Thrombus Composition, Imaging, and Outcome Prediction in Acute Ischemic Stroke

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Abstract

Background and Objectives
New imaging techniques have advanced our ability to capture thrombus characteristics and burden in real time. An improved understanding of recanalization rates with thrombolysis and endovascular thrombectomy based on thrombus characteristics has spurred interest in new therapies for acute stroke.

Methods and Results
This article reviews the biochemical, structural, and imaging characteristics of intracranial thrombi in acute ischemic stroke; the relationship between thrombus composition and response to lytic and endovascular therapies; and current and future directions for improving outcomes in patients with acute stroke based on thrombus characteristics.

Discussion
Thrombus composition, size, location, and timing from stroke onset correlate with imaging findings in acute ischemic stroke and are associated with clinical outcome. Further research across multiple domains could assist in better applying our knowledge of thrombi to patient selection and individualization of acute therapies.
Throughout the development of acute ischemic stroke therapies over the past 30 years, a focus of research has been on understanding thrombus characteristics in acute ischemic stroke, including composition, location, and size. The rationale for this line of work is that better patient selection and higher treatment efficacy can be achieved if management is tailored towards specific thrombi characteristics. Advanced imaging, new interventions, and emphasis on time to treatment has spurred a resurgence in research and interest in this topic. Here we review our current understanding of the importance of the thrombus in acute ischemic stroke, its relationship with treatments available and their effect on patient outcomes, and implications for future research in the field.

Relationship Between Thrombus Composition and Stroke Etiology

Understanding thrombus composition allows greater insight into ischemic stroke etiology, pathophysiology, and potential targets of treatment. Given the wide array of definitions used in the literature, the consensus statement by the Clot Summit Group sought to harmonize reporting research on thrombus composition in acute ischemic stroke, suggesting that thrombi should be described in terms of heterogeneity (red, white, mixed), size, shape, extraction after single or multiple passes, physical properties (elastic, stiff, friable), and imaging characteristics (hypertennuating arterial lumen on noncontrast CT head, blooming artifact on MRI, and thrombus length).1 Traditionally, erythrocyte-rich thrombi (“red thrombi”) were considered to be due to stasis in a low-pressure system, often venous, whereas platelet-rich thrombi (“white thrombi”) were active in the arterial system and often associated with high flow states and plaque rupture.2 However, this principle has not been consistent in studies of intracranial thrombi. Although the presumed origin of red thrombi is from a static cardiac source, red blood cells (RBCs) were more abundant in arteriogenic thrombi compared to cardiogenic thrombi and fibrin was higher in cardiogenic thrombi.3,4 In support of this, further studies found the highest percentage of RBCs among atherosclerotic thrombi and the lowest among cardioembolic or unknown source, but there was no difference in fibrin content between these subtypes.5,6 There is also uncertainty surrounding calcification, which is less common in cerebral thrombi and may be due to a large artery source although this association has not been clearly established.7,8 In contrast, white blood cell content has been associated with cardioembolic etiology.4,9 These findings are dampened by other histologic studies demonstrating no specific pattern among thrombi from atherogenic or cardioembolic sources.7,10 A study of 105 thrombi from proximal occlusions included in the Stroke Thromboembolism Registry of Imaging and Pathology (STRIP) found no difference in any thrombus component except platelets, which were associated with large artery atherosclerotic etiology.11 Similarly, others found that in arteriogenic thrombi, platelet aggregates covered fibrin layers and were localized at the edge of thrombi, whereas in cardiogenic clots, platelets were clustered within rich fibrin thrombi.12 A recent meta-analysis on this issue also did not demonstrate a significant difference in proportion of RBC-rich thrombi between cardioembolic and large artery atherosclerosis.13 There is therefore no clear evidence that red thrombi in the cerebral circulation originate from a cardioembolic source. Of note, biases may have been introduced into studies of histologic composition due to the use of IV thrombectomy altering thrombus composition, investigations for stroke etiology directed by thrombus or imaging features, variations in assessing etiology based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, patients with the poorest outcomes not having retrievable thrombi, or only the most stable components of the thrombi being retrieved.4,13 In addition, lack of uniformity in thrombus preservation techniques, histopathologic descriptions, quantification methods and software, and immunohistochemistry methods, as well as small sample sizes, have further contributed to the heterogeneity in findings.1 DNA content has recently been associated with cardioembolic thrombi and may be a promising novel marker of cardioembolic stroke in patients with embolic stroke of undetermined source.14

Relationship Between Thrombus Composition and Recanalization With Thrombolysis or EVT

Human fibrinogen is a soluble glycoprotein produced by the liver, which is converted to insoluble fibrin by thrombin. Conformational changes and polymerization propagate through fibrin, forming protofibrils, which in turn bundle together to form fibers. Polymerized fibrin is insoluble. However, the conversion of fibrinogen to fibrin is thought to expose binding sites for plasminogen and tissue plasminogen activator (tPA). tPA binds to fibrin through a finger domain, and in the presence of fibrin increases its activity from 100- to 1,000-fold, further activating plasmin. Plasmin is the activated form of plasminogen, and is the primary fibrinolytic
agent, with at least 34 different plasmin cleavage sites identified on fibrin. Thus, through a negative feedback loop, fibrin provides the mechanism of its own lysis. Further clot lysis is dependent on thickness of fibers, pore size between fibers, crosslinking by additional factors such as FXIIIa, and penetration of clot by fibrinolysis.15

Fibrinogen also binds to platelets at the site of the thrombus, and dynamic changes occur over time. In acute myocardial infarction, fresh coronary thrombi have the highest concentration of platelets, but as ischemic time increases, platelet concentration decreases and fibrin increases, further stabilizing the thrombus.16 Similar processes occur in the intracranial circulation, but are less well characterized. For example, changes in biochemical and hemodynamic factors in an occluded vessel could allow for dissolution of less stable thrombus and further strengthening of the fibrin meshwork.17-20 Higher fibrin content among thrombi retrieved with endovascular thrombectomy (EVT) is associated with increased thrombus stiffness,21 and a scoping review found that fibrin-rich clots were associated with increased recanalization maneuvers, longer procedure time, and less favorable clinical outcomes compared to RBC-rich clots.22

A recent study evaluated 100 thrombectomy-retrieved thrombi with immunohistology and scanning electron microscopy.23 The authors found that the thrombus composition is heterogenous, but 24/30 thrombi had an outer shell made of densely compacted thrombus components including fibrin, von Willebrand factor (vWF), and aggregated platelets, forming a continuous layer and showing decreased susceptibility to tPA-mediated thrombolysis compared to the inner core. The inner core contained less aggregated and clearly identifiable RBCs, fibrin fibers, and platelets. Staining of the thrombus shell was positive for direct inhibitors of tPA: plasminogen activator inhibitor 1 and protease nexin 1 (PN-1). In thrombectomy-retrieved thrombi there was also reduced in vitro thrombolysis for those with intact shell compared to thrombi with a ruptured external shell. This is compatible with the fact that dense fibrin thrombi are known to have reduced lysability, and higher serum D-dimer release rate in patients receiving thrombolysis—suggesting lysability—was associated with good outcome at 3 months.24

Compared to fibrin, the role of RBCs in lysability is less clear. In vitro studies show that higher RBC content is related to increased thrombolytic resistance due to an interaction with fibrinogen and modification of fibrin structure.25,26 However, this relationship is not seen in vivo. Historical experiments of coronary artery occlusion show that infusion of recombinant tPA lysed erythrocyte-rich thrombi, but was significantly less efficient for reperfusion of platelet-rich material.27 Furthermore, in a rabbit model of thromboembolic stroke, recombinant tPA enhanced lysis of erythrocyte-rich but not fibrin-rich thrombi.28 In swine extracranial arteries occluded with fibrin-rich thrombi, recanalization after thrombectomy was lower with longer mean recanalization times as compared to erythrocyte-rich thrombi.29 In humans, RBC fraction was an independent predictor of responsiveness to IV thrombolysis, measured as a decrease in clot burden from baseline CT angiography (CTA) to digital subtraction angiography during EVT.30 The proportion of RBC-rich components was higher in thrombi retrieved from reperfused compared to non-reperfused patients, with or without IV thrombolysis, with thrombi having >2/3rd erythrocyte components being associated with successful reperfusion.31 Higher RBC content may also be related to reduced number of maneuvers and decreased procedure time in patients undergoing EVT.32 This is consistent with the finding that compared to thrombi with high RBC content, fibrin-rich thrombi had a significantly higher static friction between the thrombus and the vessel, resulting in resistance to sliding, which could reduce speed and efficacy of thrombectomy.33 Conversely, one study demonstrated high RBC content in thrombolysis-resistant thrombi obtained through thrombectomy.34 However, the latter study was small and considered all thrombi obtained at EVT to be thrombolysis resistant, rather than measuring changes in clot burden between CTA and angiography. At present, although RBC content may be related to lytic responsiveness, a definitive relationship cannot be established due to the small number of studies available, variability in timing of EVT and thrombus retrieval, and sampling biases introduced by an inability to obtain and analyze thrombi that rapidly resolve with IV thrombolysis.

Other factors besides RBCs, fibrin, and platelets have been found to be implicated in the formation and maintenance of thrombi and resistance to lysis. Calcium may render a thrombus stiffer and less amenable to thrombolysis and stent retriever thrombectomy.35,36 White blood cell content has also been related to less favorable recanalization and worse clinical outcome.37 vWF is found in thrombi obtained from humans, recruiting platelets at the site of vascular injury and linking platelets with each other and with the exposed components of the endothelium, stabilizing the platelet aggregation.38 As such, thrombi with vWF demonstrate resistance to lysis from tPA,39 high vWF concentration is associated with high NIH Stroke Scale (NIHSS) score at admission, and greater platelet content of thrombi is associated with higher thrombus stiffness, more severe stroke, and worse revascularization outcomes.40 Platelet factor 4 is involved with transformation and polymerization of fibrin, which may contribute to sealing thrombi.41 PN-1 inhibits plasminogen activators, plasmin, and thrombin. PN-1 also inhibits generation and activity of plasmin by tPA and blockade or deficiency of PN-1 results in increased lysis of thrombi.42 Neutrophil extracellular traps are DNA extracellular networks produced by activated neutrophils, and also contribute to the composition of thrombi, especially the outer layers.43 Platelet factor XIII forms fibrin crosslinks and enhances the resistance of platelet-rich thrombi to fibrinolysis.44,45 Lastly, immune cells also may play a role, with the number of CD4+ and CD68+ monocytes increased in erythrocytic and red clots compared to white thrombi, which...
were instead rich in vWF+, or mixed clots. However, the role of these immune markers is unclear.40 The many proteins discussed here are additional targets to study for new thrombolytic therapies. Despite these various associations, it should be noted that in most in vitro studies of thrombolysis, tPA is added into an already formed fibrin network and therefore heavily dependent on permeation of tPA into the thrombus, whereas in vivo fibrinolysis occurs concurrently with polymerization of the fibrin network.51

**Thrombus Imaging: The Hyperdense Artery Sign**

The hyperdense artery sign (HAS), first recognized in 1983, has long been established as an indicator of acute occlusion by thrombus on noncontrast CT (NCCT)52 and tends to be at main trunks of cerebral arteries. The association between HAS and stroke etiology has been mixed.53-55 Increased sensitivity for HAS can be achieved with thinner slices, ideally close to 1 mm for optimal sensitivity rather than traditional 5–10 mm thickness, due to volume averaging of intraluminal thrombus and surrounding CSF space with thicker slices.56,57 The HAS is correlated with “early phase” pathology (RBC dominant or RBC proportion equal to fibrin) rather than “late phase” (fibrin dominant and organized fibrin), and with cardioembolic etiology.32,55,58 In a meta-analysis, patients with HAS were more likely to have RBC-rich thrombi.13

The association of HAS with outcomes is less clear. On the one hand, HAS is associated with worse outcomes. In an analysis of the IST-3 trial, presence of HAS was associated with worse outcome at 6 months, and outcome was worse with more extensive HAS, although it did not modify the benefit of alteplase.59 Improved patient outcome is shown when the HAS shrinks or disappears.60,e1 On the other hand, multiple studies have shown that higher density of thrombi predicts better recanalization, and thrombi with lower density were more resistant to treatment.31,e2,e3 Lower thrombus density on preintervention CT was an indicator of lower recanalization (Thrombolysis in Cerebral Infarction score 2b/3),13,e20,e21 early clinical improvement, and good outcome at 3 months,e13,e22 after mechanical thrombectomy. As with HAS, this apparent contradiction can be explained by the fact that acute dense thrombi portend worse outcomes, but may be amenable to revascularization with thrombectomy.53

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sensitivity than T2* for the SVS, obtaining MRA would limit practicality even further. Furthermore, in a study done on 35 manufactured thrombi with varying histologic compositions, the diagnostic accuracy of SVS varied significantly among MRI scanners. Another disadvantage is the need for postcontrast MRI to accurately assess the terminal thrombus and SVS length, given the overestimation of thrombus length on SWI.

Imaging: Thrombus Permeability

CTA has become the most useful tool for assessing acute arterial occlusion in patients with stroke given its more widespread availability and detailed characterization of intracranial occlusions, particularly on multiphase CTA. CTA has very high sensitivity, specificity, and positive predictive value for occlusion when compared to digital subtraction cerebral angiogram as the gold standard. Attenuation of the thrombus itself has been evaluated on CTA in relation to outcomes. In one study, an HU ratio comparing site of occlusion with the contralateral artery was the strongest predictor of recanalization, and specifically a HU ratio of <1.382 was associated with optimal sensitivity and specificity in predicting absence of recanalization after IV tPA. In 199 patients from the MR CLEAN trial, relative thrombus density on CTA was independently associated with functional outcome. However, no treatment effect modification was seen by any thrombus CT characteristics. In another study, recanalization in the endovascular group was better with higher CTA thrombus density, analogous to what is seen with HAS.

Although simple thrombus contrast enhancement is independently related to clinical outcome, additional measures were developed that compare thrombus attenuation on CT and CTA to assess movement of contrast through and around the thrombus. Complete occlusions were characterized on multiphase CTA as blood flowing away from the distal thrombus interface (occult anterograde flow) or within the same artery towards the thrombus (retrograde flow). Occult anterograde flow was found to be predictive of early recanalization. Permeability, or how well blood flows through a thrombus (also called perviousness), is thought to relate to enhanced effect of thrombolysis due to increased surface area of contact between the lytic agent and thrombus. This is supported by simulations of lysis in fibrin, where the lytic agent progressed fastest in regions where initial permeability was highest. Permeable thrombi have been associated with cardioembolic source, but with both higher and lower RBC fraction. Permeability can be assessed using multiple techniques, including thrombus attenuation increase or a void fraction on CTA. Thrombus attenuation increase is measured as the increase in mean attenuation for the thrombus on CTA compared with NCCT. Void fraction represents the ratio of the volume of blood within the thrombus to the total thrombus volume. A multiphase CTA, which allows visualization of the thrombus in arterial, venous, and delayed phases, was shown to be better than a snapshot CTA in assessing thrombus permeability. In a recent study, 4 methods of measuring thrombus permeability were compared, and found that automated, semi-automated, manual, and visual methods of thrombus permeability did not differ significantly in identifying early recanalizers.

In the INTERRSeCT study, patients with a higher grade of residual flow around the thrombus (grade 0: thrombus with no permeation; grade I: thrombus appearing denser than surrounding parenchyma; grade II: hairline or streak of well-defined contrast across the length of the thrombus) were more likely to recanalize. Similarly, in a study of dichotomized thrombus permeability, complete recanalization after IV tPA was much higher for those with pervious thrombus. Indeed, in a study including only those who...
received IV tPA, patients with residual flow within the thrombus were 5 times as likely to reperfuse than those without it. Those who had shorter thrombus length (≤15 mm), distal thrombi, or low clot interface ratio (measured by dividing the proximal clot interface HU by the distal clot interface HU; a high clot interface ratio indicates low permeability) were most likely to reperfuse. Proximal M1 clots without residual flow reperfused only 8% of the time and carotid T or L occlusions only 1.7%. Only one study found no association between thrombus permeability and recanalization. Thrombus permeability is also associated with reduced final infarct volume and improved functional outcome.

It should be considered that thrombus attenuation increase (or permeability) is also significantly associated with collateral score, or presence and extent of leptomeningeal collaterals. Low regional leptomeningeal score is a significant predictor of hyperacute infarct growth. Thrombus permeability was associated with better functional outcome only in patients with good to moderate collaterals, possibly due to the strong relationship of poor collaterals with poor outcome, wherein thrombus permeability is no longer relevant.

### Imaging: Location and Length of Thrombi

The location of the thrombus is clearly one of the most important factors in recanalization and outcomes. More proximal occlusions result in lower rates of recanalization. Early studies of tPA demonstrated that only 8% of extracranial internal carotid artery (ICA), 26% of proximal middle cerebral artery (MCA), and 38% of distal MCA occlusions recanalized. In terms of outcome, dramatic recovery was seen in 33% of distal MCA, 16% of proximal MCA, 15% of basilar, and no terminal ICA occlusions. Ninety-day outcomes were similarly poor with proximal occlusions. Consistent with this, in the INTERRSeCT study, the more distal the thrombus, moving from ICA, to proximal M1, to distal M1, to M2, and finally to M3, the more likely it was to recanalize (with 5 times higher odds of recanalization for an M3 thrombus compared to ICA). Only 10% of patients with ICA and 21% of patients with M1 thrombi had recanalization with IV tPA alone. Of note, not all cases present with single occlusive thrombi, and patients with multiple intracranial thrombi are more likely to have severe symptoms, larger ischemic area, lower collaterals, and unfavorable outcome.

More extensive thrombus is associated with low early recanalization. Thrombus length was >8 mm in 94% of ICA terminus occlusions, 73% of M1 occlusions, and 22% of M2 occlusions, which may partly explain the historically low published rates of recanalization with IV tPA for proximal thrombosis. Various cutoffs have been reported in relation to thrombus size and poor outcome. Hyperdense MCA sign of length >10 mm infrequently disappears on repeat NCCT after IV tPA; in one study, thrombi <10 mm disappeared 85% of the time, those 10–20 mm disappeared 37.5% of the time, and no cases of >20 mm disappeared. In other estimates, only 1% of patients recanalized with IV tPA alone when thrombus length was >8 mm on NCCT, and no patient achieved recanalization when thrombus length exceeded 14 mm. Another study found an optimal cutoff value of

### Table 1: Advantages and Disadvantages of Select Imaging Modalities in Identifying Thrombus Characteristics in Patients With Acute Stroke

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Noncontrast CT   | • Rapidly performed and universally available  
  • Hypodense artery sign  
  • Identify bleed or early infarct | • Subjectivity of hyperdense artery sign  
  • No confirmation of thrombus, no data on collaterals or flow |
| CT angiography   | • Accurately identify location and length of thrombus or multiple thrombi  
  • Simultaneously assess for intracranial and extracranial occlusions or dissections  
  • Can be used to measure thrombus permeability, collaterals, and clot burden score | • No universal or automated method to calculate permeability or clot burden  
  • Variability in use of multiphase CTA  
  • Contrast allergies or contrast-induced nephropathy |
| GRE MRI         | • Identification of susceptibility vessel sign (blooming artifact)  
  • Confirmation of evolving/established infarct  
  • Rule out other etiology | • Lost time; not generally available rapidly  
  • Low yield for occlusion without MRA |
| Catheter angiography | • Gold standard  
  • Immediately transition from diagnosis to treatment | • Invasive  
  • Time consuming  
  • Assess one hemisphere at a time  
  • Does not image thrombus directly |
| TCD             | • Dynamic bedside tool to assess for flow or microemboli | • Does not assess thrombus composition directly  
  • Usually cannot assist with acute treatment decisions  
  • Highly operator-dependent  
  • Dependent on bone windows |

Abbreviations: CTA = CT angiography; MRA = magnetic resonance angiography.
11–12 mm thrombus length in predicting poor outcomes and recanalization.\textsuperscript{63,67} Median thrombus length was significantly longer in patients with poor outcome (modified Rankin Scale \textit{[mRS]}>3) vs good outcome (mRS <2).\textsuperscript{2,6} High thrombus grade (large thrombus measuring >2 vessel diameters) and thrombus volume were independently associated with nonrecanalization and poor outcomes.\textsuperscript{58,69} In an analysis of 108 patients included in the THERAPY trial, longer thrombi were independently associated with worse clinical outcomes (90-day mRS); there was a 33% relative increase in the likelihood of a worse outcome with every 5-mm increase in thrombus length.\textsuperscript{66} Furthermore, the relative benefit of thrombectomy compared with IV thrombolysis alone increased with thrombus length. Some studies could not find an association between thrombus length and recanalization or functional neurologic outcomes.\textsuperscript{4,61,62}

## Clot Burden Score

The assessment of attenuation and permeability can be difficult in real time. The thrombus burden score is a tool developed to simply quantify extent and burden of ipsilateral intracranial thrombus to assist in patient stratification and decision making. Ten points are assigned for full ipsilateral contrast opacification and 2 points each are subtracted for absence of contrast opacification in the cross-section of any part of the proximal M1 segment, distal M1 segment, or suprachinoid ICA, and 1 point subtracted for each M2 branch, A1 segment, and infrachinoid ICA. Partial filling defects are rated as patent. A score of 10 indicates absence of visible occlusion on CTA and 0 indicates occlusion of all major intracranial anterior circulation arteries.\textsuperscript{63} The score supports the idea that not only occlusion site but amount of thrombus burden in different vascular segments is a major determinant of stroke severity and outcome in anterior circulation stroke.

Patients with higher clot burden score (CBS) were more likely to have better angiographic and clinical outcomes.\textsuperscript{31,63} Hemorrhagic infarct transformation and mortality increased in patients with low CBS, and CBS was an independent predictor of good functional outcome even after adjusting for age, sex, and thrombolytic therapy.\textsuperscript{63} Lower CBS was associated with early ischemia on CT and increased risk of ICH in patients undergoing EVT\textsuperscript{64} and CBS was a predictor of early dramatic recovery and favorable prognosis.\textsuperscript{65} In a systematic review and meta-analysis, higher CBS was associated with better functional outcome and higher rate of recanalization.\textsuperscript{66} In the INTERRSeCT study, higher CBS was independently associated with successful recanalization, with an odds ratio of 3.3 for CBS 5–7 and 3.8 for CBS 8–10 when compared to 0–4.\textsuperscript{65} However, among 500 patients in the MR CLEAN study, there was no evidence that CBS modified the effect of intraarterial treatment.\textsuperscript{67}

CBS has been modified to be used with MRI (T2*–CBS) and is associated with 24-hour recanalization and better 3-month outcome on the mRS after IV tPA.\textsuperscript{68} The relative benefit of mechanical thrombectomy over IV tPA seemed to increase with lower T2*CBS score and longer thrombus.\textsuperscript{61} The above discussed factors were all shown to be important in an analysis of 408 patients from the MR CLEAN study; thrombus with distal location, higher CBS, and shorter length, as well as thrombus permeability, were associated with better outcomes.\textsuperscript{69} Leptomeningeal collateral score and CBS are predictors of good outcome, final infarct volume, and recanalization.\textsuperscript{42,70,71} Those who have CBS >6 are 4 times more likely, and those with good collaterals 5 times more likely, to have a good clinical outcome.\textsuperscript{70}

The utility of the CBS is unclear in the current era. In an analysis of the SWIFT PRIME trial, IV tPA+ solitary stent retriever was effective in achieving recanalization and good clinical outcome throughout the entire range of CBS values.\textsuperscript{72} Therefore, a higher thrombus burden, although portending a worse outcome without treatment, would create an additional incentive to proceed with EVT in appropriate patients given the low likelihood of spontaneous recanalization.

### Table 2 Imaging Features, Recanalization, and Clinical Outcomes Associated With Various Thrombus Compositions

<table>
<thead>
<tr>
<th>Clot composition</th>
<th>Imaging features</th>
<th>Recanalization with thrombolysis</th>
<th>Recanalization with EVT</th>
<th>Clinical outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>HAS, SVS</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>WBC</td>
<td>?</td>
<td>Decreased</td>
<td>?</td>
<td>Decreased</td>
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<tr>
<td>Calcium</td>
<td>Hyperdense</td>
<td>Decreased</td>
<td>?</td>
<td>Decreased</td>
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<tr>
<td>Platelets</td>
<td>No SVS</td>
<td>Decreased</td>
<td>?</td>
<td>?</td>
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<tr>
<td>vWF</td>
<td>?</td>
<td>Decreased</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Fibrin</td>
<td>No HAS</td>
<td>Decreased</td>
<td>Decreased</td>
<td>?/Decreased</td>
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</table>

Abbreviations: EVT = endovascular thrombectomy; HAS = hyperdense artery sign; RBC = red blood cell; SVS = susceptibility vessel sign; vWF = von Willebrand factor; WBC = white blood cell.
See Figure 1 for examples of using CT to assess thrombus characteristics and Table 1 for advantages and disadvantages of select imaging modalities discussed above.

**Thrombolysis, Thrombectomy, and Implications for Future Treatment**

Identifying factors that promote recanalization is paramount to patient selection and individualization of therapy, as recanalization is associated with favorable outcome. In the INTERRSeCT study, among 575 patients with intracranial occlusion on CTA, recanalization occurred in 30% of patients who received IV alteplase and 13% of patients who did not. Among those who received alteplase, the following factors were associated with recanalization: longer time from treatment start, more distal thrombus, and higher residual flow (thrombus permeability). Early studies demonstrated that most tPA-related recanalization occurred within the first hour after treatment and the rate of thrombus resolution with thrombolysis decreases as time interval from symptom onset increases, in both mouse model and patients. Therefore, regardless of treatment approach, the priority remains developing strategies to achieve complete, early recanalization for optimization of favorable outcomes.

Compounds besides tPA have been experimentally shown to enhance reperfusion. Administering eptifibatide, a glycoprotein 2b/3a inhibitor, along with tPA has shown comparable safety. Argabotran augments recanalization by tPA in animal models with similar safety profile. ADAMTS13 can dissolve occlusive thrombi but without the hemorrhagic side effects of tPA. IV N-acetylcysteine (NAC) promotes lysis of arterial thrombi that are resistant to tPA, direct thrombin inhibitor, or antiplatelet treatments. The molecular target of NAC is vWF that crosslinks platelets in arterial thrombi. In a thromboembolic stroke model in mice, cotreatment of NAC and a glycoprotein 2b/3a inhibitor improved ischemic lesion size and neurologic outcome without worsening hemorrhagic stroke outcome. These compounds have not yet been tested in humans.

Closer to clinical practice is the use of tenecteplase (TNK) for acute stroke. Early studies identified TNK as having a longer half-life, minimizing fibrin-independent plasminogen activation, and allowing a simpler administration with single bolus of the agent, as compared to tPA. The ATTEST trial was a phase 2 trial comparing TNK with tPA in acute stroke and demonstrated no difference in neurologic outcome or incidence of symptomatic intracranial hemorrhage. Venous blood samples were retrieved pretreatment, 3–12 hours, and 24 hours post thrombolysis, and tPA was shown to induce more hypoﬁbrinogenemia compared to TNK. TNK consumed less plasminogen and ﬁbrinogen compared to tPA, consistent with lower rate of ICH. More recently, the EXTEND-IA TNK study demonstrated that TNK administered prior to thrombectomy resulted in improved recanalization and better 90-day outcomes as compared to tPA. Ongoing trials like ATTEST-2, TASTE, and AcT are likely to provide conclusive evidence on the use of TNK vs alteplase in patients with acute ischemic stroke.

Interventional procedures may also be enhanced by considering characteristics of the thrombus, such as thrombus shape.
Consoli et al. divided M1 thrombi at the occlusion site into regular phenotype (occlusion was smooth and straight with abrupt cutoff) and irregular phenotype (modification of the linearity of the occlusion, including concave or convex shapes or multiple contrast filling defects). Regular thrombi achieved recanalization more often with contact aspiration vs stent retrievers, and irregular thrombi had the opposite relationship. However, the sample size was small and the findings preliminary. The COMPASS and ASTER trials demonstrated similar revascularization and clinical outcomes with contact aspiration compared to stent retriever techniques for large vessel occlusion, and raise the question of whether techniques would be differentially effective for specific thrombus compositions and biomechanical properties. One study suggested that new devices should consider materials with greater radial forces and oval stent struts to penetrate stiff clots with high fibrin/platelet content, potentially with the addition of novel antiplatelet regimens. Another study separated 210 patients into 2 groups: waiting or nonwaiting after stent deployment. The waiting group had higher rate of 1-pass procedures, lower number of stent passages, and shorter procedure time. The proportion with successful recanalization was higher in the waiting group, and in multivariate analysis "waiting" was a predictor of successful recanalization. Similarly, in an in vitro thrombus model, pushing the Trevo stent during device delivery into hard thrombi enhanced integration, and waiting 5 minutes increased integration in both hard and soft thrombi. Furthermore, clots retrieved from later passes were associated with higher fibrin content compared to earlier passes, possibly due to a greater number of passes needed to overcome the static coefficient of friction between the clot and vessel wall for fibrin-rich components. Overall, the effect of thrombus characterization on acute clinical care has thus far been limited, due to the inability to accurately assess thrombus composition in vivo. Further understanding of individual thrombus characteristics in real time could assist in selecting the most effective endovascular strategy. See Table 2 for a summary of associations between thrombus composition, imaging features, and outcomes based on the available data.

Thrombus characteristics could also be useful to design future noninvasive trials of thrombolysis. The CLOTBUST trial randomized patients receiving IV tPA within 3 hours to receive continuous 2 MHz transcranial Doppler ultrasonography or placebo. The end point was complete recanalization or dramatic clinical recovery and occurred in 49% of the treatment group vs 30% of the placebo group. At 3 months, there was a nonsignificant trend to improved functional outcome. In 2019, another trial testing sonothrombolysis recruited patients with NIHSS ≥10 who received IVT. There were 335 patients randomly allocated to intervention and 341 to the control group. Despite earlier reports of higher rate of ICH with ultrasound, the treatment was shown to be feasible and safe, but no clinical benefit was detected. However, the treatment was unfocused ultrasound without localization or targeting of treatment based on thrombus characteristics such as size or permeability that could be substrates for patient selection in future trials.

The Dynamic Nature of Intracranial Thrombi and Future Directions

Some inconsistencies highlighted above in the studies on thrombus composition, relationship with imaging findings, and outcomes are yet to be clarified. For example, studies on relationship between thrombus composition and stroke etiology, imaging findings, and outcomes have conflicting results. On one hand, a true relationship may exist, although it is
masking the heterogeneity in studies, including differences in patient population, imaging technology, and timing of imaging in relation to onset of symptoms, as well as treatment choice and timing of thrombus removal. On the other hand, thrombus characteristics may only be a small part of the story, contingent on time since stroke onset, individual arterial anatomy, and presence of collaterals. We have previously suggested that stasis of thrombus in an intracranial vessel may rapidly produce a secondary thrombus around the original one that is RBC rich, particularly if there are poor collaterals or local angioarchitecture. Consistent with this, slow collateral flow can be associated with thrombus extension in large artery occlusions. This may explain the inability to identify a relationship between RBC composition and arteriogenic or cardioembolic stroke etiology, as the new thrombus components may constitute a significant portion of the overall thrombus, with the proportion of RBC in thrombus depending on timing from stroke onset.

The RBC-rich nature of a static, proximal, hyperacute thrombus is correlated with increased hyperdensity on NCCT and blooming artifact on MRI; longer clots may have more hyperdensity and blooming due to freshly formed clot extension within the intracranial vessel. However, we also know there is a time-dependent loss of density in occluded M1 segments within the first few hours. Therefore, depending on timing of patient presentation and imaging, thrombi may have very different composition and imaging characteristics. The lower stiffness and friction and increased permeability of the initial RBC component may facilitate higher penetration of thrombolytic agent and easier extraction. However, over time, more extensive fibrin deposition and crosslinking between RBC and fibrin may occur to facilitate stabilization of thrombus, as evidenced by RBC projections that allow interaction with each other and with fibrin fibers. Inhibitors of tPA may also play more of a role over time. All these factors may work to delay fibrinolysis and increase lytic resistance (see Figure 2 for a summary of dynamic changes in thrombus composition and imaging findings from time of stroke onset and Figure 3 for visualization of these alterations on clot lysability).

Advancing understanding of intracranial thrombus will require parallel advancement in both in vitro and in vivo studies. The Clot Summit Group highlighted the high heterogeneity between in vitro thrombus composition studies and outlined several ways to improve study of intracranial thrombi for consistency and reliability. These measures include improving quantitative analysis of thrombus composition, studying the whole thrombus specimen, standardizing methods, and building a central repository for retrieved clots. Thrombus registries are underway to better understand thrombus formation in the modern EVT era (BACTRAC, STRIP, COMPO-CLOT). Together with recent published best practice guidelines for thrombus retrieval, processing, and analysis, further insights may soon be gained on thrombus composition in stroke. Advanced imaging methods that more directly assess the thrombus in vivo are emerging, such as use of nanoparticles or automated thrombus segmentation, electron microscopy, or radiomics. Proteomics may also be an important new method to detect and characterize novel biomarkers related to the pathophysiology of thrombus formation in stroke, particularly in cryptogenic stroke, and complement histologic analyses. Furthermore, machine learning and artificial intelligence are promising approaches that may improve the accuracy, reliability, and efficiency of clot characterization. Ultimately, the most effective and revealing studies will simultaneously evaluate detailed radiologic features, in vitro thrombus analysis, radiologic outcomes (i.e., recanalization and infarct volume), and clinical outcomes (i.e., disability).

The advent of rapid care pathways for acute stroke towards thrombolysis and EVT has modified our understanding of thrombus characteristics and treatment selection. As treatment moves away from strictly time-based to imaging-based patient selection and therapeutics, we need to improve our ability to prognosticate, select appropriate patients for treatment, and employ the correct treatment for each patient in the most rapid fashion possible. Despite many advances in our knowledge of thrombus characteristics and imaging, clear recommendations have yet to emerge on how to apply this knowledge in the individualized selection of reperfusion therapy. Improving decision-making will require continued parallel progress throughout the entire spectrum of stroke research, including thrombus characteristics, effective use of imaging modalities, and expansion of interventional tools and technology.

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**Appendix**

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