Clinical and Population Studies

Elevated Soluble fms-Like Tyrosine Kinase-1 Levels in Acute Coronary Occlusion


Objective—Early recognition of an acute coronary occlusion (ACO) improves clinical outcomes. Soluble fms-like tyrosine kinase-1 (sFLT1) is an endothelium-derived protein induced by hypoxia. We tested whether sFLT1 levels are elevated in ACO.

Methods and Results—Serum sFLT1 levels were measured by enzyme-linked immunosorbent assay in patients with ST-segment elevations and angiographically confirmed ACO, unstable angina/non ST-segment elevation myocardial infarction, and 2 control groups. To further explore sFLT1 release, a mouse model of ACO and in vitro human coronary artery endothelial cell injury were used. sFLT1 levels were increased in ACO compared with unstable angina/non-ST-elevation myocardial infarction, catheterized controls, or healthy volunteers (200.7±15.5 versus 70.7±44.0 versus 10.2±4.0 versus 11.7±1.7 pg/mL respectively, P<0.001 versus ACO). At presentation, all ACO patients had elevated sFLT1 levels (>15 pg/mL, 99th percentile in controls), whereas 57% had levels of the MB isoform of creatine kinase levels >10 ng/mL (P<0.01) and 85% had ultrasensitive troponin I levels >0.5 ng/mL (P<0.05). Within 60 minutes after symptom onset, sFLT1 was more sensitive than the MB isoform of creatine kinase or ultrasensitive troponin I for ACO (100% versus 20% versus 20% respectively; P=0.01 for each). Within 60 minutes of ACO in mice, sFLT1 levels were elevated. Hypoxia and thrombin increased sFLT1 levels within 15 minutes in human coronary artery endothelial cells.

Conclusion—sFLT1 levels may be an early indicator of endothelial hypoxia in ACO. (Arterioscler Thromb Vasc Biol. 2011;31:443-450.)

Key Words: acute coronary syndromes ▪ coronary artery disease ▪ coronary heart disease ▪ endothelium ▪ thrombosis

Acute myocardial infarction (AMI) is a major cause of morbidity and mortality worldwide. During acute coronary occlusion (ACO), a delay in diagnosis beyond 2 hours after symptom onset reduces the benefits of reperfusion therapy by ≈50%. In most cases, ACO is clinically diagnosed based on electrocardiographic (ECG) evidence of ST-segment elevation (STE) and symptoms consistent with myocardial ischemia. However, STE alone is not specific to coronary occlusion. Conversely, patients with ACO may have no evidence of STE. The difficulty of timely identification of ACO is increased further because currently used biomarkers for the diagnosis of AMI, such as the MB isoform of creatine (CK) kinase (CK-MB), myoglobin, and standard or ultrasensitive troponin require cardiomyocyte damage and may not be specific to coronary occlusion. Therefore, relying on the ECG or biomarkers of myocyte necrosis to distinguish ACO from noncardiac causes of chest pain may be accompanied by both false-positive and negative results.

Human vascular endothelial growth factor receptor-1, also known as fms-like tyrosine kinase-1 (FLT1), was originally identified in human placental tissue and later characterized as a receptor for the angiogenic factors vascular endothelial growth factor-A, vascular endothelial growth factor-B, and placental growth factor. FLT1 is highly expressed by endothelial cells, vascular smooth muscle cells, and monocytes. A truncated form of FLT1 that circulates in the blood is known as soluble FLT1 (sFLT1). Several investigators have reported increased circulating sFLT1 levels in acute coronary syndromes (ACSs), preeclampsia, and sepsis. Hypoxia increases sFLT1 expression in placental explants, peripheral blood monocytes, and aortic vascular rings. Hypoxia induces sFLT1 expression by either generation of a splice variant of FLT1 or by proteolytic cleavage of the FLT1 ectodomain. Given the well-noted hypoxia that occurs during ACO, we postulated that circulating levels of sFLT1 may be elevated in ACO. To explore this hypothesis, we prospectively measured sFLT1 serum levels in patients referred for emergent percutaneous coronary revascularization with active chest pain, STE, and angiographically confirmed ACO. We identified significantly elevated sFLT1 serum...
levels during the acute phase of coronary artery occlusion and further studied the timing of sFLT1 release in a mouse model of left coronary occlusion and in human coronary artery endothelial cells (HCAECs) in vitro.

Methods

Patient Population

In this prospective, observational study, we enrolled 60 patients presenting to Tufts Medical Center with either STE (n = 30) or unstable angina/non-ST-elevation myocardial infarction (UA/NSTEMI; n = 30) within 12 hours of symptom onset who were referred for emergent percutaneous coronary angiography and intervention. All STE patients who were eligible for revascularization were enrolled to arrival to the catheterization laboratory and angiographically confirmed ACO, which was defined as thrombolysis in myocardial infarction flow (TIMI) ≤1. Subjects with UA/NSTEMI were defined by American College of Cardiology/American Heart Association guidelines2 and were required to have had chest pain within 12 hours of catheterization. Other inclusion criteria were as follows: age ≥18 years and <90 years, sinus rhythm, and ability to provide informed consent. Patients with hemodynamic or clinical instability, perceived interference with standard clinical care of patients, unsuccessful reperfusion, pregnancy, active or remote cancer, renal failure (estimated glomerular filtration rate ≤30), or liver transaminases ≥2 times the upper limit of normal were excluded. All eligible STE patients who agreed to enroll had blood sampled at the time of arterial sheath insertion and at 24 and 48 hours after revascularization.

Two control groups were enrolled. First, 25 healthy volunteers with no prior medical history and currently taking no medications as determined by a screening questionnaire were studied. Second, 20 subjects referred for elective cardiac catheterization without evidence of an ACS (non-ACS), documented heart failure, or angiographic evidence of clinically significant coronary obstruction were studied. Control subjects were required to be between 21 and 80 years of age. For healthy volunteers, serum samples were obtained as a 1-time laboratory draw in our clinical research center. For elective catheterization controls, serum samples were obtained after femoral artery sheath insertion.

All physicians were blinded to the results of the serum analysis. Patients received standard clinical care for AMI during their index hospitalization, including serial ECGs and cardiac biomarkers, and pharmacological therapy including aspirin, clopidogrel, anticoagulation, β-blockade, and angiotensin-converting enzyme inhibition as clinically indicated. The institutional review board of Tufts Medical Center approved this study, and all patients provided written informed consent. Patients with hemodynamic or clinical instability, perceived interference with standard clinical care of patients, unsuccessful reperfusion, pregnancy, active or remote cancer, renal failure (estimated glomerular filtration rate ≤30), or liver transaminases ≥2 times the upper limit of normal were excluded. All eligible STE patients who agreed to enroll had blood sampled at the time of arterial sheath insertion and at 24 and 48 hours after revascularization.

Real-Time Quantitative Polymerase Chain Reaction

RNA from harvested cultured cells was extracted using Trizol reagent (Invitrogen, Carlsbad, Calif). Real-time quantitative polymerase chain reaction was performed with a 7900HT sequence detection system (Applied Biosystems, Foster City, Calif). Triplicate samples were subjected to reverse transcription and real-time polymerase chain reaction with TaqMan One-Step RT-PCR Master Mix reagents with gene-specific primers and probes designed by Premier Express software (Applied Biosystems) (human sFLT: forward primer, AGGGAGACACTGCGGCA; reverse primer, ATGAGTCC-TTTAATGTTGTGAC; HIF-1α: forward primer, CGTCCCTCGATCGTTGTC; reverse primer, TCAGTGTCGACAGTGTTAGT). Target gene expression was normalized to total RNA content by quantification of GAPDH gene expression.

Biomarker Assays

For sFLT1 and myoglobin ELISA, blood samples were collected using serum separator tubes and allowed to clot for 30 minutes before centrifugation at 2000 g for 15 minutes. Serum samples were immediately stored at −80°C. sFLT1 and myoglobin levels were measured in duplicate for each serum sample using commercially available quantitative sandwich ELISA kits (sFLT1: R&D Systems; myoglobin: Calbiotech, Inc) according to the manufacturers’ instructions. Time to completion of the human sFLT1 ELISA was 4.5 hours. The total CK, CK-MB, and ultra-sensitive troponin I (t-tropho-I) levels were measured by the central clinical laboratory using the Advia Centaur Tn1 Ultra Immunoassay System (Siemens). For mouse sFLT1 analysis, serum sFLT1 levels were measured using a mouse Soluble FLT1 ELISA kit (R&D Systems). For in vitro sFLT1 analysis, conditioned media from incubated HCAECs was isolated, spun at 10 000 rpm for 5 minutes to remove cellular debris, and then analyzed using a human Soluble FLT1 ELISA kit (R&D Systems). All biomarker assays were performed by blinded observers. The intraassay and interassay coefficients of variation for the sFLT1 assays were 3% and 6%, respectively.

Statistical Analysis

Data are expressed as means±SE. Normality of distribution was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests and Q-Q plots. Pairwise comparisons were made using analysis of variance (ANOVA) for continuous variables and the χ²/Fisher’s exact tests for categorical values. Post hoc comparisons were made with the Scheffe method where appropriate. Analysis of covariance was used to adjust for potential confounders where appropriate. ANOVA with repeated measures examined change in outcome variables over time. When a significant main effect was detected, appropriate post hoc comparisons were made. Pearson’s correlation coefficients assessed relationships between variables of interest. Stepwise multiple regression analysis examined correlates of sFLT1 in our cohort with ACO. Variables entered into the model included cardiovascular risk factors (age, gender, presence/absence of hypertension, diabetes, hyperlipidemia, family history, smoking status) and history of coronary artery disease. The quality-of-fit of regression models was checked by the Hosmer-Lemeshow test. Area under the receiver operating characteristic curve was examined to determine the predictive power of sFLT1 for detecting presence of ACO. For in vitro data, 2-tailed t tests and 1-way ANOVA with a Bonferroni correction for intergroup comparisons were used. All statistical analyses were performed using SigmaStat Version 3.1 (Systat Software, Inc) and Statistical Package for the Social Sciences (SPSS, version 16.0.1, SPSS, Inc, Chicago, Ill). P<0.05 denotes significant differences.
sFLT1 Expression in ACO

The baseline characteristics of the overall study groups are provided in Table 1. Groups did not differ in age, gender, or race. Clinical characteristics of ACO, UA/NSTEMI, and non-ACS patients are presented in Table 2. Among the 30 patients presenting with ACO, intracoronary thrombus was evident by angiography in 55%, whereas TIMI 0 or 1 flow was observed in all subjects. Mean Killip classification score (reference) for the ACO group was 1.7±0.9. Unfractionated heparin and eptifibatide infusions were administered in 27 of 30 patients (90%), with bivalirudin used as the primary anticoagulant in the remaining 3 patients (10%). Among the 30 patients presenting with ACO, intracoronary thrombus was observed in all subjects. Mean Killip classification score (reference) for the ACO group was 1.7±0.9. Unfractionated heparin and eptifibatide infusions were administered in 27 of 30 patients (90%), with bivalirudin used as the primary anticoagulant in the remaining 3 patients (10%).

sFLT1 levels were most significantly elevated in subjects with ACO compared with UA/NSTEMI, non-ACS controls, or healthy volunteers (200.7±15 pg/mL versus 70.7±43 versus 10.2±4 versus 11.7±1.7, respectively, P<0.001 for each group versus ACO; Figure 1A). According to our univariate analysis, there was a significant main effect for elevated sFLT1 levels in ACO compared with the other 3 groups (P<0.001, F-statistic=110.4). Posthoc comparisons using the Scheffe method revealed that ACO patients had higher values compared with all other groups, including UA/NSTEMI (P<0.001). UA/NSTEMI patients also had higher values compared with both healthy controls and non-ACS patients (P=1.000). Observed power for this 4-group comparison was high (100%), with a partial estimated time of arrival of 0.77. Group differences in sFLT1 remained after covarying for demographic and medication differences between controls and study subjects (P<0.05).

Results

At presentation, 100% of ACO patients had elevated sFLT1 levels greater than the 99th percentile of values measured in normal healthy controls (>15 pg/mL), whereas only 57% (P<0.01 versus sFLT1) and 85% (P=0.03 versus sFLT1) had significantly elevated levels of CK-MB or us-Tn-I (>10 and >0.05 ng/mL, respectively; both are previously established clinical cut points; Figure 1B). Using a threshold sFLT1 value of 15 pg/mL, the area under the receiver operating characteristic curve for sFLT1 discriminating ACO from healthy controls was 1.00 (P<0.001). Among ACO patients, admission sFLT1 levels inversely correlated with peak CK (r = −0.510, P<0.01), peak CK-MB (r = −0.375, P<0.05), and echocardiographic left ventricular ejection fraction (r = −0.388, P<0.05). Among UA/NSTEMI patients, admission sFLT1 levels did not correlate with TIMI risk score or TIMI flow.

As a first step toward understanding the potential utility of sFLT1 for identifying ACO, we used the time from symptom onset as a clinical marker for the onset of coronary occlusion. Across all time points after symptom onset, sFLT1 levels were significantly elevated above the 99th percentile of normal values (P<0.001) (Figure 2A). sFLT1 levels were significantly higher in subjects who presented within 60 minutes of symptom onset compared with patients who presented more than 360 minutes after symptom onset (P<0.05). In contrast, levels of CK-MB and us-Tn-I were not
significantly increased above normal values (CK-MB >10 and us-Tn-I >0.05 ng/mL) in subjects presenting within 120 minutes after symptom onset. CK-MB and us-Tn-I levels were higher at >360 minutes from chest pain onset compared with all other time points (P<0.05). Across all time points following chest pain onset, sFLT1 levels exhibited 100% sensitivity for diagnosing an ACO (Figure 2B). In contrast, mean values of CK-MB and us-Tn-I were slightly increased above normal values (CK-MB >10 and us-Tn-I >0.05 ng/mL), but these values did not reach statistical significance in subjects presenting within 120 minutes after symptom onset. The proportion of subjects presenting within 60 minutes who had positive sFLT1 levels (100%) was higher than the same individuals with positive CK-MB (20%; P<0.001) or us-Tn-I (20%; P=0.01) levels. In subjects presenting 60 to 120 minutes after symptom onset, the sensitivity of sFLT1 levels remained higher than CK-MB (100% versus 17%, respectively, P<0.01). No statistically significant difference was observed between the sensitivity of sFLT1 and us-Tn-I (100% versus 83%) at 60 to 120 minutes after symptom onset. These data support that sFLT1 may have clinical utility as a biomarker of ACO, especially within the first 60 to 120 minutes after symptom onset.

Stepwise multiple regression analysis showed that cardiovascular risk factors were not significant correlates of sFLT1 levels in patients with ACO, because none of the selected variables (age, gender, age, gender, presence/absence of hypertension, diabetes, hyperlipidemia, family history, smoking status, coronary artery disease) entered into the linear model (P>0.05). Moreover, sFLT1 values at the time of presentation were not significantly influenced by the absence or presence of cardiovascular risk factors. There was no correlation between sFLT1 levels at the time of presentation, indices of renal function, left ventricular ejection fraction on admission, or peak CK-MB in patients with ACO (P>0.05).

According to stepwise multiple regression, hypertension was a significant predictor of sFLT1 levels when patients with ACO and UA/NSTEMI were combined for analysis (standardized β=−0.35; R²=0.12, P=0.008). sFLT1 levels were significantly lower in patients with hypertension compared patients without hypertension (118.3±88.7 versus 194.8±90.5 pg/mL, P<0.05). In a separate model, medication history was examined. According to stepwise multiple regression, the only significant medication that predicted sFLT1 levels were β blockers (standardized β=−0.54; R²=0.29, P<0.001). After adjusting for β blocker use with blockwise multiple regression, hypertension was no longer a predictor of sFLT1. Moreover, covarying for β blocker use abolished group differences in sFLT1 between hypertensives versus normotensives (P>0.05). Thus the lower sFLT1 levels seen in patients with hypertension appear to be driven by their significantly higher use of β blockers.

sFLT1 levels were highest at time of presentation and decreased significantly at both 24 and 48 hours following restoration of coronary artery patency by percutaneous coro-
In contrast to the reduction in sFLT1 levels following revascularization, myocyte-based markers increased at 24 hours postrevascularization after restoration of coronary artery patency. For example, CK-MB values at 24 hours were higher than values at the time of presentation (P < 0.05). Similarly, us-Tn-I values were significantly higher at 24 hours compared with the values at presentation (P < 0.05).

**sFLT1 Release in Coronary Occlusion**

To study the timing of sFLT1 release after coronary occlusion, healthy adult male mice underwent left coronary ligation followed by serum sampling at various time points. Serum sFLT1 levels were significantly increased within 30 minutes of coronary occlusion as compared with sham-operated controls (Figure 4; ANOVA P < 0.01). These findings support our observation that circulating sFLT1 levels are elevated during the acute phase coronary occlusion.

**sFLT1 Expression in Coronary Endothelial Cells**

To explore the timing of sFLT1 expression in vitro, hypoxic HCAECs and HVSMCs were independently studied in the presence and absence of exogenous thrombin. sFLT1 protein detected in the conditioned media of hypoxic HCAECs by ELISA increased by 60 minutes of exposure (ANOVA P < 0.01). Thrombin alone increased sFLT1 levels by 60 minutes in HCAECs; however, levels were significantly increased within 15 minutes of costimulation with both hypoxia and thrombin (Figure 5A). As a control, HIF-1α mRNA levels increased in a time-dependent manner in hypoxic HCAECs (ANOVA < 0.05) (Figure 5A).

In HCAECs, hypoxia induced a time-dependent increase in sFLT1 mRNA expression within 30 minutes (ANOVA P < 0.01). Thrombin and costimulation with both hypoxia and thrombin induced a time-dependent increase in sFLT1 mRNA expression over hours (Figure 5B). No increase in sFLT1 mRNA was observed in HVSMCs treated with hypoxia, thrombin, or their combination. No change in sFLT1 protein release was observed with hypoxia in HVSMCs (Figure 5C). These data suggest that sFLT1 protein release from HCAECs occurs within minutes in the presence of both hypoxia and thrombin.
Discussion

Our findings indicate that ACO leads to rapid release of sFLT1 into the systemic circulation. We have shown that serum sFLT1 levels are increased in patients with ACO compared with control subjects. During the early period after symptom onset, sFLT1 levels were elevated and more sensitive for the diagnosis of ACO than CK-MB or ultrasensitive troponin I. Furthermore, we observed rapid release of sFLT1 protein in a mouse model of ACO and by coronary endothelial cells exposed to hypoxia and thrombin. These data suggest that sFLT1 is an early indicator of endothelial origin for ACO.

These data may have clinical implications that require further study in a larger population of patients. First, the ability to rapidly identify an ACO could improve clinical outcomes by allowing therapeutic interventions with less delay. Second, because sFLT1 originates from the endothelium, myocyte necrosis is not required for detection in the serum. As a result, sFLT1 may potentially be able to discriminate between ACO and other causes of direct myocyte injury such as myocarditis. The role of sFLT1 in the context of direct myocyte injury requires further study. Third, circulating sFLT1 retains its ability to bind vascular endothelial growth factor and placental growth factor, suggesting that during ACO, sFLT1 may attenuate the benefit of proangiogenic cytokines. Our data support the need for further investigation of sFLT1 in patients with ACS.

Early detection of ACO with a highly sensitive biomarker in conjunction with the ECG has the potential to improve clinical outcomes by reducing treatment delay.2,23 Given the risk and cost associated with emergent coronary angiography, reducing the number of procedures performed for false-positive ECGs may be important. Finally, field diagnostics for patients with suspected ACO may be limited in underdeveloped regions where an ECG is unavailable. In these cases, the development of a rapid, diagnostic test with high sensitivity within minutes after symptom onset may affect clinical outcomes.

At present, CK-MB, troponins, and myoglobin exhibit excellent sensitivity (above 90%) for the diagnosis of AMI within 24 hours of presentation.2 However, within the first 1 hour of symptom onset, we observed lower sensitivity for biomarkers of myocardial necrosis (myoglobin, 75%; CK-MB, 20%; us-Tn-I, 20%). These levels are consistent with reports showing poor sensitivity for myocyte-based markers in the acute phase of coronary occlusion.24–29 Recently, ultrasensitive troponin assays have demonstrated improved sensitivity for detecting an AMI.6 However, patients presenting with myocardial injury from any cause may yield a positive ultrasensitive troponin test result, compromising the value of these tests for identifying patients presenting specifically with an ACO. Furthermore, despite advancements in troponin assay development, myocyte necrosis is still required for these assays to be useful. As a result, an endothelium-derived protein such as sFLT1 may promote rapid diagnosis and earlier intervention and thus improve clinical outcomes. To realize the potential benefits of sFLT1 in clinical practice,
however, rapid assay kits as point-of-care diagnostics are needed. The specificity of sFLT1 for coronary occlusion remains unknown, as circulating levels of sFLT1 may be elevated in any other disease state that is associated with hypoxic endothelium.

Of note, elevated sFLT1 in ACO was not associated with traditional cardiovascular disease risk factors per se. We noted that β-blocker use was inversely associated with sFLT1 levels in patients with ACO and UA/NSTEMI. This effect has been described in pregnant women at high risk for developing preeclampsia but requires further study in ACS.

The current study has several limitations. First, the findings require further validation in a larger population to clarify the clinical utility of sFLT1 in ACS. Second, as a prospective, observational study, the prognostic role of sFLT1 levels remains undetermined. Third, we were unable to determine whether prior episodes of chest pain before presentation influence sFLT1 levels. Fourth, we were unable to control for anticoagulant administration in subjects referred for cardiac catheterization for STE myocardial infarction or UA/NSTEMI.

In summary, we identify sFLT1 as a sensitive biomarker of endothelial origin that is released during the early phase of ACO. sFLT1 may also serve as a sensitive marker of vascular occlusion and endothelial dysfunction beyond the coronary artery to include cerebrovascular, peripheral vascular, and pulmonary vascular beds. Future studies are required to investigate the prognostic implications of sFLT1 in acute vaso-occlusive syndromes.

Disclosures

None.

References

2. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, et al. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina) [published correction...
Elevated Soluble fms-Like Tyrosine Kinase-1 Levels in Acute Coronary Occlusion


*Arterioscler Thromb Vasc Biol.* 2011;31:443-450; originally published online November 11, 2010;
doi: 10.1161/ATVBAHA.110.215897

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/31/2/443

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/