Characterization of the ‘White’ Appearing Clots that Cause Acute Ischemic Stroke

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Research article

Keywords: thrombi, platelets, macroscopic appearance

DOI: https://doi.org/10.21203/rs.3.rs-45700/v1

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Abstract

Background:

Most clots retrieved from patients with acute ischemic stroke are ‘red’ in color and are predominantly composed of red blood cells and fibrin. ‘White’ clots represent a less common entity and their histological composition is largely unknown.

The aim of this study was to investigate the composition, imaging and procedural characteristics of ‘white’ clots retrieved by mechanical thrombectomy.

Materials and Methods:

Nineteen ‘white’ thrombi selected by visual inspection from 293 cases were collected as part of the multi-institutional RESTORE registry. Non-contrast computed tomography (NCCT), histological and immunohistochemical analyses were performed. Components were quantified using Orbit Image Analysis.

Results:

Quantification of Martius Scarlett Blue stain identified platelets/other as the major component in ‘white’ clots’ (63%) followed by fibrin (26%), red blood cells (7%) and white blood cells (4%). ‘White’ clots presented significantly more platelets/other and less red blood cells compared to the ‘red’ clots which showed a mean of 23% and 44%, respectively. The mean platelet (CD42b) content in ‘white’ clots was 43%; von Willebrand Factor (vWF) mean expression was 38%.

Collagen and calcification were associated in one case. Fatty acid binding protein 4 was expressed in two cases.

‘White’ clots were also significantly smaller (9.5 versus 12 mm) and less hyperdense (52 versus 61 Hounsfield Units) on NCCT compared to the other cases.

Conclusions:

‘White’ clots represented 6% of our cohort and are platelet and vWF-rich. Calcification, collagen and adipocytes were found occasionally. ‘White’ clots differ from other clots in composition, size and density on NCCT.

Introduction

Several studies have highlighted the variations in the histopathological features of acute ischemic stroke clots (AIS) related to etiology and the impact of thrombus composition on the effectiveness of thrombolysis and mechanical thrombectomy (MT).[1–7] However, only a few papers have analyzed the homogeneous/heterogeneous aspect of color (‘red’, ‘white’, mixed), size and shape of the retrieved clots;
most AIS clots are mainly ‘red’ and histologically show a red blood cells and fibrin-dominant pattern.[4–7] ‘White’ clots account for a small subset of thrombi and their exact composition has been less characterized. It has been shown that ‘white’ coronary thrombi are smaller, predominantly composed of fibrin compared to ‘red’ ones and are associated with lower mortality while the AIS ‘white’ clots are significantly correlated with atypical etiologies.[8, 9]

Therefore, we investigated ‘white’ clots retrieved by MT focusing on their composition, imaging and procedural parameters.

Materials And Methods

Clot collection

This study was approved by the regional hospital ethics committees and National University of Ireland Galway research ethics committee (16-SEPT-08) in accordance with the ethical standards of the Declaration of Helsinki.

A total of 293 patients with AIS underwent MT between March and November 2018 at two centers within the multi-institutional RESTORE registry: Beaumont Hospital (Dublin, Ireland) and Sahlgrenska University Hospital (Gothenburg, Sweden).

Clot length and mean Hounsfield Units (HU) density were evaluated on NCCT prior to mechanical thrombectomy.

Clot analysis

Clots were immediately placed in formalin, shipped to core laboratory and upon arrival, gross photos of each clot were taken. Clots retrieved from 19 patients out of 293 cases were described as ‘white’ based on their gross appearance (Fig. 1). The clots were paraffin-embedded, sectioned and stained with Martius Scarlett Blue (MSB) to identify main components [10], von Kossa and Alizarin Red stainings to confirm calcification and mineralization when suspected and immunostained for platelets (rabbit monoclonal anti-CD42b, Abcam ab227669), von Willebrand Factor (monoclonal mouse anti-human vWF, Dako M0616) and fatty-acid binding protein 4 (anti-FABP4 rabbit polyclonal, Abcam ab13979) for adipocytes.

Stained slides underwent whole slide scanning (Olympus VS120) at 20x magnification. Histological and immunohistochemical quantification was performed using Orbit Image Analysis software (www.orbit.bio).[11]

Statistical analysis

Data were analyzed using IBM SPSS-25 software. Nonparametric Kruskal-Wallis H-test was applied to compare basic characteristics between ‘white’ clots and other cases. Results were expressed as mean ± SD, median [IQ1-IQ3] or number and % of cases, as appropriate.
Results

Study cohort

‘White’ clots represented 6% of our cohort and were selected based on the macroscopic appearance (Fig. 1). Clinical and procedural information for the entire cohort are summarized in Table 1.
Table 1
Clinical and intervention characteristics of study population.

| Characteristic                              | No of patients (%) |
|--------------------------------------------|--------------------|
| **Stroke Subtype:**                        |                    |
| Cardioembolic\(^a\)                        | 9(47)              |
| Large Artery Atherosclerosis               | 2(11)              |
| Other determined pathogenesis\(^b\)        | 2(11)              |
| Cryptogenic\(^c\)                          | 6(31)              |
| **NIHSS:**                                 |                    |
| Baseline, mean ± SD                        | 15 ± 6             |
| **mRS at 90 days:**                        |                    |
| 0–2                                        | 5(26)              |
| 3–6                                        | 13(69)             |
| Not available                              | 1 (5)              |
| **IV rtPA:**                               |                    |
| Yes                                        | 10(53)             |
| No                                         | 9(47)              |
| **Occlusion Location:**                    |                    |
| MCA                                        | 14(73)             |
| Carotid/ICA                                | 3(16)              |
| Tandem occlusion                           | 2(11)              |
| **Occlusion Length on NCCT:**              |                    |
| < 5 mm                                     | 7(37)              |

NIHSS Score: National Institutes of Health Stroke Scale Score; mRS: Modified Rankin Score; IV rtPA: intravenous recombinant tissue plasminogen activator; MCA: middle cerebral artery; ICA: internal carotid artery; mTICI Score: modified Thrombolysis in Cerebral Infarction Score.

\(^a\)Three patients had history of myocardial infarction. One patient presented with atrial fibrillation.

\(^b\)One patient had history of breast cancer. One patient had history of stroke.

\(^c\)A hypercoagulable state due to metastatic cancer was associated in one case. One patient underwent hip surgery 9 days before stroke occurred.
| Characteristic | No of patients (%) |
|----------------|-------------------|
| 5–10 mm        | 4(21)             |
| > 10 mm        | 7(37)             |
| Not available  | 1(5)              |

**Final mTICI Score:**

| mTICI Score | No of patients |
|-------------|----------------|
| 1           | 2(11)          |
| 2a          | 0              |
| 2b          | 4(21)          |
| 2c          | 3(16)          |
| 3           | 10(52)         |

**No of Passes:**

| No of Passes | No of patients |
|--------------|----------------|
| 1            | 7(37)          |
| 2            | 6(32)          |
| 3            | 0              |
| 4            | 1(5)           |
| 5+           | 5(26)          |

**Technique:**

| Technique      | No of patients |
|----------------|----------------|
| Aspiration only| 12(63)         |
| Stentriever only| 6(32)         |
| Combination    | 1(5)           |

NIHSS Score: National Institutes of Health Stroke Scale Score; mRS: Modified Rankin Score; IV rtPA: intravenous recombinant tissue plasminogen activator; MCA: middle cerebral artery; ICA: internal carotid artery; mTICI Score: modified Thrombolysis in Cerebral Infarction Score.

- Three patients had history of myocardial infarction. One patient presented with atrial fibrillation.
- One patient had history of breast cancer. One patient had history of stroke.
- A hypercoagulable state due to metastatic cancer was associated in one case. One patient underwent hip surgery 9 days before stroke occurred.

Platelets/other represent the main component of ‘white’ clots
The quantitative analysis of MSB staining (Fig. 2; Table 2) identified platelets/other as the major component in ‘white’ clots accounting for 63% followed by fibrin (26%), red blood cells (7%) and white blood cells (4%). ‘White’ clots contained significantly more platelets/other and less red blood cells compared to the ‘red’ clots (274 cases) which showed 23% platelets/other (H₁ = 37.95, p < 0.001) and 44% red blood cells (H₁ = 42.09, p < 0.001).

| MSB stain* | Immunohistochemistry |
|------------|----------------------|
|            | Platelets/other (%) | Fibrin (%) | RBC (%) | WBC (%) | CD42b (%) | vWF (%) |
| Mean ± SD  | 63 ± 22              | 26 ± 19    | 7 ± 9    | 4 ± 3    | 43 ± 29    | 38 ± 27  |
| Range      | 25-95.7              | 3.6–62     | 0.4–39.5 | 0.04–10.1| 2-93.2     | 2.1–94.3 |

MSB: Martius Scarlett Blue; RBC: Red Blood Cells; WBC: White Blood Cells; vWF: von Willebrand factor.

*In one case, collagen and calcification were identified representing 9.4% and 5.9%, respectively.

Platelets (CD42b) represented 43% of ‘white’ clots composition while the mean vWF expression was 38% (Figs. 3B, 3C; Table 2). Platelet-rich regions identified by MSB stain were also CD42b and vWF-rich regions (Fig. 3A).

**Calcification, collagen and adipocytes are present in ‘white’ clots**

Calcification suspected on gross examination in one case (Fig. 4A) was confirmed by von Kossa and Alizarin Red (Fig. 4C). Collagen was identified on MSB staining in the same case (Fig. 4B).

Adipocyte-like structures were noticed in two ‘white’ clots (Fig. 4B). Since the lipid content is lost during processing of clots through xylene, the FABP4 antibody was used as a marker for adipocytes. FABP4 was expressed by adipocytes in Case 16 while in Case 15, it was detected only in the nuclei in proximity to adipocytes (Fig. 4D).

**‘White’ clots are smaller and less hyperdense on NCCT**

We found that ‘white’ clots were significantly smaller on NCCT compared to the other clots (9.5 mm [4.25–16.5] versus 12 mm [8.0–20.0]; H₁ = 6.19, p < 0.05).

Mean HU density was available in 12 cases (63%). ‘White’ clots were significantly less hyperdense compared to the other clots (52 HU [40.5–58.0] versus 61 HU [57-68.5]; H₁ = 8.12, p < 0.05).

**Discussion**
We carried out histopathological analysis of 19 ‘white’ clots selected by their macroscopic aspect. We used the MSB stain which allows for a significantly better differentiation of the major components of clots than the traditional hematoxylin & eosin stain.[10] ‘White’ clots were platelet-rich compared to ‘red’ thrombi. We confirmed the presence of calcification in one case using von Kossa stain. Mineralization was demonstrated by Alizarin Red suggesting that arterial mineralization and calcification are governed by morphogenetic signals involved in skeletal mineralization.[12]

It has been shown that emboli with large artery atherosclerosis etiology as well as a high proportion of cryptogenic stroke cases are platelet-rich.[13] In our cohort, two ‘white’ clots had a large artery atherosclerosis source while six cases were cryptogenic. The high platelet content of ‘white’ clots may support the use of antiplatelet therapy for secondary prevention in cryptogenic stroke.

We acknowledge that there are also other components in the platelets/other-rich areas identified by MSB so we performed immunohistochemistry to distinguish between platelets and platelet-related factors such as vWF.[14] The high expression of CD42b and vWF in our ‘white’ clots highlights their critical role in ‘white’ clots formation. Recent studies have described important histological features that may explain the recombinant tissue plasminogen activator (rtPA)-resistance of AIS thrombi: the presence of a dense outer shell containing mainly platelets, vWF and extracellular DNA as well as the presence in the platelet-rich thrombi of dense fibrin structures lined with vWF, extracellular DNA and filled with platelets.[15, 16] Given these observations, we suggest that platelet and vWF-rich composition of ‘white’ clots may impair thrombolysis and therefore, antiplatelet treatment could be used in these cases to target non-fibrin components and improve rtPA efficacy.

Increased levels of platelets and calcium may also render ‘white’ clots stiffer and impact the MT outcome. [14, 17] However, despite the platelet-rich composition of ‘white’ clots compared to ‘red’ clots in our cohort, there was no significant difference in terms of number of passes during MT and final modified Thrombolysis in Cerebral Infarction (mTICI) score.

We identified also the presence of adipocyte-like structures in two ‘white’ clots. Originally described as an adipocyte marker, FABP4 plays an important role in atherogenesis. In particular, FABP4 expression within the carotid atherosclerotic plaque is associated with its vulnerability and adverse outcome.[18] FABP4 immunofluorescence confirmed the presence of adipocytes in one case which may represent a histological marker of fat embolism or a vulnerable atherosclerotic plaque.

Imaging may hint to clot composition.[5, 6, 19] A recent study has demonstrated that platelet-rich thrombi identified by MSB staining are isodense on NCCT.[10] We found that ‘white’ thrombi are smaller and less hyperdense compared to the other clots.

Our study has limitations. The low number of ‘white’ clots may lead to under or overrepresentation of some characteristics presented. Formalin fixation may change the macroscopic appearance and processing through xylene causes the loss of lipid content.
Conclusions

‘White’ clots represent a distinct subset of clots extracted from AIS patients. They are characterized by platelet and vWF-rich composition, are smaller and less hyperdense on NCCT than the other clots. The platelet-rich structure of ‘white’ clots may support the potential use of antiplatelet treatment to prevent stroke recurrence in these cases.

LIST OF ABBREVIATIONS:

AIS: acute ischemic stroke; MT: mechanical thrombectomy; NCCT: Non-contrast computed tomography; HU: Hounseld Units; rtPA: recombinant tissue plasminogen activator; mTICI Score: modified Thrombolysis in Cerebral Infarction Score; MSB = Martius Scarlett Blue; RBC: Red Blood Cells; WBC: White Blood Cells; vWF = von Willebrand Factor; FABP4 = fatty-acid binding protein 4.

DECLARATIONS

Declarations

Ethics approval and consent to participate

This study was approved by the regional hospital ethics committees and National University of Ireland Galway research ethics committee (16-SEPT-08) in accordance with the ethical standards of the Declaration of Helsinki.

A waiver of consent was granted.

Consent for publication

Not applicable

Availability of data and materials

Data are available upon reasonable request.

Competing interests

None declared

Funding

This work has emanated from research conducted with the financial support of Science Foundation Ireland and European Regional Development Fund (Grant Number 13/RC/2073) and Cerenovus.

Authors’ contributions
OMM, and KD were involved in all stages of the manuscript from concept design to drafting the manuscript. AO’H, SP, PB, JA, BM, MD, AB, AA, TG, TT, AR and JT were responsible for collecting and recording the clinical and procedural information from patients. All authors reviewed, edited, and approved the final manuscript prior to submission.

**Acknowledgements**

The authors wish to acknowledge the invaluable contributions made by the Interventional/Nursing and Clinical coordination teams at each of the sites included in the RESTORE registry.

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Figures
Figure 1

Macroscopic features of ‘white’ clots.
Figure 2

‘White’ clots composition. Main components identified by MSB staining in two representative platelet-rich (A) and fibrin-dominant (B) clots: red blood cells (RBC, yellow), white blood cells (WBC, blue), fibrin (red), platelets/other components (grey). Magnification: 50x (A, B). (C) Quantification of main components for each patient.
Figure 3

Immunostaining reveals potential key components of ‘white’ clots. Sequential sections of a representative case stained with MSB, anti-CD42b and anti-vWF (A). Quantification of CD42b (B) and vWF (C) expression for each clot. Magnifications: 30x (A).
Figure 4

Calcification, collagen and adipocytes are present in ‘white’ clots. Gross photos of two representative cases (A). MSB staining (B) showing adipocyte-like structures (star) and collagen (bright blue, arrow). Magnifications: 20x, 40x. Calcification suspected on gross examination (A, red square), confirmed by von Kossa staining (C, brown). Alizarin Red staining demonstrating mineralization (C, orange-red). Magnification: 20x. FABP4 expressed by adipocytes in Case 16 and nuclei adjacent to adipocytes in Case 15 (D, green fluorescence). Nuclei stained with DAPI (blue fluorescence). Magnification: 100x.