Relationship between coagulation cascade, cytokine, adhesion molecule and aortic aneurysm☆

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Received 30 September 2002; received in revised form 13 February 2003; accepted 6 March 2003

Abstract

Objectives: Patients with aortic aneurysm (AA) were in the chronic inflammatory condition and are often combined with disseminated intervascular coagulation. Recent studies demonstrated that atherosclerosis was inflammatory disease. AA and severe atherosclerosis with ulcer formation contain macrophages and T lymphocytes and accelerate the production of interleukin (IL)-2, which activates lymphocytes and lead to further adhesion of leukocytes. This study was designed to clarify the coagulation condition, cytokine, adhesion molecule, and collagen turnover in patients with AA and finally their relationship with the aneurysmal size. Methods: Thrombin–antithrombin III complex (TAT), plasma D-dimer, serum type III procollagen peptide (PIIIP), serum soluble IL-2 receptor (sIL-2R), Free tissue factor pathway inhibitor (TFPI), and soluble intercellular adhesion molecule (ICAM-1) were measured preoperatively around the same period when computed tomography (CT) was taken in 17 patients with AA (mean age: 72.2 years). Age-matched (mean age:70 years) volunteers were served as control. Maximum aneurysmal size was measured by CT and aneurysmal volume was also calculated from CT.

Results: AA patients showed significantly higher level in preoperative TAT and D-dimer compared to control (TAT: control 2.5 ± 1.2 ng/ml, pre 7.2 ± 4.5 ng/ml; P = 0.0001; D-dimer: control 107 ± 46 U/ml, pre 420 ± 256 U/ml; P = 0.0001). Cytokine also showed higher level preoperatively (sIL-2R: control 398 ± 7.2 ng/ml; AA 460 ± 132 U/ml, pre 735 ± 260 U/ml; P = 0.0001). TFPI showed higher value preoperatively (control 22.9 ± 4.9 ng/ml, pre 30.4 ± 6.9 ng/ml; P = 0.003). PIIIP (collagen turnover) showed no difference between the groups (P = 0.0057) and neither did ICAM-1 (P = 0.0087). TAT (r = 0.799, P = 0.0001), D-dimer (r = 0.56, P = 0.0193), sIL-2R (r = 0.709, P = 0.0021), PIHP (r = 0.56, P = 0.00239), and sICAM-1 (r = 0.505, P = 0.046) level showed positive correlation with aortic aneurysmal size and also TAT D-dimer, and sIL-2R levels were positively correlated with aneurysmal volume (r = 0.714 P = 0.0013, r = 0.556 P = 0.00204, r = 0.693 P = 0.0029, respectively). Conclusions: AA patients were in the hypercoagulation and inflammatory condition. Aneurysmal size was well correlated with TAT, D-dimer, sIL-2R, PIHP, and sICAM-1, suggesting that these markers could be good diagnostic and monitoring tool for the disease progression.

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Keywords: Coagulopathy; Aortic aneurysm; Cytokine; Adhesion molecule, Collagen turnover

1. Introduction

Aortic aneurysm is a chronic degenerative condition associated with aging, atherosclerosis, male gender, cigarette smoking, pulmonary emphysema, and hypertension [1]. The risk of rupture increases proportionately with aneurysmal size up to about 5.0 cm in diameter, when the chance of rupture is 20–40%, and tends increase nearly exponentially with any further enlargement [2]. Knowledge of etiology and pathophysiology of aneurysmal degeneration is still incomplete, yet considerable progress is being made through the application of cellular and molecular biology approaches to human aortic aneurysm tissue.

Aortic aneurysm has been considered to be direct consequence of atherosclerosis, because atherosclerosis is starting at the intima and then progressing through all layers with transmigration of leukocytes and focal necrosis [3]. Moreover, recently there is an increasing evidence that inflammatory response could play a major role in the
pathogenesis of atherosclerosis [4,5]. The earliest type of lesion, so-called fatty streak, is a pure inflammatory lesion, consisting only of monocyte derived macrophages and T lymphocytes [6]. T-lymphocyte activation is mediated by tumor necrosis factor (TNF)-α, interleukin (IL)-2, and granulocyte-monocyte colony-stimulating factor. Continued inflammation results in increased numbers of macrophages and lymphocytes, which both emigrate from blood and infiltrated leukocytes change their phenotype (foam cells) and release cytokines which are chemoattractive to further leukocytes. Activation of these cells leads to the release of cytokines (IL-1), and release cytokines which are chemoattractive to further leukocytes. Activation of these cells leads to the release of cytokines (IL-1b, IL-2, IL-4, IL-6, IL-10, TNF-α, and tissue factor (TF) [7–10]).

During last decade, attention on the pathogenesis of aortic aneurysm has focused largely on the role of chronic inflammation. On the other hand, from the viewpoint of coagulation, Fisher and colleagues [11] reported that patients with aortic aneurism were in a hypercoagulation status and often combined with disseminated intravascular coagulation. Severe atherosclerosis with atheroma or ulcer formation contains T lymphocyte and this T lymphocyte induces as well as accelerates production of IL-2 [12,13]. Then IL-2 activates and promotes proliferation of T lymphocyte and promotes coagulation [14,15]. Adhesion molecules such as intercellular adhesion molecule (ICAM)-1 was expressed on the endothelium as well as adventitia and promoted adhesion of monocytes, neutrophils, and lymphocytes [16,17]. As a matter of fact, these findings were closely related to atherosclerosis. Thus, expansion of the aneurysm could be modulated by the connective tissue metabolism such as elastin degradation and collagen turnover.

This study was designed to clarify the coagulation condition, cytokine level, collagen turnover, and adhesion molecule production in patients with aortic aneurysm and to evaluate their relationship to the aneurysmal size.

2. Subjects and methods

From January 2000 to March 2002, 17 patients with aortic aneurysm (TAA: 10; AAA: 5; TAAA: 2) were studied. The mean age of this group was 72.2 years. Age-matched volunteer were served as control, volunteer numbers were ranging from 15 cases to 72 cases, and mean age was 70 years old. Blood samples were taken from the patients with consent to measure thrombin–antithrombin III (TAT ng/ml, by ELISA), plasma D-dimer (U/ml by ELISA), serum type III procollagen peptide (PIIIP U/ml, by IRMA CIS Bio International), sIL-2 receptor (sIL-2R U/ml, by cell free IL-2R ELISA, EURO/DPC Ltd.), free tissue factor pathway inhibitor (TFPI ng/ml, by ELISA), soluble ICAM-1 (sICAM-1 ng/ml, by ELISA). The timing of blood sampling was around the same period preoperatively when computed tomography was examined. Aortic aneurysmal size was measured with computed tomography, which was sliced in 1-cm widths. Aortic aneurysmal volume was calculated by summation of the area of the aneurysm in computed tomography.

Results were expressed as mean ± SD. Data were analyzed with Student’s t-test. The relationship between markers and aneurysmal size as well as volume was assessed with a linear regression analysis. P < 0.05 was considered to be statistically significant.

3. Results

3.1. TAT and D-dimer levels (Fig. 1)

TAT showed significantly higher levels preoperatively in patients with aortic aneurysm compared to the control group (control: n = 66, 2.5 ± 1.2 ng/ml; pre: n = 17, 7.2 ± 4.5 ng/ml; P = 0.0001). D-dimer showed also significantly higher levels in preoperatively in patients with aortic aneurysm compared to control (control: n = 72, 104 ± 46 U/ml; pre: n = 17, 420 ± 256 U/ml; P = 0.0001).

3.2. sIL-2 receptor and sICAM-1 levels

sIL-2R (Fig. 2) clearly showed higher levels preoperatively in patients with aortic aneurysm compared to control (control: n = 39, 398 ± 132 U/ml; pre: n = 17, 735 ± 260 U/ml; P = 0.0001), sICAM-1 (Fig. 2) showed no significant difference between the groups (control: n = 26, 211 ± 48 ng/ml; pre: n = 17, 240 ± 60 ng/ml; P = 0.087).

3.3. PIIIP and TFPI levels in aortic aneurysm (Fig. 3)

Collagen turnover was measured as PIIIP level, the difference of which did not reach statistical significance between the groups (control: n = 15, 0.58 ± 0.13 U/ml;
3.4. Correlation between aneurysmal size and volume, and TAT, D-dimer, sIL-2R, sICAM-1, and PIIIP (Figs. 4–6)

Aortic aneurysmal size was significantly positively correlated with TAT, D-dimer, sIL-2R, sICAM-1 (Fig. 4), and PIIIP (Fig. 5) (TAT: $r = 0.714$, $P = 0.0013$; D-dimer: $r = 0.556$, $P = 0.0204$; sIL-2R: $r = 0.709$, $P = 0.0021$; sICAM-1: $r = 0.505$, $P = 0.046$; PIIIP: $r = 0.561$, $P = 0.0239$). Furthermore, aneurysmal volume showed significant positive correlation between TAT, D-dimer, sIL-2R (Fig. 6) (TAT: $r = 0.714$, $P = 0.0013$; D-dimer: $r = 0.556$, $P = 0.0204$; sIL-2R: $r = 0.693$, $P = 0.0029$).

4. Discussion

During last decade, attention on the pathogenesis of aortic aneurysm has focused largely on the role of chronic inflammation and on various connective tissue proteinases capable of degrading elastin and collagen [18,19]. In particular matrix metalloproteinases (MMPs) such as...
MMP-9 have been focused on, which is prominently expressed by a subset of macrophages in aortic aneurysm tissue and in atherosclerosis [20] and selectively increased to a great extent in aortic aneurysm than in aortic occlusive disease (atherosclerosis). Moreover, MMP-9 could correlate with aortic diameter of aortic aneurysm [21].

Recent advances showed increasing evidences that inflammatory response could play a major role in the pathogenesis of atherosclerosis [4]. Endothelial dysfunction [22] or infection would be the initial cause of inflammation. Then, the continuing entry, survival, and replication of mononuclear cells in lesions depend in part on factors such as macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor for monocytes and IL-2 for lymphocytes. The ability of macrophages to produce cytokines (such as TNF-α, IL-1, and transforming growth factor-β), proteolytic enzymes (particularly metalloproteinases), and growth factors (such as platelet-derived growth factor and insulin-like growth factor I) may be critical in the role of these cells in damage and repair that ensue as the lesions progress. Aneurysm-infiltrating macrophages play a key role in connective tissue destruction in outer aortic wall. Thus, variety of proinflammatory cytokines are also produced in aortic aneurysm tissue, and the local production of soluble mediators and adhesion molecules (ICAM-1, VCAM-1) likely participate in activating macrophages toward a matrix-degrading phenotype [8,9,16,17,23,24]. The aortic wall inflammatory response induced B and T lymphocytes, suggesting that immune-mediated mechanism participate in aneurysmal degeneration [13]. At least part of this may involve cell–cell interaction between lymphocytes and mononuclear phagocytes that promote cellular expression of matrix metalloproteinases. This pathway may have relevance for aneurysm disease and for atherosclerosis and other chronic immune-mediated or inflammatory conditions. The aortic wall mainly consists of elastin, collagen, and fibrillar matrix protein.

Loss of elastin and the relative increase in collagen account for the diminished compliance of aortic aneurysm wall and probably are responsible for the initial stage of aneurysmal dilatation, whereas the degradation of collagen is a prerequisite to aneurysmal rupture [25]. On the other hand, hemodynamic wall stress and shape of aneurysm are nonetheless likely to have a major influence on development and site of aneurysm rupture. Type III collagen is responsible for tensile strength of the aortic wall. Serum procollagen type III (PIIIP) levels were significantly higher in aortic aneurysm patients than in aortic occlusive disease patients, and furthermore they correlated positively with aortic diameter [26]. Our study clearly confirmed positive correlation between PIIIP level and aortic diameter (correlation = 0.561; P = 0.0239), indicating that markers of connective tissue metabolism including collagen turnover have a strong correlation with progressive aneurysm expansion.

This study demonstrated that patients with aortic aneurysm were in a chronic inflammatory condition and also in the hypercoagulation state before aortic aneurysmal repair because of higher cytokine levels, higher TAT level, and higher D-dimer levels preoperatively. Macrophage accumulation may be associated with increased plasma concentration of both fibrinogen and C-reactive protein, and these two markers of inflammation are thought to be early signs of atherosclerosis [27]. In terms of inflammation, however, we did not measure the high-sensitivity C-reactive protein in this series, which is under investigation in subgroup.

Macrophages as well as T lymphocytes gathered on the atherosclerotic area and cytokines produced by these T lymphocytes stimulated endothelial cell, smooth muscle cell and macrophages. Aortic aneurysm was closely linked to the atherosclerosis and in the status of coagulopathy.

Limitation of this study was of course the limited number of aortic aneurysm patients in the preoperative condition. However, using the statistical method, we were able to draw some important information. We did not measure possible relevant cytokines such as IL-1β, IL-4, IL-6 and so on, nor MMP-9 as an indicator of elastin degradation; however, we obtained some information on cytokine involvement and connective tissue metabolism. It is still open to question regarding a relationship between aortic aneurysm and atherosclerosis, because the severity of atherosclerosis did not always correlate with aneurysmal formation. This needs to be clarified in the near future.

Preoperative TAT, D-dimer, PIIIP, sIL-2R, and sICAM-1 level showed positive correlations with aortic aneurysmal size and aneurysmal volume, which have never been
reported except PIIIP with aneurysmal size. Although we can easily find aortic aneurysm by computed tomography, circulating markers such as IL-1β, IL-6, MMP-9, PIIIP [13], TAT, D-dimer, sIL-2R and sICAM-1 levels might be useful in the management of aortic aneurysm as a diagnostic screening tool and as a simple means for monitoring disease progression. Computed tomography together with these markers could help the follow-up.

Acknowledgements

This study was partially supported by Mitsui Life Social Welfare Foundation.

References


Appendix A. Conference discussion

Dr L. Eijssen (Amsterdam, The Netherlands): The question arises, of course, why the thrombin—antithrombin complex arises. There can be several reasons, but let’s start with the preoperative situation. Thrombin formation means that the coagulation cascade goes on at least to the level of II-A, yes?

Dr Nomura: Yes.

Dr Eijssen: Did you measure on your aneurysm, for example, thrombomodulin, because that is the defense system of the aneurysm against the thrombin formation at the aneurysm site?

Dr Nomura: Unfortunately we don’t measure those kinds of things, but, as you know, there are already a lot of papers concerning the preoperative coaguolopathy. So we have no data about what concerns you, but at least we can
find that there are atherosclerotic changes which develop some inflammation or which lead to beginning of the coagulation cascade somehow.

**Dr Eijsman**: Your thoughts in the direction of a general inflammatory process are very interesting, especially since you mentioned interleukins and things like that, but did you also take into account more general inflammatory markers like C-reactive protein, perhaps other ones, and also the anti-inflammatory interleukins?

**Dr Nomura**: About C-reactive protein: we measured it in the usual way, but we didn’t measure the high-sensitive C-reactive protein. In small series I did measure it, but it is a very, very small level. I didn’t count it in this study, but in the future maybe I can present some data about C-reactive protein. There are very interesting aspects concerning that protein and the atherosclerotic indices.

**Dr S. Martens** (Frankfurt, Germany): Just a question regarding the progression of disease and timing of the operation. These are very interesting data, but I doubt that the timing of the operation can be really based on this correlation, which is, in my opinion, not good enough. I think the timing for the operation should be based on aneurysm size and the risk of rupture, and I don’t really think that the correlation is strong enough to take this into account.

**Dr Nomura**: I agree, absolutely I agree with you. This is not a marker directly to indicate timing for the operation. Of course, operation timing is mostly decided by the CT scan. But if you see the patient and if you don’t have any chance to have the CT scan for that patient for a long time, and then at a later date the patient comes back, then you can have some small blood test. So this may be an indication to have the CT scan right at that time. Together with the CT scan, it must be considered.

**Dr Martens**: I think a patient with an expanding aneurysm should be followed by CT scans in, say, 1-year follow-up or so. This correlation is very impressive and for me surprising, but I think it is not strong enough to really base the operative timing on it. But they are very interesting data.

**Dr J. Firk** (Prague, Czech Republic): I would like to ask you about the localization of the aneurysms.

**Dr Nomura**: Ten of them were thoracic and two of them were thoracoabdominal, and the rest were abdominal aortic aneurysms so spreading all through.