Elevated Whole-Blood Tissue Factor Procoagulant Activity as a Marker of Restenosis After Percutaneous Transluminal Coronary Angioplasty and Stent Implantation

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Background—Experimental data suggest that tissue factor (TF) may induce neointimal hyperplasia after arterial injury. In this study, we investigated the hypothesis that elevated levels of TF in the circulation contribute to the development of restenosis after percutaneous transluminal coronary angioplasty (PTCA) or stent implantation.

Methods and Results—Whole-blood TF procoagulant activity (TF-PCA) was measured using a previously described assay before, at 3 hours after, and at 24 hours after the intervention in 61 patients with stable angina undergoing PTCA (n=20) or stent implantation (n=41). Coronary angiography was performed 4 to 6 months after the intervention, and luminal narrowing ≥50% was defined as restenosis. Whole-blood TF-PCA levels did not correlate with intracellular monocyte tumor necrosis factor-α expression, a marker of activation of these cells. Baseline levels and time course of whole-blood TF-PCA after the intervention were compared in patients who did or did not subsequently develop restenosis. Whole-blood TF-PCA levels did not change significantly in the 24 hours after either intervention. However, in both the PTCA and stent groups, initial TF-PCA was significantly higher in patients who subsequently developed restenosis (P=0.018 and 0.039 compared with those who did not develop restenosis for PTCA and stent groups, respectively).

Conclusions—Higher baseline values of whole-blood TF-PCA may be a predictor of restenosis after PTCA and stent implantation. **(*Circulation*. 2003;108:1581-1584.)**

Key Words: angina ■ angioplasty ■ stents ■ restenosis

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Percutaneous transluminal coronary angioplasty (PTCA) and stent implantation are commonly used procedures in the management of coronary artery stenosis; however, restenosis still remains their most important limitation. Although restenosis after PTCA mainly results from negative (constrictive) geometric remodeling, neointimal hyperplasia is the dominant mechanism after stent implantation.[1]

In normal blood vessels, tissue factor (TF) is predominantly localized to fibroblasts of the adventitia and is variably present in the outer layer of medial smooth muscle cells. However, in pathological arteries, the intima, neointima, and atherosclerotic plaque have all been demonstrated to contain abundant active TF, which may be found in both cellular (mainly in smooth muscle cells and foam cells) and extracellular locations.[2,3] Apart from its role in the initiation of coagulation, TF can promote the release of certain growth factors, such as vascular endothelial growth factor and migration of vascular smooth muscle cells, both of which are believed to be important steps in the genesis of neointimal hyperplasia.[4,5] Moreover, it has been demonstrated that tissue factor pathway inhibitor can prevent acute thrombosis and intimal hyperplasia after angioplasty in experimental models.[6] For these reasons, it may be expected that increased TF expression may cause development of restenosis by inducing neointimal hyperplasia after PTCA or stent implantation.

Although upregulation of TF synthesis in cells located within the plaque (for example, smooth muscle cells) may be important in restenosis, it is also plausible that TF in the damaged vessel wall may originate from an intravascular pool of circulating TF that has recently been described by us[7] and by others.[8] In this study, we therefore evaluated the possibility that elevated levels of circulating whole-blood TF procoagulant activity (PCA), present at the time of the intervention, may be a marker for restenosis risk after PTCA or stent implantation.

Methods

Patients

Sixty-one consecutive patients with a diagnosis of stable angina pectoris who underwent elective coronary angioplasty were enrolled in the study. Twenty patients underwent successful PTCA and constituted the PTCA group. Successful PTCA was defined as residual stenosis of ≤50% and the presence of TIMI 3 flow.

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Forty-one patients underwent stent implantation because of either residual stenosis (>50%) or flow-compromising dissection after PTCA, and these patients constituted the stent group. All patients were administered 300 mg/d acetylsalicylic acid. Ticlopidine was started at a dose of 250 mg twice daily 5 days before the intervention and continued for 4 weeks in patients who underwent stent implantation.

Coronary Angioplasty and Follow-Up

Coronary angioplasty was performed using the standard Judkins technique via the femoral approach. An 8F-guiding catheter was used for the procedure. At the beginning of the intervention, all patients were intravenously administered 10 000 U of unfractionated heparin. PTCA and stent implantation were performed at 8- and 12-atmosphere balloon inflation pressure, respectively. All patients underwent follow-up coronary angiography at 4 to 6 months after the intervention to detect angiographic restenosis, and ≥50% stenosis at the site of the intervention was considered indicative of restenosis.

Assay of TF-PCA Levels

Blood samples were drawn from all patients just before and at 3 and 24 hours after the coronary intervention. Blood was collected into Vacutainer tubes containing EDTA using a 21-gauge needle. Anticoagulated whole-blood samples were immediately transferred to polypropylene tubes and frozen at −70°C. Whole-blood TF-PCA was then measured exactly as previously described.[7]

Flow Cytometry of Monocytes

Three-color immunofluorescence was performed to quantify monocyte intracellular tumor necrosis factor (TNF)-α expression as a measure of cellular activation.[9] The following monoclonal antibodies or appropriate isotype-matched irrelevant monoclonal antibodies (all from Immunotech Coulter, France) were used: PE-conjugated TNF-α, PC5-conjugated CD45, and FITC-conjugated CD14. Antibodies were incubated with the blood sample for 20 minutes at room temperature. Notably, intracellular TNF-α labeling was performed after a washing step and cell permeabilization. Cells were then fixed in 0.5% formaldehyde and analyzed in a FacSort flow cytometer (BDIS) using Cell Quest software. A minimum of 60 000 events was acquired. Monocytes were gated according to CD45-PC5 versus side scatter and CD14-FITC intensity. Isotypic controls were used to set a marker for calculating the percentage of cells that expressed TNF-α.

Statistical Analysis

Whole-blood TF-PCA seems to have a substantially skewed distribution in the healthy population[7] and in the study groups. For this reason, logarithmic transformation of the raw data for this variable was performed. All TF-PCA data were then presented as geometric means with 95% CIs. All other data are presented as arithmetic mean±SD. Categorical variables were compared by χ^2 analysis, and continuous variables were compared by Student *t* test. Comparisons of time course and levels of TF-PCA in patients with and without restenosis were made by ANOVA for repeated measures. Results of this analysis are expressed with 3 *P* values; the first refers to TF-PCA at each time point for the 2 groups, whereas the second refers to the change in TF-PCA across the measured time points (baseline, 3rd hour, and 24th hour). The third *P* value is relevant to the analysis of whether there exists any interaction between restenosis and time. The means of 6 measurements (2 groups at 3 time points) are included in this analysis. Correlation between whole-blood TF-PCA and monocyte TNF-α expression was calculated using the Pearson correlation method. $P<0.05$ was considered to be statistically significant. Statistical analysis was performed with SPSS (version 10.0).

Results

Baseline Characteristics

Clinical characteristics of the patients are summarized in Table 1. Restenosis was demonstrated in 20 (33%) patients, 9 of whom were in the PTCA group (restenosis rate, 45%), with the remaining 11 in the stent group (restenosis rate, 27%).

There were no differences in target vessel diameter, frequency of type B or C lesion, or diabetes mellitus, all of which are known to be associated with high rate of restenosis, between patients who did or did not develop restenosis (Table 2).

TABLE 1. Baseline Characteristics of Study Subjects

	Subjects (n=61)
Age, mean±SD	57±10
Male/female	49/12
Diabetes mellitus	15 (25%)
Hypertension	28 (46%)
Smoking	32 (53%)
Total cholesterol >160 mg/dL	44 (72%)

TF-PCA Levels in the Whole Group (PTCA and Stent Groups)

In a previous study, we demonstrated that whole-blood TF-PCA levels in healthy individuals (n=65) ranged from 1.6 to 84.0 U/mL, with geometric mean values of approximately 11 to 12 U/mL.[7] Therefore, TF-PCA levels were significantly higher in this group of subjects with stable angina (23.27±1.33 U/mL [geometric mean±95% CI]; n=61). Within the overall group, we found that TF-PCA levels were significantly higher in subjects who subsequently developed restenosis. Specifically, TF-PCA values for patients who did versus those who did not develop restenosis were (respectively) as follows: at baseline, 29.40±8.87 versus 22.67±3.66 U/mL; at 3 hours, 30.29±4.74 versus 24.15±3.38 U/mL; and at 24 hours, 29.48±5.19 versus 23.87±2.93 U/mL ($P=0.006$). Somewhat contrary to our expectations, TF-PCA levels did not change significantly after the intervention either in patients who were or were not destined to develop restenosis ($P=0.651$) (Figure). Furthermore, there was no interaction between restenosis and time ($P=0.4$).

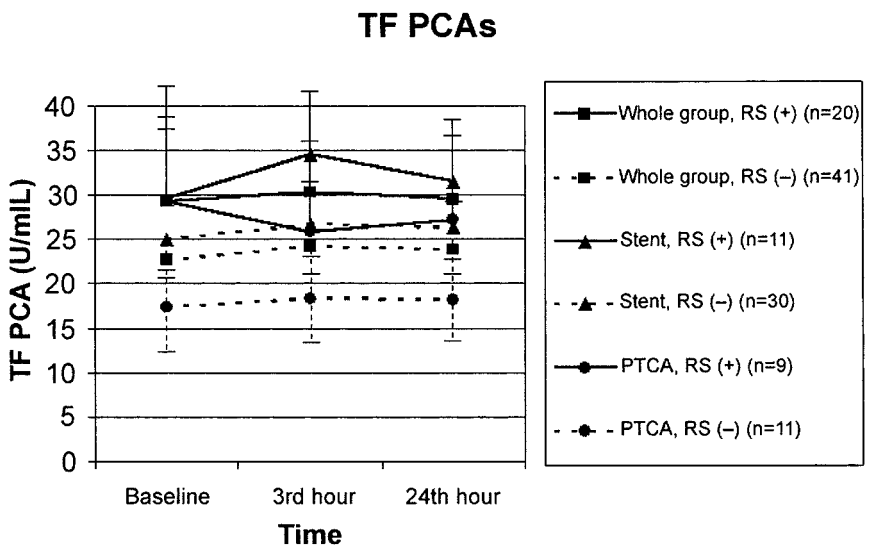
TF-PCA Levels in the Stent Group

Within this subgroup, TF-PCA levels were significantly higher in patients who proceeded to develop restenosis (baseline values for the patients with versus without subsequent restenosis were 29.50±9.61 versus 25.01±4.48 U/mL; at 3 hours, 34.48±6.60 versus 26.65±4.21 U/mL; and at 24 hours, 31.51±7.40 versus 26.31±3.63 U/mL; $P=0.039$). TF-PCA levels did not change significantly after stent implantation either in patients with or without subsequent

TABLE 2. Comparison of Selected Angiographic and Clinical Parameters Determining Restenosis in Subjects With and Without Subsequent Restenosis

	Restenosis (+) (n=20)	Restenosis (−) (n=41)	*P*
Balloon diameter, mm	2.7±0.3	2.9±0.4	NS
Type B and C lesion (%)	5 (25)	7 (17)	NS
Diabetes mellitus (%)	6 (30)	9 (22)	NS

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TF-PCA in the whole group (angioplasty [PTCA] and stent groups combined), stent group alone, and PTCA group alone. TF-PCA levels were significantly higher in patients who subsequently developed restenosis (P=0.006, 0.039, and 0.018, respectively) and did not change significantly after the intervention in any of the 3 groups (P=0.651, 0.456, and 0.456, respectively). There was no interaction between 2 factors (restenosis and time) in any of the groups (P=0.4, 0.801, and 0.470, respectively). The data are expressed as geometric means with 95% CIs.

restenosis (P=0.456) (Figure). There was no interaction between restenosis and time (P=0.801).

TF-PCA Levels in the PTCA Group

Also in this subgroup, TF-PCA levels were significantly higher in patients who later developed restenosis (at baseline, 29.27±16.55 versus 17.26±5.48 U/mL; at 3 hours, 25.77±6.46 versus 18.36±4.02 U/mL; and at 24 hours, 27.14±7.46 versus 18.22±3.05 U/mL; P=0.018). Similar to the group of patients who underwent stenting, TF-PCA levels did not significantly change after the PTCA (P=0.456) (Figure). There was no interaction between restenosis and time (P=0.470).

Relationship Between TF-PCA and Monocyte Expression of Intracellular TNF-α

Monocyte activation has been described to be a risk factor for restenosis after PTCA.[10] Therefore, to determine whether whole-blood TF-PCA is simply a surrogate marker of monocyte activation, we determined whether whole-blood TF-PCA levels correlated with intracellular TNF-α expression in CD14(+) cells in 12 subjects with stable angina pectoris and 8 healthy individuals. No significant correlation was noted (r=−0.219; P=0.49), indicating that whole-blood TF-PCA levels are independent of the degree of monocyte activation.

Discussion

This study was conducted to test a possible association between blood-borne TF-PCA measured at the time of coronary revascularization and the subsequent development of restenosis after PTCA or stent implantation. Mizuno et al[11] demonstrated that elevated levels of TF antigen in the coronary sinus blood after PTCA in patients with ischemic heart disease (both stable and unstable angina) correlated with late restenosis. Our study extends this observation by showing that functionally active TF measured in the peripheral blood, which is apparently not simply a surrogate marker of monocyte activation, is also a prognostic factor for late restenosis. Elevated monocyte TF-PCA in the peripheral blood in subjects with ischemic heart disease has previously been documented, although not in the context of examining TF as a prognostic factor for late restenosis. Specifically, whereas monocyte TF levels are increased in patients with acute myocardial infarction and unstable angina, they are generally normal in patients with stable angina.[12–14] The cause of the increased monocyte TF activity in these acute coronary artery syndromes is unknown.

At first glance, our finding of increased whole-blood TF-PCA in patients with stable angina is inconsistent with the existing literature. However, because our assay measures the sum of all cell- and microparticle-associated TF, one possibility is that we are detecting elevated intravascular TF expression in nonmonocytic cells or in microparticles in this patient population, which has not previously been assessed. Giesen et al[8] demonstrated that cell-free plasma from healthy subjects contains circulating TF that may be deposited on a growing thrombus, additionally supporting the possibility that microparticle-associated TF accounts for some fraction of the activity measured by our assay. Studies that are expected to clarify the relative proportions of active TF in monocytes, platelets, and microparticles in health and in disease states are presently underway in our laboratory.

We did not observe any change in whole-blood TF-PCA after PTCA or stenting, consistent with previous studies showing that monocyte TF activity also did not increase after stenting in patients with stable angina.[15] It should be noted that because we measured TF in the systemic circulation, a transient increase in TF in coronary sinus blood may have been overlooked. Furthermore, because the whole-blood assay detects total cell and microparticle-associated TF, we cannot exclude the possibility that a transfer of TF occurred between one circulating pool and another, for example between monocytes and activated platelets.[16] Nonetheless, our findings suggest that it is the initial level of TF-PCA in the peripheral blood, rather than any acute change in TF-PCA level after the intervention, that determines the future risk of restenosis.

Because the mechanism of restenosis varies with the procedure, we evaluated our PTCA and stent groups separately. However, we found the same relationship between

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intravascular TF expression and risk of restenosis in both groups (Figure).

Although TF promoting thrombosis at a site of vessel injury may originate from the vessel wall,[17] an alternative (and not mutually exclusive) possibility is that TF accumulation at the site of a growing thrombus may originate from a circulating intravascular pool. This activity may originate from blood-borne TF-bearing microparticles, probably derived from leukocytes, that may be transferred to platelets.[8,16,18] In support of this hypothesis, extracellular TF (likely blood-borne) could be detected on the intimal surface of a balloon-injured rat aorta.[19] Therefore, it is possible that TF present in circulating whole blood may be a potential source of TF accumulation in the vessel wall (with subsequent neointimal hyperplasia) in subjects undergoing interventional procedures in the coronary vasculature. If this is indeed the case, the results of this study suggest that the levels present in blood may be a determinant of the future risk of restenosis. However, although our data do demonstrate an association between increased whole-blood TF-PCA at the time of the acute intervention with subsequent restenosis, they do not establish causality. Indeed, as noted above, it is quite possible that a significant fraction of the TF activity in our whole-blood assay may have originated from nonmonocyte sources, such as the atherosclerotic plaque itself, which is known to be rich in microparticle-associated TF.[3] In that case, higher levels of TF-PCA may merely represent a surrogate marker for more extensive atherosclerotic disease burden, which may be the true risk factor for development of late restenosis.

In conclusion, this study demonstrates that levels of whole-blood TF-PCA at the time of PTCA or stent implantation may be a predictive marker for the development of restenosis. We postulate that TF originating from the intravascular compartment may be deposited in the injured vessel wall after PTCA or stent implantation, in which location it may contribute to the process of restenosis.

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