Higher Coated-Platelet Levels in Acute Stroke are Associated with Lower Cognitive Scores at Three Months Post Infarction

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Background: Coated-platelets are a subset of highly procoagulant platelets observed after dual agonist stimulation with collagen and thrombin. Coated-platelet levels are increased in acute stroke compared to controls, and higher levels are associated with stroke recurrence. We examined whether coated-platelet levels measured at the time of the stroke correlate with cognitive scores at 3 months following the brain infarction. *Methods*: Coated-platelets were assayed in consecutive patients with non-lacunar stroke. Cognitive screening was performed using the Mini-Mental State Examination (MMSE) at 3 months after discharge. Linear regression, with adjustment for individual covariates, was used to model the association between coated-platelet levels and MMSE scores. *Results*: One hundred and twenty-eight patients with a mean MMSE score of 26 points (range 14-30, standard deviation [SD] 3.1) and mean coated-platelet levels of 40.9% (range 5.2-76.2, SD 13.3), completed cognitive screening. An inverse linear association was found between coated-platelet levels and MMSE score, with higher levels seen in patients with lower MMSE scores ($r = -.34$, $R^2 = .12$, $P < .0001$). This association remained despite adjustment for potential confounding factors. In the final model, higher coated-platelet levels (coefficient −.078, 95% confidence interval [CI]: −.12 to −.041, $P < .0001$), presence of hypertension (coefficient −2.42, 95% CI: −3.90 to −.95, $P = .0015$), and anticoagulant use at discharge (coefficient −1.48, 95% CI: −2.56 to −.39, $P = .0079$) were predictive of lower MMSE. *Conclusions*: These findings support a link between increased platelet procoagulant potential at the time of the stroke and development of cognitive impairment following cerebral infarction.

Key Words: Platelets—vascular cognitive impairment—stroke—thrombosis
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Introduction

Coated-platelets are a subpopulation of procoagulant platelets observed only after dual agonist stimulation with collagen and thrombin.[1] Upon activation, coated-platelets express surface phosphatidylserine and retain several procoagulant proteins on the cell surface, such as fibrinogen, factor V, and von Willebrand factor.[1-6] They represent approximately 30% of all platelets in healthy controls. These levels remain stable over time in the absence of significant medication changes or new cardiovascular comorbidities.[1]

Coated-platelet levels are increased in patients with non-lacunar stroke and transient ischemic attack (TIA) as compared to stroke-free controls, and higher levels obtained at the time of the initial ischemic event correlate with an elevated risk for subsequent stroke.[7-10] In addition to recurrent stroke, we found that increased coated-platelet potential was associated with incident brain infarction in

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patients with asymptomatic carotid stenosis, where individuals with greater than or equal to 45% coated-platelets had a 10-fold higher risk for incident stroke as compared to those with less than 45% coated-platelets.[11] As patients with carotid stenosis have a heightened risk for cognitive impairment,[12] we conducted a pilot study in patients with asymptomatic carotid atherosclerotic disease and found that coated-platelet levels are significantly elevated in patients with cognitive impairment as compared to those without cognitive impairment.[13]

Prompted by the increased recognition that ischemic stroke is increasing the risk for development of cognitive impairment,[14] we now investigate whether coated-platelet levels measured at the time of the ischemic stroke correlate with cognitive screening scores obtained at approximately 3 months following the initial brain infarction.

Methods

Study Patients

The study was approved by the Institutional Review Board of our University. Individual written informed consent was obtained for all participants.

We recruited consecutive patients with nonlacunar ischemic stroke from the Neurology service between January 1, 2015 and July 30, 2017. All patients were diagnosed based on TOAST criteria[15] and underwent brain CT followed by MRI studies within 24 hours to confirm cerebral ischemia. Additional investigations included ECG, carotid ultrasound, CT angiogram or magnetic resonance angiogram studies, echocardiogram, cardiac telemetry, complete blood count, coagulation studies, lipid profile, and serum chemistry.

Exclusion criteria at the time of enrollment were determined a priori and consisted of: (1) administration of anticoagulants or thrombolytics prior to blood sampling, (2) imaging evidence of lacunar stroke, (3) primary intracerebral hemorrhage or tumor, (4) greater than 96 hours between onset of the symptoms and blood sampling, (5) end-stage renal disease, (6) dementia, (7) prolonged coagulation tests, or (8) ongoing hemorrhage or bleeding diathesis. We based our exclusion criteria on prior data showing that physiological manipulation in animals may change coated-platelet production after a minimum of 4 days,[1] differences in coated-platelet levels between lacunar and nonlacunar stroke,[7] published coated-platelet abnormalities in Alzheimer disease and end-stage renal disease,[16,17] and the potential for heparin and thrombolytics to confound coated-platelet measurements.

After discharge, all patients were offered a follow-up visit in the outpatient stroke clinic at approximately 3 months. The visit included a complete history and neurological examination, including cognitive screening with the Mini-Mental State Examination (MMSE),[18,19] performed by a neurologist. Additional exclusion criteria for patients seen at 3 months included: (1) interval stroke or TIA, (2) presence of aphasia, or (3) absence of cognitive screening results.

Demographic information included gender, race, and ethnicity (based on criteria defined by the US census), age, and educational level. Additional clinical information included smoking status and use of medications that may influence coated-platelet levels, such as selective serotonin reuptake inhibitors, HMG-CoA reductase inhibitors (statins), or antiplatelets,[20,21] (recorded at the time of enrollment for each patient and reflecting prehospitalization status) and a history of diabetes, hypertension, atrial fibrillation, large-artery stenosis, hypercholesterolemia, obesity, coronary artery disease, myocardial infarction, stroke, TIA, National Institutes of Health Stroke Scale (NIHSS) score, the subtype of nonlacunar stroke based on TOAST criteria, and a diagnosis of depression and the MMSE score at the time of follow-up. The following hematological parameters obtained on the same day with the coated-platelet measurement were recorded for all patients: total platelet count, mean platelet volume, white blood cell count, and hemoglobin.

The MMSE, a widely used test of cognitive function, is a 30-point questionnaire that includes tests of orientation, attention, memory, language, and visual-spatial skills. A score of less than or equal to 24 points on MMSE is suggestive of cognitive impairment; a higher cutoff ($\leq$26) may suggest a cognitive impairment in individuals with a higher level of education. The NIHSS score is a routinely used tool to quantify stroke-related neurological deficits, most often in the acute stage of the stroke. It consists of 11 elements and has a score range of 0-42, with higher scores indicating more severe neurological deficits.

Clinical and laboratory data were obtained at the time of discharge and/or follow-up visit through review of electronic medical records. The physicians establishing the diagnosis during the admission or providing outpatient care during the 3-month follow-up visit in the stroke clinic were not aware of the coated-platelet levels.

Coated-Platelet Assay

After informed consent and with the patient in a resting position for at least 30 minutes, 5 mL of blood was drawn into acid citrate dextrose, platelet rich plasma was prepared, and coated-platelets were assayed as previously described.[1,22] Briefly, the coated-platelets assay is performed with 1 μL of platelet rich plasma in a 100 μL assay with the following reagents (final concentrations): 1.0 μg/mL biotin-fibrinogen, .4 mM gly-pro-arg-pro-amide, 500 ng/mL convulxin, .5 U/mL bovine thrombin, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 150 mM NaCl, and 10 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-(4-butanesulfonic acid) (HEPES), pH 7.5. Reaction is initiated by addition of convulxin/thrombin (premixed). After 5 min at 37°, .8 μg of phycoerythrin-streptavidin and .5 μg of FITC-abciximab were added. After an additional 5 min at 37°, the reaction is stopped with .2 mL of

1.5% (w/v) formalin in 150 mM NaCl, 10 mM HEPES, pH 7.5. Flow cytometric analysis is used to identify platelets as abciximab-positive events, and platelets which were also positive for bound biotin-fibrinogen are quantitated as coated-platelets.[1] Results are reported as percent of cells converted to coated-platelets.[1,22] The methodology for the coated-platelet assay, including the source and manufacturer for all reagents, has been described in detail as part of the on-line Supporting Information section in a previous publication.[22] Individuals performing the coated-platelet assay were not aware of any clinical data corresponding to the blood sample analyzed.

Statistical Analysis

Data were analyzed using SAS (SAS System for Windows, ver. 9.4, SAS Institute Inc., Cary, NC). Descriptive statistics were used to summarize the distribution of

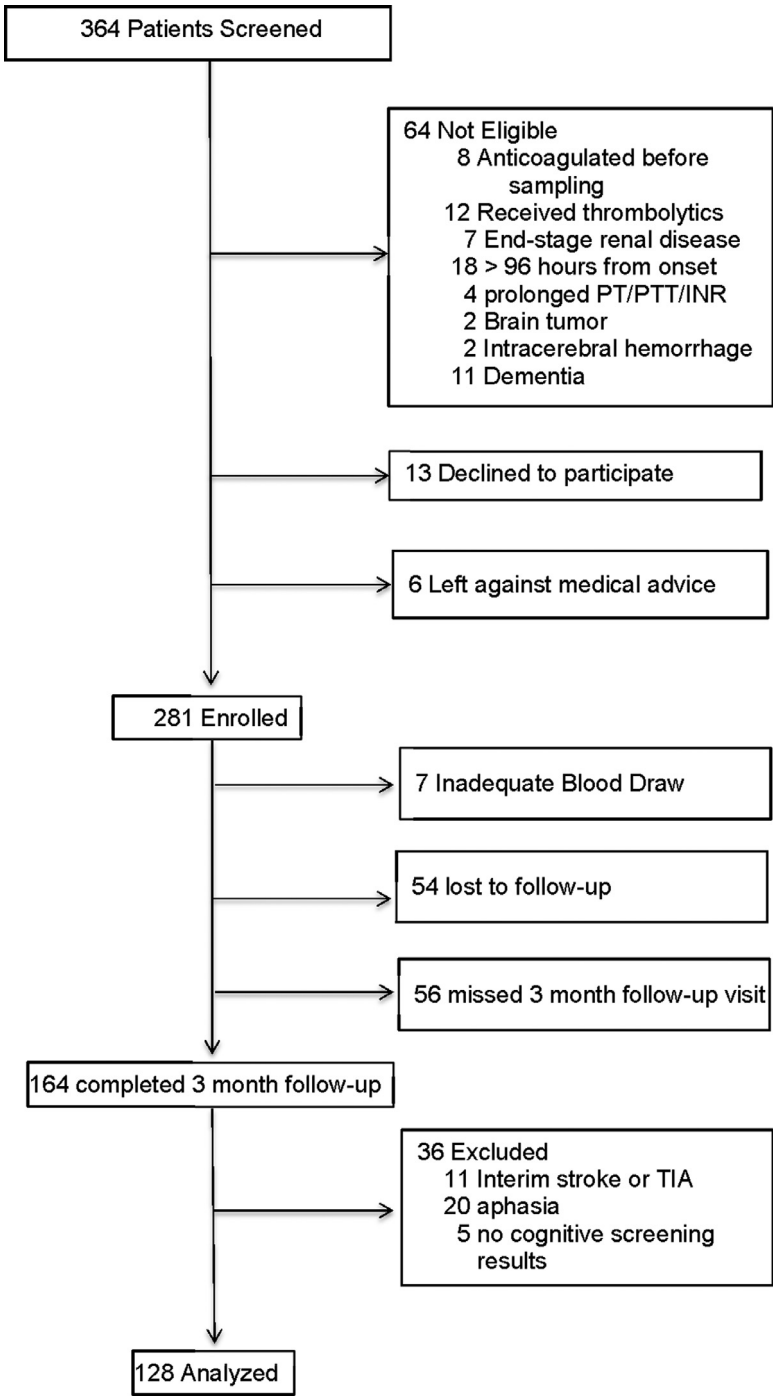

Figure 1. *Flow diagram of subjects screened, enrolled, followed, and analyzed.*

coated-platelet levels, MMSE, and demographic and clinical variables. Comparisons of means between 2 groups were made using a *t* test, or Wilcoxon rank sum test when a group size less than 20. Comparisons among 3 or more groups were made using a Kruskal-Wallis test for continuous measures and a Cochran-Armitage trend test for categorical measures. Pearson correlation coefficients, or Spearman's rank correlation coefficient for skewed distributions, were used to quantify the strength of the linear association between 2 continuous measures. Linear regression, with adjustment for individual covariates, was used to model the association between coated-platelet level (independent factor) and MMSE (dependent variable). A multivariate model was fit that included coated-platelet level, confounding factors, and factors associated with MMSE at .10 alpha level. A confounder was defined as a factor that, with adjustment, resulted in at least a 10% change in the coated-platelet coefficient when modeling MMSE.[23] The full model was reduced by deleting nonsignificant factors until all coefficients were significant at .05 alpha level. A two-sided .05 alpha level was used to define statistical significance.

We estimated that a sample size of 125 achieves 80% power to detect a correlation coefficient of .247 units or more from the null hypothesis correlation of 0, when investigating the linear association between MMSE and

Table 1. *Summary of demographic variables, coated-platelet levels, laboratory measures, relevant comorbidities, and medications for all patients*

Baseline values*	All subjects (n = 128)			
	Min	Max	Mean	SD
Age (y)	37	88	66	10.5
Coated-platelets (%)	5.2	76.2	40.9	13.3
Platelet count (K/mm^3)	79	684	227.9	70.6
Mean platelet volume (fl)	5.9	11.5	8.6	1
White blood cell count (K/mm^3)	4.2	40.8	8.9	4
Hemoglobin (g/dL)	6.8	17.3	14.6	9.1
NIHSS score	0	13	4.5	3
Male		118 (92%)		
Ethnicity (not Hispanic or Latino)		125 (98%)		
Race				
White		105 (82%)		
Black		20 (16%)		
Other		2 (2%)		
Education				
<12 years		12 (9%)		
12 years		83 (65%)		
>12 years		33 (26%)		
Smokers		51 (40%)		
Antiplatelet use		61 (48%)		
SSRI use		23 (18%)		
Statin use		68 (53%)		
Diabetes		57 (45%)		
Hypertension		112 (88%)		
Large-artery stenosis		55 (43%)		
Atrial fibrillation		28 (22%)		
Hypercholesterolemia		97 (76%)		
Coronary artery disease		61 (48%)		
Obesity		39 (30%)		
Prior stroke		29 (23%)		
Prior TIA		14 (11%)		
Prior myocardial infarction		12 (9%)		
Stroke subtype				
Large-artery		63 (49%)		
Cardioembolic		34 (27%)		
Indeterminate		25 (20%)		
Other		6 (5%)		
Depression at 3 months		31(24%)		

Abbreviation: SSRI, selective serotonin reuptake inhibitors.
*Data summarized using minimum (Min), maximum (Max), mean and standard deviation (SD) or count (%).

coated-platelet levels, using a two-sided hypothesis test with a significance level of .05. This calculation is based on published research data on MMSE score at 3 months poststroke[24,25] and coated-platelet levels in ischemic stroke.[9] Sample size calculations were performed using PASS software.[26]

Results

Three hundred and sixty-four consecutive patients admitted with a diagnosis of ischemic stroke were screened over a period of 30 months, 281 were enrolled, 164 completed the 3-month follow-up visit, and 128 (10 women and 118 men) were analyzed. A flow chart illustrating the enrollment and follow-up, and reasons for ineligibility and/or exclusion at each step is presented in Figure 1. Ninety-one of the final 128 patients (71%) were military veterans, resulting in a larger percentage of men because of the composition of the US armed forces. Demographic and clinical data for all patients analyzed are listed in Table 1.

Among all patients who completed follow-up at 3 months (N = 128), coated-platelet measures ranged from 5.2% to 76.2% with a mean (standard deviation [SD]) of 40.9% (13.3%). MMSE scores ranged from 14 to 30 points with a mean (SD) of 26 (3.1) points, with 36 individuals (28%) scoring less than or equal to 24 points. Tables 2 and 3 summarize the distribution of MMSE scores, modeled as a continuous measure, relative to the demographic variables, hematological parameters, stroke etiology and severity, pertinent medications, and relevant comorbidities.

MMSE scores were negatively associated with coated-platelet levels (regression coefficient = −.08, 95% confidence interval [CI]: −.12 to −.04, $P < .0001$, Fig 2). Mean MMSE

Table 2. *Summary of the distribution of MMSE scores relative to the demographic characteristics, stroke etiology and severity, and hematological measures*

Characteristic*	N	Correlation		*P* Value
Age (y)	128	−.029		.74
Coated-platelet (%)	128	−.34		<.0001
Platelet count (K/mm^3)	128	−.024		.79
Mean platelet volume (fl)	128	.047		.60
White blood cell count (K/mm^3)	128	.0086		.92
Hemoglobin (g/dL)	128	−.043		.63
NIHSS score	128	−.081		.36
	n	Mean	SD	
Gender				.97
Male	118	26.0	3.1	
Female	10	26.0	2.9	
Education				.24†
<12 years	12	25.2	2.7	
12 years	83	26.4	2.9	
>12 years	33	25.5	3.6	
Ethnicity				.33†
Hispanic/Latino	2	28.0	2.8	
Not Hispanic/Latino	125	26.0	3.1	
Race				.023‡
White	105	26.2	2.8	
Black	20	24.6	3.9	
Other	2	30.0	0.0	
Smoking status				.73
Smoker	51	24.6	3.9	
Nonsmoker	77	30.0	0.0	
Stroke cause				.48†
Large-artery	63	26.3	2.6	
Cardioembolic	34	25.7	3.7	
Indeterminate	25	26.1	3.1	
Other	6	24.3	3.1	

*Data summaries include the Pearson or Spearman's rank correlation coefficient for continuous measures and the mean and standard deviation of MMSE scores for categorical measures. Between-group comparison of means based on *t* test unless otherwise indicated.

†*P* values based on Wilcoxon rank sum test (comparing 2 groups) or Kruskal-Wallis test (comparing more than 2 groups).

‡*P* value based on Black versus other/White comparison.

Table 3. *Summary of the distribution of MMSE scores relative to pertinent medications and relevant comorbidities*

Characteristic*	Medication users			No medication use			*P* Value
Medications at baseline	N	Mean	SD	N	Mean	SD	
Statin use	68	26	3.3	60	26.1	2.8	.83
SSRI use	23	26.7	2.7	105	25.9	3.1	.23
Antiplatelet use	61	25.9	3.3	67	26.2	2.9	.55
Treatment at discharge							
Statin use	126	26.1	3.1	2	25.0	5.7	.79†
SSRI use	23	26.6	2.8	105	25.9	3.1	.37
Antiplatelet use	117	26.1	3.1	11	25.6	2.8	.49†
Anticoagulants	34	24.9	3.8	94	26.5	2.7	.028
Antihypertensives	128	26.0	3.1	0			NP
Comorbidities	With comorbidity			Without comorbidity			
	N	Mean	SD	N	Mean	SD	
Diabetes	57	26	3.4	71	26.1	2.8	.94
Hypertension	112	25.7	3.1	16	28.2	1.8	<.0001
Large-artery stenosis	55	26.1	2.8	73	26	3.3	.74
Atrial fibrillation	28	25.6	4.0	100	26.2	2.8	.46
Hypercholesterolemia	97	26.2	3.1	31	25.6	2.9	.34
Obesity	39	26.2	2.7	89	26	3.2	.64
Coronary artery disease	61	25.5	3.4	67	26.5	2.7	.057
Prior TIA	14	27.5	1.3	114	25.9	3.2	.0012
Prior stroke	29	25.8	2.6	99	26.1	3.2	.64
Prior myocardial infarction	12	24.6	5.2	116	26.2	2.8	.31
Depression at 3 months	31	26.4	2.9	97	25.9	3.1	.43

Abbreviations: NP, not performed given no variability in the data; all were receiving antihypertensives at discharge; SSRI, selective serotonin reuptake inhibitors.

*Data summaries include the mean and standard deviation of MMSE scores. *P* values based on the *t* test unless otherwise specified.

†*P* values based on the Wilcoxon rank sum test.

scores were noted to be lower among black participants compared to those who were white or other race ($P = .023$), among those with hypertension versus those without hypertension ($P < .0001$), among those with coronary artery disease versus those without coronary artery disease ($P = .057$), and among those who were treated with anticoagulants at the time of discharge versus those not receiving anticoagulants at the time of discharge ($P = .028$). Those with a prior TIA tended to have higher mean MMSE scores compared to those without prior TIA ($P = .0012$). No other associations were statistically significant.

The distribution of coated-platelet levels relative to the demographic variables, hematological parameters, stroke etiology and severity, pertinent medications, and relevant comorbidities showed that only the MMSE score was negatively associated with coated-platelets ($P < .0001$, see above). No other associations were statistically significant.

The association between coated-platelet levels (independent factor) and MMSE scores at 3 months (dependent variable) was adjusted for each factor listed in Table 1 to identify confounding factors. The association between coated-platelet level and MMSE scores remained largely unchanged, with none of the potential confounding factors resulting in greater than 10% change in the coated-platelet coefficient with adjustment.

The 5 factors associated with MMSE scores at .10 alpha level (race, prior TIA, coronary artery disease, hypertension, and anticoagulation use at discharge) were included in a regression model with coated-platelet levels as an independent factor and MMSE scores as the outcome. Nonsignificant factors were removed one at a time while retaining coated-platelets. The final model retained coated-platelet levels, hypertension, and anticoagulation use at discharge. After adjustment for hypertension status and anticoagulation use at discharge, a 10% higher coated-platelet value (absolute difference) is associated with a reduction in mean MMSE score of .76 points (95% CI: −1.1 points to −.4, $P < .0001$) when comparing across patients. After adjustment for coated-platelet level and anticoagulant use at discharge, those with hypertension have a mean MMSE that is 2.4 points lower than those without hypertension (95% CI: −3.8 to −1.0 points, $P = .0013$). With adjustment for coated-platelet level and hypertension, those using anticoagulation at discharge have a mean MMSE that is 1.5 points lower than those not using anticoagulation at discharge (95% CI: −2.6 to −.4, $P = .0079$).

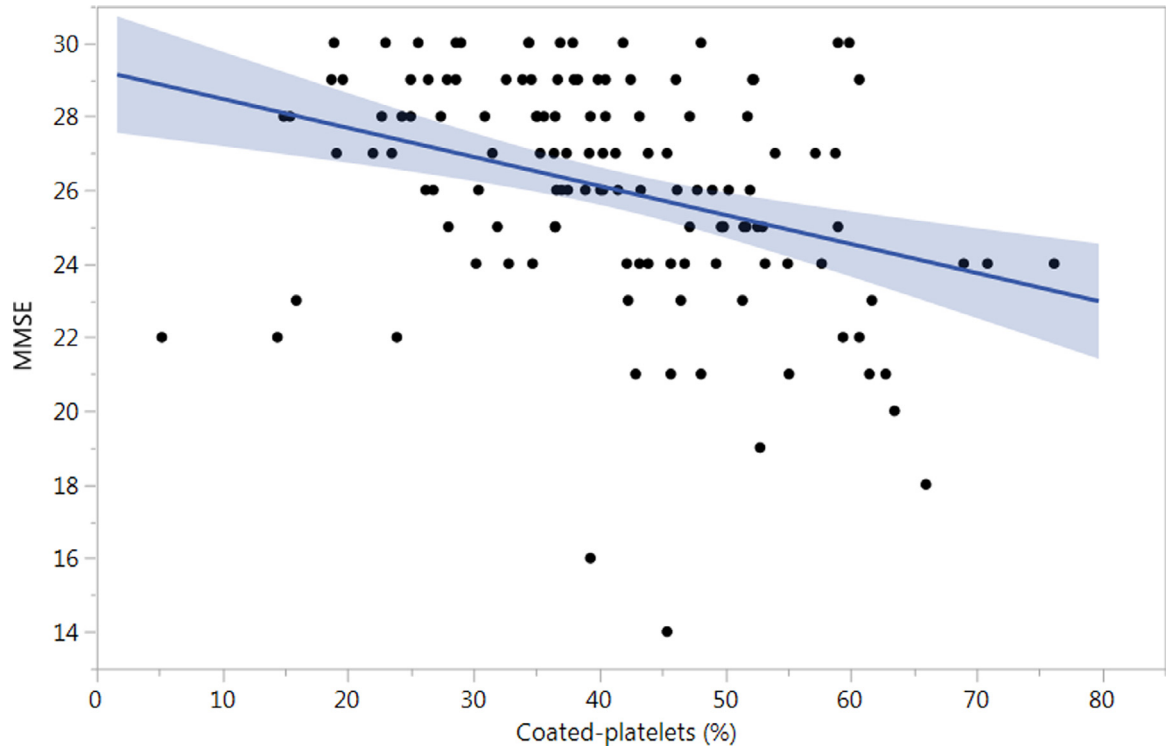

Figure 2. *Association between initial coated-platelet and MMSE scores at 3 months post-stroke. The scatter plot represents coated-platelets levels (horizontal axis) upon admission and MMSE scores at 3 months after discharge (vertical axis) in 128 patients with nonlacunar ischemic stroke. The blue line represents the best-fit linear association between the measures. The shaded area reflects a 95% confidence region around the predicted regression line. Coated-platelets explain 12% of the variation in MMSE. There is a significant negative association between coated-platelet levels and MMSE scores at 3 months (regression coefficient: −.08, 95% CI: −.12 to −.04, P < .0001).*

Discussion

The current study shows for the first time that higher levels of coated-platelets, measured at the time of a nonlacunar ischemic stroke, are associated with lower cognitive screening scores at 3 months after the brain infarction (Fig 2), even after adjusting for potentially confounding variables and after excluding patients with a prior diagnosis of dementia, interval recurrence of stroke or with aphasia.

Although prior studies have shown that higher levels of coated-platelets in nonlacunar stroke, TIA, and asymptomatic carotid stenosis were linked with increased risk for incident or recurrent stroke,[9-11] these results represent the first specific data regarding an impact on poststroke cognition. These data are compatible with 2 preliminary studies in patients with asymptomatic carotid atherosclerotic disease and subarachnoid hemorrhage showing a correlation between increased coated-platelet potential and lower cognitive screening scores.[13,27] Of note, the stroke subtype did not influence the association between coated-platelet levels and cognitive scores in the current study, suggesting that large-artery disease is unlikely to act as a confounder for our results.

The key characteristic of coated-platelets is the ability to support a robust prothrombinase activity due to both surface expression of phosphatidylserine and enhanced retention of procoagulant proteins.[1,2] Our findings support a link between an increase in platelet prothrombotic potential at the time of the stroke and development of subsequent vascular cognitive impairment following cerebral infarction, independent of clinical stroke recurrence.

Mechanisms underlying this finding are yet to be identified, but we hypothesize that inflammatory pathways are involved, based on animal and human studies showing a potentiating effect of inflammation on coated-platelet potential[1,17] and research linking inflammation with subsequent development of vascular dementia.[14,28,29] As the impact of vascular disease on cognitive impairment has become increasingly recognized, a convergence of pathologic mechanisms has been proposed to cause impairment of cognition[14,30,31] and these mechanisms are likely to involve both thrombosis and inflammation.

In addition to coated-platelets, hypertension and anticoagulant use at discharge were also associated with lower MMSE scores, without having an impact on the relationship between coated-platelet potential and MMSE at 3 months. While hypertension has been associated with an increased risk for cognitive impairment,[14,32] prior studies examining cognitive scores using MMSE after stroke have not found such a link despite reporting similar proportions of patients with scores less than 24 at 3 months postinfarction.[24,25] We suspect that this finding may be

related to a relatively high percentage of patients with hypertension in our group and also to the diagnosis of hypertension being historical rather than based on repeated blood pressure measurements. Patients were anticoagulated at discharge for either confirmed atrial fibrillation or a suspected cardioembolic source for stroke in the absence of documented atrial fibrillation. An increased risk for cognitive impairment has been described for both these conditions.[14] An additional small group of patients (N = 6) were not anticoagulated despite these diagnoses because of bleeding risk, adding variability to the any potential association between cardioembolic stroke and lower MMSE. We suspect that the association between anticoagulant use at discharge and lower MMSE scores is explained by the underlying diagnoses warranting anticoagulation. Conversely, the lack of an association between atrial fibrillation or cardioembolic stroke and MMSE score in our study is likely due to small samples sizes as our study was not specifically powered to investigate these conditions.

Limitations of the current pilot study include a short follow-up time, an over-representation of men and a limited racial and ethnic diversity. These limitations should be addressed in future studies with extended follow-up. Despite the limitations, our results support the need for additional research aimed at deciphering the mechanisms responsible for the association between increased platelet procoagulant potential and lower poststroke cognitive scores.

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Disclosures

The authors have no potential conflicts of interests.

Conflict of Interest

The authors have no conflict of interest to declare.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2019.06.033.

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