Coated-platelets predict stroke at 30 days following TIA

ABSTRACT

Objective: To examine the potential for coated-platelets, a subset of highly procoagulant platelets observed on dual agonist stimulation with collagen and thrombin, for predicting stroke at 30 days in patients with TIA.

Methods: Consecutive patients with TIA were enrolled and followed up prospectively. ABCD2 scores were obtained for each patient. Coated-platelet levels, reported as percent of cells converted to coated-platelets, were determined at baseline. The primary endpoint was the occurrence of stroke at 30 days. Receiver operator characteristic (ROC) analysis was used to calculate area under the curve (AUC) values for a model including coated-platelets to predict incident stroke at 30 days.

Results: A total of 171 patients with TIA were enrolled, and 10 strokes were observed at 30 days. A cutoff of 51.1% for coated-platelet levels yielded a sensitivity of 0.80 (95% confidence interval [CI] 0.55–1.0), specificity of 0.73 (95% CI 0.66–0.80), positive predictive value of 0.16 (95% CI 0.06–0.26), and negative predictive value of 0.98 (95% CI 0.96–1.0). The adjusted hazard ratio of incident stroke in patients with coated-platelet levels $\geq 51.1\%$ was 10.72 compared to those with levels $<51.1\%$. ROC analysis showed significant improvement in the predictive ability of the coated-platelet model compared to ABCD2 score (AUC 0.78 ± 0.07 vs 0.54 ± 0.07, $p = 0.01$).

Conclusions: These findings suggest a role for coated-platelets in risk stratification for stroke at 30 days after TIA. Neurology® 2017;89:1–4

GLOSSARY

CI = confidence interval; ROC = receiver operator characteristic.

Although neurologic deficits in TIA are transient, the short-term risk for subsequent stroke may be in excess of 10% at 90 days. The ABCD2 score is widely used for very early risk stratification of patients with TIA, with scores $\geq 4$ indicating a much higher risk for stroke than scores $<4$. With the advent of novel treatment strategies, improved risk stratification is of critical importance.

Coated-platelets constitute a subset of procoagulant platelets observed on dual agonist stimulation with collagen and thrombin or with thrombin and convulxin, a collagen receptor agonist. These activated platelets retain high levels of procoagulant proteins on the cell surface, including factor V, thrombospondin, fibronectin, fibrinogen, and von Willebrand factor. In healthy controls, coated-platelet levels represent $\approx 30\%$ of the platelet population.

In previous studies, we found that mean coated-platelet levels were elevated in patients with nonlacunar ischemic stroke and TIA compared to controls and that individuals with higher coated-platelet levels have higher rates of recurrent stroke. We also noted a positive linear correlation between coated-platelet levels and ABCD2 scores in TIA. These findings prompted us to investigate whether coated-platelet levels can serve as a potential risk stratification tool for ischemic stroke after TIA.
METHODS Patients. Consecutive patients with TIA were recruited from the Neurology service. Inclusion criteria consisted of a diagnosis of TIA based on the tissue-based definition and normal diffusion-weighted MRI studies. Primary outcome was stroke at 30 days. All stroke diagnoses were confirmed by a neurologist. Clinicians were not aware of coated-platelet measurements.

We excluded patients with dementia, end-stage renal disease, prolonged prothrombin time/partial thromboplastin time/international normalized ratio, or intracerebral hemorrhage or tumor; those with >24 hours between symptom onset and enrollment; those treated with anticoagulation or thrombolytic therapy; or patients for whom MRI was not obtained or did show diffusion-weighted changes. These exclusion criteria were prompted by any potential confounding effects of thrombolytics or heparin on coated-platelet measurements, the definition of TIA, and previously published coated-platelet abnormalities in Alzheimer disease, end-stage renal disease, and intracerebral hemorrhage.

Demographics, ABCD2 score, smoking status, medications that may alter coated-platelet levels, including selective serotonin reuptake inhibitors, HMG-CoA reductase inhibitors (statins), and antplatelets, platelet count, mean platelet volume, and pertinent comorbidities were recorded for each patient. We also recorded the presence of symptomatic ipsilateral carotid stenosis, severe intracranial stenosis, atrial fibrillation, or other cardiac source of embolism because these factors were previously associated with increased stroke risk even with ABCD2 scores <4.

Standard protocol approvals, registrations, and patient consents. This study was approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center. Signed informed consent was obtained for all participants.

Coated-platelet assay. After informed consent, 5 mL blood was drawn into acid citrate dextrose, and coated-platelets were assayed as previously described by our group. Results are reported as percent of platelets converted to coated-platelets. Individuals performing the coated-platelet measurements were not aware of any clinical diagnosis.

Statistical analysis. Statistical analyses were performed with SAS (SAS System for Windows, version 9.2, SAS Institute Inc, Cary, NC) with significance set at $p < 0.05$. Descriptive statistics for relevant demographic variables, pertinent comorbidities, medical use, and clinical characteristics were generated. Comparisons were made with independent-sample $t$ tests, Pearson $χ^2$ tests (or Fisher exact tests), or Wilcoxon rank-sum test, as appropriate. Receiver operator characteristic (ROC) analysis was used to calculate area under the curve values for a model including coated-platelets to predict incident stroke at 30 days. The ROC curve for this model was compared to the ABCD2 score with a non-parametric approach. Cox proportional hazard regression was used to calculate the hazard ratio of stroke recurrence according to coated-platelet level after adjustments for age; diabetes mellitus; hypertension; smoking; history of stroke/TIA; atrial fibrillation/ cardioembolism; carotid stenosis; intracranial stenosis; use of statins, selective serotonin reuptake inhibitors, or antplatelets; mean platelet volume; and ABCD2 score. Variables having a value of $p < 0.05$ were retained in the model.

A sample size of 162 participants was targeted to achieve 90% power to detect hazard ratios $≥1.2$ for incident stroke at 30 days associated with a 1-SD decrease in coated-platelet levels. This calculation assumes an SD of 13% in coated-platelet levels, a rate of stroke of 5%, and a one-sided $α$ level of 0.05. The estimates were based on published research data for stroke after TIA and coated-platelet levels in ischemic stroke and TIA.

RESULTS Consecutive patients with TIA (n = 251) were screened over 31 months, and 179 were enrolled. Three patients without adequate blood samples and 5 patients lost to follow-up were excluded (figure 1). The remaining 171 patients were analyzed. Eighty-nine of these patients (52%) were veterans of the US military, resulting in a larger percentage of men in our study. Demographic variables, relevant medications, and pertinent comorbidities are included in the table.

There were 10 strokes during follow-up (5.8%). ROC analysis showed significant improvements in the predictive ability of the coated-platelet model compared to ABCD2 score (area under the curve $0.78 ± 0.07$ vs $0.54 ± 0.07$, $p = 0.01$).

A cutoff of 51.1% for coated-platelet levels yielded a sensitivity of 0.80 (95% confidence interval [CI] 0.55–1.0), specificity of 0.73 (95% CI 0.66–0.80), positive predictive value of 0.16 (95% CI 0.06–0.26), and negative predictive value of 0.98 (95% CI 0.96–1.0) for stroke.

There were 51 patients with coated-platelet levels $≥51.1$. These patients had higher ABCD2 scores and were more likely to have hypertension, elevated blood pressure, carotid stenosis, and intracranial
stenosis than those with levels $<$51.1% (table). The survival curves for the 2 groups differed significantly [$\log$-rank $\chi^2(1) = 12.9, p = 0.0003$, figure 2]. Multivariate Cox regression analysis, after controlling for all potentially confounding variables, showed that the adjusted hazard ratio of incident stroke in patients with coated-platelet levels $\geq51.1\%$ was 10.72 (95% CI 1.73–66.27) compared to those with levels $<51.1\%$, with stroke rates at 30 days of 16% vs 2%, respectively ($p = 0.001$, table).

**DISCUSSION** The current study indicates that patients with TIA with higher levels of coated-platelets have higher 30-day stroke rates. The incidence of stroke at 30 days reached 16% in patients with coated-platelet levels $\geq51.1\%$, resulting in a 10-fold increased rate compared to those with levels $<51.1\%$. The findings suggest a potential role for coated-platelets in stroke risk, highlighting the need for further research to understand the underlying mechanisms and the potential for targeted therapeutic interventions.
showing increased coated-platelet potential associated with intracranial stenosis, consistent with prior studies of prothrombotic platelets may improve the predictive potential of the ABCD2 score for stroke at 30 days after a TIA.

These results are consistent with previous studies showing elevated coated-platelet levels in stroke patients with infarct recurrence. The cutoff of 51.1% is similar to previous results showing that patients with symptomatic carotid stenosis and coated-platelet levels $\geq 50\%$ have a rate of early recurrent stroke 6- to 7-fold higher than those with coated-platelet levels $< 50\%$. As noted in the table, patients in the high-risk group were more likely to have hypertension, elevated blood pressure, carotid stenosis, or intracranial stenosis, consistent with prior studies showing increased coated-platelet potential associated with hypertension and large artery disease.

Limitations of the current pilot study include a limited number of patients and outcomes, an over-representation of men, and a limited follow-up time. These limitations should be addressed in future, larger studies with extended follow-up. Despite these limitations, our results support the evolving paradigm of coated-platelet involvement in thrombosis and suggest a role for coated-platelets in stroke risk stratification after TIA.

**AUTHOR CONTRIBUTIONS**

Angelia C. Kirkpatrick participated in study design, data collection and classification, patient recruitment, interpretation of results, and manuscript preparation. Andrea S. Vincent participated in study design, statistical analyses and data presentation, interpretation of results, and manuscript preparation. George L. Dale participated in study design, coated-platelet measurements, interpretation of results, and manuscript preparation. Calin I. Prodan participated in study design, data collection and classification, patient recruitment, interpretation of results, and manuscript preparation.

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**DISCLOSURE**

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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**REFERENCES**


