Clot permeability and histopathology: is a clot’s perviousness on CT imaging correlated with its histologic composition?

John Charles Benson, 1 Sean T Fitzgerald, 2,3 Ramanathan Kadirvel, 2 Collin Johnson, 2 Daying Dai, 2 Doyle Karen, 3 David F Kallmes, 1 Waleed Brinjikji 1

ABSTRACT

Background Clot perviousness in large vessel occlusion has been shown to be associated with improved recanalization outcomes with mechanical thrombectomy and intravenous thrombolysis.

Objective To evaluate the association between clot perviousness based on thrombus attenuation increase (TAI) on CT, and histologic composition of clots in acute ischemic stroke (AIS).

Methods A retrospective review was completed of patients with AIS secondary to large vessel occlusion, non-contrast CT (NCCT) and CT angiography (CTA) images, and histologic analysis of the retrieved clot. TAI was measured by subtracting clot attenuation on NCCT from the attenuation on CTA. Up to 3 regions of interest (ROIs) were evaluated on each clot; the average attenuation was used for analysis if multiple ROIs were assessed. Pervious clots were defined as TAI ≥10 Hounsfield units (HUs); impervious clots had TAI <10 HU. Histopathologic analyses of clots were assessed for relative compositions of red blood cells (RBCs), white blood cells (WBCs), fibrin, and platelets/other.

Results 57 patients were included. Pervious clots were more likely to be RBC rich (p=0.04); impervious clots were more likely to be fibrin and WBC rich (p=0.01 for both). Pervious clots also had greater RBC density than impervious clots (49.8% and 33.0%, respectively; p=0.006); fibrin density of pervious clots was lower than that of impervious clots (17.8% and 23.2%, respectively; p=0.02).

Conclusion Clot perviousness, assessed on NCCT and CTA imaging, is associated with higher RBC density and lower fibrin density, offering a possible explanation for the higher rates of successful thrombectomy and favorable clinical outcome seen in such patients.

INTRODUCTION

The use of endovascular thrombectomy in patients with acute ischemic stroke (AIS) has allowed for histopathological analysis of the inciting clots. 1 The bulk of these retrieved clots is made up of four major components: red blood cells (RBCs), white blood cells (WBCs), fibrin, and platelet conglomerations. 2 Histologically, however, significant diversity is seen among these clots; relative densities of the major components, type of fibrin network, and presence or absence of atheromatous gruel can vary widely. 1, 3 This cellular variability has implications for treatment: RBC-rich clots are thought to be more amenable to thrombolysis and thrombectomy, whereas fibrin-rich clots are less responsive to revascularization attempts. 4

Clot permeability (also known as perviousness), the degree to which blood is able to flow through a clot’s structure, is increasingly being recognized as an important predictor of responsiveness to therapy. 5, 6 CT imaging can be used to estimate clot perviousness by comparing clot attenuation on non-contrast CT (NCCT) with that on CT angiography (CTA)—known as ‘thrombus attenuation increase’ (TAI). 6 High TAI implies increased perviousness, and is associated with better functional outcome and response to recanalization in AIS. 6–8 To date, however, scant research has directly compared the degree of TAI with the histologic composition of a clot retrieved during mechanical thrombectomy.

The purpose of this study was to assess if TAI is associated with the histologic characteristics of retrieved clots. Because both increased perviousness on CT imaging and high levels of RBCs within clots predict better responses to therapy and favorable patient outcomes, we hypothesize that clots with increased TAIIs will have RBC-rich compositions.

MATERIALS AND METHODS

Patient selection

Institutional review board approval was obtained for this study. A retrospective review was completed of consecutive patients who presented to our institution with stroke-like symptoms between January 1, 2016 and October 1, 2018. Included patients: (1) had AIS caused by an occlusive intracranial clot, (2) had pre-thrombectomy NCCT and CTA imaging and (3) underwent successful mechanical thrombectomy with histopathological analysis of the retrieved clot available. Patients were excluded if the inciting clot was too small to be visualized on NCCT or if diagnostic quality was poor (eg, degraded by motion artifact).

CT imaging

The vast majority of NCCT and CTA imaging was completed on a 128-slice multidetector scanner (SOMATOM Definition Flash; Siemens Healthcare, Erlangen, Germany). Slice thickness for both NCCT and CTA was 0.75 mm. Tube voltage and tube current were set to 120 kV and 350 mA for NCCT, respectively, and to 120 kV and 415 mA for CTA. CTA was performed almost immediately.
after acquisition of NCCT images, according to our institution’s stroke code protocol. In cases where IV thrombolysis was administered, it was used to treat all patients after acquisition of CTA images.

**Imaging review and interpretation**

A neuroradiology fellow, who had been blinded to the clot histology, reviewed the images. TAI was used to assess the degree of clot permeability based on NCCT and CTA imaging, as this technique has been used in many previous studies. To measure the TAI, the Hounsfield units (HUs) of multiple regions of interest (ROIs) within each clot were measured on both CTA and corresponding NCCT images. NCCT and CTA images were not co-registered, but instead read side-by-side within the picture archiving and communication system (PACS) system to ensure accurate placement of ROIs within the thrombus location on the NCCT images. The placement, and number, of ROIs was based on a subjective analysis of how well the artery and/or clot could be visualized on both CTA and NCCT images. Up to three ROIs were placed over each clot; fewer (one or two) ROIs were used for smaller clots. The diameters of ROIs measured approximately 2–3 mm. If multiple ROIs were obtained, the average value of the measured HUs was used. Intra-clot attenuation on NCCT was subtracted from that measured on CTA to yield the TAI (figures 1–3). Clot perviousness on NCCT/CTA was based on absolute TAI: pervious clots were defined as having TAI ≥10 HU; impervious clots had TAI <10 HU. The level of 10 HU was chosen as the cut-off point for clot perviousness using Youden’s index in conjunction with a receiver operating characteristic analysis.

**Thrombectomy and histologic analysis**

Thrombolysis in Cerebral Infarction (TICI) scores were obtained by local operators within our institution. All histopathological

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**Figure 1** Elderly patient who presented with right hemiplegia, gaze preference, homonymous hemianopsia, found to have an occlusion of the left middle cerebral artery (MCA). Acquired (A) and digitally zoomed (B) non-contrast CT and CT angiography (C and D)—also acquired and digitally zoomed, respectively) images show an occlusive clot in the M1 segment of the left MCA.

**Figure 2** Elderly patient with a history of hyperlipidemia and atrial fibrillation who presented with right-sided weakness. CT angiography (CTA) images demonstrated an occlusive clot in the distal M1 segment of the left middle cerebral artery. Regions of interest of the clot were measured on CTA and non-contrast CT (arrows and circles in A and B, respectively).
specimens were immediately fixed in 10% phosphate-buffered formalin, processed using a standard tissue processing protocol, and embedded in paraffin. The formalin-fixed paraffin-embedded clot material was cut into 3–5 µm sections and representative slides from each clot were stained with hematoxylin and eosin and Martius scarlet blue. Representative Martius scarlet blue stained slides from each case were sent for whole slide scanning (Aperio Scanscope AT Turbo, Leica Biosystems). Histologic quantification was performed using Orbit Image Analysis Software (Orbit Image Analysis, Idorsia Ltd) and clots were assessed for relative densities of RBCs, WBCs, fibrin, and platelets. The median of each clot component was calculated and if the proportion of a given component in a clot was higher than the overall median value the clot was considered rich in that component. Thus, RBC-rich clots were defined as having >49% RBC composition; WBC-rich clots were composed of >3.9% of WBC, fibrin-rich clots were composed of >28.7% of fibrin, and platelet-rich clots were composed of >16.3% of platelets.

**Statistical analysis**

Statistical analyses were performed with the SAS-based statistical software package JMP 13.0 (http://www.jmp.com, Cary, North Carolina, USA). Clot histologic compositions and perviousness based on TAI were statistically analyzed using Student’s t-test for continuous variables and χ² tests for categorical variables. R² values were calculated examining the association between TAI and clot composition. The threshold for significance was set to p<0.05. The data were normally distributed for the Student’s t-test. No expected counts of 0 were noted in the χ² test.

**RESULTS**

**Baseline characteristics**

Fifty-seven patients were included, all of whom underwent both pre-thrombectomy NCCT and CTA imaging before mechanical thrombectomy (figure 4); 27/57 (47.4%) were female. Average patient age at time of presentation was 68.3±12.8. All patients were treated with IV tissue plasminogen activator (tPA) before endovascular thrombectomy. The location of most clots was in the middle cerebral artery (MCA) (n=38; 66.7%), followed by the internal carotid artery (ICA; n=6; 10.5%), both the ICA and MCA (n=5; 8.8%), basilar artery (n=3; 5.3%), posterior cerebral artery (n=2; 3.5%), the ICA, MCA, and anterior cerebral artery; n=2; 3.5%), and extending from the common carotid artery to the MCA (n=1; 1.8%). Of clots within part of the MCA, the most common arterial segment involved was the M1 (35/46; 76.1%). The most common etiology of observed clots was cardiac origin (n=34; 59.6%),
followed by large artery atherosclerosis (n=12; 21.1%), other (n=7; 12.3%), and unknown (n=4; 7.0%).

Five patients (8.8%) were treated with stent retriever alone, 39 patients (68.4%) were treated with aspiration alone, and 13 patients (22.8%) were treated with a combination of the two. The mean number of passes made during thrombectomy was 2.2±1.6. Following thrombectomy, the most frequently observed thrombolysis in cerebral infarction (TICI) grade was 2b (n=31, 54.3%), followed by 3 (n=20; 35.1%), 2a (n=3; 5.3%), 0 (n=2; 3.5%), and 2c (n=1; 1.8%). Both patients with TICI 0 had some clot aspirated and available for histologic analysis, but had residual clot without revascularization of the affected territory.

**Clot perviousness and composition**

For all clots, the average attenuation was 46.4±15.6 HU on NCCT and 65.6±21.3 HU on CTA. Multiple ROIs were used in 33 clots and a single ROI was used in 24 clots. When multiple ROIs were used on the NCCT, the median difference in attenuation between various clot segments was 9 (IQR 4.5–14). For CTA, the median difference was 10 (IQR 7–21). Measured TAI's ranged from 0 to 81 HU.

Thirty-eight of the 57 clots (66.7%) were pervious based on TAI≥10. Clots that appeared pervious on imaging had higher RBC density than impervious clots (49.8% vs 33.0%, respectively; p=0.006); fibrin density within pervious clots was lower than in impervious clots (17.8% and 23.2%, respectively; p=0.02). Pervious clots were more likely to be RBC-rich (23/38; 60.5%) than impervious clots (6/19; 31.6%) (p=0.04). Conversely, impervious clots were more likely to be WBC-rich (14/19; 73.7%) and fibrin-rich (14/19; 73.7%) than pervious clots (15/38 (39.5%) and 15/38 (39.5%), respectively) (p=0.01 for both). No significant difference was found between the frequency of pervious and impervious platelet-rich clots, although there was a trend towards increased platelet content in impervious clots (p=0.06). When examining the linear association between clot perviousness and composition, we found a positive association between TAI and RBC composition (p=0.01), and no association between TAI and platelet or WBC composition (table 1).

No association was noted between clot density on NCCT and density on CTA (p=0.22). In addition, no association was found between clot perviousness and clot age (p=0.36). No association was found between the first-pass effect and clot perviousness (p=0.99) or between the first-pass effect and TAI (p=0.65).

The majority of thrombi were treated with aspiration thrombectomy; there was insufficient statistical power to detect a difference in the composition of thrombi retrieved with and without a stent retriever.

**DISCUSSION**

This study sought to find an association between clot perviousness based on TAI and clot histologic composition. The results indicate that there is an association between clot permeability and relative density of RBCs and fibrin within a clot. An absolute arterial attenuation increase of ≥10 HU on CTA compared with NCCT was associated with relatively higher RBC density and lower fibrin density within the retrieved clots.

The relative densities of these RBCs, WBCs, and fibrin/platelet conglomerations within a clot are predictive of its responsiveness to treatment: clots with denser fibrin have impaired lysability during attempted revascularization with tPA, while RBC-rich clots have better responsiveness to IV revascularization therapy.4 9 10 There also seems to be an emerging consensus in the neurointerventional literature that fibrin-rich clots are more difficult to remove than RBC-rich clots. This is due to factors such as a higher static coefficient of friction, resulting in increased adherence to the vessel wall and decreased...
compressibility of the fibrin rich clots, which leads to poorer device integration with stent retrievers.

Multiple prior studies have established a correlation between clot histology and clot appearance on NCCT.11-12 RBC-rich clots tend to have higher attenuation than those that are platelet or fibrin-rich, probably owing to the hemoglobin concentration within the clot.15 In addition, the presence of a hyperdense middle cerebral artery sign (HMCAS) on NCCT is predictive of a RBC-rich clot and early-phase clot composition; according to Liebeskind et al, absence of a HMCAS is an indicator that the clot may be fibrin-predominant.12,23 These findings have implications for clot retrieval: increased clot density on NCCT and high RBC density on histologic analysis have been shown to be associated with successful recanalization after thrombectomy and reduced thrombectomy procedure time.16-18 Conversely, Sporns et al found that fibrin-rich clots are associated with increased intervention time, and are more susceptible to embolism during thrombectomy, possibly reflecting fragility of clots with high fibrin levels.19

Most previous investigations of clot permeability based on CT imaging have focused on the association between clot imaging characteristics and responsiveness to treatment or clinical outcome. Correlations found between such variables have been attributed to multiple factors: clots that appear pervious on CT may be relatively porous, allowing the passage of residual arterial flow and preserving at least some oxygenation to downstream tissues.7 Additionally, pervious clots may be more amenable to IV thrombolysis as the perviousness of a clot to contrast may be a reasonable biomarker of tPA drug delivery to the clot itself. For endovascular recanalization therapy, most studies examining the association between perviousness and thrombectomy outcomes have concluded that patients with pervious clots are more likely to be successfully recanalized and will have better outcomes. However this notion has been refuted in at least one subsequent study.5,20,21 By correlating the TAI with clot composition, our study offers a histologic explanation for the association between clot perviousness on imaging and favorable outcomes.

Only one prior study, by Berndt et al, has directly investigated the association between clot perviousness based on TAI and the histologic composition of clots.22 The results of that study, which performed histological analysis on 32 clots, were opposite to those reported here. The authors found an inverse correlation between TAI and RBC density, and a positive correlation between clot perviousness and fibrin/platelets. Such contrasting conclusions question the reproducibility of the results, and further research into this topic is required before reaching a finalized conclusion. Nevertheless, the results of the current study offer a reasonable association between two variables previously shown to be associated with positive response to recanalization: RBC-rich histology and high perviousness based on CTA.

Our study has limitations. First, clinical outcome was not assessed as part of this study as our main interest was in finding an association between perviousness and histopathology. Hence, it is uncertain if the observed relationship between TAI and clot histopathology also correlates with clinical outcome, as has been reported in prior studies. Also, the majority of cases were treated with aspiration thrombectomy. Possibly, the relatively high number of thrombi treated with aspiration treatment could affect the composition of the retrieved clots. Similarly, only clots that were successfully retrieved could be histologically analyzed, which is a potential source of bias. Next, patients were excluded if their clot was below the level of NCCT imaging, and thus the results from this study are confined to patients with intracranial arterial occlusion. Finally, as stated above, the conflicting results obtained between the recent study of Berndt et al and this study suggests that further research is needed.

CONCLUSIONS

Increased clot perviousness based on NCCT and CTA imaging is associated with higher RBC density and lower fibrin density of mechanically retrieved clots. This histologic association offers a possible explanation for the superior recanalization rates with IV thrombolysis and mechanical thrombectomy and favorable clinical outcomes of patients with permeable clots seen on CT imaging (Supplemental Files 1-4).

Correction notice Since this paper was first published online, the funding statement has been updated.

Contributors JCB participated in the data collection and imaging review, and drafted the manuscript. STF, RK, CJ, DD, and DK participated in the review and interpretation of imaging and pathology data. DFK and WB participated in the conceptualization and design of the study, performed the statistical analysis, assisted in the interpretation of data, and revised the manuscript.

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Ischemic Stroke


