.5–2.0)	2.3±0.6 2.2 (2.0–2.5)	2.3±0.9 2.2 (1.8–2.6)	2.1±0.7 2.2 (1.6–2.4)	2.0±0.9 1.9 (1.5–2.4)	1.7±0.7 1.7 (1.2–2.0)
Monocytes (x 10 ⁹ /l)	0.61±0.22 0.54 (0.48–0.72)	0.51±0.24 0.47 (0.41–0.75)	0.42±0.18 0.40 (0.33–0.56)	0.47±0.24 0.43 (0.33–0.60)	0.46±0.16 0.46 (0.41–0.52)	0.40±0.18 0.39 (0.26–0.58)
Platelets (x 10 ⁹ /l)	311±105 336 (216–385)	249±67 241 (212–293)	278±84 254 (234–330)	293±98 277 (230–310)	295±84 325 (253–338)	271±68 268 (246–314)

RA – rheumatoid arthritis, SD – standard deviation, p25–p75 – 25th–75th percentile, ESR – erythrocyte sedimentation rate, WBC – white blood count. Clinical evaluations were performed and blood samples were obtained at weeks 0, 2, 6, 14, and 38 prior to infusion, and at week 62. Data are presented as means ±SD, median (p25–p75). Significances of the differences between weeks were described in the Results section.

sICAM-1, 0.9 ng/ml for sVCAM-1, 0.5 ng/ml for sE-selectin, and 9.0 pg/ml for VEGF.

Statistical analysis

The normally distributed data were compared by the paired Student's *t*-test. The Wilcoxon signed rank test was used to evaluate the differences between such non-normally distributed data as the number of tender joints, number of swollen joints, and ESR, sICAM-1, and sVCAM-1 values. Data were correlated by the Spearman rank order test. *p* values lower than 0.05 were considered statistically significant.

RESULTS

Clinical and laboratory findings

In 11 RA patients studied we observed a decrease of disease activity. ESR values significantly decreased after the first infusion of infliximab as evaluated at week 2, prior to the second infliximab infusion (*p*<0.001), and remained stable during the rest of the study (Table 1). Also the number of tender joints and the number of swollen joints were reduced after initial infliximab

administration (*p*<0.001 for both comparisons). However, the number of tender joints and the number of swollen joints were especially reduced after the second infliximab infusion (assessment done at week 6 prior to the third drug administration) (*p*<0.001 for both comparisons), even when compared with the effect of the first infusion evaluated at week 2 prior to infliximab infusion (*p*<0.05; Table 1).

White blood counts showed a tendency to increase following the infliximab infusions, but these changes were not significant. The number of lymphocytes markedly increased after the first infliximab infusion (*p*<0.05). However, this effect did not last to the end of the study. Compared with the baseline, infliximab treatment induced a significant decrease in the number of monocytes after the first (*p*<0.05) and subsequent infusions (*p*<0.01). The number of platelets decreased significantly only after the initial drug infusion (*p*<0.05; Table 1).

Serum soluble cell adhesion molecule profiles

As shown in Fig. 1 and Table 2, the concentrations of sICAM-1 in the serum of the RA patients studied decreased significantly after the first infliximab infusion.

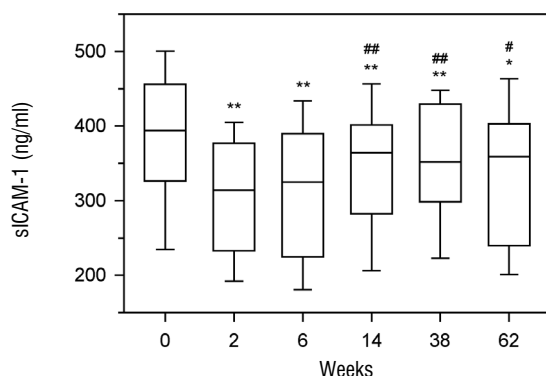


Figure 1. Serum concentrations of soluble intercellular adhesion molecule (sICAM)-1 in 11 RA patients studied, assessed by the ELISA technique. Patients were treated with infliximab (3 mg/kg) on weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54. Blood samples were obtained at weeks 0, 2, 6, 14, and 38 prior to infusion, and at week 62. Box plots represent median (line), 25th and 75th percentiles (box), and 10th and 90th percentiles (whiskers). Significance of differences between pre-infusion sICAM-1 values at week 0 and following weeks were expressed as: * $p < 0.01$, ** $p < 0.001$. Significance of differences between pre-infusion sICAM-1 values at week 2 and following weeks were expressed as: # $p < 0.05$, ## $p < 0.01$.

Repeated infliximab administration also markedly reduced sICAM-1 levels, although they were less effective than the first two infusions. The concentration of sVCAM-1 was also diminished following the initial drug infusion (Fig. 2 and Table 2). This reduction persisted throughout the study, although it was less significant. Serum sE-selectin levels also decreased after infliximab administration (Fig. 3 and Table 2). The concentration of sE-selectin was especially suppressed after the second drug infusion, as evaluated on week 6 prior to the third infliximab administration. Repeated infliximab infusion also significantly diminished sE-selectin levels, although with less effect than the first two drug administrations.

Serum VEGF concentrations

Serum VEGF decreased after the initial infliximab infusion (Fig. 4 and Table 2). VEGF was found at the lowest levels after the second drug administration, as assessed on week 6 prior to infusion. VEGF reduction persisted throughout the study, though it was less significant.

Correlations between serum levels of soluble adhesion molecule and/or VEGF and clinical findings

Before the first infliximab infusion, serum concentrations of sICAM-1, sVCAM-1, sE-selectin and VEGF correlated with markers of disease activity, such as ESR levels, the number of tender joints and the number of swollen joints (Table 3). After each drug administration, such correlations were also revealed, but were less significant (data not shown). No correlations between

patient age, disease duration or rheumatoid factor and any soluble adhesion molecule or VEGF levels were noticed.

DISCUSSION

Endothelium and adhesion molecules are involved in the pathogenesis of RA. They are engaged in the process of leukocyte attachment and migration out of the circulation into the rheumatoid synovium^{3, 24, 25, 37}. Increased ICAM-1, VCAM-1 or E-selectin (endothelial leukocyte adhesion molecule-1) expressions in rheumatoid synovium, even in an early stage of the disease, emphasize the role of adhesion molecules in RA^{25, 32, 35, 37}. Although the pathogenetic role of the soluble forms of cell adhesion molecules (sCAMs) remains incompletely understood, several studies have shown elevated levels of sCAMs in the serum of RA patients^{13, 16, 19, 27}. It has been proposed that sCAMs might regulate the contact between leukocytes and the cell-surface forms of these molecules. Furthermore, sCAM binding may modulate leukocyte activation prior to their interaction with the endothelial cells. Some studies indicate that elevated sCAM levels could simply reflect endothelial activation^{3, 5, 24, 25}.

Single doses of the anti-TNF- α monoclonal antibody infliximab not only cause a suppression in disease activity^{7, 18, 21, 23}, but also diminish the concentrations of sICAM-1 in the serum of RA patients^{20, 29}. In our study we also observed that sICAM-1 levels in the serum of the patients studied strongly decreased after the first infliximab infusion. Furthermore, we demonstrated that repeated infliximab administration also significantly reduced sICAM-1 levels, although with less effect than the first two infusions.

Recently it was revealed that another sCAM, sVCAM-1, also mediates monocyte chemotaxis in RA¹¹. Moreover, sVCAM-1 demonstrated angiogenic activity, increasing mononuclear cell ingress into inflamed RA synovial tissue¹⁵. The concentration of sVCAM-1 was also significantly reduced following initial drug infusion. This reduction persisted throughout the study; however, after repeated infliximab administrations, sVCAM-1 suppression was less marked. Others did not observe significant changes in sVCAM-1 levels in serum after a single infliximab infusion²⁹.

The soluble form of E-selectin also seems to mediate chemotaxis of endothelial cells¹⁵. Moreover, sE-selectin demonstrates angiogenic activity and may enhance leukocyte delivery into rheumatoid synovium^{15, 36}. E-selectin, present in very early stages of the rheumatoid process, may be important in initiating neoangiogenesis in RA⁸. In the present study, serum sE-selectin levels

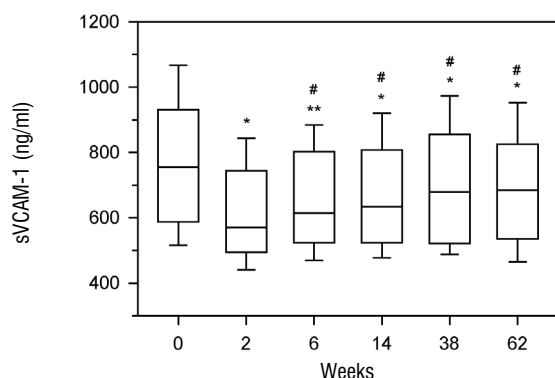


Figure 2. Serum concentrations of soluble vascular cell adhesion molecule (sVCAM)-1, determined and presented as described in the legend to Fig. 1.

also decreased after infliximab administration. Others also revealed sE-selectin suppression following anti-TNF- α monoclonal antibody treatment with single doses of infliximab²⁹. Furthermore, we showed that the concentration of sE-selectin decreased especially after the second drug administration, as evaluated at week 6 prior to the third infusion. Repeated infliximab infusions also significantly reduced sE-selectin levels, although they were less effective.

One of the earliest features observed in rheumatoid synovitis is neovascularization, which promotes the transport of inflammatory cells and mediators into the synovium^{4, 9}. Recently it was demonstrated that anti-TNF- α treatment reduces the formation of new vessels in RA synovium²². It was suggested that suppression of angiogenesis might be a result of VEGF down-regulation after infliximab treatment³⁰. VEGF is a potent

mediator of endothelial proliferation, angiogenesis, and capillary hyperpermeability, the production of which is regulated by TNF- α ^{10, 28}. Numerous reports have revealed raised VEGF levels in serum of RA patients^{10, 17, 30}. In our study, serum VEGF concentration was also suppressed after the initial infliximab infusion. However, the lowest VEGF level was noticed after the second drug administration, as assessed at week 6 prior to infusion. Further drug administrations were less effective in reducing VEGF serum concentration. Taken together, repeated infliximab infusions caused and sustained the suppression of circulating adhesion molecules and of VEGF. Therefore, infliximab, through TNF- α neutralization, may suppress endothelium activation and inflammatory cell migration into rheumatoid synovium.

Numerous investigators have shown significant associations between serum concentrations of sICAM-1, sVCAM-1, or sE-selectin and ESR^{3, 6, 16, 19} or tender and swollen joint count⁶. However, others did not find the correlations between the serum levels of sCAMs and clinical markers of disease activity²⁷. Associations were also demonstrated between VEGF and ESR, and the number of tender or swollen joints¹⁷. In the present study, we also revealed that before the initial infliximab infusion, serum concentrations of sICAM-1, sVCAM-1, sE-selectin and VEGF correlated with markers of disease activity such as ESR levels or the number of swollen joints. After further drug administrations, such correlations were also found, but were less significant. Therefore, soluble adhesion molecules and VEGF might be useful markers of RA activity even during anti-TNF- α monoclonal antibody treatment with multiple infliximab infusions.

Table 2. Serum concentrations of soluble adhesion molecules and VEGF in 11 RA patients treated with infliximab (3 mg/kg) at weeks 0, 2, 6, 14, 22, 30, 38, 46 and 54

	Week 0	Week 2	Week 6	Week 14	Week 38	Week 62
sICAM-1 (ng/ml)	382.3 $\pm$ 95.8 394.0 (326.3–455.8)	302.5 $\pm$ 83.6 314.0 (232.8–376.8)	312.1 $\pm$ 96.8 325.0 (224.5–389.5)	343.9 $\pm$ 89.0 364.0 (282.5–401.5)	348.6 $\pm$ 84.1 352.0 (298.5–429.5)	336.9 $\pm$ 99.8 359.0 (240.0–402.8)
sVCAM-1 (ng/ml)	768.3 $\pm$ 213.5 755 (587.0–931.0)	621.4 $\pm$ 159.2 570.0 (494.3–744.5)	656.8 $\pm$ 162.5 615.0 (523.8–802.3)	671.9 $\pm$ 172.0 634.0 (523.8–807.5)	712.5 $\pm$ 197.4 679.0 (521.8–854.8)	698.9 $\pm$ 185.8 685.0 (536.0–825.3)
sE-selectin (ng/ml)	69.7 $\pm$ 19.3 68.1 (54.4–88.0)	58.1 $\pm$ 13.8 58.4 (47.3–64.0)	57.3 $\pm$ 13.6 53.7 (49.0–65.4)	57.8 $\pm$ 14.6 55.9 (46.7–64.0)	60.9 $\pm$ 15.4 56.5 (49.3–68.9)	63.6 $\pm$ 16.3 61.5 (51.3–72.4)
VEGF (pg/ml)	513.2 $\pm$ 182.8 524.0 (353.5–619.0)	383.4 $\pm$ 148.0 365.0 (268.8–504.8)	359.6 $\pm$ 131.9 326.0 (256.8–491.5)	384.5 $\pm$ 129.8 371.0 (305.3–486.8)	419.6 $\pm$ 133.6 359.0 (318.3–550.5)	441.3 $\pm$ 132.7 435.0 (333.8–509.5)

RA – rheumatoid arthritis, SD – standard deviation, p25–p75 – 25th–75th percentile, sICAM-1 – soluble intercellular adhesion molecule 1, sVCAM-1 – soluble vascular cell adhesion molecule 1, sE-selectin – soluble E-selectin, VEGF – vascular endothelial growth factor.

Serum samples were obtained at weeks 0, 2, 6, 14, and 38 prior to infusion, and at week 62. Data are presented as means $\pm$ SD, median (p25–p75). Significances of the differences between weeks are shown on Fig. 1–4.

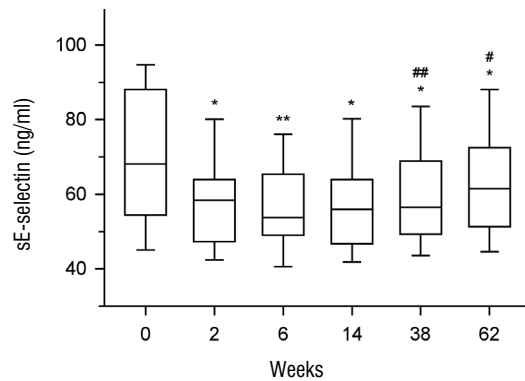


Figure 3. Serum concentrations of soluble E-selectin (sE-selectin), determined and presented as described in the legend to Fig. 1.

There was no association of soluble adhesion molecule levels with leukocyte count (data not shown). Total white blood cell counts showed a tendency to increase after infliximab infusions, but these changes were not significant. Only the number of lymphocytes increased remarkably after infliximab infusion. However, this effect was transient and did not last to the end of the study. It was suggested that the observed increase in the number of circulating lymphocytes in RA patients might be the effect of a reduction in the trafficking of lymphocytes into the rheumatoid synovium following anti-TNF- α treatment^{20, 29, 34}. Neutralization of TNF- α by infliximab seems to suppress the cytokine cascade responsible for endothelium activation. Deactivation of the endothelium limits interactions between adhesion molecules and leukocytes. Therefore, migration of inflammatory cells into the rheumatoid synovium might be reduced^{22, 29}. The observed decrease of the monocytes might be caused by the antibody- and complement-dependent cytotoxicity induced by the interaction between infliximab and TNF- α on the monocyte membrane. This can contribute to the infections during therapies with TNF- α blockers^{2, 29}.

No associations between the sex, age, and disease duration of the patients and the serum concentrations of

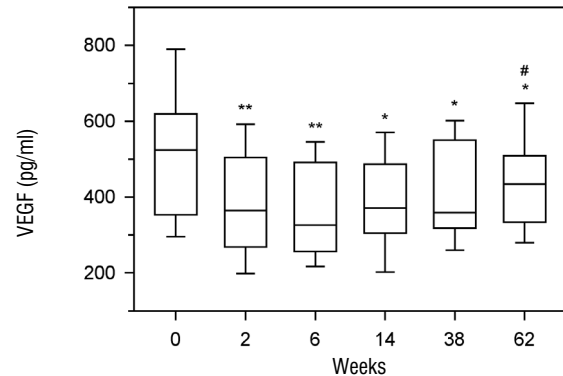


Figure 4. Serum concentrations of vascular endothelial growth factor (VEGF), determined and presented as described in the legend to Fig. 1.

sICAM-1, sVCAM-1, sE-selectin or VEGF were demonstrated (data not shown). Others also did not observe such correlations^{3, 19}.

In our study, besides a rapid reduction in disease activity, we demonstrated a significant decrease in serum sICAM-1, sVCAM-1, sE-selectin and VEGF levels in RA patients after the initial dose of infliximab. Moreover, we observed that repeated infliximab administration prolonged the suppression of serum soluble adhesion molecule and VEGF concentrations, although with less effect than the first two infusions. Down-regulated soluble adhesion molecule and VEGF levels in serum following TNF- α neutralization with infliximab may reflect a suppressed activation of the endothelium, resulting in a decreased migration of leukocytes into the rheumatoid synovium. Furthermore, we found that serum concentrations of sICAM-1, sVCAM-1, sE-selectin and VEGF correlated with markers of disease activity such as the number of swollen joints and ESR levels prior to the first infliximab infusion and subsequent drug administrations. Therefore, these molecules might be useful as markers of disease activity in patients undergoing anti-TNF- α monoclonal antibody therapy.

Table 3. Correlations between serum concentrations of soluble adhesion molecules and/or VEGF and clinical parameters in 11 RA patients

	sVCAM-1	sE-selectin	VEGF	ESR	No. of tender joints	No. of swollen joints
sICAM-1	0.673*	0.718*	0.755**	0.811***	0.502	0.712*
sVCAM-1		0.273	0.645*	0.774**	0.653*	0.685*
sE-selectin			0.482	0.633*	0.306	0.616*
VEGF				0.724**	0.301	0.703*
ESR					0.618*	0.803**
No. of tender joints						0.789**

RA – rheumatoid arthritis, sICAM-1 – soluble intercellular adhesion molecule-1, sVCAM-1 – soluble vascular cell adhesion molecule-1, sE-selectin – soluble E-selectin, VEGF – vascular endothelial growth factor, ESR – erythrocyte sedimentation rate.

Data expressed as *r* values (correlation coefficient) according to the Spearman rank correlation: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Clinical evaluations were performed and blood samples were obtained at week 0, prior to the first infliximab infusion.

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