



Published in final edited form as:

Stroke. 2019 July ; 50(7): 1907–1910. doi:10.1161/STROKEAHA.118.024543.

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

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Abstract

Background and Purpose: Nearly 30% of large vessel occlusion (LVO) acute ischemic stroke (AIS) 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 etiology.

Methods: As part of the multi-institutional STRIP registry, consecutive emboli retrieved during mechanical thrombectomy were stained using Martius Scarlett Blue (MSB) 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 etiology (Large-artery atherosclerosis, cardioembolism and stroke of undetermined etiology) 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 ($22.1\pm 4.2\%$ vs. $13.9\pm 14.2\%$, $P=0.03$) was significantly higher in the large artery atherosclerosis (LAA) group compared to 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 to cardioembolic cases. 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 stroke etiology and the other major clot components.

Conclusion: High platelet content of emboli is associated with a large artery atherosclerosis etiology of LVO.

Keywords

Acute Ischemic Stroke; Cryptogenic Stroke; Histology Quantification; Mechanical Thrombectomy

Introduction

Mechanical thrombectomy has revolutionized the treatment of acute ischemic stroke (AIS) 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 etiology of cryptogenic strokes. The widespread use of mechanical thrombectomy devices has resulted in availability of clot material for histopathological analysis. We assessed histological clot composition in a series of patients with large vessel occlusion and investigated correlations between clot composition and stroke etiology 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 upon reasonable request. This study included clot samples collected from multiple sites in the STRIP registry 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, had undergone mechanical thrombectomy treatment for AIS 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 lab for standard tissue processing and embedded in paraffin. The formalin-fixed paraffin-embedded clot material was cut into 3–5 μ m sections. Representative slides from each clot were stained with Hematoxylin and Eosin (H&E) and Martius Scarlet Blue (MSB). Representative MSB stained slides were sent for whole slide scanning (Aperio Scanscope AT-Turbo, Leica Biosystems). Histologic quantification was performed using Orbit Image Analysis Software (www.Orbit.bio) as per the standard operating procedure

(See Supplemental Methods). 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 RBC-rich (>41.9%), Fibrin-rich (>38.2%) and WBC-rich (>3.2%) clots.

Data Collection

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

Statistical analysis

Categorical variables were compared using the Chi-Squared test. Continuous variables were compared using the Wilcoxon test due to non-normal distribution. All statistical correlations were assessed using IBM SPSS Statistics 22.

3. Results

Baseline Characteristics

One-hundred-five patients were included. Median age was 68 (Range=25–93). Forty-nine-percent were treated with recombinant tissue Plasminogen Activator (rtPA). Successful reperfusion (TICI 2b/3) rate was 98% with an overall mean number of passes 2.1. Stroke etiologies were Cardioembolic ($n=52$, 50%), Large Artery Atherosclerotic ($n=20$, 19%) Cryptogenic ($n=21$, 20%) and Other (e.g. dissections, hypercoagulable) ($n=12$, 11%).

Histological Analysis

The quantified histological composition of the 105 cases is shown in Figure 1. Mean platelet, RBC, Fibrin and WBC content were 16.7%, 41.9%, 38.2% and 3.2% respectively. Pre-treatment with rtPA did not significantly affect the mean content of any clot component. LAA strokes had a significantly mean platelet content than cardioembolic stroke ($22.1\% \pm 18.6\%$ vs. $13.9\% \pm 14.3$, $P=0.03$) and non-significantly higher platelet content than cryptogenic stroke ($22.1\% \pm 18.6\%$ vs $17.2\% \pm 14.8\%$, $P=0.74$). Cardioembolic strokes had a non-significantly lower platelet content than cryptogenic stroke ($13.9\% \pm 14.3$ vs $17.2\% \pm 14.8\%$, $P=0.11$).

A significantly higher proportion of LAA strokes were platelet-rich than cardioembolic cases (55.0% vs 21.2%, $p=0.005$). Similarly, a significantly larger proportion of cryptogenic stroke cases were platelet-rich than cardioembolic cases (50.0% vs 21.2%, $p=0.024$). LAA and cryptogenic cases had a similar proportion of platelet-rich clots (55.0% vs 50.0%, $P=0.636$). There was no significant difference between strokes etiologies in relation to any of the other major clot components (Table 1).

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 etiology 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 etiology have focused specifically on RBCs, WBCs and Fibrin/platelet compositions with, largely inconclusive results (3, 4). *Sporns et al.* suggested that cardioembolic emboli had significantly fewer RBCs and higher proportions of fibrin/platelets than non-cardioembolic thrombi, however there was no accounting for specific fibrin and platelet compositions (5).

The findings of this study have implications for management of cryptogenic stroke LVO patients. At present the majority of cryptogenic stroke patients receive anti-platelet therapy for the secondary prevention of stroke. Many recent clinical trials are testing the efficacy of anti-coagulation assuming that most of these lesions have a cardiac source. However, there is growing data suggesting that non-stenotic atheromatous plaque is the culprit in up to 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 while 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 etiology 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 MSB 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 a large artery atherosclerosis etiology of large vessel occlusion. Approximately half of cryptogenic strokes had high platelet content, possibly supporting current treatment paradigms in cryptogenic stroke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contributions made by the Interventional, Nursing and Clinical coordination teams at each of the STRIP registry sites and our Industrial Partners Cerenovus.

FUNDING STATEMENT

This work was supported by the National Institutes of Health grant number (R01 NS105853) and the European Regional Development Fund and Science Foundation Ireland grant number (13/RC/2073).

COMPETING INTERESTS STATEMENT

The authors declare competing interests (Funding, Employment or Personal financial interests) in relation to the work described herein. Dr Waleed Brinjikji declares competing interests in the form of Research Grants; National Institute of Health (No Compensation), Ownership Interest; Marblehead Medical LLC (Significant Compensation) and Other Research Support from Johnson and Johnson (No Compensation). Dr David F. Kallmes declares competing interests in the form of Research Grants; National Institute of Health (No Compensation) and Ownership Interest in Marblehead Medical LLC (Significant compensation). Dr Raul G Nogueira declares competing interests in the form of Stryker Neurovascular (DAWN Trial Principal Investigator - No Compensation, TREVO Registry Steering Committee - No Compensation, Trevo-2 Trial Principal Investigator - Modest Compensation, Consultant – Modest Compensation); Medtronic (SWIFT Trial Steering Committee – Modest Compensation, SWIFT-Prime Trial Steering Committee - No Compensation, STAR Trial Angiographic Core Lab – Significant Compensation); Penumbra (No Compensation), Cerenovus/ Neuravi (ENDOLOW Trial Principal Investigator, EXCELLENT Registry Principal Investigator, ARISE-2 trial Steering Committee - No Compensation, Physician Advisory Board - Modest Compensation), Phenox (Physician Advisory Board - Modest Compensation), Anaconda (Physician Advisory Board - Modest Compensation), Genentech (Physician Advisory Board – Modest Compensation), Biogen (Physician Advisory Board – Modest Compensation), Prolong Pharmaceuticals (Physician Advisory Board – Modest Compensation), IschemaView (Speaker, Modest Compensation), Brainomix (Research Software Use – No Compensation), Somsome (Research Device Use – No Compensation), Viz-AI (Physician Advisory Board - Stock Options), Philips (Research Software Use – No Compensation, Speaker - Modest Compensation), Corindus Vascular Robotics (Physician Advisory Board - Stock options). Dr Albert J. Yoo declares competing interests in the form of Research Grants (All Significant Compensation); Medtronic, Cerenovus, Penumbra, Stryker, Genentech; Employment (Modest Compensation); Cerenovus, Genentech and Personal Financial Interests (Significant Compensation); Inera Therapeutics. Dr Josser A. Delgado declares competing interests in the form of Employment (Modest Compensation) from Medtronic Neurovascular and Penumbra Inc. Dr Andrew M. Demchuk received honoraria from Medtronic for Continuing Medical Education (CME) events. All other authors declare no competing interests (Funding, Employment or Personal financial interests) in relation to the work described herein.

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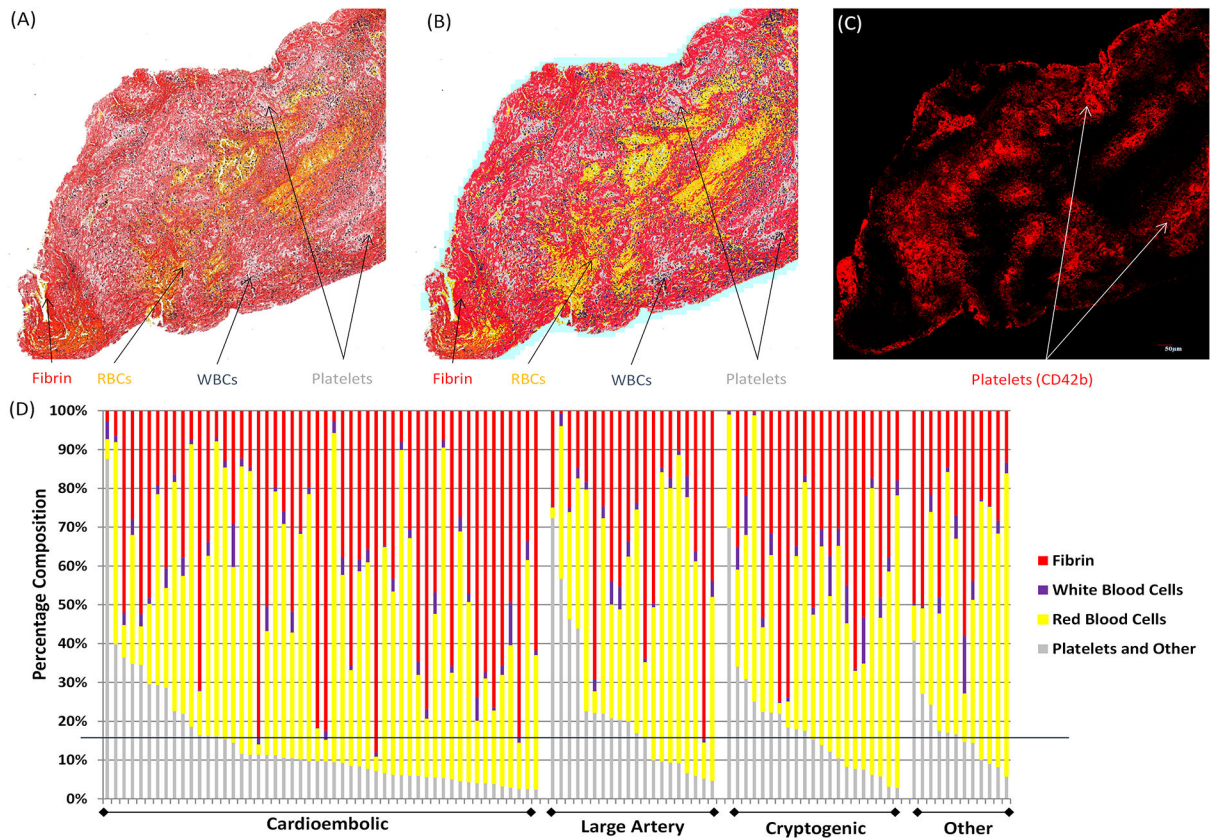


Figure 1: Clot composition of the patient cohort.

(A) Low magnification image (4x) of an MSB-stained slide demonstrating the presence of RBCs (Yellow), WBCs (Blue), Fibrin strands (Red) and Platelets (Grey). (B) The corresponding output image from Orbit Image analysis demonstrating its ability to identify RBCs (Yellow), WBCs (Blue), Fibrin strands (Red) and Platelets (Grey). (C) Immunofluorescence image demonstrating the presence of Platelets (CD42b, Red). (D) Graphical representation of the clot composition of each patient in the cohort.

Table 1.

Histopathological composition of thrombi from Various Stroke Etiologies

Average Composition	Cell Type			
	RBCs	WBCs	Fibrin	Platelets/Other
All Cases	41.9%	3.2%	38.2%	16.7%
Etiology:	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			
Etiology:	RBC-Rich		WBC-Rich	
	Yes(%)	No(%)	Yes(%)	No(%)
Cardioembolic	26(50%)	26(50%)	19(37%)	33(63%)
Large Artery	9(45%)	11(55%)	6(30%)	14(70%)
Cryptogenic	8(38%)	13(62%)	11(52%)	10(48%)
Other	7(58%)	5(42%)	6(50%)	6(50%)
	Fibrin-Rich		Platelet-Rich	
	Yes(%)	No(%)	Yes(%)	No(%)
Cardioembolic	26(50%)	26(50%)	11(21%)	41(79%)
Large Artery	7(35%)	13(65%)	11(55%)	9(45%)
Cryptogenic	8(38%)	13(62%)	10(48%)	11(52%)
Other	5(42%)	7(58%)	6(50%)	6(50%)