

## CLINICAL IMPLICATIONS OF SERUM THROMBOMODULIN IN PR3-ANCA-ASSOCIATED VASCULITIS

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### Abstract

**Background:** Vascular injury is the main mechanism in pathophysiology of PR3-ANCA-associated vasculitis. Soluble serum thrombomodulin (sTM) is a membrane-bound receptor for thrombin expressed by vascular endothelial cells.

**Objective:** The aim of study was to determine the blood levels of sTM in patients with PR3-ANCA-associated vasculitis.

**Material and methods:** Twenty five patients with Wegener's granulomatosis (WG), 13 with generalized WG and 12 with limited WG, with histologically proven disease, and 15 healthy subjects as a control were investigated. An ELISA for detection of sTM and PR3-ANCA was performed. The disease activity was evaluated according to BVAS and DEI indexes.

**Results:** Significant increases in sTM were found in both active generalized and limited active WG compared with control values:  $108 \pm 12$ ,  $56 \pm 2$ , and  $12 \pm 4$  ng/ml, respectively. Elevated ANCA titer correlated with disease activity, but more weakly than sTM levels did. Elevated sTM concentration is a result of vascular endothelial injury in the course of PR3-ANCA associated vasculitis.

**Conclusions:** Soluble serum thrombomodulin is a promising, both diagnostic and therapeutic, marker of endothelial cell injury in relation to disease activity and progression in autoimmune disorders, reflecting the degree of endothelial cell damage.

**Key words:** thrombomodulin, protein C receptor, Wegener's granulomatosis

### INTRODUCTION

Endothelial cell damage, probably mediated by polymorphonuclear cells (PMN) and PR3-ANCA, plays the main role in the pathogenesis of Wegener's granulomatosis (WG). Elevated soluble thrombomodulin (sTM) is an accepted marker of endothelial damage [1]. Factors reflecting endothelial cell damage, or response to injury, are of considerable interest in patients with PR3-ANCA positive vasculitis. They may be potential markers of vasculitic disease activity and progression [2]. Thrombomodulin (TM) is an integral membrane protein made up of 557 aminoacids that bears some structural resemblance to the LDL receptor. Expressed by endothelial cells and functioning as a thrombin receptor, TM is one of the factors responsible for the anticoagulant properties of the vascular endothelium [3, 4]. TM is an endothelial cell transmem-

brane co-factor for thrombin-mediated protein C activation. There are 30000-50000 thrombomodulin molecules expressed on an endothelial cell, representing 50-60% of all thrombin binding sites. This glycoprotein is present on all endothelial cells, except the sinusoidal hepatic lining cells and postcapillary endothelial cells in lymph node veins [4, 5]. It is also found in the mucosal mesothelium and lining of the body cavities, blood plasma, platelets, neutrophils, monocytes, urine, and placenta. Membranes and large arteries respond to exposed TM much more strongly than veins and capillaries. A strongly positive response in blood vessels does not depend on the investigated organ, but rather is related to the quality of blood flow [6]. TM plays a major role in maintaining blood in a liquid state and preventing intravascular coagulation. Furthermore, thrombin complexed with thrombomodulin loses its pro-coagulation properties in the conversion of fibrinogen into fibrin, activation of factors V, VII, and XIII, inactivation of protein S, and induction of platelet aggregation [7].

A soluble form of thrombomodulin (sTM) found in plasma and urine is released mainly due to endothelial cell damage, but not due to physiological activation [6, 7]. The aim of the study was to determine the relevance of sTM as a predictor of endothelial cell damage in Wegener's granulomatosis (WG) and as a marker of disease activity (progression and remission).

### MATERIAL AND METHODS

The study was approved by a local Ethics Committee. Twenty five serum samples obtained from 13 patients with active generalized biopsy-proven WG and from 12 patients with limited WG were tested. The patients' mean age was  $46.8 \pm 12.5$ SD. All patients fulfilled the American College of Rheumatology criteria for classification of WG and the Chapel Hill Consensus Conference definition, and also EUVAS ANCA-associated vasculitis definition for WG. Disease activity was confirmed by clinical scoring, laboratory variables, and imaging procedures. DEI and BVAS indexes were determined to measure organ involvement and disease activity. An infective reason of inflammation was ruled out in the patients by routine blood and urine culture, bronchoalveolar lavage fluid culture, and serological tests. Another fifteen serum samples obtained from 8 women and 7 men, aged 32-68, without any clinical symptoms of vasculitis, were tested as control. CRP was measured with a routine turbidime-

try assay (ILAD-900): a value greater than 10 mg/l was considered to be abnormally high. An ELISA test for detection of PR3-ANCA and sTM in plasma was performed. The concentration of TM was determined using the Imubind® Thrombomodulin ELISA Kit assay that is able to recognize the intact and partially degraded forms of TM (reference values: women-age-dependent: 2.73 ng/ml for the age 21-30, than increased up to 4.79 ng/ml for the age 61-79 ng/ml; men-age-independent: 4.00-5.35 ng/ml).

All data were expressed as means  $\pm$  SE. The Wilcoxon and Spearman tests were used for statistic analysis.  $P < 0.05$  was considered statistically significant.

## RESULTS

Thrombomodulin levels were markedly elevated in 13 sera from the active generalized WG group and in 12 sera from the limited WG group. Baseline characteristics in both generalized and limited WG are shown in Table 1.

Significant increases in sTM levels were found in both active and limited active WG compared with control values; the respective values were  $108 \pm 12$ ,  $56 \pm 2$ , and  $12 \pm 4$  ng/ml. Elevated ANCA titer also correlated with disease activity, but more weakly than the sTM levels. There was a significant correlation between sTM level and BVAS index ( $r=0.35$ ), DEI index ( $r=0.50$ ), erythrocyte sedimentation rate ( $r=0.60$ ), c-ANCA titer ( $r=0.40$ ), PR3-ANCA ( $r=0.50$ ), C-reactive

protein ( $r=0.45$ ), white blood cell count ( $r=0.22$ ), and thrombocyte count ( $r=0.50$ ) (Table 2).

CRP levels were significantly elevated in the patients with both generalized ( $28.0 \pm 0.5$  mg/100ml) and limited WG ( $1.8 \pm 0.6$  mg/100ml). PR3-ANCA titers were significantly different from the controls in both groups. In addition, there was a significant difference in the ANCA titer between the groups of patients with active generalized and limited WG. There was no correlation between specific organ involvement and sTM concentration, except for renal involvement. The mean values of other variables in active generalized and limited WG and in the control group were presented in Table 3.

Table 2. Correlation table in Wegener's Granulomatosis group.

Characteristics	sThrombomodulin	
	n	Correlation coefficient
BVAS	25	0.35
DEI	25	0.50
ESR	25	0.60
c-ANCA	16	0.40
PR3-ANCA	18	0.50
CRP	25	0.45
WBC	25	0.40
Thrombocytes	25	0.22

Table 1. Baseline characteristics in generalized and limited Wegener's granulomatosis (WG).

Baseline characteristics	Generalized WG (n=13)	Limited WG (n=12)	Control (n=15)
Sex (M/F)	6/7	5/7	8/7
Median age (yr)	48.5 (23.6-52.8)	54.4 (25.4-67.4)	48.3 (32.3-68.4)
Disease duration (mo)	66 (6-148)	58 (8-116)	0

Table 3. Laboratory data in generalized and limited Wegener's granulomatosis (WG).

	n	Generalized WG	Limited WG	Control	P
sTM (ng/ml)	40	$108 \pm 12$	$56 \pm 2$	$12 \pm 4$	0.0015
ESR (mm/h)	40	$79.1 \pm 26.6$	$27.3 \pm 10.7$	$8.4 \pm 4.2$	0.01
CRP (mg/dl)	40	$13.7 \pm 7.8$	$1.5 \pm 0.98$	$1.5 \pm 0.98$	0.1
Fibrinogen (mg/dl)	40	$426.5 \pm 164.2$	$212.6 \pm 21.6$	$160.6 \pm 22.6$	0.04
Leucocytes / $\mu$ l	40	$9900 \pm 3500$	$5400 \pm 1500$	$4400 \pm 1500$	0.008
Thrombocytes / $\mu$ l	40	$398.7 \pm 134.1$	$223.4 \pm 57.5$	$130.4 \pm 59.5$	0.0021
Hg (g/%)	40	$11.4 \pm 2.4$	$12.2 \pm 1.8$	$13.2 \pm 1.8$	0.004
GFR (ml/min)	40	$64.21 \pm 16.8$	$76.8 \pm 9.4$	$96.8 \pm 8.4$	0.005
c-ANCA	14	$640 \pm 160$	$80 \pm 40$	0	0.24
p-ANCA	3	$160 \pm 80$	$80 \pm 20$	0	0.19
PR3-ANCA	16	$100 \pm 80$	$8.2 \pm 4.6$	0	0.001
MPO-ANCA	2	$40 \pm 14$	$9.4 \pm 2.1$	0	0.28
DEI	25	12 (8-14)	6 (3-9)	0	0.004
BVAS-WG	25	21 (16-32)	14 (12-16)	0	0.0001

## DISCUSSION

Clinical studies have shown elevated levels of soluble thrombomodulin in various pathological conditions such as: diffuse intravascular coagulation (DIC) syndrome, pulmonary embolism, chronic renal failure (MIA-syndrome), diabetic microangiopathy, systemic lupus erythematosus (SLE), peripheral and coronary atherosclerosis, Schönlein-Henoch purpura and malignancies (lymphomas, leukemias, carcinomas) [3, 8]. Endothelial cell activation and damage also seem to be a pathophysiological mechanism of PR3-ANCA positive vasculitis [9].

Previous *in vitro* studies have shown that autoantibodies, such as PR3-ANCA, activate cytokine-primed neutrophils and monocytes leading to release of proteolytic enzymes and the production of the oxygen radicals and pro-inflammatory cytokines. PR3-ANCA may play a role in vascular injury by attracting leucocytes to the inflammatory site. Interaction of PR3-ANCA with their target antigens may aggravate an inflammatory process [9-11]. In the present study, we demonstrated a significant correlation between Wegener's granulomatosis disease activity and sTM levels. This finding is in accord with a study by Boehme et al [12] in which 197 serum samples obtained from 102 patients with WG, of different disease activity, and 41 samples from patients with other active vasculitides: 12 patients with Takayasu arteritis (TA), 7 with giant cell arteritis (GCA), 10 with polyarteritis nodosa (PAN), and 12 with Behcet's disease (BD) were assessed [12].

In the present study, in limited WG, sTM levels were elevated in more patients than the PR3-ANCA titers were. This finding is in agreement with other studies in which elevated levels of sTM were demonstrated in patients with active WG [13, 14]. In our study, patients with active disease and elevated sTM levels had a higher prevalence of renal involvement, and higher serum creatinine levels, the findings being similar to those of Boomsma et al's [15]. The expression of TM is decreased by inflammatory cytokines, such as TNF- $\alpha$ , due to suppression of TM transcription and translation. Moreover, *in vitro* granulocyte-derived proteases, such as cathepsin, elastase, and hydrogen peroxide have been shown to induce the release of TM from the endothelial cell surface as a result of endothelial cell damage. Thus, this pathway would explain a good correlation between the disease activity in PR3-ANCA positive vasculitis and thrombomodulin concentration, as a predictor of vascular damage and injury.

We conclude that patients with PR3-ANCA positive vasculitis, such as Wegener's granulomatosis, had elevated sTM levels, which may result from vascular endothelium damage and injury. Higher sTM concentrations were observed in patients with active generalized Wegener's granulomatosis. Thus, sTM may be considered as a serological marker and clinically useful predictor of disease activity and course in vasculitis and angiitis directly correlating with the degree of endothelial cell damage. On the other hand, determination of sTM may be helpful in assessing the disease activity in patients who are negative for c-ANCA or PR3-ANCA, or in whom ANCA titers do not reflect clinical disease activity.

*Conflicts of interest:* No conflicts of interest were declared by the authors in relation to this article.

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