Soluble Endothelial Cell Adhesion Molecules and Their Relationship to Disease Activity in Takayasu's Arteritis

NARESH K. TRIPATHY, VINOD CHANDRAN, NAVEEN K. GARG, NAKUL SINHA, and SONIYA NITYANAND

ABSTRACT. ***Objective.*** To investigate soluble (s) E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular cell adhesion molecule-1 (ICAM-1), and their relationship to disease activity in Takayasu's arteritis (TA).

Methods. Levels of adhesion molecules were measured by enzyme immunoassay in the sera of 35 patients with TA, 17 healthy controls, and 15 patients with 12 months followup.

Results. Compared to controls, patients had elevated levels of sE-selectin (54.5 ± 35.0 vs 36.4 ± 13.0 ng/ml, $p < 0.05$), sVCAM-1 (280.9 ± 267.6 vs 141.2 ± 76.1 ng/ml, $p < 0.05$), and sICAM-1 (261.3 ± 168.1 vs 198.3 ± 74.3 ng/ml, $p < 0.05$). Compared to controls, patients with inactive TA also had elevated levels of sE-selectin (67.4 ± 45.9 vs 36.4 ± 13.0 ng/ml, $p < 0.02$), sVCAM-1 (327.6 ± 327.8 vs 141.2 ± 76.1 ng/ml, $p < 0.02$), and sICAM-1 (321.9 ± 179.5 vs 198.3 ± 74.3 ng/ml, $p < 0.02$). There was no difference between active TA and controls. sE-selectin had a trend towards increased levels in inactive versus active TA (67.4 ± 45.9 vs 44.9 ± 20.3 ng/ml $p = 0.059$), but there was no difference in sVCAM-1 and sICAM-1 levels between the groups. No adhesion molecule levels showed a change among followup patients.

Conclusion. Patients with inactive TA have elevated levels of sE-selectin, sVCAM-1, and sICAM-1 that might indicate persistent vasculopathy in clinically inactive disease. (First Release June 15 2008; J Rheumatol 2008;35:1842–5)

Key Indexing Terms:
TAKAYASU'S ARTERITIS DISEASE ACTIVITY
SOLUBLE ENDOTHELIAL CELL ADHESION MOLECULES

The infiltration of large elastic arteries by circulating leukocytes is one of the pathological hallmarks of Takayasu's arteritis (TA), and is mediated by several endothelial cell adhesion molecules (ECAM) such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular cell adhesion molecule-1 (ICAM-1) expressed on the target vessels[1,2]. During vascular inflammation, these ECAM are released in the circulation in the soluble forms representing their expression on inflamed vessels[3,4]. These soluble ECAM (sECAM) thus may serve as an index of endothelial inflammation in an inflammatory vascular disease like TA and may be related to disease activity.

From the Departments of Hematology, Immunology, and Cardiology; Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow, India.

Supported by an intramural grant of SGPGIMS, Lucknow-226014, India to Dr N.K. Garg.

N.K. Tripathy, PhD, Technical Officer, Department of Hematology; V. Chandran*, MD, DM, Student, Department of Immunology; N.K. Garg, MD, DM, DNB, Professor; N. Sinha, MD, DM, Professor, Head, Department of Cardiology; S. Nityanand, MD, PhD, Professor, Head, Department of Hematology.*

**The first 2 authors contributed equally to the work.*

Address reprint requests to Dr. S. Nityanand, Department of Hematology, SGPGIMS, Raebareli Road, Lucknow-226014, India.
E-mail: soniya@sgpgi.ac.in

Accepted for publication April 14, 2008.

MATERIALS AND METHODS

Subjects. The study included 35 patients with TA (28 females, 7 males; mean age 28.5 ± 10.5 yrs; range: 8–48 yrs) fulfilling 1990 American College of Rheumatology criteria for TA[5], and 17 age and sex matched healthy controls with no signs or symptoms of cardiovascular disease. The patients were classified into active TA (n = 20) and inactive TA (n = 15) according to described criteria[6]. All patients with active TA were prescribed immunosuppressive therapy (1 mg/kg/day) and azathioprine (2 mg/kg/day) and prospectively followed up; 15 of these patients who completed their 12 month followup were included in the followup study.

Detection of sECAM. Serum levels of sE-selectin, sVCAM-1, and sICAM-1 were detected by enzyme immunoassay (R&D Systems, Minneapolis, MN, USA) per manufacturer's instructions. The intra- and inter-assay coefficient of variation was < 5.0% and < 8.0%, respectively. An elevated value was calculated as > mean ± 2 SD of controls.

Statistical analysis. Statistical analysis was done using Student's t-test and Fisher's exact probability test and a p value (2-tailed) < 0.05 was considered to be significant.

RESULTS

Serum levels of sECAM. The patients compared to controls had elevated levels of sE-selectin (54.5 ± 35.0 vs 36.4 ± 13.0 ng/ml, $p < 0.05$), sVCAM-1 (280.9 ± 267.6 vs 141.2 ± 76.1 ng/ml, $p < 0.05$), and sICAM-1 (261.3 ± 168.1 vs 198.3 ± 74.3 ng/ml, $p < 0.05$). The patients with inactive TA compared to controls also had elevated levels sE-selectin (67.4 ± 45.9 vs 36.4 ± 13.0 ng/ml, $p < 0.02$), sVCAM-1 (327.6 ± 327.8 vs 141.2 ± 76.1 ng/ml, $p < 0.02$), and sICAM-1 (321.9

± 179.5 vs 198.3 ± 74.3 ng/ml, p < 0.02). However, there was no difference between active TA and controls (sE-selectin: 44.9 ± 20.4 vs 36.4 ± 13.0 ng/ml, p = 0.148; sVCAM-1: 245.9 ± 214.5 vs 141.2 ± 76.1 ng/ml, p = 0.065; and sICAM-1: 260.9 ± 167.9 vs 198.3 ± 74.3 ng/ml, p = 0.165). sE-selectin had a trend towards increased levels in inactive versus active TA (67.4 ± 45.9 vs 44.9 ± 20.3 ng/ml, p = 0.059), but there was no difference in the levels of sVCAM-1 (327.6 ± 327.8 vs 245.9 ± 214.5 ng/ml, p = 0.379) or sICAM-1 (321.9 ± 179.5 vs 260.9 ± 167.9 ng/ml; p = 0.309) between the groups (Figure 1).

Followup study of sECAM. There was no difference between baseline and followup levels of sE-selectin (47.8 ± 20.6 vs 52.4 ± 17.9, p = 0.453), sVCAM-1 (253.7 ± 231.6 vs 173.0 ± 111.8, p = 0.235) and sICAM-1 (277.0 ± 174.9 vs 221.2 ± 108.2, p = 0.263) in the patients (Figure 2). Most patients had type-III disease, and there was no specific correlation of sECAM levels with extent of disease.

DISCUSSION

Our study shows elevated levels of sE-selectin, sVCAM-1, and sICAM-1 in inactive TA compared to controls, but no difference between active TA and controls or between active and inactive TA. Noguchi, *et al*[7] showed elevated levels of sVCAM-1 and sICAM-1 in older (> 50 yrs) compared to younger (< 39 yrs) TA patients, who had normal levels of fibrinogen and thus were supposed to have inactive disease. Hoffman and Ahmad[8] have shown no difference in the levels of sE-selectin, sVCAM-1, and ICAM-1 between patients, and healthy controls or between active and inactive TA. However, they observed elevated levels of soluble platelet endothelial cell adhesion molecule (sPECAM-1) in patients

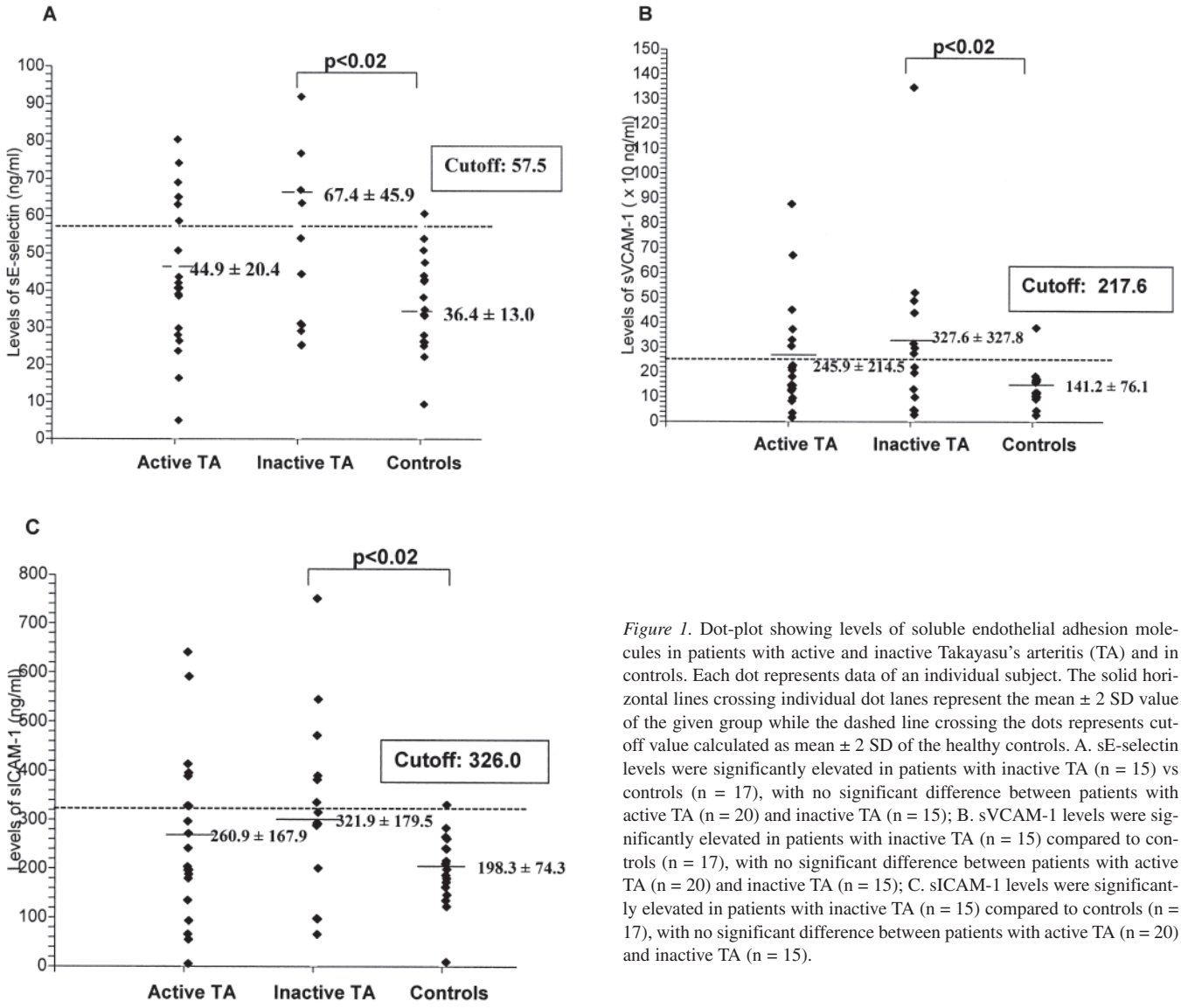

Figure 1. Dot-plot showing levels of soluble endothelial adhesion molecules in patients with active and inactive Takayasu's arteritis (TA) and in controls. Each dot represents data of an individual subject. The solid horizontal lines crossing individual dot lanes represent the mean ± 2 SD value of the given group while the dashed line crossing the dots represents cut-off value calculated as mean ± 2 SD of the healthy controls. A. sE-selectin levels were significantly elevated in patients with inactive TA (n = 15) vs controls (n = 17), with no significant difference between patients with active TA (n = 20) and inactive TA (n = 15); B. sVCAM-1 levels were significantly elevated in patients with inactive TA (n = 15) compared to controls (n = 17), with no significant difference between patients with active TA (n = 20) and inactive TA (n = 15); C. sICAM-1 levels were significantly elevated in patients with inactive TA (n = 15) compared to controls (n = 17), with no significant difference between patients with active TA (n = 20) and inactive TA (n = 15).

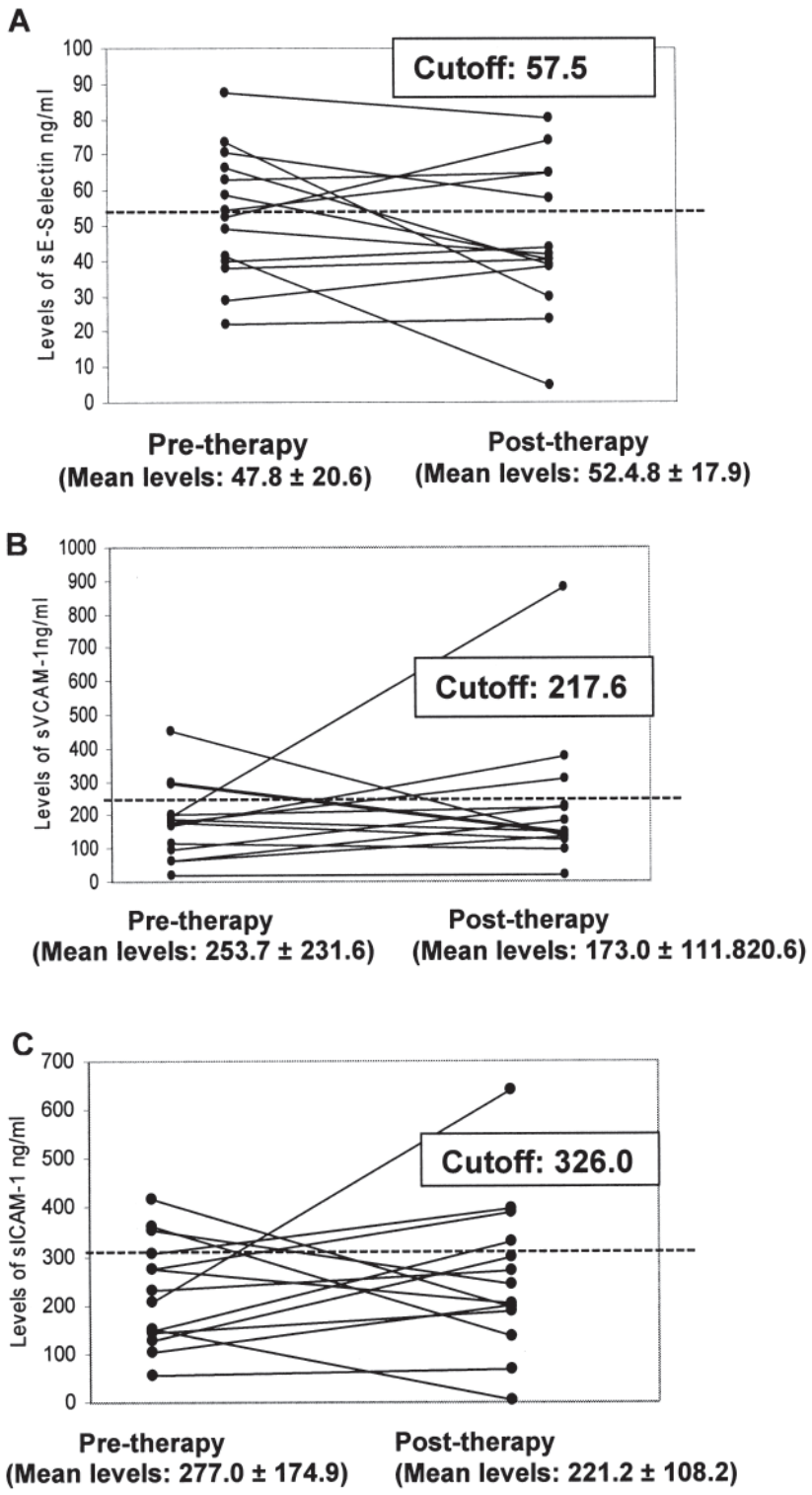

Figure 2. Line-plot showing levels of soluble endothelial adhesion molecules in pre- and post-therapy patients with Takayasu's arteritis (TA) (n = 15) with 12 months' followup. All pre-therapy patients had active TA and most of the post-therapy patients had inactive TA (n = 13). Each line represents data of an individual patient. The dashed line crossing the plot represents the cutoff value calculated as mean ± 2 SD of the healthy controls. A. sE-selectin levels in pre-therapy and post/therapy patients; B. sVCAM-1 levels in pre-therapy and post therapy patients; C. sICAM-1 levels in pre-therapy and post/therapy patients.

with inactive TA compared to active TA. Our observation of elevated levels of sE-selectin, sVCAM-1, and ICAM-1 in patients, particularly in those with inactive disease compared to healthy controls supports the study of Noguchi, *et al*[7]. We also noticed a trend towards elevation of sE-selectin in inactive TA compared to active TA, which is in line with the pattern of sPECAM-1 reported in TA[8]. Since E-selectin is exclusively expressed on activated endothelium, a trend towards elevated levels of sE-selectin in inactive TA indicates persistence of subclinical vascular inflammation during remission of the disease. Our previous study in patients with inactive TA showing immunoinflammatory activity as measured with a variety of inflammatory cytokines including tumor necrosis factor-α and interferon-γ, which are potent mediators of expression of ECAM, lends support to persistent vascular inflammation during inactive disease[9]. Moreover, subclinical disease activity in clinical remission has also been reported in other immune inflammatory diseases such as lupus[10].

Similar to our cross sectional data, we observed no difference in the levels of sE-selectin, sVCAM-1, and ICAM-1 between pre- and post-therapy patients of our followup study showing persistence of elevated levels of sECAM during inactive stage of disease. The frequent relapses after therapy in longterm followup[11] and histopathological evidence of active vasculitis in > 40% patients with inactive TA[12] substantiate these findings. In addition, similar followup studies in Wegener's granulomatosis[13] and polyarteritis nodosa[14] showing elevated levels of one or more of sECAM in patients under remission point towards their role in detection of a subclinical disease activity. Whether elevated sECAM, particularly sE-selectin in TA, may have a role in detecting subclinical disease activity from true remission needs further study, including analysis of sECAM in disease controls and correlation of followup data with vascular imaging, which is an important modality for diagnosis and monitoring of disease progression.

In conclusion, we have demonstrated that patients with inactive TA have elevated levels sE-selectin, sVCAM-1, and sICAM-1, which may indicate persistent vasculopathy in clinically inactive disease.

REFERENCES

1. Inder SJ, Bobryshev YV, Cherian SM, et al. Accumulation of lymphocytes, dendritic cells and granulocytes in the aortic wall affected by Takayasu's disease. Angiology 2000;51:565-79.
2. Kevil CG, Bullard DC. Roles of leukocyte/endothelial cell adhesion molecules in the pathogenesis of vasculitis. Am J Med 1999;106:677-87.
3. Pigott R, Dillon LP, Hemingway LH, et al. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys Res Commun 1992;187:584-9.
4. Nakai K, Itoh C, Kawazoe K, et al. Concentration of soluble vascular cell adhesion molecule –1(VCAM-1) correlated with expression of VCAM-1 mRNA in the human atherosclerotic aorta. Coron Artery Dis 1995;6:497-502.
5. Arend WP, Michel BA, Bloch DA, et al. American College of Rheumatology criteria for the classification of Takayasu arteritis. Arthritis Rheum 1990;33:1129-34.
6. Tripathy NK, Sinha N, Nityanand S. Anti-annexin-V antibodies in Takayasu's arteritis and their relationship with disease activity. Clin Exp Immunol 2003;134:360-4.
7. Noguchi S, Numano F, Gravanis MB, Wilcox JN. Increased levels of soluble forms of adhesion molecules in Takayasu arteritis. Int J Cardiol 1998;66 Suppl 1:S23-33.
8. Hoffman GS, Ahmed AE. Surrogate markers of disease activity in patients with Takayasu arteritis. A preliminary report from The International Network for the Study of the Systemic Vasculitides (INSSYS). Int J Cardiol 1998;66 Suppl 1:S191-4.
9. Tripathy NK, Chauhan SK, Nityanand S. Cytokine mRNA repertoire of peripheral blood mononuclear cells in Takayasu's arteritis. Clin Exp Immunol 2004;138:369-74.
10. Wais T, Fierz W, Stoll T, et al. Subclinical disease activity in systemic lupus erythematosus: immunoinflammatory markers do not normalize in clinical remission. J Rheumatol 2003;30:2133-9.
11. Talwar KK, Vasan RS, Sharma S, et al. Non-specific aorto arteritis: long term follow-up on immunosuppressive therapy. Int J Cardiol 1993;39:79-84.
12. Hoffman GS. Takayasu arteritis: lessons from the American National Institutes of Health experience. Int J Cardiol 1996;54 Suppl:83-6.
13. Stegeman CA, Tervaert JW, Huitema MG, et al. Serum levels of soluble adhesion molecules intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin in patients with Wegener's granulomatosis. Relationship to disease activity and relevance during follow up. Arthritis Rheum 1994;37:1228-35.
14. Coll-Vinent B, Grau JM, Lopez-Soto A, et al. Circulating soluble adhesion molecules in patients with classical polyarteritis nodosa. Br J Rheumatol 1997;36:1178-83.