Coated-Platelets Improve Prediction of Stroke and Transient Ischemic Attack in Asymptomatic Internal Carotid Artery Stenosis

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Background and Purpose—Coated-platelets, a subset of procoagulant platelets observed on dual agonist stimulation with collagen and thrombin, support a robust prothrombinase activity and provide a unique measure of platelet thrombotic potential. Coated-platelet levels are increased in large artery stroke, and higher levels are associated with early stroke recurrence, suggesting a potential role for risk stratification in asymptomatic patients with carotid artery stenosis.

Methods—Three-hundred twenty-nine consecutive patients with technically adequate carotid Doppler evaluation without stroke or transient ischemic attack (TIA) in the previous 6 months were enrolled as part of a prospective cohort study conducted during a 40-month period. The main outcome was occurrence of stroke or TIA according to coated-platelet levels and internal carotid stenosis severity at enrollment. The optimal cutoff value of coated-platelet levels was determined by recursive partitioning analysis. Event-free survival was estimated using Kaplan–Meier and Cox proportional hazards regression analyses.

Results—A cutoff of $\geq45\%$ for coated-platelet levels in combination with stenosis $\geq50\%$ yielded a sensitivity of 0.78 (95% confidence interval, 0.51–1.0), specificity of 0.92 (0.89–0.95), positive predictive value of 0.21 (0.07–0.34), and a negative predictive value of 0.99 (0.98–1.0) for ipsilateral stroke or TIA. The incidence rate of ipsilateral stroke or TIA for patients with $\geq50\%$ stenosis and $\geq45\%$ coated-platelets was 21.5 per 100 person-years versus 1.27 per 100 person-years for patients with $\geq50\%$ stenosis and $<45\%$ coated-platelets ($P<0.0001$).

Conclusions—Coated-platelet levels identify asymptomatic carotid stenosis patients at high risk for stroke or TIA, which suggests a role for coated-platelets in risk stratification before revascularization. (Stroke. 2014;45:2995-3001.)

Key Words: blood platelets ■ carotid arteries ■ stroke ■ thrombosis

Stroke is a leading cause of death and disability in the United States. Although early evaluation and treatment of acute stroke have improved outcomes, the best approach to reducing stroke burden is prevention. Several stroke prevention strategies have proven effective, including aggressive management of vascular risk factors with antiplatelet drugs, statins, and antihypertensive medications, as well as endarterectomy or angioplasty and stent placement.

A reduction in both recurrent and incident stroke has been documented in carotid endarterectomy trials among patients with symptomatic and asymptomatic high-grade stenosis. However, the benefit of carotid endarterectomy among asymptomatic carotid stenosis patients reflects a modest absolute reduction of fatal or disabling stroke of $\approx0.5\%$ per year. Moreover, stroke rates among patients with asymptomatic carotid stenosis have fallen to $<1\%$ per year because of improvement in medical therapy. In light of a perioperative complication rate of 4.7% to 6.7% with endarterectomy and a relative reduction of fatal or disabling stroke of 4.1% to 6.7%, the benefit of revascularization in asymptomatic patients has been questioned, and calls for methods to identify patients at highest risk for stroke have increased.

Previous research has identified and characterized coated-platelets, a subset of platelets produced after coactivation with collagen and thrombin. These activated platelets express high levels of procoagulant proteins on their surface and support a robust prothrombinase activity. Although control populations produce $\approx30\%$ coated-platelets, patients with nonlacunar stroke have higher levels of coated-platelets (mean, 39.4%) when compared with patients with lacunar strokes (21.8%).

Also, patients with nonlacunar ischemic strokes who have higher levels of coated-platelets are more likely to have recurrent ischemic stroke than patients with lower coated-platelet levels, suggesting that coated-platelets may serve as a biomarker for stroke risk. In addition, patients with symptomatic large-artery atherosclerosis and coated-platelet levels of $\geq50\%$ have a rate of early recurrent stroke 6- to 7-fold higher than those with coated-platelet levels of $<50\%$. We sought...
to determine whether coated-platelet levels may improve risk stratification for stroke or transient ischemic attack (TIA) among patients with asymptomatic carotid stenosis.

Methods

Study Design and Oversight

The Coated-Platelets in Asymptomatic Carotid Stenosis (CoPACS) study was a prospective cohort study adhering to prospective-specimen-collection, retrospective-blinded-evaluation design conducted at the Oklahoma City Veteran’s Affairs Medical Center during a 40-month period from February 2010 to June 2013. The study was approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center. Individual informed consent was obtained for all subjects before enrollment.

Study Population

We recruited consecutive patients referred for carotid Doppler evaluation who met the following inclusion criteria: (1) no history or symptoms of stroke or TIA in the preceding 6 months, (2) technically adequate carotid Doppler evaluation, (3) capacity for informed consent, and (4) no known comorbidity likely to be fatal within 6 months. Exclusion criteria consisted of (1) current use of anticoagulants, (2) any transfusion within 2 weeks before screening, and (3) history of dementia.

Smoking status, sex, race, age, and the use of medications that may influence coated-platelet levels, such as selective serotonin reuptake inhibitors, statins, or antithrombolytic medications, were recorded at the time of enrollment for each patient. Additional information recorded included a history of hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease, peripheral arterial disease, or end-stage renal disease (ESRD).

Carotid Doppler Evaluation

Carotid Doppler studies were performed at the Oklahoma City Veteran’s Affairs vascular laboratory using the Philips iU22 ultrasound and L9-3 linear transducer. Carotid arteries were categorized as normal, having <50%, 50% to 69%, 70% to 79%, 80% to 99% stenosis, or occluded. A recent audit of Doppler ultrasound results at the Oklahoma City Veteran’s Affairs vascular laboratory using the Philips iU22 ultrasound and L9-3 linear transducer.

Study Outcomes

The primary outcome was occurrence of a new ipsilateral stroke or TIA during the follow-up period. Secondary outcomes included ipsilateral stroke, any stroke or TIA, and any stroke or vascular death, including fatal stroke or fatal myocardial infarction. TIA was defined as any transient neurological deficit without imaging evidence of infarction.

Stroke was defined as a new, sudden-onset, persistent focal neurological deficit lasting >24 hours, confirmed by imaging, that could not be attributed to a mass or hemorrhage. All diagnoses of stroke or TIA were established at time of occurrence by the treating physician, confirmed by a consulting neurologist not participating in the study, and recorded in the medical record. Brain imaging studies (brain magnetic resonance imaging, or computed tomography if magnetic resonance imaging was contraindicated) were obtained for all patients with a diagnosis of stroke or TIA. The interpreting neuroradiologist was blinded to coated-platelet measurements. For study purposes, diagnosis of stroke or TIA was determined at time of outcomes adjudication by the review of Veteran’s Affairs inpatient records, clinic notes, and medical records from outside facilities. At adjudication, if a clinician had not assessed the subject within a 1-month period, the subject was interviewed by telephone for neurological symptoms. Individuals performing chart review, outcomes adjudication, and data entry were unaware of coated-platelet levels.

Coated-Platelet Assay

Detailed methodology for the coated-platelet assay has been published. Briefly, 5 mL of blood was drawn into a plastic syringe containing 0.5 mL of acid citrate dextrose and platelet-rich plasma was prepared as described. Coated-platelets were assayed with 1 μL of platelet-rich plasma in a 100-μL assay with the following reagents (final concentrations): 1.0 μg/mL biotin-fibrinogen, 0.4 mmol/L glyc-pro-arg-pro-amide, 500 ng/mL convulxin, 0.5 μL bovine thrombin, 2 mmol/L CaCl₂, 1 mmol/L MgCl₂, 150 mmol/L NaCl, and 10 mmol/L HEPES, with pH 7.5. After 5 minutes at 37°C, 0.8 μg of phycoerythrin-streptavidin and 0.5 μg of fluorescein isothiocyanate-abciximab were added. After an additional 5 minutes at 37°C, the reaction was stopped with 0.2 mL of 1.5% (wt/vol) formalin in 150 mmol/L NaCl, 10 mmol/L HEPES, pH 7.5. The percentage of abciximab-positive events (platelets) with bound biotin-fibrinogen was quantitated by flow cytometry. Results are reported as the percentage of cells converted to coated-platelets. All individuals performing the coated-platelet measurements were unaware of any clinical diagnoses corresponding to the sample analyzed.

Sample Size and Statistical Analysis

Data analyses were generated using SAS software, version 9.2 of the SAS System for Windows (SAS Institute Inc, Cary, NC). We assumed that 30% of subjects would be classified as high risk and 70% as low risk with a rate of events in the low-risk group of 1%, with a hazard ratio (HR) of 3 for the high-risk group. Using a 2-tailed test, P value of 0.05, and power of 0.8, our estimated sample size was 594.

To identify risk groups, we used a recursive partitioning method using a graphical approach to prune unnecessary splits. Baseline characteristics were compared between carotid stenosis groups using either t test for continuous variables or χ² or Fisher exact test for categorical variables. Receiver operator characteristic analysis was used to calculate area under the curve values for a model including coated-platelets and carotid stenosis ≥50% to predict ipsilateral stroke or TIA. The receiver operator characteristic curve for this model was compared with a model including only ≥70% carotid stenosis, based on guidelines suggesting consideration for revascularization for patients with ≥70% asymptomatic carotid stenosis, using a nonparametric approach. Differences in survival times between risk groups were determined using Kaplan–Meier analysis. Subjects were censored at time of stroke, TIA, revascularization, or death. Follow-up comparisons were performed using Tukey–Kramer adjustment to control for type I error. Cox proportional hazards regression analysis was used to examine medication use, comorbidities, and vascular risk factors associated with primary and secondary outcomes and to determine HRs and their 95% confidence intervals. Only variables having a P<0.05 were retained in the model.

At 50% of enrollment, we conducted an interim analysis, and levels of significance were calculated according to the α spending function by O’Brien-Fleming. The results for the primary outcome were statistically significant, and the difference in rate of stroke or TIA between the high-risk and low-risk groups reached the prespecified stopping boundary (P=0.00517). Therefore, we decided to close recruitment to present our results.

Results

Study Patients and Follow-Up

Between February 5, 2010, and May 1, 2013, 925 patients referred for carotid Doppler evaluation were screened and 380 were enrolled. Of these, 37 without adequate blood samples and 14 with either unilateral or bilateral carotid artery occlusions by ultrasound without angiographic confirmation were excluded from final analysis. Thus, 329 patients were analyzed (Figure 1).

Baseline characteristics are included in Table 1. Follow-up ranged from 4 days to 39.8 months (median, 10.1 months). Rates of stroke, TIA, vascular death, and revascularization by carotid Doppler result are reported in Table 2. Thirteen subjects experienced either a stroke or TIA during follow-up, and
9 events were secondary to an ipsilateral carotid stenosis. Four of the ipsilateral events were strokes, 1 of which was fatal. One ipsilateral stroke occurred in a patient who had <50% stenosis at time of enrollment but was found to have progressed to 50% to 69% stenosis at the time of stroke. The overall incidence rate of ipsilateral stroke or TIA and ipsilateral stroke for subjects with ≥50% stenosis was 7.2 per 100 person-years and 2.7 per 100 person-years, respectively.

Four strokes and TIsAs unrelated to carotid stenosis occurred during follow-up: 3 among patients with <50% carotid stenosis and 1 in a patient with 50% to 69% unilateral stenosis. Three vascular-related deaths occurred, 1 fatal stroke and 2 fatal myocardial infarctions.

Receiver operator characteristic analysis showed significant improvement in the predictive ability of ≥50% carotid stenosis plus coated-platelet levels when compared with a model using only ≥70% carotid stenosis (area under the curve, 0.90±0.05 versus 0.71±0.09, respectively; P=0.03). Recursive partitioning analysis identified ≥45% as the discriminating cutoff value for coated-platelets in combination with ≥50% stenosis for incident ipsilateral stroke or TIA, which yielded a sensitivity of 0.78 (95% confidence interval, 0.51–1.0), specificity of 0.92 (0.89–0.95), positive predictive value of 0.21 (0.07–0.34), and negative predictive value of 0.99 (0.98–1.0).

**Primary Outcome**

Table 3 and Figure 2 include the survival analysis results by risk group for the primary outcome of ipsilateral stroke or TIA. The HR for the risk of ipsilateral stroke or TIA for patients with ≥50% carotid stenosis and ≥45% coated-platelets when compared with those with <50% stenosis was 45.2 (95% confidence interval, 5.6–367.4; P=0.0004), with an incidence of ipsilateral stroke or TIA of 21.54 (10.27–45.18) per 100 person-years. The HR for patients with ≥50% stenosis and <45% coated-platelets was not different than the HR for
those with <50% stenosis (HR, 2.50; 95% confidence interval, 0.20–40.6; \( P = 0.68 \)), with an incidence of ipsilateral stroke or TIA of 1.27 (0.18–9.05) per 100 person-years. The survival curves of the 3 groups differed (log-rank \( \chi^2 = 46.0; P < 0.0001 \)). Follow-up comparisons indicate that the survival curve for the ≥54% coated-platelets and ≥50% stenosis group differed from both the ≥50% stenosis and the <45% coated-platelets (\( P < 0.0001 \)) and the <50% stenosis (\( P < 0.0001 \)) groups. There were no differences between the survival curves for the ≥50% stenosis and <45% coated-platelets group and the <50% stenosis group (\( P = 0.36 \)). Age, medication use, history of stroke >6 months before enrollment, smoking status, hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease, peripheral arterial disease, and ESRD were not significant predictors of ipsilateral stroke or TIA.

### Secondary Outcomes

Table 3 and Figure 2 include the survival analysis results by risk group for the secondary outcomes of ipsilateral stroke, any stroke or TIA, and the combined risk of any stroke or vascular death. The HR for the risk of ipsilateral stroke for patients with ≥50% carotid stenosis and ≥45% coated-platelets was 13.2 (1.2–145.5; \( P = 0.04 \)), with an incidence of ipsilateral stroke of 6.15 (1.54–24.61) per 100 person-years. The HR for patients with ≥50% stenosis and <45% coated-platelets was 2.50 (0.20–39.5; \( P = 0.52 \)) with an incidence of ipsilateral stroke of 1.27 (0.18–9.05). The survival curves did not differ (log-rank \( \chi^2 = 7.62; P = 0.02 \)).

For the outcome of any stroke or TIA, multivariate analysis revealed that ESRD was a significant predictor while controlling for risk group using log-rank tests (HR, 3.31; 3.6–308.1; \( P < 0.0001 \)), thus it was included in the final model. The HR for the risk of any stroke or TIA for patients with ≥50% carotid stenosis and ≥45% coated-platelets was 13.2 (1.2–145.5; \( P = 0.04 \)) with an incidence of 24.62 (12.31–49.23) per 100 person-years. The HR for patients with ≥50% stenosis and <45% coated-platelets was 0.6 (0.10–5.7; \( P = 0.68 \)) with an incidence of any stroke or TIA of 1.27 (0.18–9.05). The survival curves differed (log-rank \( \chi^2 = 39.0; P < 0.0001 \)). Follow-up comparisons indicate that the survival curve for the ≥45% coated-platelets and ≥50% stenosis group differed from both the ≥50% stenosis and <45% coated-platelets (\( P < 0.0001 \)) and the <50% stenosis (\( P < 0.0001 \)) groups. There were no differences between the survival curves for the ≥50% stenosis and <45% coated-platelets and the <50% stenosis groups (\( P = 0.78 \)).

For the outcome of any stroke or vascular death, multivariate analysis revealed that ESRD was a significant predictor while controlling for risk group (HR, 72.8; 10.9–487.5; \( P < 0.0001 \)), and so ESRD was included in the final model. The HR for the risk of any stroke or vascular death for patients with ≥50% carotid stenosis and ≥45% coated-platelets was 6.2 (1.0–40.1; \( P = 0.05 \)) with an incidence of 6.2 (1.54–24.61) per 100 person-years. The HR for patients with ≥50% stenosis and <45% coated-platelets was 2.2 (0.5–11.0; \( P = 0.35 \)) with an incidence of any stroke or vascular death of 3.8 (1.23–11.85). The survival curves did not differ (log-rank \( \chi^2 = 3.2; P = 0.20 \)).

Other than the significant predictive ability of ESRD for any
stroke or TIA and for any stroke or vascular death, age, medication use, history of stroke >6 months before enrollment, smoking status, hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease, peripheral arterial disease, and ESRD were not significant predictors of any other secondary outcomes.

**Discussion**

In this prospective study, elevated coated-platelet levels in asymptomatic subjects with ≥50% carotid stenosis by ultrasound criteria strongly correlated with subsequent ipsilateral stroke and TIA. Specifically, we were able to stratify asymptomatic patients with ≥50% stenosis into a low-risk category if they had <45% coated-platelet levels and into a high-risk category if they had ≥45% coated-platelet levels, with rates of stroke or TIA of 1.27 and 21.54 per 100 person-years, respectively. These findings suggest that coated-platelet levels may be useful in this setting to identify a subgroup of patients at high risk for stroke for whom revascularization would be warranted or who may qualify for investigation of novel primary prevention strategies. Our results also showed that ESRD is an independent predictor of any stroke or TIA and any stroke or vascular death. This finding is in agreement with previous studies demonstrating that elevated creatinine is an independent predictor of ischemic stroke, and that cardiovascular mortality is increased among dialysis patients.
This is the first prospective study that assesses the validity of platelet reactivity as a potential biomarker to predict stroke or TIA in asymptomatic carotid stenosis. The association between increased coated-platelet production and stroke or TIA in patients with carotid stenosis is consistent with the prothrombotic potential of these activated platelets, and with previous studies reporting increased coated-platelet potential in patients with ischemic stroke and a higher risk for infarct recurrence. The phenotypic differences between coated- and noncoated-platelets center on the presence of several prohemostatic proteins on the surface of coated-platelets resulting in a robust prothrombinase activity associated with coated-platelets but not with noncoated-platelets. The unique synthetic and activation pathways associated with retention of prohemostatic proteins on coated-platelets have been detailed elsewhere.

Our study was conducted among veterans of the US military, with high rates of smoking, hypertension, hyperlipidemia, and diabetes mellitus. Accordingly, overall rates of events were 2 to 3× higher than those reported recently. These findings support the need for collection of quality observational data for carotid stenosis patients among local practice networks to better inform treatment decisions.

Limitations include a relatively small sample size with a limited number of events for the derivation of our predictive model, which has yet to be validated externally. Although we have no previous findings to suggest that coated-platelet levels differ by sex or race, limited data for women and minorities are available for this study because of the structure of the veteran population. Additional larger studies are warranted to address these limitations, to validate our predictive model, and to determine whether coated-platelets are predictive of ipsilateral stroke and vascular death in asymptomatic carotid stenosis. Nevertheless, these findings suggest that coated-platelets are one determinant of new carotid-related stroke or TIA in the population studied.

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Disclosures

None.

References


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