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# Soluble Thrombomodulin Levels are Related to Inflammation after Coronary Bypass Surgery

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## Abstract

**Background:** Soluble Thrombomodulin (sTM) is released from endothelial cells after injury by activated leukocytes in inflammatory states and it has been associated with worse prognosis in inflammatory conditions. The purpose of this study was to analyze the association between levels of sTM and inflammation and to describe the possible explanations about association between sTM and post-operative complications.

**Methods and Materials:** We measured levels of sTM, IL6 and leukocytes in different moments during the first month in 18 patients after coronary by-pass graft surgery. Chisquare test and Fisher test were used for categorical variables. For continuous variables we used either Student t test or Wilcoxon rank-sum test depending on whether the data were normally distributed.

**Results:** The levels of sTM increased during the first post-surgery week, and then decreased to values similar to those recorded pre-operatively. IL6 peaked at after 24 hours, and significantly correlated with white blood cell count (Spearman 0.69, p=<0.0001). There was a significant association between sTM at T3 and white blood cells count at T1 (p=0.01224). Two patients had three or more post-operative complications, and they presented higher levels of sTM.

**Conclusions:** We found a transient increase in sTM during the first week after CABG associated with an inflammatory response and leukocytosis. These changes could imply endothelial dysfunction after surgery and may represent prognostic factors for outcomes.

**Keywords:** Thrombomodulin; CABG; Inflammation; Interleukin 6; Endothelium

## Introduction

Thrombomodulin (TM) is a membrane-bound protein which is normally expressed on the surface of endothelial cells. It captures circulating thrombin and changes it from a fibrin-forming enzyme to an active catalytic protein that activates the protein C anticoagulant pathway, and hence neutralizing clotting activity [1]. In addition to membrane-bound TM, there is a soluble TM (sTM) form in plasma, which results from endothelial damage. It is released into serum by proteolytic degradation of endothelial cells after injury by oxidative stress products, such as hydrogen peroxide, or by activated leukocytes [2,3]. Clinically, elevated sTM has been found to predict events in patients surviving an acute coronary syndrome [4], and increased sTM level has been reported in patients with multiple organ dysfunction syndrome (MODS), disseminated intravascular coagulation induced by sepsis or blunt trauma. In all these conditions, sTM has been associated with increased mortality [5-7]. Therefore, it has been suggested that the elevation of sTM levels might be associated with a higher inflammatory state [4,8,9]. Cardiac surgery with cardiopulmonary bypass (CPBP) is associated with a systemic inflammatory response similar to that observed in all these conditions, and leukocytosis with neutrophilia frequently occurs in this scenario [10]. We performed an observational study analyzing the variability of sTM level during the first month after CPBP. Our goal was to analyze the association between levels of sTM and inflammation, measured by levels of interleukin 6 (IL6) and leukocyte count. A secondary objective was to describe associations between sTM and post-operative complications.

## Material and Methods

### Study population

We prospectively included 18 patients undergoing elective coronary artery by-pass grafting (CABG) with CPBP in two centers in Argentina. Exclusion criteria were: CABG without CPBP, consent withdrawal, concomitant carotid or valvular surgery, discontinuation or contraindication to aspirin, treatment with clopidogrel or other non steroidal anti-inflammatory drugs, acute myocardial infarction and creatinine clearence ≤ 30 ml/min/kg.

Informed consent was obtained from all participating subjects before any study-related procedure. The study was conducted in accordance with the Declaration of Helsinki, and approved by the institution's Ethics Committee.

### Blood samples

We measured levels of sTM, IL6, white blood cell count and leukocyte formula within 2 days previous to surgery (T0), and 24 hours (T1), 72 hours (T2), one week (T3), and one month after the procedure (T4). These time points, although arbitraries were taken to represent different but critical moments during the first month after surgery. sTM and IL6 were measured by ELISA according to manufacturer's instructions. Results are expressed in ng/ml for sTM, pg/ml for IL6 and cells/mm3 for white blood cells.

### Statistical analysis

Continuous variables are expressed as median with 25-75%

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interquartile range (25-75% IQR). Categorical variables are expressed as frequencies and percentage. For comparison of continuous variables, we used either Student t test or Wilcoxon rank-sum test, according to data distribution.

Chi-square test and Fisher's exact test were used for categorical variables. A P value less than 0.05 was considered statistically significant.

## Results

A total of 90 samples from 18 patients at times T0-T4 were collected and analyzed. Table 1 shows the demographic characteristics of the included population. The median number of anastomosis performed was 3 (11 patients received 3 bypasses; 3 patients received 4 bypasses and another 4 received 2 bypasses). Bypass time was 43 (39-51) minutes. Cardiopulmonary bypass was carried out in normothermia (rectal temperature > 35°C).

The level of sTM increased gradually from T0 to T3, when it peaked. At T3, the level of sTM was significantly higher than pre-operatively (median 13 ng/ml (25-75% IQR: 5-38 ng/ml) vs 3 ng/ml (25-75% IQR: 1-7 ng/ml); p=0.0011).

IL6 peaked at T1, decreasing to values similar to those recorded pre-operatively by T4 (Figure 1).White blood cell count peaked at T1, with marked increase of neutrophils, and then decreased by T4 to a similar value to those recorded pre-operatively. There was a significant correlation between levels of IL6 and WBC count, with a Spearman correlation coefficient of 0.69 (p<0.0001).We found also a significant association between sTM at T3 and white blood cells count at T1 (p=0.01224), but not to the other time points (Figure 2).

Regarding clinical events, there were neither deaths nor myocardial infarctions during the follow up period. Seven patients (38%) presented Systemic Inflammatory Response Syndrome defined as two or more of the following: temperature >38°C or <36°C, tachycardia, tachypnea with hypocapnia and leukocytosis >12000/mm3 or leukopenia <4000/mm3, or >10% immature forms. Only one patient presented shock parameters. Two patients had a minor infectious process (infection of the saphenectomy that had a favorable outcome with antibiotics), two had acute renal failure (creatinine >2 mg/dl), two had atrial fibrillation that required special treatment and one patient had respiratory failure. Two patients presented significant bleeding in the postoperative period, one requiring re-operation. Two patients (11%) had three or more of these complications, and they had high levels of sTM.

## Conclusions

This observational study shows that serum sTM levels are increased and fluctuate in the postoperative period of CABG. We found an association between inflammation and high levels of sTM. We also found an association between leukocytosis in T1 and high levels of sTM in T3. This temporal association may represent a pathophysiological link between early leukocyte activation and subsequent endothelial release of sTM. This pattern of association is similar to previous studies that found that neutrophil products cleave TM from endothelial cell membrane [8,11-13], loss of surface TM coupled with poorly regulated tissue factor activity changes the endothelium to a more procoagulant inflammatory surface, increaseing at the same time the amount of sTM release into the plasma [13]. TM is normally expressed on the endothelial cell surface bound to the endothelium. It neutralizes thrombin clotting activity and accelerates thrombin-catalyzed activation of protein C to activated protein C (protein Ca), thus converting thrombin from a procoagulant protease to an anticoagulant protein. The TM/thrombin complex and protein Ca, together with protein S (a cofactor of protein C), are potent inhibitors of the coagulation factors Va and VIIIa [14]. Soluble TM is released from the surface of endothelial cells secondary to increased proteolytic cleavage when endothelial damage occurs [7]. Increased sTM level has been associated with mortality in patients with trauma, acute respiratory distress syndrome, major surgery and sepsis, expressing a correlation with multiple organ failure [5-7]. Moreover,

| | |
|---|---|
| Age | 64.1 +- 4 |
| Hypertension | 13(72) |
| Diabetes | 4(22) |
| Smokers | 6(33) |
| Previous smokers | 8(44) |
| PCI | 1(6) |
| CABG | 1(6) |
| Statins | 1(11) |
| Angiotensin Inhibitors | 11(61) |
| By pass (n± SD) | 2.94+-0.64 |
| CPBP time (minutes) | 76(64-88) |
| Cross clamp time (minutes) | 43(39-51) |

PCI: Percutaneous Coronary Intervention
CABG: Coronary artery bypass graft

**Table 1:** Baseline characteristics.

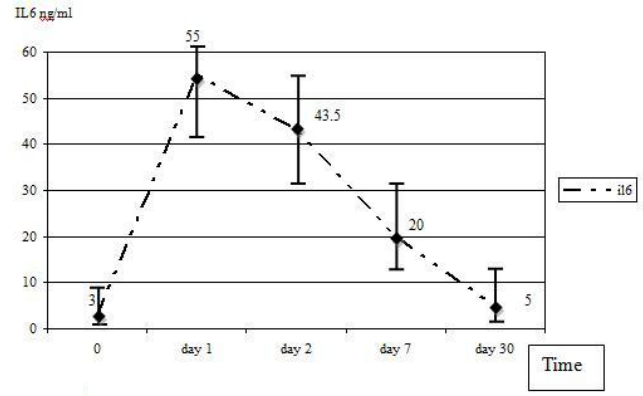


**Figure 1:** Median values of IL6 (median (pg/ml), 25-75% IQR); IL6: Interleukin 6.

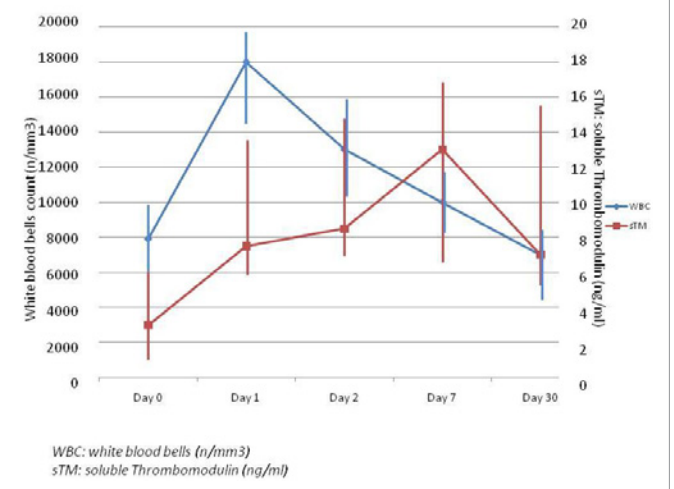


**Figure 2:** Levels of white blood cells count and sTM; sTM= soluble Thrombomodulin; WBC= White blood cells.

increased sTM in patients with atherosclerosis is a predictor of poor cardiovascular outcome and increased levels of sTM, C-reactive protein (CRP) and another inflammatory markers have been reported after percutaneous coronary interventions [15] and have been found to be a predictor of poor outcome in patients surviving acute coronary syndromes [4].

Previous studies have shown the relationship between levels of ILs and TM in endothelial cell alterations. In the presence of IL6, TM surface activity decreases, resulting in an enhancement of the thrombotic properties of the endothelium [16]. Proinflammatory cytokines promote the synthesis and up-regulation of adhesion molecules, leading to adherence of neutrophils and activation of the endothelium. After adherence, neutrophils secrete several enzymes, such as myeloperoxidase and elastase, which cause endothelial injury [8,17]. Moreover, previous reports showed that cardiopulmonary bypass can increase plasma levels of sTM, specially under hypothermic CPBP. In our study CPBP was carried out in normothermia [1,14,18,19]. We found that the increase in sTM levels is preceded by a correlated increase in leukocytes after surgery, which may indicate a causal relationship. An increased level of circulating sTM is a biological marker of the prothrombotic antifibrinolytic state associated with patient poor outcomes and possibly contributing to graft thrombosis [20,21].

We could not conclude that an association between complications and levels of sTM exists because the study lacks adequate statistical power for that purpose. However, levels of sTM were higher than described for patients with high mortality due to sepsis in the study by Lin SM et al. [7]. In this study, early sTM levels had good discriminative power to predict the occurrence of disseminated intravascular coagulation, multiorgan dysfunction, and mortality in patients with sepsis.

The major limitation of this study is the reduced number of patients enrolled, which does not allow for assessing differences in the rates of events between patients. On the other hand, samples were not collected on a daily basis, but we considered different representative moments. T0 represents pre-operative situation; T1 shows possible effects of CPBP and surgery; samples for T3 were obtained one week after surgery, as different studies consider this moment particularly high risk for bypass thrombosis, and in accordance with the hypothesis that leukocytes stimulate the release of sTM. Samples for T4 were collected one month after CABG to obtain remote results. This timeframe is representative of the postoperative course in this population.

In conclusion, there is a transient increase in sTM during the first week after CABG associated with inflammatory response and leukocytosis that could imply endothelial dysfunction. Further research in order to establish its association with adverse outcomes after surgery is needed.

### Aknowledgments

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