Longitudinal study of rheumatoid arthritis patients discloses sustained elevated serum levels of soluble CD106 (V-CAM)

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Abstract

Objective

To appreciate the evolution of serum angiogenic and/or adhesion molecules levels during a long term follow-up of rheumatoid arthritis (RA) patients.

Methods

Serum levels of 5 soluble adhesion/angiogenesis glycoproteins (VEGF, CD31, CD54, CD62E, CD106) were measured in Elisa in samples collected over 6 years in a cohort of 43 RA patients with monitored clinical parameters of disease activity and severity.

Results

RA patients had significantly higher levels ($p < 0.0001$) of sCD106 (VCAM-1) than control subjects. Conversely, the levels of soluble VEGF, CD31, CD54 and CD62E were normal or lower than normal. No statistically significant time effect was noted. No effect either was noted as related to the therapeutic agents taken by the patients.

Conclusion

The sustained elevated serum levels of sCD106 observed here imply that this molecule might be related to the chronicity and progression of RA.

Key words

Rheumatoid arthritis, adhesion molecules, angiogenesis, CD106, CD31.

This work was supported by a CIRC grant from CRC-CHU Nancy 1996 and by a PHRC program from the French Ministère de la Santé 1995-97.

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Received on July 26, 2000; accepted in revised on December 6, 2000.

Introduction

The articular synovium in rheumatoid arthritis (RA) is characterised by neoangiogenesis phenomena allowing the colonisation of this tissue by proinflammatory immunocompetent cells, eventually leading to synovial proliferation and joint destruction (1). Neovascularisation is a physiological process in embryogenesis and wound repair, and may be involved in pathological conditions. It depends both on locally produced and soluble factors (2). Soluble proangiogenic growth factors such as the vascular endothelial growth factor (VEGF) have been described (3). Cell adhesion molecules, expressed as transmembrane glycoproteins on endothelial cells and/or migrating leukocytes, are involved in cell recruitment. In soluble forms, resulting from alternative splicing or shedding, they can also act as angiogenesis mediators, as has been shown in a few experimental models (4).

In RA, the expression of angiogenic mediators and adhesion molecules in the synovial tissue appears to be crucial for leukocyte extravasation (5,6). Such factors can also be found in soluble forms in the synovial fluid or serum (7). Although Patel and Haynes (8) pointed to the need for more studies into the potential value of soluble adhesion molecules as surrogate markers for autoimmune disease, only a few studies so far have tackled this issue. Indeed, the limited data available are controversial regarding the correlation of soluble adhesion molecules levels with indicators of disease activity (7). Moreover, most studies have been performed as single time-point assays, with cross-sectional comparisons of RA patients to controls or patients with other rheumatologic diseases. Since RA is a chronic and progressive disorder (1), it would appear interesting to investigate whether or not the circulating levels of angiogenic/ adhesion soluble factors vary over time during disease progression.

Here we report data of such a follow-up study, where angiogenic and adhesion soluble molecules were assayed repeatedly over 6 years in serum samples from a cohort of 43 RA patients.

Patients and methods

Patients

43 subjects (30 women, 13 men) enrolled in the prospective EURIDISS project (9) were included in this study. All patients met the American College of Rheumatology 1987 revised criteria for RA (10) and had suffered from RA for less than 5 years at enrolment. Following the EURIDISS protocol, all subjects were medically examined regularly on a yearly basis over 6 years (except on year 5), and blood was drawn on these occasions. Clinical and biological criteria to assess disease activity and severity were collected as well: the Health assessment questionnaire (HAQ, 11), nodules, extra-articular localisations, Ritchie's index (12), Steinbrocker's stages, Larsen score (13), Karnofsky's index, overall estimation of health, erythrocyte sedimentation rate, and rheumatoid factor.

Serum samples were stored at -30°C until they were assayed; all were assayed at the same time.

ELISA assays

The angiogenic or adhesion glycoproteins assayed were soluble VEGF, sCD31, sCD54, sCD62E, and sCD106. The methods used were sandwich ELISA tests performed according to the distributor's specifications (R&D, Abingdon, UK). Briefly, thawed serum samples were incubated in microtitration plates coated with a monoclonal antibody specific for the molecule tested. After incubation and a series of washes, a second antibody, specific for a different epitope of the same molecule and conjugated to horseradish peroxidase, was added to the wells. The optical density, proportional to the amount of soluble factor assayed, was developed by addition of the enzyme's substrate and a chromogen (tetramethylbenzidine), and measured in spectrophotometry at 450 nm.

Data were expressed according to calibration curves set up for each test on the same microtitration plate as the samples tested.

Reference values for this laboratory were established by applying the same assays to a series of serum samples from a control group of 35 healthy sub-

jects (14). All had been submitted to clinical examination, which disclosed no inflammatory rheumatologic disease nor other pathologies.

Statistical analysis

Clinical characteristics and biological variables were described using mean and standard deviation for continuous variables and percentages for categorical data. The distribution of biological variables satisfied normality assumption tested by Kurtosis. Patients' levels were compared to the reference values for healthy subjects for each of the molecules assayed using Student's t-tests. An analysis of the relationship between clinical characteristics and biological data was conducted using analysis of variance and the Pearson correlation coefficient where appropriate. This analysis was repeated at each assessment time. The variation of biological marker levels over time was assessed using ANOVA with repeated measures testing for any time effect and to handle missing data. Visual examination of graphic data displays, plotted for each angiogenic/adhesion molecule, was used to identifypotential subgroupsof patients. Where appropriate, further testing of time effects and of the relationships with clinical characteristics was conducted between subgroups. P values <0.05 were considered statistically significant.

Results

Analysis of the clinical criteria of disease activity and severity showed that the Ritchie's index increased over time, as illustrated in Figure 1, while the HAQ index remained relatively stable.

Figure 2 reports the mean levels of the glycoproteins assayed in RA samples collected at the time of the patients' inclusion in the EURIDISS project, compared with reference levels for each molecule. Significantly higher ($p < 0.00001$) levels of sCD106 were observed in the patient samples, while sCD62E ($p = 0.00002$) and VEGF ($p = 0.01$) levels were significantly lower than in controls. sCD31 and sCD54 levels did not differ from normal.

All mean levels appeared stable over time, as shown in Table I, without any

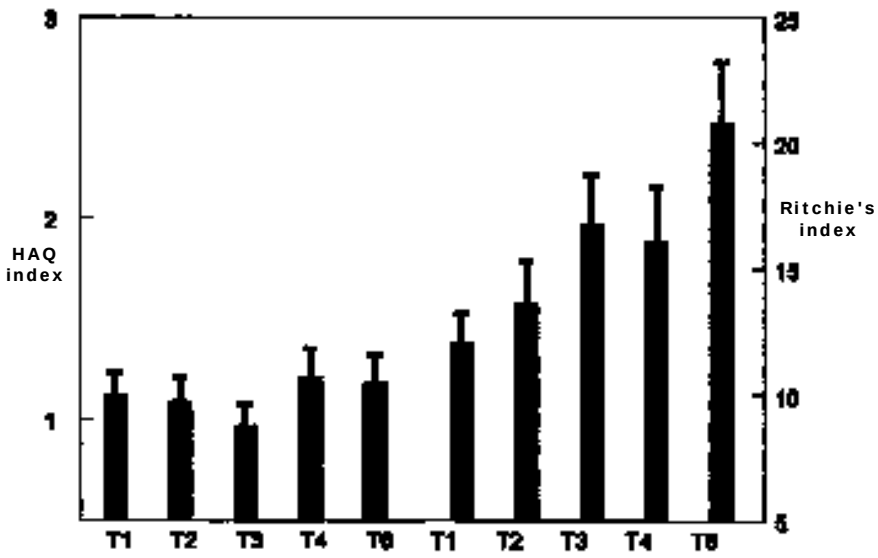

Fig. 1. Evolution of the mean (+ S.E.M.) HAQ (left ordinate, black bars) and Ritchie's (right ordinate, striped bars) indexes over time in the cohort of 43 RA patients studied.

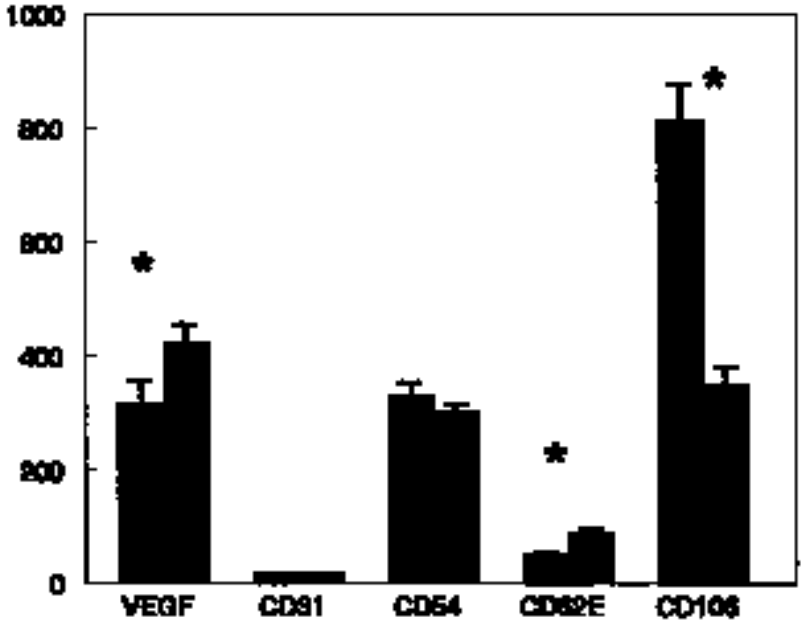

Fig. 2. Mean (+ S.E.M.) serum levels, at inclusion, of VEGF (pg/L), CD31 (ng/L), CD54 (ng/L), CD62E (ng/L) and CD106 (ng/L) in RA patients (black bars) compared to the laboratory's normal levels as assessed in a cohort of 35 healthy subjects (striped bars). Asterisks indicate statistically significant differences.

Table I. Mean ± S.D. values of soluble adhesion or angiogenic molecules in serum samples at the time points tested i.e. every year except year 5. ANOVA with repeated measures disclosed no statistically significant variation over time.

	Year 1	Year 2	Year 3	Year 4	Year 6
VEGF (pg/L)	315.2 ± 230.5	324.7 ± 223.8	312.0 ± 186.8	387.9 ± 264.0	355.9 ± 205.7
CD31 (ng/L)	18.7 ± 6.9	19.3 ± 5.9	19.0 ± 6.6	18.7 ± 7.7	19.5 ± 6.6
CD54 (ng/L)	330.6 ± 120.9	346.5 ± 127.7	332.5 ± 97.0	308.1 ± 89.1	322.2 ± 71.2
CD62E (ng/L)	52.9 ± 23.0	55.2 ± 24.8	60.5 ± 31.1	62.4 ± 28.0	54.1 ± 20.3
CD106 (ng/L)	814.2 ± 345.1	850.6 ± 380.0	845.0 ± 353.3	944.9 ± 573.6	920.9 ± 487.5

Table II. Correlation coefficients (r) and p values obtained when comparing soluble VEGF levels and clinical factors of disease activity or severity at the time points tested, i.e. every year except year 5.

	Baseline value	Year 1	Year 2	Year 3	Year 4	Year 6
HAQ	1.12 ± 0.74	r = 0.44 (0.003)	r = 0.18 (0.20)	r = 0.29 (0.05)	r = 0.68 (<0.001)	r = 0.46 (0.02)
Ritchie's index	12.0 ± 7.9	r = 0.16 (0.30)	r =-0.01 (0.9)	r = -0.15 (0.3)	r = 0.47 (0.01)	r = 0.31 (0.03)
Larsen score	18.6 ± 20.7	r = 0.37 (0.03)	NA	r = 0.61 (0.002)	NA	r = 0.12 (0.64)
Karnofsky index	76.4 ± 11.9	r =-0.38 (0.01)	r = -0.13 (0.39)	r = -0.33 (0.02)	r =-0.58 (0.002)	NA
ESR	26.3 ± 23.4	r = 0.57 (< 0.001)	r = 0.36 (0.02)	r = 0.48 (0.01)	NA	r = 0.36 (0.02)

NA : not available.

Table III. Evolution of serum sCD62 levels and the HAQ index over time. Anova with repeated measures. P=0.009.

Time point	sCD62	HAQ
Year 1	46.86	1.12
Year 2	56.64	1.15
Year 3	63.64	1.06
Year 4	62.46	1.29
Year 6	55.00	1.34

statistically significant time effect. High mean levels of sCD106 therefore were noted at each time point tested. This was confirmed by visual examination of graphical data display for each patient, confirming that the stable mean levels noted were not related to compensated variations between patients.

Investigating for relationships between clinical and biological parameters, we observed a significant link of sVEGF with higher age (r = 0.40; p = 0.01) and a correlation between sVEGF levels, disease activity and severity as shown in Table II. Interestingly, correlations between sVEGF levels and the Karnofsky index were negative. We also noted significantly higher (p = 0.003) levels of sVEGF in older patients and significantly higher (p = 0.009) sCD62E levels in patients with increasing disability over time (Table III).

No relationships were observed between the parameters measured and therapy, using ANOVA to test for the effect of steroids, AINS or DMARD, except between DMARD and CD31 levels at year 1 (p = 0.009).

Close examination of sequential assays disclosed a peculiar distribution pattern of sCD106 levels. At inclusion, the population tested appeared to be partitioned into two groups, with sCD106 levels higher (n = 13) or lower (n = 30) than 1000 ng/mL. Interestingly, this partition persisted significantly over time, suggesting that RA patients can belong to one of these two groups of subjects with very high ($sCD106^{hi/hi}$) or high ($sCD106^{hi}$) serum levels of sCD106, both groups displaying significantly increased levels (p < 0.001), respectively about 3 times and twice normal levels (Fig. 3).

Comparing $sCD106^{hi/hi}$ and $sCD106^{hi}$ patients for all the criteria tested, at all time points, we found no correlation with known markers of disease activity or severity. However, significant differences (p = 0.03) were noted over time between subgroups for the mean sCD-31 level which, interestingly, was higher in the sCD106hi subgroup. No significant relationship between drug intake of either slow-acting drugs, corticosteriods or NSAIDS, and CD106 levels, and no therapeutic difference between patients of the $CD106^{hi}$ and $CD106^{hi/hi}$ groups, as assessed by Fisher's exact test, were seen at any measurement time.

Discussion

This study reports on the longitudinal assay, over a 6-year period, of a series of soluble molecules potentially reflecting angiogenesis and/or endothelial cell activation, in serum samples from RA patients. All levels appeared remarkably stable over time, sCD106 remaining significantly higher in patients than in controls over the whole period tested. Moreover, two subgroups of RA patients were identified based on their sCD106 serum levels, sCD31 levels also being steadily and significantly elevated in $sCD106^{hi}$ patients.

This study was undertaken on the basis of previously reported single time-point assessments investigating one or a few of these molecules. One of the objectives was to assess whether serum samples could reflect events occurring

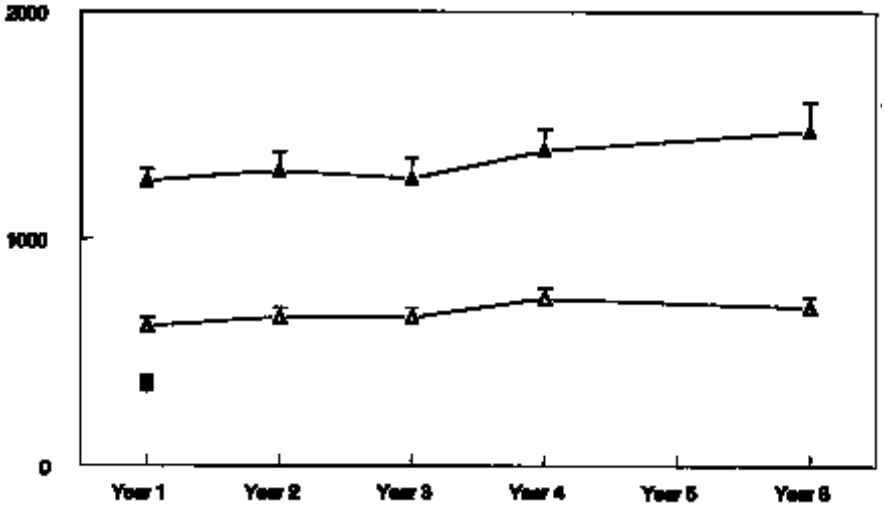

Fig. 1. Evolution of the mean (+ S.E.M.) HAQ (left ordinate, black bars) and Ritchie's (right ordinate, white bars) indexes over time in the cohort of 43 RA patients studied.

mostly in the synovium, and provide some information about the joints' inflammatory activity in terms of cell recruitment and angiogenesis. Although the high levels of sCD106 could indeed reflect increased production of this molecule in RA, the highly stable levels we observed are more in favour of a role for these molecules in the chronicity of the disease. Moreover, no correlation could be found between the parameters measured and the patients'therapy. This would also suggest a remote relationship between serum samples and synovial events.

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and an angiogenesis inducer released by a variety of tumour cells and expressed in human tissues *in situ* (3). In RA, significant amounts of VEGF have been reported in the synovial fluid (15,16) and a role of VEGF in the angiogenesis concomitant to synovial growth and inflammation has been suggested in several studies (17, 18). We observed significantly lower levels of VEGF in the serum samples we tested than in normal controls. However, VEGF was the only parameter which showed any correlation with clinical criteria, indicating that although low, the serum VEGF levels observed could nevertheless be related to variations in synovial activity. The negative correlation noted with Karnofsky's index indeed shows that the lower the VEGF levels, the better the patient feels.

CD31/PECAM is expressed at high levels on activated endothelial cells, and appears to be upregulated on blood vessels, lining cells and macrophages of the synovial membrane (19). Moreover, CD31 has been proposed to play a role in angiogenesis based on its expression in newly formed blood vessels (20). Soluble CD31 levels do not seem to have been investigated in the peripheral blood of RA patients. Here we observed similar levels of sCD31 in the whole group of RA patients compared to controls. However, there appeared to be some degree of co-regulation between sCD31 and sCD106 as discussed below.

CD54/ICAM-1 is involved in leukocyte transmigration, and its expression is upregulated in such inflammatory tissues as the rheumatoid synovium (21). Soluble CD54 levels have been determined in several series of RA patients (22, 23). The levels reported by Littler *et al.* (23) and Cush *et al.* (21) are comparable to those we observed. However, their series of controls were much smaller (7 and 10 individuals respectively) which might account for their different interpretation of patients/reference data. In the series of Mason et al. (22), the methodology was different, but the large series of controls showed highly dispersed values, and only 5 RA patients with very high sCD54 levels accounted for mean increased levels in their patients' group. Our data provide the complementary information of highly stable levels in our 43 patients (215 assays).

CD62E/E-selectin and CD106/VCAM-1 have been reported in the literature as angiogenic factors in experimental models (24). They appear to be potent inducers of endothelial cell chemotaxis *in vitro* and of angiogenesis in a model of rat cornea vascularisation (24). Soluble levels of CD62E were assessed by Littler *et al*. (23) in serum samples from RA patients, and found not to differ from those of 10 controls. We observed less dispersion of the values, and significantly lower stable levels of CD62E in our patients sample compared to the reference level determined from 35 healthy controls. Interestingly, CD62E expression is mostly reported as a transient event on activated endothelia (25). CD62E expression has been reported on endothelial cells in synovial tissue from RA patients (5). The low serum levels we noted could be related to the decreased shedding of CD62E in the inflamed tissue of active patients.

Soluble CD106 levels have been previously reported increased in both RA and lupus erythematosus (23, 26). This molecule is strongly expressed on several cell types in the articular synovium of RA patients (5, 27) and could be involved both in angiogenesis and synoviocyte differentiation. It could also play a role in modulating the lymphocyte dysregulation of RA patients, as it was shown to bind T-cells, which carry the CD106 ligand VLA4, and induce their anergy (28).

A novel finding reported here is the description of two subsets of RA patients differing in terms of serum levels of sCD106, yet presenting similar clinical criteria. The smaller series reported in the literature did not allow others to identify these two patterns, although 2 of the 22 patients tested by Littler *et al.* (23), with the same technique as that used by us, could belong to the group of $CD106^{hi/hi}$ RA patients. Six years follow-up were not enough to tell us in which way CD $106^{hi/hi}$ and $CD106^{hi}$ patients might differ but it would certainly be interesting to confirm this observation in other cohorts and to continue investigating what could be a new independent parameter of RA. The relationship between sCD106 and sCD31 levels, the latter being significantly raised in $CD106^{hi}$ patients, suggests that these two soluble molecules could be concomitantly involved in vascular or immunological regulation mechanisms in slowly progressing RA. Indeed, CD31 has also been reported to be able to interact with T-cells and downregulate their activity (29). Soluble CD106 alone or a concerted action of sCD106 and sCD31 may therefore be suspected as tentative regulatory mechanisms of disease progression in this chronic disorder.

Acknowledgements

The authors are grateful to Sandrine Engel for her help with the statistical analysis and to Renaud Valette and Céline Hagen for their invaluable technical assistance.

References

1. HARRIS ED JR: Rheumatoid arthritis: Pathophysiology and implications for therapy. *N Engl J Med* 1990; 332: 1277-87.
2. FOLKMAN J, KLAGSBURN M: Angiogenic factors. *Science* 1987; 235: 442-7.
3. FERRARA N, HOUCK K, JAKEMAN L, LEUNG DW: Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocrine Rev* 1992; 13: 18-32.
4. POLVERINI PJ: Cellular adhesion molecules. Newly identified mediators of angiogenesis. *Am J Pathol* 1996; 148: 1023-9.
5. SZEKANECZ Z, SZEGEDI G, KOCH AE: Cellular adhesion molecules in rheumatoid

arthritis: Regulation by cytokines and possible clinical importance. *J Invest Med* 1996; 44: 124-35.

6. SZEKANECZ Z, SZEGEDI G, KOCH AE: Angiogenesis in rheumatoid arthritis: Pathogenic and clinical significance. *J Invest Med* 1998; 46: 27-41.
7. MOJCIK CF, SHEVACH EM: Adhesion molecules. A rheumatologic perspective. *Arthritis Rheum* 1997; 40: 991-1004.
8. PATEL DD, HAYNES BF: New surrogate markers for autoimmune disease activity. *J Clin Invest* 1998; 106: 1075-6.
9. GUILLEMIN F, SUURMEIJER T, KROLL B *et al.*: Functional disability in early rheumatoid arthritis: Description and risk factors. *J Rheumatol* 1994; 21: 1051-5.
10. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.*: The American Rheumatism Association 1987 criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
11. RAMEY DR, RAYNAULD JP, FRIES JF: The Health Assessment Questionnaire 1992: Status and review. *Arthritis Care Res* 1992; 5: 119-29.
12. RITCHIE DM, BOYLE JA, MCINNES JM *et al.*: Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968; 37: 393-406.
13. LARSEN A, DALE K, ECK M: Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. *Acta Radiologica* 1977; 18: 481-91.
14. KOLOPP-SARDA MN, BÉNÉ MC, MASSIN N, WILD P, FAURE GC: Altered partition of T cell subsets in the peripheral blood of healthy workers exposed to flour dust. *Am J Ind Med* 1995; 28: 497-504.
15. KOCH AE, HARLOW LA, HAINES GK *et al.*: Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. *J Immunol* 1994; 152:4149-56.
16. FAVA RA, OLSEN NJ, SPENCER-GREEN G *et al.*: Vascular permeability factor/endothelial growth factor (VPF/VEGF): Accumulation and expression in human synovial fluids and rheumatoid synovial tissue. *J Exp Med* 1994; 180: 341-6.
17. NAGASHIMA M, YOSHINO S, ISHIWATA T, ASANO G: Role of vascular endothelial growth factor in angiogenesis of rheumatoid arthritis. *J Rheumatol* 1995; 22: 1624-30.
18. PALEOLOG EM: Angiogenesis: A critical process in the pathogenesis of RA :A role for VEGF ? *Br J Rheumatol* 1996;35:917-9.
19. SZEKANECZ Z,HAINES GK,HARLOW LA *et al.*: Increased synovial expression of the adhesion molecules CD66a, CD66b and CD31 in rheumatoid and osteoarhtritis. *Clin Immunol Immunopathol* 1995; 76: 180-6.
20. DELISSER HM, BALDWIN HS, ALBELDA SM: Platelet endothelial cell adhesion molecule 1 (PECAM-1/CD31):A multifunctional vascular cell adhesion molecule. *Trends Car - diovasc Med* 1997; 7: 203-10.
21. CUSH JJ, ROTHLEIN R, LINDSLEY HB, MAINOLFI EA, LIPSKY PE: Increased levels of circulating intercellular adhesion molecule 1 in the sera of patients with rheumatoid arthritis. *Arthritis Rheum* 1993; 36: 1098-102.
22. MASON JC, KAPAHI P, HASKARD DO: Detection of increased levels of circulating intercellular adhesion molecule in some patients with rheumatoid arthritis but not in patients with systemic lupus erythematosus. Lack of correlation with levels of circulating vascular cell adhesion molecule 1. *Arthritis Rheum* 1993; 36: 519-27.
23. LITTLER AJ, BUCKLEY CD, WORDSWORTH P, COLLINS I, MARTINSON J, SIMMONS DL: A distinct profile of six soluble adhesion molecules (ICAM-1, ICAM-3, VCAM-1, E-selectin, L-selectin and P-selectin) in rheumatoid arthritis. *Br J Rheumatol* 1997; 36:164-9.
24. KOCH AE, HALLORAN MM, HASKELL CJ, SHAH MR, POLVERINI PJ: Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature* 1995; 376: 517-9.
25. READ MA, NEISH AS, GERRITSEN ME, COLLINS T: Postinduction transcriptional repression of E-selectin and vascular cell adhesion molecule-1. *J Immunol* 1996; 157: 3472-9.
26. WELLICOME SM, KAPAHI P, MASON JC, LEBRANCHU Y, YARWOOD H, HASKARD DO: Detection of a circulating form of vascular cell adhesion molecule-1:Raised levels in rheumatoid arthritis and systemic lupus erythematosus. *Clin Exp Immunol* 1993; 92: 412-8.
27. WILKINSON LS, EDWARDS JC, POSTON RN, HASKARD DO: Expression of vascular cell adhesion molecule-1 in normal and inflamed synovium. *Lab Invest* 1993; 68: 82-8.
28. MATSUYAMA T, KITANI A: The role of VCAM-1 molecule in the pathogenesis of rheumatoid synovitis. *Hum Cell* 1996; 9: 187-92.
29. PRAGER E, SUNDER-PLASSMANN R, HANSMANN C *et al.*: Interaction of CD31 with a heterophilic counterreceptor involved in downregulation of human T cell responses. *J Exp Med* 1996; 184: 41-50.