Thrombosis

## Risk of acute coronary artery disease associated with functional thrombin activatable fibrinolysis inhibitor plasma level

**To our knowledge, there is little information about functional thrombin activatable fibrinolysis inhibitor (TAFI) levels and the risk of acute coronary artery disease (CAD). We investigated the risk of acute CAD related to plasma levels of functional TAFI. We found that functional TAFI levels in plasma (above 126%), increased the risk of acute CAD almost 4-fold.**

haematologica 2004; 89:880-881
(*http://www.haematologica.org/2004/7/880*)

Thrombin activatable fibrinolysis inhibitor (TAFI) leads to potent inhibition of t-PA-induced fibrinolysis.[1,2] Functional TAFI, described by Mosnier *et al.*,[2] is based on the activation of TAFI with thrombin-thrombomodulin and the measure of the TAFI activity generated. Although the precise relevance of TAFI in thromboembolic diseases is unclear, high plasma levels of TAFI may be related to thrombotic disorders.[3-5] Here we report the results of our investigation of the association of plasma levels of functional TAFI with the risk of acute CAD.

A total of 385 consecutive individuals under the age of 80 years were included in our case-control study; 174 cases were admitted to the Cardiology Unit with a diagnosis of acute CAD and 211 individuals (spouses and friends of patients) served as controls. Acute CAD was confirmed on the basis of definitive ischemia or necrosis of the myocardium. The control subjects had no previous history of thromboembolism and were matched for age and gender with patients. Informed consent was obtained from all participants. The interview included questions on personal and family history of cardiovascular diseases and the conventional cardiovascular risk factors. Blood samples were obtained from the antecubital vein at least six months after the acute thrombotic episode. Plasma functional TAFI was measured as described by Mosnier *et al.*[2] using an assay that measures TAFI activity after *in vitro* activation of pro-enzyme by thrombin-thrombomodulin complex; the result therefore represents the amount of activatable TAFI with hippuryl-arginine substrate, which corresponds to the amount of pro-enzyme. Statistical analyses were performed using conventional software. Values are expressed as mean ± standard deviation. Student's T-test was used to calculate the mean differences between groups. Odds ratios (OR) were calculated by conditional logistic-regression analysis. We used the 90$^{th}$ percentile to determine the cut-off values. The cut-off of functional TAFI levels used in order to calculate the OR in relation to the reference group was 126%. To determine the adjusted OR for different variables, we converted factor VIII plasma levels and fibrinogen levels into binary variables. Cut-offs for factor VIII and fibrinogen were 175% and 3.5% g/L, respectively. The basic characteristics of the sample population are given in Table 1. All of the major cardiovascular conventional risk factors were associated with a significantly increased risk of acute CAD. Hypertension, a family history of arterial disease and diabetes were the most relevant risk factors in patients with acute CAD.

Functional TAFI levels tended to be higher in patients with acute CAD [100.8% (range: 49-177)] than in controls [96.9% (range: 50-165)], but this difference did not reach statistical significance ($p$<0.07). The variation of functional TAFI levels in controls and in patients was not correlated with either sex or age. Only women younger than 30 years showed lower levels. No correlation among TAFI levels and conventional cardiovascular risk factors or hemostatic risk factors were found.

**Table 1. Basic characteristics of patients with acute CAD and the controls.**

| | *Acute CAD (n=174) N (% )* | *Controls (n=211) N (% )* | *Unadjusted OR (95%CI) (p value)* |
|---|---|---|---|
| Sex (F/M) | 54/120 | 91/120 | ns |
| Age (mean, range, yr) | 57 (21-78) | 56 (23-80) | ns |
| Smoking | 96 (55) | 78 (37) | 2.1 (1.4-3.1)* |
| Hypercholesterolemia | 92 (53) | 44 (21) | 4.3 (2.7-6.7)* |
| Family history of arterial disease | 84 (48) | 38 (18) | 4.3 (2.7-6.7)* |
| Hypertension | 92 (53) | 40 (19) | 4.8 (3.0-7.6)* |
| Morbid obesity | 19 (11) | 7 (3) | 3.6 (1.5-8.7)* |
| Alcohol intake | 11 (6) | 11 (5) | Ns |
| Diabetes mellitus | 34 (20) | 11 (5) | 4.4 (2.2-9.0)* |
| Factor VIII levels | 186.9 (74-526) | 159.4 (48-360) | $p$<0.01 |
| Fibrinogen (g/L) | 3.7 (1.9-6.8) | 3.5 (2.-7.8) | $p$<0.05 |
| Antiphospholipds antibodies | 16 (9) | 4 (2) | 5.2 (1.7-15.9)* |
| Functional TAFI <126% | 147 | 203 | 1° |
| Functional TAFI >126% | 27 | 8 | 4.7 (1.3-8.7)* |

**p<0.0005. ns: not significant; F: female; M: male; °Reference group.*

To determine the risk of acute CAD, we used the cut-off level of 126% for functional TAFI. Eight controls and 27 patients showed functional TAFI levels higher than 126%, and 203 controls and 147 patients showed functional TAFI levels lower than 126%. Unadjusted and adjusted OR were calculated using the subjects with functional TAFI levels less than 126% as the reference group. The unadjusted OR for functional TAFI levels was 4.0 (95% CI: 1.6-10.0) in acute CAD patients compared with controls. When we analyzed the risk adjusted by sex, age and other covariables such as hypercholesterolemia, family history of arterial disease, diabetes, antiphospholipids, fibrinogen and factor VIII levels, the adjusted OR was 3.5 (95% CI 1.3-8.7) for acute CAD patients with plasma functional TAFI levels greater than 126%.

In conclusion, we found that a high level of functional

TAFI in plasma confers a significant risk of acute CAD. Thus, functional TAFI plasma levels above the 126% cut-off increased the risk of acute CAD almost 4-fold. To our knowledge, this is the first case-control study that unequivocally establishes that high levels of functional TAFI are associated with an increased risk of acute CAD in patients under the age of 80 years. We hypothesize that high functional TAFI levels may represent a significant thrombotic biomarker for the risk of acute CAD. Knowledge of the pathophysiological role of functional TAFI should lead to a better understanding of the mechanism of thrombotic disease.

*Amparo Santamaría,* Antonio Martínez-Rubio, Montserrat Borrell, José Mateo, Rosa Ortín, Jordi Fontcuberta*

*Hemostasis and Thrombosis Unit, Haematology Department, *Cardiology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona, Barcelona, Spain*

*Aknowledgments. We are indebted to Professor WH Stone for his help in the revision and discussion of the manuscript.*

*Keywords: TAFI, arterial, thrombosis, coronary*

*Correspondence: Dr. Amparo Santamaría Ortiz, Department of Hematology, Hospital de la Santa Creu i Sant Pau, C/ Sant Antoni Mª Claret, 167, 08025-Barcelona, Spain. Phone: international +34.93l.2919193. Fax: international +34.93.2919192. E-mail: msantamaria@hsp.santpau.es*

## References

1. Bouma BN, Meijers JCM. Thrombin-activatable fibrinolysis inhibitor (TAFI, plasma procaroxypeptidase B, procarboxypeptidase R, procarboxypeptidase U). J Thromb Haemost 2003;1: 1566-74.
2. Mosnier LO, van der Borne PA, Meijers J, Bouma BN. Plasma TAFI levels influence the clot lysis time in healthy individuals in the presence of an intact intrinsic pathway of coagulation. Thromb Haemost 1998;80:829-35.
3. Juhan-Vague I, Morange PE, Aubert H, Henry M, Aillaud MF, Alessi MC, et al. Plasma thrombin-activatable fibrinolysis inhibitor antigen concentration and genotype in relation to myocardial infarction in the North and South of Europe. Arterioscler Thromb Vasc Biol 2002;22:867-73.
4. Morange PE, Juhan-Vahue I, Scarabin PY, Alessi MC Luc G, Arveiler D, et al. Association between TAFI antigen and Ala I 47Thr polymorphism of the TAFI gene and the angina pectoris incidence. (The PRIME study). Thromb Haemost 2003;89:554-60.
5. Brouwers GJ, Leebeek FG, Tanck MW, Wouterjukema J, Kluft C, de Maat MM. Association between thrombin-activatable fibrinolysis inhibitor (TAFI) and clinical outcome in patients with unstable angina pectoris. The APRAIS study. Thromb Haemost 2003;90:92-100.



Stem Cell Transplantation

### High-dose granulocyte colony-stimulating factor mobilizes a higher proportion of *early* CD34⁺CD33⁻ hemopoietic progenitors in children receiving treatment for solid tumors

**A relationship between dose of granulocyte colony-stimulating factor (G-CSF) and maturational stage of the progenitors mobilized in healthy adult donors has been suggested.[1] In this study we characterize the progenitors mobilized by 2 different dosages of G-CSF in children receiving autologous grafts after intensive treatment for solid tumors.**

haematologica 2004; 89:881-882
(*http://www.haematologica.org/2004/7/881)*

From 1997 to 2001, 55 children received an autologous peripheral blood progenitor cells (PBPC) transplant as consolidation treatment for solid tumors at The Royal Marsden Hospital. Indications for transplant were: neuroblastoma (27 cases), rhabdomyosarcoma (8), Hodgkin's disease (7), Wilm's tumor (4), non-Hodgkin's lymphoma (2), Ewing's sarcoma (4), germ cell tumor (2) and synovial sarcoma (1). Data on the mobilization and harvest procedures were available in 51/55 cases (31 boys, 20 girls, median age 6.0±4.4 years). All children received G-CSF (5 μg/Kg in 35 cases and 10 μg/Kg in 16 cases) for four consecutive days. The first harvest session was performed on the 5th day. If an insufficient number of CD34⁺ cells was harvested (<2.5x10⁶ CD34⁺ cells/Kg), the patient received a 5th dose of G-CSF on that day and a second harvest session was performed on the 6th day. Overall, a second harvest was performed in 45 cases. In addition, 24 patients received *priming* with cyclophosphamide (1.5 g/m²) prior to mobilization with G-CSF. The average time from the last course of chemotherapy to the first harvest session was 28.3±23.9 days.

Conditioning regimens included melphalan (33 cases), busulphan plus melphalan (10), thiotepa plus etoposide (2), carboplatin alone (9), and carboplatin plus melphalan (1).

Endpoints for this study were: numbers of CD34⁺, CD34⁺CD33⁺ and CD34⁺CD33⁻ cells harvested, time to neutrophil and platelet engraftment and influence of harvest timing and cyclophosphamide priming on the maturation stage of these progenitors. High doses of G-CSF appear to mobilize a higher proportion of *early* CD34⁺CD33⁻ progenitors.

The most relevant data on the qualitative contents of harvests are shown in Table 1. There were no significant differences in overall number of CD34⁺ or CD34⁺CD33- cells harvested after mobilization with either 5 or 10 μg/Kg of G-CSF. However, the percentage of CD34⁺CD33⁻ cells within the CD34⁺ population was significantly ($p$<0.05) higher in patients receiving 10 mg/Kg of G-CSF. A similar dose-dependent effect has been reported in healthy adult donors.[1,2] A possible explanation is that high doses of G-CSF

**Table 1. Most relevant results of the mobilization/harvest procedures according to the dosage of G-CSF. All values are expressed as number of cells×10⁶ per Kilogram body weight. In the last column, values are expressed as percentages. 1st: first harvest; 2nd: second harvest; total: first plus second harvests.**

| | *CD34⁺* | | | *CD34⁺CD33⁻* | | | *% CD33⁻ within overall CD34⁺* | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | total | 1st | 2nd | total | 1st | 2nd | total |
| 5 mg/Kg | 4.9±8.0 | 4.4±8.3 | 8.6±14.8 | 1.3±1.4 | 1.6±1.7 | 2.7±2.8 | 47.6±30.4 | 57.9±26 | 50.9±27.2 |
| 10 mg/Kg | 2.3±2.2 | 2.0±1.4 | 4.2±3.5 | 2.2±2.1 | 1.7±1.4 | 3.7±3.5 | 73.8±23.4 | 82.8±16 | 74.3±23.5 |