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Brief Report

Platelet-Rich Emboli in Cerebral Large Vessel Occlusion Are Associated With a Large Artery Atherosclerosis Source

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Background and Purpose—Nearly 30% of large vessel occlusion acute ischemic stroke clots are from an unknown source. We assessed histological clot composition in a series of patients with large vessel occlusion and investigated correlations between clot composition and stroke pathogenesis.

Methods—As part of the multi-institutional STRIP registry (Stroke Thromboembolism Registry of Imaging and Pathology), consecutive emboli retrieved during mechanical thrombectomy were stained using Martius Scarlett Blue and analyzed using machine learning software. We assessed proportions of red blood cells, fibrin, platelets, and white blood cells. Correlations between clot components and stroke pathogenesis (large artery atherosclerosis, cardioembolism, and stroke of undetermined pathogenesis) were assessed using SPSS22.

Results—One hundred five patients were included. The proportion of platelet-rich clots (55.0% versus 21.2%; *P*=0.005) and percentage of platelet content (22.1±4.2% versus 13.9±14.2%; *P*=0.03) was significantly higher in the large artery atherosclerosis group compared with the cardioembolic group. The proportion of platelet-rich clots (50.0% versus 21.2%; *P*=0.024) was also significantly higher in the cryptogenic group compared with cardioembolic cases. Large artery atherosclerosis and cryptogenic cases had a similar proportion of platelet-rich clots (55.0% versus 50.0%; *P*=0.636). There was no significant difference between stroke pathogenesis and the other major clot components.

Conclusions—High platelet content of emboli is associated with a large artery atherosclerosis etiology of large vessel occlusion. **(*Stroke*. 2019;50:00-00. DOI: 10.1161/STROKEAHA.118.024543.)**

Key Words: arteries ■ blood platelets ■ fibrin ■ humans ■ software

Mechanical thrombectomy has revolutionized the treatment of acute ischemic stroke secondary to large vessel occlusion (LVO). However, recurrent stroke rates remain high.[1] The cause of the recurrent stroke is typically directly related to the etiology of the primary occlusion, and therefore effective secondary stroke prevention depends on determining the initial stroke mechanism.[2]

There remains an unmet clinical need to accurately diagnose the cause of cryptogenic strokes. The widespread use of mechanical thrombectomy devices has resulted in availability of clot material for histopathologic analysis. We assessed histological clot composition in a series of patients with LVO and investigated correlations between clot composition and stroke pathogenesis with an emphasis on platelet content.

Methods

Patient Selection and Clinical Data

The data that support the findings of this study are available from the corresponding author on reasonable request. This study included clot samples collected from multiple sites in the STRIP registry (Stroke

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Thromboembolism Registry of Imaging and Pathology) collected from September 2016 to November 2018. The study was institutional review board approved, and a waiver of consent was granted. Patients were included if they were >18 years of age, had undergone mechanical thrombectomy treatment for acute ischemic stroke, and clot material was retrieved.

Clot Processing and Histological Characterization

Each embolus was immediately fixed in 10% phosphate buffered formalin. Emboli were shipped to a central core laboratory for standard tissue processing and embedded in paraffin. The formalin-fixed paraffin-embedded clot material was cut into 3- to 5-µm sections. Representative slides from each clot were stained with hematoxylin and eosin and Martius Scarlett Blue. Representative Martius Scarlett Blue–stained slides were sent for whole slide scanning (Aperio ScansScope AT-Turbo, Leica Biosystems). Histological quantification was performed using Orbit Image Analysis Software (www.Orbit.bio) as per the standard operating procedure (Methods in the online-only Data Supplement). The mean value of each clot component was calculated. Platelet-rich clots were defined as those with platelet content greater than the mean platelet content (>16.7%). The same was true for red blood cell–rich (>41.9%), fibrin-rich (>38.2%) and white blood cell–rich (>3.2%) clots.

Data Collection

Data regarding patient demographics, clinical presentation, treatment strategies, outcome, imaging findings, and stroke pathogenesis were collected using a data abstraction form. Stroke pathogenesis was classified using the TOAST (Trial of Org 10172 in Acute Stroke Treatment) system into 5 subtypes: (1) large artery atherosclerosis (LAA), (2) cardioembolism, (3) small-vessel occlusion, (4) stroke of other determined pathogenesis, and (5) stroke of undetermined pathogenesis. All data were self-reported by the included centers.

Statistical Analysis

Categorical variables were compared using the χ^2 test. Continuous variables were compared using the Wilcoxon test because of nonnormal distribution. All statistical correlations were assessed using IBM SPSS Statistics 22.

Results

Baseline Characteristics

One hundred five patients were included. Median age was 68 (range, 25–93) years. Forty-nine percent were treated with r-tPA (recombinant tissue-type plasminogen activator). Successful reperfusion (TICI [thrombolysis in cerebral infarction score] 2b/3) rate was 98% with an overall mean number of passes 2.1. Stroke pathogeneses were cardioembolic (n=52, 50%), large artery atherosclerotic (n=20, 19%), cryptogenic (n=21, 20%), and other (eg, dissections, hypercoagulable; n=12, 11%).

Histological Analysis

The quantified histological composition of the 105 cases is shown in the Figure. Mean platelet, red blood cell, fibrin, and white blood cell contents were 16.7%, 41.9%, 38.2%, and 3.2%, respectively. Pretreatment with r-tPA did not significantly affect the mean content of any clot component. LAA strokes had a significantly higher mean platelet content

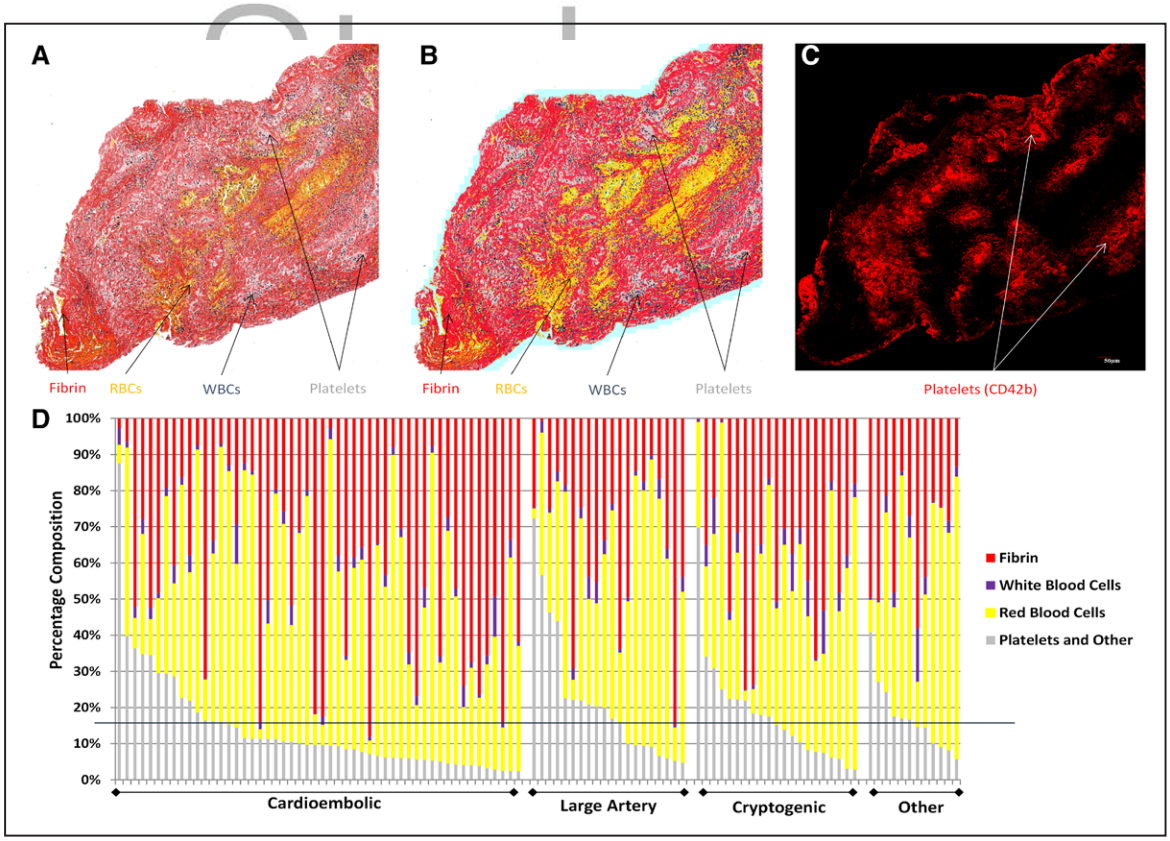

Figure. Clot composition of the patient cohort. **A**, Low-magnification image (×4) of an Martius Scarlett Blue–stained slide demonstrating the presence of red blood cells (RBCs; yellow), white blood cells (WBCs; blue), fibrin strands (red) and platelets (gray). **B**, The corresponding output image from Orbit Image analysis demonstrating its ability to identify RBCs (yellow), WBCs (blue), fibrin strands (red) and platelets (gray). **C**, Immunofluorescence image demonstrating the presence of platelets (CD42b [cluster of differentiation 42b], red). **D**, Graphical representation of the clot composition of each patient in the cohort.

Table. Histopathologic Composition of Thrombi From Various Stroke Pathogeneses

Average composition	Cell type			
	RBCs	WBCs	Fibrin	Platelets/other
All cases	41.9%	3.2%	38.2%	16.7%
Pathogenesis	RBCs	WBCs	Fibrin	Platelets/other
Cardioembolic	41.4%	2.9%	41.8%	13.9%
Large artery	42.2%	2.6%	33.1%	22.1%
Cryptogenic	40.8%	4.2%	37.1%	17.9%
Other	45.6%	3.6%	33.5%	17.2%

Cases ≥mean	Cell type							
Pathogenesis	RBC rich		WBC rich		Fibrin rich		Platelet rich	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Cardioembolic	26 (50%)	26 (50%)	19 (37%)	33 (63%)	26 (50%)	26 (50%)	11 (21%)	41 (79%)
Large artery	9 (45%)	11 (55%)	6 (30%)	14 (70%)	7 (35%)	13 (65%)	11 (55%)	9 (45%)
Cryptogenic	8 (38%)	13 (62%)	11 (52%)	10 (48%)	8 (38%)	13 (62%)	10 (48%)	11 (52%)
Other	7 (58%)	5 (42%)	6 (50%)	6 (50%)	5 (42%)	7 (58%)	6 (50%)	6 (50%)

RBC indicates red blood cell and WBC, white blood cells.

than cardioembolic stroke (22.1±18.6% versus 13.9±14.3%; *P*=0.03) and nonsignificantly higher platelet content than cryptogenic stroke (22.1±18.6% versus 17.2±14.8%; *P*=0.74). Cardioembolic strokes had a nonsignificantly lower platelet content than cryptogenic stroke (13.9±14.3% versus 17.2±14.8%; *P*=0.11).

A significantly higher proportion of LAA strokes were platelet rich than cardioembolic cases (55.0% versus 21.2%; *P*=0.005). Similarly, a significantly larger proportion of cryptogenic stroke cases were platelet rich than cardioembolic cases (50.0% versus 21.2%; *P*=0.024). LAA and cryptogenic cases had a similar proportion of platelet-rich clots (55.0% versus 50.0%; *P*=0.636). There was no significant difference between strokes pathogeneses in relation to any of the other major clot components (Table).

Discussion

Our study of 105 retrieved emboli in LVO patients found that patients with strokes secondary to LAA were more likely to be platelet rich than cardioembolic strokes. This finding suggests a potential histological signature of LAA and cardioembolic clots that could be used to identify the pathogenesis of cryptogenic strokes in LVO patients. We also found that a substantial proportion of cryptogenic stroke cases were platelet rich, which possibly supports current treatment paradigms for cryptogenic stroke.

Previous studies examining correlations between thrombus composition and stroke pathogenesis have focused specifically on red blood cells, white blood cells, and fibrin/platelet compositions with largely inconclusive results.[3,4] Sporns et al[5] suggested that cardioembolic emboli had significantly fewer red blood cells and higher proportions of fibrin/platelets than noncardioembolic thrombi; however, there was no accounting for specific fibrin and platelet compositions.

The findings of this study have implications for management of cryptogenic stroke LVO patients. At present, the majority of cryptogenic stroke patients receive antiplatelet therapy for the secondary prevention of stroke. Many recent clinical trials are testing the efficacy of anticoagulation assuming that most of these lesions have a cardiac source. However, there are growing data suggesting that nonstenotic atheromatous plaque is the culprit in ≤40% of cryptogenic stroke.[6] Our findings support this theory, as we demonstrate that a higher proportion of cryptogenic stroke cases are platelet rich and thus similar to cases of large artery etiology.

Our findings could hypothetically help individualize secondary prevention strategies for LVO stroke patients. It may be that stroke LVO patients with platelet-rich emboli could respond better to antiplatelet therapy, whereas those with platelet-poor emboli could respond better to anticoagulation. Further studies are needed to test and validate this hypothesis.

Our study has limitations. First, the determination of suspected stroke pathogenesis was self-reported at each site, and therefore there may have been some site-to-site variability in the interpretation and implementation of the TOAST criteria. Second, the Martius Scarlett Blue stain cannot differentiate between platelets and other potentially key platelet-related factors such as von Willebrand factor that may also account for a significant proportions of clot composition.

Conclusions

High platelet content of emboli is associated with an LAA etiology of LVO. Approximately half of cryptogenic strokes had high platelet content, possibly supporting current treatment paradigms in cryptogenic stroke.

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