Studying stroke thrombus composition after thrombectomy: what can we learn?

Cover title: Novel insights from thrombus composition: a review

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Non-standard Abbreviations and Acronyms

ADAMTS13	A disintegrin and metalloprotease with thrombospondin type-1 repeats, member 13)
CD31	Cluster of differentiation 31
CE	Cardioembolic
CT	Computed tomography
HAS	Hyperdense artery sign
IVT	Intravenous thrombolysis
LAA	Large artery atherosclerosis
MRI	Magnetic resonance imaging
mRS	Modified Ranking scale
mTICI	Modified Thrombolysis in Cerebral Infarction
NETs	Neutrophil extracellular traps
NIHSS	National Institute of Health Stroke Scale
OCT	Optical coherence tomography
PAI-1	Plasminogen activator inhibitor-1
RBC	Red blood cell
rt-PA	Recombinant tissue plasminogen activator
SVS	Susceptible vessel sign
TOAST	Trial of ORG 10172 in Acute Stroke Treatment

Abstract

The composition of ischemic stroke thrombi has gained an increasing amount of interest in recent years. The implementation of endovascular procedures in standard stroke care has granted researchers the unique opportunity to examine patient thrombus material. Increasing evidence indicates that stroke thrombi are complex and heterogenous, consisting of various biochemical (e.g. fibrin, von Willebrand factor and neutrophil extracellular traps) and cellular (e.g. red blood cells, platelets, leukocytes and bacteria) components. This complex composition may explain therapeutic limitations and also offer novel insights in several aspects of stroke management. Better understanding of thrombus characteristics could therefore potentially lead to improvements in the management of stroke patients. In this review, we provide a comprehensive overview of the lessons learned by examining stroke thrombus composition after endovascular thrombectomy and its potential relevance for thrombectomy success rates, thrombolysis, clinical outcomes, stroke etiology, and radiological imaging.

Introduction

Thrombectomy has in recent years dramatically changed acute ischemic stroke care, following several successful thrombectomy trials in 2015.[1–5] Besides the enormous clinical impact, endovascular procedures have also instigated a novel subfield in stroke research. By mechanically, and usually en bloc, removing the occluding thrombus from the patient vasculature, endovascular thrombectomy is providing the opportunity to collect thrombus material for research purposes.[6,7] Better understanding of thrombus composition may help to overcome the current limitations of both pharmacological and mechanical revascularization therapies. As stroke thrombus material is increasingly available, a growing number of studies is revealing the multifaceted composition of endovascularly-retrieved cerebral thrombo-emboli. Whereas the first reports mainly focused on the presence of red blood cells (RBCs), fibrin and platelets[6,7], subsequent research showed that also other components contribute to the complexity of ischemic stroke thrombi, including leukocytes, von Willebrand factor, neutrophil extracellular traps (NETs) and extracellular DNA.[8–11] The variable composition of such thrombi may present obstacles for recanalization therapies but also new opportunities in the management of ischemic stroke patients. In this review, we cover the most important associations between thrombus composition and endovascular treatment parameters (such as thrombectomy recanalization rates), effect of pharmacological thrombolysis, clinical outcome, stroke etiology, and radiological imaging. This review is based on as good as all reports (until December 2020) that used patient stroke thrombi to study these associations. For a more elaborate and in-depth description of the composition and internal architecture of ischemic stroke thrombi we refer to recently published reviews.[12,13]

Thrombus composition and endovascular procedural success

The main goal of endovascular treatment is to establish recanalization of the affected blood vessel by removing the occluding thrombus, which can be achieved by a stent retriever, by aspiration or by a combination of both techniques. Several factors are known to influence thrombectomy success rates, including thrombus location, size and vascular access.[7,14] First-pass complete reperfusion has in recent years become the preferred goal in endovascular therapy since the number of thrombectomy attempts

needed to achieve good recanalization is inversely correlated with clinical outcome.[15–18] Such first-pass effect is not always achieved and multiple attempts, often using various devices, are required in 60-75% of patients to achieve complete recanalization.[15,16] In 10-20% of the patients, the attempts remain futile due to failure to remove the thrombus and establish reperfusion.[14] Procedural success rates are likely to be influenced by thrombus characteristics such as stiffness, stickiness, deformability and mechanical friction, all of which may be defined by thrombus composition. Various studies examined whether and how thrombus composition affects thrombus removability and thrombectomy success rates. A summary of these studies is presented in Table 1 and online Supplemental Table I and II (online supplement, please see https://www.ahajournals.org/journal/str).

Emerging evidence indicates that the amount of RBCs is an important determinant of thrombus removability as RBC-rich thrombi require lower amounts of passes to establish recanalization.[19,20] Interestingly, Duffy et al. showed that the composition of thrombus material retrieved in the first two thrombectomy attempts contains significantly more RBCs and less fibrin compared to thrombus material retrieved in subsequent attempts, indicating that RBC-rich thrombus material is easier to remove than fibrin-rich thrombi.[19] Several factors could contribute to this observation. RBC-rich thrombi have a lower coefficient of friction compared to fibrin-rich thrombi.[19,21] Higher amounts of RBCs also reduce thrombus stiffness and are associated with better stent strut integration into the thrombus and also probably lead to better conformability into an aspiration catheter in case such a technique is applied.[22,23] Of note, such increased deformability and reduced friction potentially also explains why RBC-dominant thrombi are more prone to pre-interventional thrombus migration[24,25]. This phenomenon, in which the entire thrombus migrates more distally in the intracranial blood vessel before intervention, was reported to reduce subsequent thrombectomy success rates.[26]

Other thrombus components may also influence thrombus removability. Boeckh-Behrens et al. showed that higher amounts of leukocytes in the thrombus have a tendency to require more passes to be needed for removal of the thrombus.[27] Along the same lines, the presence of neutrophil-derived NETs, was shown to be associated with a higher number of thrombectomy attempts to remove the

thrombus.[11] NETs components such as DNA and histones can modify the structure of fibrin and render it resistant to mechanical deformation, which could account for the thrombectomy resistance.[28] Similarly, bacteria increase thrombus stiffness by altering the fibrin microstructure[29], which could explain why the presence of bacteria negatively affects septic thrombus removability.[30] Components of the vascular wall in a thrombus, such as collagen, have also been shown to reduce thrombus removability, although such presence may also be attributed to vascular injury induced by multiple passes required to remove a difficult thrombus.[31]

Besides the number of thrombectomy attempts to achieve recanalization, the degree of reperfusion as indicated by the modified Thrombolysis in Cerebral Infarction (mTICI) scale is also a key indicator of procedural success. Various studies focused on the association between the composition of the thrombus and the mTICI score. Whereas some studies were unable to show such correlation[24,32–38], an increasing number of studies indicate that RBC-rich thrombi are associated with better recanalization outcomes (mTICI >2b) in comparison to fibrin/platelet-rich thrombi.[19,31,39–41] Similarly, the presence of leukocytes and NETs were not associated with the mTICI score in various reports[11,27,32–34,38,42,43], although some studies show that leukocytes, in particular neutrophil elastase positive cells and NETs, contribute to lower rates of complete recanalization (mTICI score < 2b).[26,44,45] In line with the need for multiple passes to remove them, both septic emboli and those containing vascular wall components are associated with worse mTICI scores.[31,46]

An important unwanted aspect of thrombectomy procedures is thrombus fragmentation, leading to a shower of small thrombo-emboli that may travel more distally in the brain where mechanical removal is impossible or at least very difficult and risky. Fragmentation is likely influenced by the biochemical and cellular make-up of the thrombus as this can greatly influence the mechanical properties. To date, reports on thrombus composition and thrombus fragmentation are scarce. Low RBC and high fibrin content[42], and higher amounts of neutrophil elastase-positive cells[47] have been suggested to be associated with the occurrence of secondary emboli.

In sum, it is clear that the composition of thrombi can influence endovascular procedural success. The growing body of literature indicates that RBC-rich thrombi are more easily retrieved which translates into better recanalization and clinical outcomes compared to fibrin/platelet-rich thrombi. Leukocytes, perhaps specifically NETs, may also influence success rates although more studies are needed to fully confirm this idea. New insights on thrombus characteristics and their impact on thrombus retrieval can help in the development of improved thrombectomy protocols and adapted device technology, such as stent retriever designs for easy to retrieve but fragment-prone thrombi[48] or to improve retrieval of difficult fibrin/platelet-dominant thrombi.[49]

Thrombus composition and thrombolysis

At present, rt-PA is the only FDA-approved thrombolytic drug to pharmacologically dissolve the thrombotic cerebral occlusion. Use of rt-PA is, however, limited to less than 15% of patients due to the short therapeutic time window of 4.5 hours after stroke onset.[50] In addition, recanalization after rt-PA is only successful in less than half of patients with a proximal artery occlusion.[51] The reasons for the latter are not well understood but it seems that thrombus length plays a major role as thrombi >8 mm respond badly or not at all to intravenous thrombolysis (IVT).[52,53] Recent evidence indicates that thrombolysis reduces the size of a thrombus retrieved by thrombectomy, but this effect is not associated with recanalization outcome.[54] Most likely, also thrombus composition influences the response to IVT and studies examining retrieved stroke thrombus material might shed some light on this so-called rt-PA resistance (summarized in Table 1 and online Supplemental Table III; online supplement, please see https://www.ahajournals.org/journal/str). The thrombolytic mechanism of rt-PA is based on the activation of plasminogen into plasmin, which degrades fibrin in the thrombus. Fibrin is an important constituent of RBC-rich as well as platelet-rich stroke thrombus material, but platelet-dominant thrombi have other specific structural features that could impair rt-PA mediated fibrinolysis. Indeed, whereas RBC-rich material mainly consists of RBCs and fibrin, platelet-dominant thrombus regions also contain various other extracellular scaffold molecules such as dense fibrin, von Willebrand factor (VWF), extracellular DNA and NETs.[8–11,43] Such non-fibrin components may

contribute to rt-PA resistance by providing additional mechanical stabilization of the thrombus, by altering the structure of fibrin or by decreasing the thrombus permeability[8,10,11], which is in line with the observation that RBC-dominant thrombi are more efficiently dissolved by rt-PA than platelet-rich thrombi.[8,37,55,56] Of note, Di Meglio et al. recently described a fibrinolysis-resistant outer thrombus shell composed of platelets, VWF and extracellular DNA, forming a barrier that hampers rt-PA-mediated thrombolysis.[57] Interestingly, this shell also contained inhibitors of fibrinolysis, such as plasminogen-activator inhibitor 1 (PAI-1).[58] Thrombus contraction, a common phase of thrombus formation mediated by contractile forces of platelets on fibrin, might also influence thrombolytic success.[59] Thrombus contraction facilitates the redistribution of platelets and RBCs into separate areas and mediates the compression of RBCs into tightly-packed polyhedrocytes[59,60], which can reduce thrombus permeability and thus the degree of thrombolysis.[61] Intravital thrombus contraction was recently demonstrated in stroke thrombi, resulting in a compact structure with a limited porosity.[62]

Taken together, insights on thrombus composition and architecture may reveal novel therapeutic avenues that can lead to improved thrombolysis. Future pharmacological treatment could include the VWF-degrading substances ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type-1 repeats, member 13) and N-acetylcysteine, the DNA-cleaving enzyme DNAse1 or inhibitors of PAI-1, all of which show promising results in pre-clinical studies.[8,10,11,63–65] Novel fibrin-targeting fibrinolytics could further add to the efficacy and safety of thrombolysis, such as Tenecteplase, which has a higher specificity, longer half-life and improved resistance to endogenous inhibitors compared to alteplase.[66]

Thrombus composition and stroke etiology

Due to the mainly embolic nature of acute ischemic stroke, thrombo-emboli can originate from different locations in the body.[67] The original hemodynamic conditions in which thrombus formation took place, such as blood flow rate, shear stress, turbulence and vasculature most likely influence the composition of the thrombus and thus the embolus causing the ischemic stroke.[68] Arterial conditions with high shear stress are typically associated with platelet-rich thrombi, whereas venous, low shear stress conditions are assumed to promote the development of more coagulation-driven thrombi.

Stroke etiology is classified according to the TOAST criteria (Trial of ORG 10172 in Acute Stroke Treatment), identifying the origin as cardioembolic, large artery atherosclerotic (LAA), small vessel occlusion, other (e.g. carotid dissection or paradoxical embolisms) or a cryptogenic origin.[69] To prevent recurrent stroke, good knowledge of the underlying risk factors and potential pathogenesis is crucial in patient follow-up and treatment. Recurrent strokes still occur in approximately 25% of all stroke patients within 5 years after the initial event, highlighting the need for improved primary and secondary prevention.[70] Cardioembolic strokes are commonly caused by atrial fibrillation and are mainly treated using anticoagulants, while large artery atherosclerotic strokes are mostly treated using antiplatelet agents (e.g. aspirin).[71] Cryptogenic strokes, which comprise approximately a third of all ischemic strokes, pose a significant problem as the appropriate secondary prevention strategy is difficult to select in the absence of a known underlying pathogenesis.

To better understand the variable stroke thrombus pathogenesis, numerous studies have investigated the link between the histological composition of retrieved thrombi and the origin of the thrombus.[72] A summary of these studies is shown in Table 1 and online Supplemental Table IV (online supplement, please see https://www.ahajournals.org/journal/str).

The majority of reports mainly focused on the quantity of RBCs and fibrin and show inconsistent results. Whereas several studies found that cardioembolic thrombi are characterized by higher amounts of RBCs and lower amounts of fibrin compared to LAA thrombi[34,36,39,73–76], other studies reported the opposite[20,38,77–79] or found no association at all.[19,33,44,80] Forming the main target in antiplatelet therapy, platelets are an important factor in thrombosis and are thought to play a particular role in high-shear conditions. Two studies found that cardioembolic thrombi contain higher amounts of platelets in comparison to LAA thrombi.[38,76] whereas the opposite was reported by others.[32,81] Various studies showed no association between etiology and platelet content.[34,44,73,79] Similarly, VWF has been shown to be present in all thrombi regardless of their origin, with amounts ranging from 0.1% to 95%.[8,41,43] Initial reports, using low sample sizes, showed no link between VWF content and stroke etiology.[8,43] Data on leukocytes also remain inconsistent. Various reports indicate

that leukocyte content is not related to stroke etiology[19,20,33,34,44,78], whereas several other studies did find an association between higher leukocyte content and cardioembolic origin.[27,77,78] Different leukocyte subtypes, including neutrophils, eosinophils, monocytes/macrophages, T-cells and B-cells, have also been linked with stroke etiology, but the overall findings remain fragmentary and not conclusive at this moment (Table 1 and online Supplemental Table IV; online supplement, please see https://www.ahajournals.org/journal/str).[10,43,44,74,78,82] NETs or extracellular DNA seems to be particularly present in cardioembolic thrombi.[10,44,74,81] While it is common to see calcium deposition in many LAA lesions using imaging modalities, radiological studies indicate that only 1.3% of thrombi are calcified.[83,84] At this point, only one study, using a specific histological staining method, has evaluated the presence of calcifications in a limited subset of stroke thrombi[85], highlighting the need for additional large scale studies to evaluate this aspect.

Besides the quantitative determination of thrombus components, various studies have also evaluated the internal architecture of thrombi from different etiologies. The presence of serpentine and layered fibrin was not linked with etiology[80,86], but LAA thrombi were found to more frequently consist of an inner RBC-rich core surrounded by platelets along the thrombus surface, in contrast to cardioembolic thrombi in which platelets were typically found to be interspersed with RBCs.[38,87]

Taken together, various studies attempted to link thrombus composition and stroke etiology, but the overall outcome remains largely inconclusive. The reported inconsistencies are most likely related to the low sample sizes used in the majority of studies, as underlined by a recent meta-analysis.[6] Nevertheless, two of the largest patient thrombus cohorts, indicate that cardioembolic thrombi contain higher amounts of fibrin/platelet aggregates and lower amounts of RBCs compared to other etiological subtypes.[77,78] Based on the histological analysis, these large studies suggest that the majority of cryptogenic thrombi most likely originate from a cardioembolic etiology.[77,78] Additional large-scale studies will be needed to further clarify if and how thrombus composition, organization and structure can reveal information on stroke etiology and guide treatment using anticoagulant therapy, antiplatelet therapy or other strategies to prevent secondary events.[7,88] Another uncertainty in this

context is that it is currently unknown if the composition of the original thrombus, mostly located in the heart or the carotid bifurcation, is reflected in the composition of the emboli found in the brain vasculature. For instance, no evidence is available whether certain parts of the parental thrombus are more prone to embolize, highlighting the potential differences between the parental and embolized thrombi. Stroke etiology has typically been classified according to the TOAST criteria. Currently, newer classification methods are available such as the ASCOD criteria that assign a degree of likelihood to a patient-specific etiological classification.[89] Potentially, such improved etiology classifications will further strengthen the link between thrombus characteristics and stroke etiology. Finally, histological thrombus analysis might also be used to identify less common etiologies such as septic emboli and atrial myxomas. Using Gram-staining, two studies revealed the presence of Gram-positive bacteria in thrombi from patients suffering from infective endocarditis or other infectious diseases.[46,90] Since diagnosis of stroke due to an infectious disease is often not straightforward, early identification of a septic embolus might help to initiate early antibiotic treatment.

Prediction of thrombus composition by radiological imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are the primary imaging modalities used to exclude cerebral hemorrhage, to assess the extent of infarction and the at-risk penumbra, to grade the collateral circulation and to identify the location of the arterial occlusion. Apart from identifying thrombus location and its size, imaging also has the potential to allow early characterization of thrombus composition and permeability, which could guide procedural decisions such as selection of thrombus-specific retrieval protocols or device technologies. A summary of studies addressing the link between thrombus composition and radiological imaging is given in Table 1 and online Supplemental Table V (online supplement, please see https://www.ahajournals.org/journal/str).

At the site of the cerebral occlusion a hyperdense artery sign (HAS) and a susceptible vessel sign (SVS) is detected in approximately 50% of ischemic stroke patients using CT or MRI, respectively.[91] The vast majority of studies indicate that both the presence and the density, typically measured in Hounsfield units, of HAS on CT is associated with RBC-dominant thrombi, while the absence of this radiological

sign is indicative of fibrin/platelet-rich thrombi.[20,27,92,33,34,36,37,39,42,74,79] The correlation of HAS and SVS with the presence of RBCs can be explained by the concentration of hemoglobin in the thrombus.[93–96] Current imaging modalities, however, can only discriminate between RBC-dominant and fibrin/platelet-dominant thrombi and are unable to accurately identify mixed thrombi. Brinjikji et al. recently demonstrated *in vitro* that dual energy CT can be used to improve the characterization of thrombus composition, but this remains to be evaluated in patients.[97] Apart from CT and MRI-based imaging, intravascular optical coherence tomography (OCT) can also be used to determine thrombus composition. Intravascular OCT uses a fiber optic wire that both emits and records the reflection of light while simultaneously being rotated and pulled back from the artery, giving rise to an image by measuring the backscattering of light from the vessel wall and thrombus.[98] While currently in use to evaluate the morphology of coronary plaques during coronary endovascular interventions, recent *in vitro* studies showed that OCT can be used to discriminate between RBC-dominant, fibrin-dominant and mixed blood clots as well.[99,100]

Apart from identifying thrombus composition, radiological imaging can also be used to assess thrombus permeability, also termed 'thrombus perviousness'. Thrombus perviousness is defined as the degree in which a contrast agent is able to flow through the structure of the thrombus and is measured by comparing thrombus attenuation on non-contrast CT with that on CT angiography, thereby giving an idea about residual blood flow through the thrombus.[101] An increase in thrombus attenuation between the two respective imaging techniques implies a higher thrombus perviousness as contrast media enters the thrombus.[101] Higher thrombus perviousness is associated with better functional outcome, smaller infarct volumes and improved recanalization outcomes with both thrombolytic and endovascular therapy.[101] Indeed, pervious thrombi have a porous structure that allows passage of residual arterial flow or thrombolytics. Current reports with regard to the histology of the thrombus and thrombus perviousness are conflicting.[102,103] Benson et al. have shown that RBC-dominant, fibrin/platelet-poor thrombi are associated with more permeable thrombi on CT imaging.[102] On the other hand, Berndt et al. have shown that higher amounts of fibrin/platelet conglomerates and

lower amounts of RBCs are associated with more permeable thrombi.[103] As described earlier, the degree of thrombus contraction, a process that is dependent on platelets, most likely contributes to permeability and differences in contraction might explain the conflicting results.

In the future, it will be interesting to further establish the link between radiological signs and thrombus composition and to use such insights to develop pre-treatment decision-making strategies to increase first pass recanalization success rates.

Thrombus composition, stroke severity and functional outcome

In the past decade, several studies have identified various clinical, interventional and blood parameters as independent predictors of clinical outcome. Some examples of these parameters include a higher National Institute of Health Stroke scale (NIHSS) score at admission[104], diabetes mellitus[105], multiple thrombectomy attempts[15,16,104], higher neutrophil counts[105], higher neutrophil/lymphocyte ratio[106] and a higher VWF/ADAMTS13 ratio[107]. The question whether the composition of the occluding thrombus is directly associated with functional outcome has also been addressed. Various studies have attempted to correlate thrombus composition with functional parameters such as stroke severity (NIHSS) and clinical outcome (modified Rankin Score (mRS)) (Table 1 and online Supplemental Table VI; online supplement, please see https://www.ahajournals.org/journal/str). Whereas not all studies could confirm strong associations[8,34,41 11,35,43,57], some interesting correlations have been reported. For example, stroke severity, based on admission NIHSS scores, is positively correlated with the content of RBC, platelets, fibrin, VWF and monocytes in the occluding thrombus.[37,43,62] In particular, polyhedral RBCs, a morphological marker of clot contraction, were associated with more severe strokes, most likely because high amounts of polyhedrocytes render thrombi more compact, less deformable and less porous.[62] Thrombus composition was also found to be associated with stroke outcome. Apart from lower amounts of RBCs[20], especially higher amounts of leukocytes and NETs and in the thrombus have been linked with a poor outcome.[27,44,62]

Limitations and future perspectives

Since the arrival of endovascular thrombectomy, stroke thrombi have been collected and studied. It has become clear that stroke thrombi are complex and heterogenous, consisting of various cellular and molecular components that affect endovascular/thrombolytic success rates and that are associated with stroke etiology and radiological signs. Current imaging techniques can to some extent be used to characterize the thrombus prior to therapy, providing an early sense of how the thrombus will respond. Increased knowledge on thrombus composition has instigated refined treatment strategies to improve thrombectomy first-pass recanalization rates and to increase the efficiency and safety of pharmacological thrombolysis. Yet, research on thrombi retrieved via thrombectomy is not without limitations, which should be considered. First, only thrombi from large vessel occlusions that did not dissolve spontaneously or after infusion of rt-PA and that can be successfully retrieved via mechanical thrombectomy, are available for study. Thus, a selection bias exists, excluding rt-PA-susceptible or thrombectomy-resistant thrombi. The improvement in radiological characterization of thrombi could potentially be used in the future as a surrogate to estimate the composition of these inaccessible thrombi.[97] Second, thrombus characteristics could be influenced by patient-specific variables such as pre-stroke anti-thrombotic treatment, pharmacological thrombolysis or the technique of mechanical thrombectomy itself. Little information is currently available on these aspects and should be addressed in future studies.

While early studies may have been limited by the low sample sizes, they provided proof-of-concept for thrombus-driven stroke research and laid the foundation for larger scale studies. Various national and international initiatives have in the meantime established large-scale thrombus registries, such as the EXCELLENT (NCT03685578)[108], STRIP[32,41] and the THRAPS (MR CLEAN) registries. It will be interesting to see how results from these large studies will further our understanding of ischemic stroke thrombi and potentially inspire novel ideas for optimized stroke treatment.

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Conflict of Interest

TA is a consultant for Anaconda, Amnis Therapeutics, Cerenovus – Neuravi, Rapid Medical and Stryker. KMD, OF, SFDM, SS and WB have no conflicts of interest to report.

Supplemental Material

Online Tables I – VI

References [109],[110] and [111]

Data availability

The authors declare that all supporting data are available within this review manuscript and in the supplemental files.

References

1. Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N. Engl. J. Med.* 2015;372:11–20.
2. Campbell BC V, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N. Engl. J. Med.* 2015;372:1009–1018.
3. Jovin TG, Chamorro A, Cobo E, Miquel M, Molina CA, Rovira A, et al. Thrombectomy within 8 Hours after Symptom Onset in Ischemic Stroke. *New Engl J Med.* 2015;372:2296–2306.
4. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *New Engl J Med.* 2015;372:1019–1030.
5. Saver JL, Goyal M, Bonafé A, Diener H-C, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N. Engl. J. Med.* 2015;372:2285–2295.
6. Brinjikji W, Duffy S, Burrows A, Hacke W, Liebeskind D, Majoie CBLM, et al. Correlation of imaging and histopathology of thrombi in acute ischemic stroke with etiology and outcome: a systematic review. *J. Neurointerv. Surg.* 2017;9:529–534.
7. De Meyer SF, Andersson T, Baxter B, Bendszus M, Brouwer P, Brinjikji W, et al. Analyses of thrombi in acute ischemic stroke: A consensus statement on current knowledge and future directions. *Int J Stroke*. 2017;12:606–614.
8. Denorme F, Langhauser F, Desender L, Vandenbulcke A, Rottensteiner H, Plaimauer B, et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood*. 2016;127:2337–2345.
9. Staessens S, Denorme F, François O, Desender L, Dewaele T, Vanacker P, et al. Structural analysis of ischemic stroke thrombi: Histological indications for therapy resistance. *Haematologica*. 2020;105:498–507.
10. Laridan E, Denorme F, Desender L, François O, Andersson T, Deckmyn H, et al. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann. Neurol.* 2017;82:223–232.
11. Ducroux C, Di Meglio L, Loyau S, Delbosc S, Boisseau W, Deschildre C, et al. Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke. *Stroke*. 2018;49:754–757.
12. Staessens S, Meyer SF De. Thrombus heterogeneity in ischemic stroke. *Platelets*. 2020;1–9.
13. Fitzgerald S, Mereuta OM, Doyle KM, Dai D, Kadirvel R, Kallmes DF, et al. Correlation of

imaging and histopathology of thrombi in acute ischemic stroke with etiology and outcome. J. Neurosurg. Sci. 2019;63:292–300.

14. Yoo AJ, Andersson T. Thrombectomy in Acute Ischemic Stroke: Challenges to Procedural Success. *J Stroke*. 2017;19:121–130.
15. García-Tornel Á, Requena M, Rubiera M, Muchada M, Pagola J, Rodriguez-Luna D, et al. When to Stop: Detrimental Effect of Device Passes in Acute Ischemic Stroke Secondary to Large Vessel Occlusion. *Stroke*. 2019;50:1781–1788.
16. Zaidat OO, Castonguay AC, Linfante I, Gupta R, Martin CO, Holloway WE, et al. First pass effect: A new measure for stroke thrombectomy devices. *Stroke*. 2018;49:660–666.
17. Flottmann F, Leischner H, Broocks G, Nawabi J, Bernhardt M, Faizy TD, et al. Recanalization rate per retrieval attempt in mechanical thrombectomy for acute ischemic stroke. *Stroke*. 2018;49:2523–2525.
18. Seker F, Pfaff J, Wolf M, Ringleb PA, Nagel S, Schönenberger S, et al. Correlation of thrombectomy maneuver count with recanalization success and clinical outcome in patients with ischemic stroke. *Am. J. Neuroradiol.* 2017;38:1368–1371.
19. Duffy S, McCarthy R, Farrell M, Thomas S, Brennan P, Power S, et al. Per-Pass Analysis of Thrombus Composition in Patients With Acute Ischemic Stroke Undergoing Mechanical Thrombectomy. *Stroke*. 2019;50:1156–1163.
20. Maekawa K, Shibata M, Nakajima H, Mizutani A, Kitano Y, Seguchi M, et al. Erythrocyte-Rich Thrombus Is Associated with Reduced Number of Maneuvers and Procedure Time in Patients with Acute Ischemic Stroke Undergoing Mechanical Thrombectomy. *Cerebrovasc Dis Extra*. 2018;8:39–49.
21. Gunning GM, Kevin M, Mirza M, Duffy S, Gilvarry M, Brouwer PA. Clot friction variation with fibrin content; implications for resistance to thrombectomy. *J Neurointerv Surg*. 2018;10:34–38.
22. Gersh K, Nagaswami C, Weisel J. Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes. *Thromb. Haemost.* 2009;102:1169–1175.
23. Weafer FM, Duffy S, Machado I, Gunning G, Mordasini P, Roche E, et al. Characterization of strut indentation during mechanical thrombectomy in acute ischemic stroke clot analogs. *J. Neurointerv. Surg.* 2019;11:891–897.
24. Sporns PB, Jeibmann A, Minnerup J, Broocks G, Nawabi J, Schön G, et al. Histological Clot Composition Is Associated With Preinterventional Clot Migration in Acute Stroke Patients. *Stroke*. 2019;50:2065–2071.
25. Maegerlein C, Friedrich B, Berndt M, Lucia KE, Schirmer L, Poppert H, et al. Impact of histological thrombus composition on preinterventional thrombus migration in patients with

acute occlusions of the middle cerebral artery. *Interv. Neuroradiol.* 2018;24:70–75.

26. Kaesmacher J, Maegerlein C, Kaesmacher M, Zimmer C, Poppert H, Friedrich B, et al. Thrombus migration in the middle cerebral artery: Incidence, imaging signs, and impact on success of endovascular thrombectomy. *J. Am. Heart Assoc.* 2017;6:e005149.
27. Boeckh-Behrens T, Schubert M, Förschler A, Prothmann S, Kreiser K, Zimmer C, et al. The Impact of Histological Clot Composition in Embolic Stroke. *Clin. Neuroradiol.* 2016;26:189–197.
28. Longstaff C, Varjú I, Sótonyi P, Szabó L, Krumrey M, Hoell A, et al. Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, and histones. *J. Biol. Chem.* 2013;288:6946–6956.
29. Ma TM, VanEpps JS, Solomon MJ. Structure, Mechanics, and Instability of Fibrin Clot Infected with Staphylococcus epidermidis. *Biophys. J.* 2017;113:2100–2109.
30. Hernández-Fernández F, Rojas-Bartolomé L, García-García J, Ayo-Martín Ó, Molina-Nuevo JD, Barbella-Aponte RA, et al. Histopathological and Bacteriological Analysis of Thrombus Material Extracted During Mechanical Thrombectomy in Acute Stroke Patients. *Cardiovasc. Intervent. Radiol.* 2017;40:1851–1860.
31. Funatsu N, Hayakawa M, Hashimoto T, Yamagami H, Satow T, Takahashi JC, et al. Vascular wall components in thrombi obtained by acute stroke thrombectomy: Clinical significance and related factors. *J. Neurointerv. Surg.* 2019;11:232–236.
32. Fitzgerald S, Dai D, Wang S, Douglas A, Kadirvel R, Layton KF, et al. Platelet-Rich Emboli in Cerebral Large Vessel Occlusion Are Associated with a Large Artery Atherosclerosis Source. *Stroke*. 2019;50:1907–1910.
33. Liebeskind DS, Sanossian N, Yong WH, Starkman S, Tsang MP, Moya AL, et al. CT and MRI early vessel signs reflect clot composition in acute stroke. *Stroke*. 2011;42:1237–1243.
34. Kim SK, Yoon W, Kim TS, Kim HS, Heo TW, Park MS. Histologic Analysis of Retrieved Clots in Acute Ischemic Stroke: Correlation with Stroke Etiology and Gradient-Echo MRI. *Am. J. Neuroradiol.* 2015;36:1756–1762.
35. Singh P, Doostkam S, Reinhard M, Ivanovas V, Taschner CA. Immunohistochemical analysis of thrombi retrieved during treatment of acute ischemic stroke: does stent-retriever cause intimal damage? *Stroke*. 2013;44:1720–1722.
36. Simons N, Mitchell P, Dowling R, Gonzales M, Yan B, Simons N, et al. Thrombus composition in acute ischemic stroke: a histopathological study of thrombus extracted by endovascular retrieval. *J Neuroradiol.* 2015;42:86–92.
37. Choi M, Park G, Lee J, Lee S, Lee S-J, Kim J-H, et al. Erythrocyte Fraction Within Retrieved Thrombi Contributes to Thrombolytic Response in Acute Ischemic Stroke. *Stroke*.

2018;49:652–659.

38. Ahn S, Hong R, Choo I, Heo J, Nam H, Kang H, et al. Histologic features of acute thrombi retrieved from stroke patients during mechanical reperfusion therapy. *Int. J. Stroke*. 2016;11:1036–1044.
39. Shin JW, Jeong HS, Kwon HJ, Song KS, Kim J. High red blood cell composition in clots is associated with successful recanalization during intra-arterial thrombectomy. *PLoS One*. 2018;13:e0197492.
40. Hashimoto T, Hayakawa M, Funatsu N, Yamagami H, Yamagami H, Satow T, et al. Histopathologic Analysis of Retrieved Thrombi Associated With Successful Reperfusion After Acute Stroke Thrombectomy. *Stroke*. 2016;47:3035–3037.
41. Douglas A, Fitzgerald S, Mereuta OM, Rossi R, O'Leary S, Pandit A, et al. Platelet-rich emboli are associated with von Willebrand factor levels and have poorer revascularization outcomes. *J. Neurointerv. Surg.* 2020;12:557–562.
42. Sporns PB, Hanning U, Schwindt W, Velasco A, Buerke B, Cnyrim C, et al. Ischemic Stroke: Histological Thrombus Composition and Pre-Interventional CT Attenuation Are Associated with Intervention Time and Rate of Secondary Embolism. *Cerebrovasc. Dis.* 2017;44:344–350.
43. Schuhmann MK, Gunreben I, Kleinschnitz C, Kraft P. Immunohistochemical Analysis of Cerebral Thrombi Retrieved by Mechanical Thrombectomy from Patients with Acute Ischemic Stroke. *Int. J. Mol. Sci.* 2016;17:298.
44. Novotny J, Oberdieck P, Titova A, Pelisek J, Chandraratne S, Nicol P, et al. Thrombus NET content is associated with clinical outcome in stroke and myocardial infarction. Neurology. 2020;94:e2346–e2360.
45. Boeckh-Behrens T, Schubert M, Förschler A, Prothmann S, Kreiser K, Zimmer C, et al. The Impact of Histological Clot Composition in Embolic Stroke. *Clin. Neuroradiol.* 2014;26:189–197.
46. Francisco H-F, Laura R-B, Jorge G-G, Óscar A-M, D M-NJ, A B-AR, et al. Histopathological and Bacteriological Analysis of Thrombus Material Extracted During Mechanical Thrombectomy in Acute Stroke Patients. *Cardiovasc Interv. Radiol*. 2017;40:1851–1860.
47. Kaesmacher J, Boeckh-Behrens T, Simon S, Maegerlein C, Kleine JF, Zimmer C, et al. Risk of Thrombus Fragmentation during Endovascular Stroke Treatment. *Am J Neuroradiol*. 2017;38:991–998.
48. Chueh J-Y, Marosfoi MG, Brooks OW, King RM, Puri AS, Gounis MJ. Novel Distal Emboli Protection Technology: The EmboTrap. *Interv. Neurol.* 2017;6:268–276.
49. Fennell VS, Setlur Nagesh SV, Meess KM, Gutierrez L, James RH, Springer ME, et al. What to do about fibrin rich "tough clots"? Comparing the Solitaire stent retriever with a novel

geometric clot extractor in an in vitro stroke model. *J. Neurointerv. Surg.* 2018;10:907–910.

50. Aguiar de Sousa D, von Martial R, Abilleira S, Gattringer T, Kobayashi A, Gallofré M, et al. Access to and delivery of acute ischaemic stroke treatments: A survey of national scientific societies and stroke experts in 44 European countries. *Eur. Stroke J.* 2019;4:13–28.
51. Prabhakaran S, Ruff I, Bernstein RA. Acute Stroke Intervention: A Systematic Review. *Jama*. 2015;313:1451–1462.
52. Riedel CH, Zimmermann P, Ulf J-K, Stingele R, Deuschl G, Jansen O. The importance of size: successful recanalization by intravenous thrombolysis in acute anterior stroke depends on thrombus length. *Stroke*. 2011;42:1775–1777.
53. Rohan V, Baxa J, Tupy R, Cerna L, Sevcik P, Friesl M, et al. Length of occlusion predicts recanalization and outcome after intravenous thrombolysis in middle cerebral artery stroke. *Stroke*. 2014;45:2010–2017.
54. Rossi R, Fitzgerald S, Molina S, Mereuta OM, Douglas A, Pandit A, et al. The administration of rtPA before mechanical thrombectomy in acute ischemic stroke patients is associated with a significant reduction of the retrieved clot area but it does not influence revascularization outcome. *J. Thromb. Thrombolysis*. 2020;21:545–551.
55. Jang IK, Gold HK, Ziskind AA, Fallon JT, Holt RE, Leinbach RC, et al. Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis. *Circulation*. 1989;79:920–928.
56. Tomkins AJ, Schleicher N, Murtha L, Kaps M, Levi CR, Nedelmann M, et al. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. *Exp Transl Stroke Med*. 2015;7:2.
57. Di Meglio L, Desilles JP, Ollivier V, Nomenjanahary MS, Di Meglio S, Deschildre C, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis. *Neurology*. 2019;93:E1686–E1698.
58. Di Meglio L, Desilles J-P, Ollivier V, Nomenjanahary MS, Meglio S Di, Deschildre C, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis. *Neurology*. 2019;93:e1686--e1698.
59. Cines DB, Lebedeva T, Nagaswami C, Hayes V, Massefski W, Litvinov RI, et al. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood*. 2014;123:1596–1603.
60. Nechipurenko DY, Receveur N, Yakimenko AO, Shepelyuk TO, Yakusheva AA, Kerimov RR, et al. Clot Contraction Drives the Translocation of Procoagulant Platelets to Thrombus Surface. *Arterioscler. Thromb. Vasc. Biol.* 2019;39:37–47.

61. Tutwiler V, Peshkova AD, Le Minh G, Zaitsev S, Litvinov RI, Cines DB, et al. Blood clot contraction differentially modulates internal and external fibrinolysis. *J. Thromb. Haemost.* 2019;17:361–370.
62. Khismatullin RR, Nagaswami C, Shakirova AZ, Vrtková A, Procházka V, Gumulec J, et al. Quantitative Morphology of Cerebral Thrombi Related to Intravital Contraction and Clinical Features of Ischemic Stroke. *Stroke*. 2020;51:3640–3650.
63. de Lizarrondo S, Gakuba C, Herbig BA, Repessé Y, Ali C, Denis CV V, et al. Potent Thrombolytic Effect of N-Acetylcysteine on Arterial Thrombi. *Circulation*. 2017;136:646–660.
64. Wyseure T, Rubio M, Denorme F, de Lizarrondo S, Peeters M, Gils A, et al. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood*. 2015;125:1325–1332.
65. Zhu Y, Carmeliet P, Fay WP. Plasminogen activator inhibitor-1 is a major determinant of arterial thrombolysis resistance. *Circulation*. 1999;99:3050–5.
66. Tanswell P, Modi N, Combs D, Danays T. Pharmacokinetics and Pharmacodynamics of Tenecteplase in Fibrinolytic Therapy of Acute Myocardial Infarction. *Clin. Pharmacokinet.* 2002;41:1229–1245.
67. Yaghi S, Bernstein RA, Passman R, Okin PM, Furie KL. Cryptogenic Stroke: Research and Practice. *Circ. Res.* 2017;120:527–540.
68. Hathcock JJ. Flow effects on coagulation and thrombosis. Arterioscler. Thromb. Vasc. Biol. 2006;26:1729–1737.
69. Love BB, Bendixen BH. Classification of subtype of acute ischemic stroke definitions for use in a multicenter clinical trial. *Stroke*. 1993;24:35–41.
70. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation*. 2017;135:e146–e603.
71. Boehme AK, Esenwa C, Elkind MSV. Stroke Risk Factors, Genetics, and Prevention. Circ. Res. 2017;120:472–495.
72. Gounis MJ, Chapot R. Histological Composition and the Origin of the Thrombus: A New Diagnostic Assay for Secondary Stroke Prevention? *Stroke*. 2017;48:2040–2041.
73. Sato Y, Hatsue I-U, Iwakiri T, Ikeda Y, Matsuyama T, Hatakeyama K, et al. Thrombus components in cardioembolic and atherothrombotic strokes. *Thromb. Res.* 2012;130:278–280.
74. Essig F, Kollikowski AM, Pham M, Solymosi L, Stoll G, Haeusler KG, et al. Immunohistological Analysis of Neutrophils and Neutrophil Extracellular Traps in Human Thrombemboli Causing Acute Ischemic Stroke. *Int. J. Mol. Sci.* 2020;21:7387.

75. Liao Y, Guan M, Liang D, Shi Y, Liu J, Zeng X, et al. Differences in Pathological Composition Among Large Artery Occlusion Cerebral Thrombi, Valvular Heart Disease Atrial Thrombi and Carotid Endarterectomy Plaques. *Front. Neurol.* 2020;11:811.
76. Nouh A, Mehta T, Hussain M, Song X, Ollenschleger M. Clot composition of embolic strokes of undetermined source: a feasibility study. *BMC Neurol.* 2020;20:383.
77. Boeckh-Behrens T, Kleine JF, Zimmer C, Neff F, Scheipl F, Pelisek J, et al. Thrombus Histology Suggests Cardioembolic Cause in Cryptogenic Stroke. *Stroke*. 2016;47:1864–1871.
78. Sporns PB, Hanning U, Schwindt W, Velasco A, Minnerup J, Zoubi T, et al. Ischemic Stroke: What Does the Histological Composition Tell Us About the Origin of the Thrombus? *Stroke*. 2017;48:2206–2210.
79. Niesten JM, Van Der Schaaf IC, Van Dam L, Vink A, Vos JA, Schonewille WJ, et al. Histopathologic composition of cerebral thrombi of acute stroke patients is correlated with stroke subtype and thrombus attenuation. *PLoS One*. 2014;9:e88882.
80. Marder VJ, Chute DJ, Starkman S, Abolian AM, Kidwell C, Liebeskind D, et al. Analysis of Thrombi Retrieved From Cerebral Arteries of Patients With Acute Ischemic Stroke. *Stroke*. 2006;37:2086–2093.
81. Di Meglio L, Desilles JP, Solonomenjanahary M, Labreuche J, Ollivier V, Dupont S, et al. DNA Content in Ischemic Stroke Thrombi Can Help Identify Cardioembolic Strokes among Strokes of Undetermined Cause. *Stroke*. 2020;51:2810–2816.
82. Dargazanli C, Rigau V, Eker O, Bareiro C, Machi P, Gascou G, et al. High CD3+ Cells in Intracranial Thrombi Represent a Biomarker of Atherothrombotic Stroke. *PLoS One*. 2016;11:e0154945.
83. Maurer CJ, Dobrocky T, Joachimski F, Neuberger U, Demerath T, Brehm A, et al. Endovascular Thrombectomy of Calcified Emboli in Acute Ischemic Stroke: A Multicenter Study. *AJNR. Am. J. Neuroradiol.* 2020;41:464–468.
84. Bardon M, Hanson J, O'Brien B, Naeem A. Calcified cerebral emboli: Incidence and implications. *J. Med. Imaging Radiat. Oncol.* 2018;62:499–503.
85. Almekhlafi MA, Hu WY, Hill MD, Auer RN. Calcification and endothelialization of thrombi in acute stroke. *Ann. Neurol.* 2008;64:344–348.
86. Sallustio F, Arno N, Legge DS, Koch G, Martorana A, Rossi C, et al. Histological Features of Intracranial Thrombo-Emboli Predict Response to Endovascular Therapy for Acute Ischemic Stroke. *J. Neurol. Disord. Stroke*. 2015;3:1105–1110.
87. Kim B, Kim YM, Jin SC, Lee J won, Lee BI, Kim SE, et al. Development of a predictive scale for cardioembolic stroke using extracted thrombi and angiographic findings. *J. Clin. Neurosci.* 2020;73:224–230.

88. Staessens S, Fitzgerald S, Andersson T, Clarençon F, Denorme F, Gounis MJ, et al. Histological stroke clot analysis after thrombectomy: Technical aspects and recommendations. Int. J. Stroke. 2020;15:467–476.
89. Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Wolf ME, Hennerici MG. The ASCOD phenotyping of ischemic stroke (Updated ASCO Phenotyping). Cerebrovasc. Dis. 2013;36:1–5.
90. Bhaskar S, Saab J, Cappelen-Smith C, Killingsworth M, Wu XJ, Cheung A, et al. Clot Histopathology in Ischemic Stroke with Infective Endocarditis. *Can. J. Neurol. Sci.* 2019;46:331–336.
91. Mair G, Boyd E V, Chappell FM, von Kummer R, Lindley RI, Sandercock P, et al. Sensitivity and specificity of the hyperdense artery sign for arterial obstruction in acute ischemic stroke. *Stroke*. 2015;46:102–7.
92. Fitzgerald ST, Wang S, Dai D, Douglas A, Kadirvel R, Gounis MJ, et al. Platelet-rich clots as identified by Martius Scarlet Blue staining are isodense on NCCT. *J. Neurointerv. Surg.* 2019;11:1145–1149.
93. Kirchhof K, Welzel T, Mecke C, Zoubaa S, Sartor K. Differentiation of white, mixed, and red thrombi: value of CT in estimation of the prognosis of thrombolysis phantom study. *Radiology*. 2003;228:126–130.
94. New PFJ, Aronow S. Attenuation measurements of whole blood and blood fractions in computed tomography. *Radiology*. 1976;121:635–640.
95. Black DF, Rad AE, Gray LA, Campeau NG, Kallmes DF. Cerebral Venous Sinus Density on Noncontrast CT Correlates with Hematocrit. *Am Soc Neuroradiol.* 2011;32:1354–7.
96. Lee SY, Cha SH, Lee SH, Shin DI. Evaluation of the effect of hemoglobin or hematocrit level on dural sinus density using unenhanced computed tomography. *Yonsei Med. J.* 2013;54:28–33.
97. Brinjikji W, Michalak G, Kadirvel R, Dai D, Gilvarry M, Duffy S, et al. Utility of single-energy and dual-energy computed tomography in clot characterization: An in-vitro study. *Interv Neuroradiol*. 2017;23:279–284.
98. Oosterveer TTM, van der Meer SM, Scherptong RWC, Jukema JW. Optical Coherence Tomography: Current Applications for the Assessment of Coronary Artery Disease and Guidance of Percutaneous Coronary Interventions. *Cardiol. Ther.* 2020;9:307–321.
99. Ding Y, Abbasi M, Eltanahy AM, Jakaitis DR, Dai D, Kadirvel R, et al. Assessment of Blood Clot Composition by Spectral Optical Coherence Tomography: An In Vitro Study. *Neurointervention*. 2021;16:29–33.
100. Liu HC, Abbasi M, Ding YH, Polley EC, Fitzgerald S, Kadirvel R, et al. Characterizing thrombus with multiple red blood cell compositions by optical coherence tomography attenuation coefficient. *J. Biophotonics*. 2021;14.

101. Santos EMM, Dankbaar JW, Treurniet KM, Horsch AD, Roos YB, Kappelle LJ, et al. Permeable Thrombi Are Associated With Higher Intravenous Recombinant Tissue-Type Plasminogen Activator Treatment Success in Patients With Acute Ischemic Stroke. *Stroke*. 2016;47:2058–2065.
102. Benson JC, Fitzgerald ST, Kadirvel R, Johnson C, Dai D, Karen D, et al. Clot permeability and histopathology: Is a clot's perviousness on CT imaging correlated with its histologic composition? *J. Neurointerv. Surg.* 2020;12:38–42.
103. Berndt M, Friedrich B, Maegerlein C, Moench S, Hedderich D, Lehm M, et al. Thrombus Permeability in Admission Computed Tomographic Imaging Indicates Stroke Pathogenesis Based on Thrombus Histology. *Stroke*. 2018;49:2674–2682.
104. Linfante I, Starosciak AK, Walker GR, Dabus G, Castonguay AC, Gupta R, et al. Predictors of poor outcome despite recanalization: A multiple regression analysis of the NASA registry. *J. Neurointerv. Surg.* 2016;8:224–229.
105. Boisseau W, Desilles JP, Fahed R, Kyheng M, Zuber K, Sabben C, et al. Neutrophil count predicts poor outcome despite recanalization after endovascular therapy. *Neurology*. 2019;93:e467–e475.
106. Song SY, Zhao XX, Rajah G, Hua C, Kang RJ, Han YP, et al. Clinical Significance of Baseline Neutrophil-to-Lymphocyte Ratio in Patients With Ischemic Stroke or Hemorrhagic Stroke: An Updated Meta-Analysis. Front. Neurol. 2019;10:1032.
107. Taylor A, Vendramin C, Singh D, Brown MM, Scully M. von Willebrand factor/ADAMTS13 ratio at presentation of acute ischemic brain injury is predictive of outcome. *Blood Adv.* 2020;4:398–407.
108. EmboTrap® Revascularization Device Registry - ClinicalTrials.gov [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT03685578
109. Krajíčková D, Krajina A, Šteiner I, Vyšata O, Herzig R, Lojík M, et al. Fibrin Clot Architecture in Acute Ischemic Stroke Treated With Mechanical Thrombectomy With Stent-Retrievers - Cohort Study. *Circ. J.* 2017;82:CJ-17-0375.
110. Hayosh ZI, Bandora EA, Shelestovich N, Nulman M, Bakon M, Yaniv G, et al. In-thrombus thrombin secretion: A new diagnostic marker of atrial fibrillation in cryptogenic stroke. *J. Neurointerv. Surg.* 2020;neurintsurg-2020-016484.
111. Boeckh-Behrens T, Kleine J, Kaesmacher J, Zimmer C, Schirmer L, Simon S, et al. The CD31 molecule: a possible neuroprotective agent in acute ischemic stroke? *Thromb J*. 2017;15:11.

Tables

Table 1: Main associations between thrombus composition and endovascular/thrombolytic treatment success, functional outcome, stroke etiology and radiological imaging

Histological parameter	Endovascular treatment success	Thrombolytic treatment success	Stroke etiology	Radiological imaging	Stroke severity/ Functional outcome
RBCs, fibrin and platelets	RBC-thrombi are more easy to retrieve[19,20] and have improved recanalization outcomes[19,39,40] compared to fibrin/platelet-rich thrombi[19,20,39–41] RBC- rich thrombi are prone to preprocedural thrombus migration[24,25] Formation of secondary embolisms is more present in RBC-rich thrombi[47] versus secondary embolisms are more present in fibrin/platelet-rich thrombi[42] Studies showing no association[32–38,42,80]	RBC-rich areas are more susceptible to thrombolysis compared to platelet-rich areas[37] Thrombi contain a dense outer shell of platelets that is resistant to fibrinolysis[57]	CE thrombi = higher RBC and lower fibrin/platelet content [34,36,39,73–76] versus LAA thrombi = higher RBC and lower fibrin/platelet content[20,38,77–79,81] Cryptogenic thrombi resemble CE[38,42,77,79] versus LAA thrombi[39] LAA thrombi = Inner RBC core with platelets on the surface vs. CE thrombi = platelets interspersed with RBCs throughout the entire thrombus.[38,87] No association[19,33,44,80,86]	A HAS is associated with higher RBC and lower fibrin/platelet content while the absence is associated with a lower RBC and higher fibrin/platelet content. [20,33,34,36,37,39,42,74,79,92] Increased thrombus perviousness is associated with RBC-rich thrombi[102] versus perviousness is associated with fibrin/platelet-rich thrombi[103]	Higher NIHSS score at admission = Higher RBC content[37], higher platelet or fibrin bundle content[62] Higher NIHSS score 7 days post admission = Higher polyhedral RBC, platelet or fibrin bundle content[62] Favorable clinical outcome = Higher RBC content[20] Worse clinical outcome = higher polyhedral RBC content[62] No association[34,35,41]
VWF	Unknown	Higher VWF content = increased rt-PA resistance[8]	No association[8,43]	Unknown	Higher NIHSS score at admission = Higher VWF content[43] No association[8]

Leukocytes	Higher leukocyte content = improved recanalization outcomes[27] Higher leukocyte content = more passes required[27] Higher neutrophil content = more secondary embolisms[47] No association exists[32–34,38,43,78]	Unknown	CE thrombi = more leukocytes than LAA thrombi[27,77,78] No association[19,33] CE thrombi are associated with a higher neutrophil[74] and lower T-cell[82] content No association (T/B-cells, eosinophils, monocyte/macrophages, neutrophils)[10,44,78]	Unknown	Higher NIHSS score at admission/discharge = higher leukocyte content[27] or monocytes[43] No association[34,41]
NETs	Higher NET content = increasing amount of thrombectomy attempts[11,44] and a worse recanalization outcome[44]	Higher NET content = increasing rt-PA resistance[10,11]	CE thrombi = Higher NET content[10,44,74] and higher overall DNA content[81] than non-CE thrombi No association[11]	Unknown	NETS are associated with a worse NIHSS score at discharge and a worse mRS score.[44]
Bacteria	Presence of bacteria = more thrombectomy attempts[46]	Unknown	Presence of bacteria = underlying infectious pathology (e.g. infective endocarditis)[46,90]	Unknown	Unknown

CE: Cardioembolic, HAS: Hyperdense artery sign, LAA: Large artery atherosclerosis, mRS: modified Ranking scale, NETs: Neutrophil extracellular traps, NIHSS: National Institute of Health Stroke Scale, RBCs: Red blood cells, rt-PA: Recombinant tissue plasminogen activator

SUPPLEMENTAL MATERIAL

Studying stroke thrombus composition after thrombectomy: what can we learn?

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Supplemental Table I: Overview of studies describing the association of thrombus composition with procedural success

Ref.	Description	Association with the amount of thrombectomy attempts	Association with the recanalization score
Duffy et al.[19]	RBC-rich thrombi are more easily retrieved in the first two thrombectomy attempts and a trend was noticed between high RBC/lower fibrin content and better recanalization scores. No differences were found in the amount of leukocytes.	RBC-rich: more easily retrieved	RBC-rich: better recanalization scores
Boeckh-Behrens et al.[53]	Higher amounts of leukocytes have a tendency of required multiple thrombectomy attempts and are associated with a worse recanalization score.	Leukocytes ↑: tendency towards more difficult to retrieve	Leukocytes ↑: worse recanalization scores
Funatsu et al.[63]	Thrombi containing vascular wall components have a tendency of requiring multiple passes, resulting in worse recanalization scores. Higher RBC-content is an independent predictor for better recanalization scores.	Vascular wall components ↑: more difficult to retrieve	Vascular wall components ↑: worse recanalization scores RBC-rich: better recanalization scores
Novotny et al.[47]	Higher amounts of NETs are associated with more thrombectomy attempts and worse recanalization scores. Platelets, fibrin, leukocytes, eosinophils, monocytes and T/B-cells are not associated with these parameters.	NETs ↑: more difficult to retrieve	NETs ↑: Worse recanalization scores
Ducroux et al.[11]	Higher amounts of NETs are associated with multiple passes to remove the thrombus	NETs ↑: more difficult to retrieve	No association
Hernandez-Fernandez et al.[55]	Septic emboli require multiple passes to remove the thrombus and are associated with longer procedure times	Bacteria ↑: more difficult to retrieve	No association

Maekawa et al.[20]	Patients with RBC-rich thrombi had a smaller number of recanalization maneuvers and shorter procedure times.	RBC-rich: more easily retrieved	No association
Shin et al.[21]	Higher amounts of RBCs are related to better recanalization scores	NA	RBC-rich: Better recanalization scores
Hashimoto et al.[22]	RBC content is positively correlated to recanalization score whereas atheromatous gruel is inversely correlated to recanalization score	NA	RBC-rich: Better recanalization scores
Kaesmacher et al.[46]	The presence of neutrophil elastase positive cells is correlated with lower rates of complete recanalization	NA	Neutrophils: Worse recanalization scores
Krajicova et al.[106]	Thinning of fibrin, a phenomenon caused by treatment with intravenous rt-PA, is associated with worse recanalization outcomes.	NA	Fibrin thinning: worse recanalization scores
Douglas et al.[23]	Higher amounts of platelets are associated with worse recanalization outcomes.	NA	Platelets: Worse recanalization outcomes
Fitzgerald et al.[51]	RBCs, fibrin, platelets and leukocytes are not related to the amount of passes required to remove the thrombus and did not affect final recanalization score.	No association	No association
Liesbeskind et al.[36]	Thrombus histology (RBCs, fibrin, leukocytes) was not predictive for successful recanalization.	NA	No association
Kim et al.[25]	No correlation was found between clot components (RBCs, fibrin, platelets and leukocytes) and recanalization success	NA	No association

Singh et al.[50]	No significant association was found between thrombus histology (red, mixed, white) and recanalization success.	NA	No association
Simons et al.[26]	Thrombus pathology (early or late phase, individual thrombus components (RBCs and fibrin)) is not related to recanalization success.	NA	No association
Sporns et al.[33]	Recanalization success is not associated with the amount of RBCs, fibrin/platelets aggregates, leukocytes, macrophages, T/B-cells.	NA	No association
Choi et al.[24]	No link between the fraction of RBCs and successful reperfusion.	NA	No association
Ahn et al.[31]	The proportion of components (RBCs, fibrin, platelets, leukocytes) did not differ according to the recanalization score.	NA	No association
Marder et al.[48]	Histological fibrin patterns (layered versus serpentine fibrin) is not associated with recanalization scores	NA	No association
Schuhmann et al.[52]	VWF, monocytes and CD4+ T-cells are not associated with recanalization score	NA	No association
Douglas et al.[23]	The amount of VWF is not associated with successful reperfusion	NA	No association

RBCs: Red blood cells, NETs: Neutrophil extracellular DNA traps, rt-PA: Recombinant tissue-plasminogen activator, VWF: von Willebrand factor, NA: Not applicable

Supplemental Table II: Overview of studies describing the association of thrombus composition with pre-interventional thrombus migration

Pre-interventional thrombus migration is associated with higher amounts of RBCs	
Maegerlein et al.[42]	RBC-rich thrombi (more than 60% in content) are associated with thrombus migration. No significant associations with absolute thrombus content (RBCs, fibrin and leukocytes) were found.
Sporns et al.[41]	Higher amounts of RBCs, lower amounts of fibrin and a smaller thrombus lengths is related to pre-interventional thrombus migration. No associations were found with leukocytes or specific leukocyte subsets (macrophages and T/B-cells).

Supplemental Table III: Overview of studies describing the association of thrombus composition with thrombolytic success

Ref.	Description
RBCs, VWF and NETs are associated with thrombolytic success	
Choi et al.[24]	Increased intravenous thrombolysis responsiveness is associated with higher RBC fractions and lower fibrin and platelet fractions.
Di Meglio et al.[40]	Stroke thrombi contain a dense outer shells of platelets, VWF, extracellular DNA and PAI-1 that is resistant to thrombolysis
Denorme et al.[8]	Stroke thrombi contain abundant amounts of VWF which were shown to be resistant to rt-PA mediated thrombolysis in a ferric chloride-induced thrombotic MCA mouse model.
Laridan et al.[10]	NETs are important consituents of cerebral thrombi. Ex vivo thrombolysis experiments using rt-PA in combination with DNase-I promotes the lysis of stroke thrombi compared to rt-PA alone.
Ducroux et al.[11]	Stroke thrombi contain considerable amounts of NETs. Ex vivo thrombolytic experiment with patient stroke thrombi indicate that rt-PA in combination with DNase-I accelerates thrombolysis in comparison to rt-PA or DNase-I alone.

RBCs: Red blood cells, VWF: von Willebrand factor, NETs: Neutrophil extracellular DNA traps, PAI-1: plasminogen activator inhibitor-1, rt-PA: recombinant tissue-plasminogen activator, MCA: middle cerebral artery

Supplemental Table IV: Overview of studies describing the association of thrombus composition and stroke etiology

Ref.	N	Etiology	Additional descriptions	Thrombus components	Histological staining
Red blood cells ↑ and fibrin ↓ are associated with cardioembolic thrombi Red blood cells ↓ and fibrin ↑ are associated with LAA thrombi or non CE-thrombi					
Kim et al.[25]	37	CE = 22 LAA = 8 Cryptogenic = 7	No differences observed with relation to platelets and leukocytes	RBCs Fibrin Platelets Leukocytes	H&E IHC: platelets (GPIIIa)
Sato et al.[27]	17	CE = 11 LAA = 6	No differences observed with relation to platelets	RBCs Fibrin Platelets	IHC: RBCs (glycophorin A), Fibrin, platelets (GPIIbIIIa)
Simons et al.[26]	40	NR	CE stroke is related to early phase thrombus pathology (RBC-dominant thrombi and thrombi with equal amounts of RBCs and fibrin)	RBCs Fibrin Endothelial cells	H&E IHC: Endothelial cells (CD34)
Shin et al.[21]	37	CE = 22 LAA = 7 Cryptogenic = 8	Cryptogenic thrombi resemble the composition of LAA thrombi. Lower amount of leukocytes in CE thrombi compared to LAA.	RBCs Fibrin/platelets aggregates Leukocytes	H&E
Red blood cells ↓ and fibrin ↑ are associated with cardioembolic thrombi Red blood cells ↑ and fibrin ↓ are associated with LAA thrombi or non CE-thrombi					
Niesten et al.[34]	22	CE = 6 LAA = 8 Cryptogenic =5 Other = 3	No differences observed with relation to fibrin and platelets. Cryptogenic thrombi resemble CE thrombi.	RBCs Fibrin Platelets	H&E, PTAH IHC: RBCs (glycophorin A), platelets (CD31)

Maekawa et al.[20]	43	CE = 30 LAA = 5 Cryptogenic = 7 Other = 1	No differences observed with relation to leukocytes.	RBCs Fibrin Leukocytes	H&E
Boeckh-Behrens et al.[32]	145	CE = 67 LAA = 22 Cryptogenic = 36 Other = 11	Leukocytes are higher in CE thrombi. Cryptogenic thrombi resemble CE thrombi.	RBCs Fibrin/platelets aggregates Leukocytes	H&E
Sporns et al.[33]	187	CE = 77 LAA = 35 Cryptogenic = 64 Other = 11	Leukocytes are higher in CE thrombi. No differences observed in subpopulations of leukocytes. Cryptogenic thrombi resemble CE thrombi.	RBCs Fibrin/platelets aggregates Leukocytes Macrophages T-cells B-cells	H&E IHC: Macrophages (CD68), T-cells (CD3) and B-cells (CD20)
Ahn et al.[31]	36	CE = 22 LAA = 8 Cryptogenic = 6	No differences observed in platelets content. On a structural level, platelets are located on the surface of LAA thrombi. Cryptogenic thrombi resemble CE thrombi.	RBCs Fibrin Platelets	H&E, MSB IHC: platelets (CD42b)
Liao et al.[29]	88	CE = 46 LAA = 25 Cryptogenic = 11 Other = 6	Cardioembolic thrombi contain more fibrin and less RBCs than LAA thrombi. No difference in platelets and leukocytes were found. Cryptogenic thrombi are similar in composition to CE thrombi.	RBCs Fibrin Leukocytes Platelets	H&E IHC: platelets (CD31)
Nouh et al.[30]	33	CE = 14 LAA = 9 Cryptogenic = 6 Other = 4	The RBC to platelet ratio is higher in LAA than CE thrombi. cryptogenic thrombi resemble CE thrombi.	RBCs Platelets	H&E IHC: Platelets (CD61)
Platelets ↑ in LAA thrombi **Platelets ↓ in CE thrombi**					
Fitzgerald et al.[51]	103	CE = 50 LAA = 20 Cryptogenic = 21 Other = 12	No differences observed in RBCs, fibrin and leukocytes content.	RBCs Fibrin Platelets Leukocytes	H&E and MSB

Di Meglio et al.[35]	250	CE = 142 Non-CE= 33 Cryptogenic = 75	CE thrombi contain more DNA and less platelets than non-CE thrombi. Cryptogenic thrombi resemble CE thrombi	RBCs Platelets DNA	Biochemical assay against heme (RBCs), GPVI (platelets) and DNA
Fibrin ↑ in CE thrombi **Fibrin ↓ in LAA thrombi**					
Khismatullin et al.[39]	41	CE = 23 LAA = 18	A higher fibrin content and more balloon-like platelets were observed in CE versus LAA thrombi. No differences were observed in polyhedral RBC, platelets and leukocyte content.	Polyhedral RBCs Fibrin Platelets Balloon-like platelets Leukocytes	Scanning electron microscopy
Essig et al.[28]	58	CE = 42 Non-CE= 7 Cryptogenic = 9	CE and cryptogenic contain more neutrophils, fibrin and NETs than non-CE thrombi. Cryptogenic thrombi resemble CE thrombi in terms of neutrophils and fibrin.	RBCs Fibrin Neutrophils NETs	H&E IHC: neutrophils (CD66b), NETS (H3Cit in combination with neutrophil elastase)
Leukocytes ↑ in CE thrombi compared to LAA thrombi					
Boeckh-Behrens et al.[53]	34	CE = 16 LAA = 3 Cryptogenic = 9 Other = 9	No differences observed in RBCs and fibrin content.	RBCs Fibrin Leukocytes	H&E and Elastica von Gieson
Boeckh-Behrens et al.[32]	145	CE = 67 LAA = 22 Cryptogenic = 36 Other = 11	NA	RBCs Fibrin/platelets aggregates Leukocytes	H&E
Sporns et al.[33]	187	CE = 77 LAA = 35 Cryptogenic = 64 Other = 11	No differences observed in subpopulations of leukocytes.	RBCs Fibrin/platelets aggregates Leukocytes Macrophages T-cells B-cells	H&E IHC: Macrophages (CD68), T-cells (CD3) and B-cells (CD20)
Neutrophils ↑ in CE thrombi					

Essig et al.[28]	58	CE = 42 Non-CE= 7 Cryptogenic = 9	CE and cryptogenic contain more neutrophils, fibrin and NETs than non-CE thrombi. Cryptogenic thrombi resemble CE thrombi in terms of neutrophils and fibrin.	RBCs Fibrin Neutrophils NETs	H&E IHC: neutrophils (CD66b), NETS (H3Cit in combination with neutrophil elastase)
T-cells ↑ in LAA thrombi					
Dargazanli et al.[54]	54	CE = 25 LAA = 10 Other = 19	NA	T-cells	H&E IHC: T-cells (CD3)
NETs ↑ in CE thrombi					
Laridan et al.[10]	68	CE = 40 LAA = 7 Cryptogenic = 15	Neutrophils are not associated with stroke etiology	Neutrophils NETs	H&E IHC: Neutrophils (CD66b) and NETs (H3Cit in combination with neutrophil elastase)
Novotny et al.[47]	71	CE = 35 LAA = 15 Cryptogenic = 21	The other thrombus components are not associated with stroke etiology.	Platelets Fibrin Leukocytes Neutrophils Monocytes Eosinophils T-cells B-cells NETs	Luna stain (eosinophils) IHC: Leukocytes (CD45), neutrophils (NE), monocytes (CD14), T-cells (CD3), B-cells (CD20), platelets (CD41), fibrinogen, NETS (H3Cit in combination with neutrophil elastase)
Essig et al.[28]	58	CE = 42 Non-CE= 7 Cryptogenic = 9	CE and cryptogenic contain more neutrophils, fibrin and NETs than non-CE thrombi. Cryptogenic thrombi resemble CE thrombi in terms of neutrophils and fibrin.	RBCs Fibrin Neutrophils NETs	H&E IHC: neutrophils (CD66b), NETS (H3Cit in combination with neutrophil elastase)
Overall DNA content ↑ in CE thrombi					

Di Meglio et al.[35]	250	CE = 142 Non-CE= 33 Cryptogenic = 75	CE thrombi contain more DNA and less platelets than non-CE thrombi. Cryptogenic thrombi resemble CE thrombi	RBCs Platelets DNA	Biochemical assay against heme (RBCs), GPVI (platelets) and DNA
An increasing thrombin activity elution pattern is associated with a LAA-origin A decreasing thrombin activity elution pattern is associated with a CE-origin					
Hayosh et al.[107]	68	CE = 15 Non-CE=157 Cryptogenic = 17 Other = 18	Thrombi were washed to elute thrombin from the thrombus. Subsequently, thrombin activity was measured over time during this elution process. Thrombin activity in CE thrombi decreased significantly with time, while thrombin activity increases over time in LAA thrombi. The elution pattern of a cryptogenic thrombus, resembles that of a CE thrombus.	Thrombin	Thrombin activity assay
Bacterial signature is associated with an underlying infectious disease					
Bhaskar et al.[56]	4	SE = 4	Thrombi of 4 patients with infective endocarditis contained bacteria	Bacteria	Gram Stain
Hernandez-Fernandez et al.[55]	64	Non-SE = 60 SE = 40	Four patients were diagnosed with a septic embolus which were related to various underlying infectious pathologies	Bacteria	Gram Stain
Thrombus composition is not related to stroke etiology					
Liebeskind et al.[36]	50	NR	NA	RBCs Fibrin Leukocytes	H&E

Marder et al.[48]	25	CE = 16 LAA = 4 Cryptogenic = 2 Other = 3	Fibrin is described as two histological patterns: serpentine and layered fibrin. No difference was found between the two patterns and etiology.	RBCs Fibrin	H&E
Sallustio et al.[49]	28	CE = 11 LAA =17	Fibrin is described as two histological patterns: serpentine and layered fibrin. No difference was found between the two patterns and etiology.	RBCs Fibrin	H&E and PTAH
Duffy et al.[19]	60	CE = 20 LAA = 15 Cryptogenic = 22 Other = 3	No link was found with the full thrombus load and etiology. However, Pass 1 and 2 are similar between CE and cryptogenic, no difference between LAA and CE. Passes 3 and higher showed no difference.	RBCs Fibrin Leukocytes	H&E and MSB
Schuhmann et al.[52]	37	NR	NA	CD4+ T-cells Monocytes VWF	H&E IHC: CD4+ T-cells (CD4), Monocytes (CD68) and VWF
Denorme et al.[8]	36	CE = 23 LAA = 4 Cryptogenic = 6 Other = 3	VWF was the sole parameters that was investigated with relation to etiology.	RBCs Fibrin VWF	H&E and MSB IHC: VWF
Di Meglio et al.[40]	199	CE = 80 LAA = 20 Cryptogenic = 93 Other = 3	Fibrin shell thickness was the sole parameters that was investigated in relation to etiology	RBCs Fibrin Platelets VWF	H&E IHC: RBCs (Glycophorin A), Fibrin, platelets (CD42b) and VWF
Ducroux et al.[11]	108	CE = 53 LAA = 14 Cryptogenic = 37 Other = 4	NA	NETs	H&E IHC: NETs (H4Cit3 in combination with myeloperoxidase)

CE = cardioembolic, LAA = large artery atherosclerotic, RBCs = Red blood cells, H&E = Hematoxylin and eosin, IHC = Immunohistochemical stain, MSB = Martius Scarlet Blue, PTAH = Phosphotungstic acid hematoxylin, NETs = Neutrophil extracellular trap, H3Cit = Citrullinated histone 3, H4Cit3 = Histone 4 citrulline 3, SE = septic embolus, non-SE = non-septic embolus, VWF = von Willebrand factor, NR = Not reported, NA = Not applicable

Supplemental Table V: Overview of studies describing the association of thrombus composition with imaging features (radiological signs and thrombus perviousness)

Ref.	Description
Radiological signs (HAS/SVS) are associated with higher amounts of RBCs in the thrombus	
Liebeskind et al.[36]	Higher amounts of RBCs composition is an independent predictor for HAS and SVS.
Maekawa et al.[20]	RBC-rich thrombi have a higher thrombus density (in Hounsfield Units) than fibrin-rich thrombi.
Choi et al.[24]	A higher fraction of RBCs is positively correlated with SVS on MRI.
Shin et al.[21]	The total area occupied by RBCs was significantly higher in patients with clot signs than in patients without clot signs.
Simons et al.[26]	HAS is significantly associated with early phase thrombus pathology (RBC-dominant thrombi and thrombi containing equal amounts of RBCs and fibrin)
Kim et al.[25]	SVS is associated with more RBCs, less fibrin, less platelets and a trend towards less leukocytes.
Sporns et al.[37]	Higher relative Hounsfield Units are associated with higher amounts of RBCs and lower amounts of fibrin/platelet aggregates.
Niesten et al.[34]	A moderate linear correlation was found between the attenuation and percentage of RBCs, a weak negative linear correlation between attenuation and platelets and a negligible negative linear correlation for fibrin.
Essig et al.[28]	RBC content, but not fibrin, neutrophils and NETs, is associated with thrombus density (Hounsfield Units) on non-contrast CT.
Lowers amounts of platelets, but not RBCs or fibrin, is associated with HAS	
Fitzgerald et al.[38]	A significant correlation between platelet-rich clots and the absence of a HAS on non-contrast CT was identified. In addition, a significant inverse correlation between the percentage of platelets and the mean Hounsfield Units on non-contrast CT. No correlations were found with other components (RBCs, fibrin and leukocytes).
Thrombus perviousness is associated with higher RBC content and low fibrin/leukocyte content	

Benson et al.[44]	Pervious clots contain higher amounts of RBCs and lower amounts of fibrin and leukocytes compared to impervious clots. A trend towards increased platelet content in impervious clots was observed.
Thrombus perviousness is associated with low RBC and high fibrin/platelets	
Berndt et al.[45]	Permeable thrombi showed a strong correlation with lower fractions of red blood cells counts and more fibrin/platelets conglomerations.

HAS = hyperdense artery sign, SVS = susceptibility vessel sign, RBCs = red blood cells, MRI = Magnetic resonance imaging, CT = computed tomography

Supplemental Table VI: Overview of studies describing the association of thrombus composition with clinical outcome

Ref.	Description
Association with stroke severity or early neurological improvement (NIHSS)	
Choi et al.[24]	NIHSS at admission (stroke severity) is associated with increase RBC content.
Schuhmann et al.[52]	Higher amounts of VWF and monocytes are associated with higher NIHSS at admission (stroke severity).
Boeckh-Behrens et al.[53]	Higher amounts of leukocytes are associated with NIHSS at discharge, but not with NIHSS at admission or $\Delta NIHSS_{admission\text{-}discharge}$.
Boeckh-Behrens et al.[108]	Higher amounts of CD31+ cells are associated with early neurological improvement ($\Delta NIHSS_{admission\text{-}discharge}$).
Khismatullin et al.[39]	A higher content of polyhedral and polyhedral-like RBCs are associated with a worse NIHSS score 24 hours and 7 days after admission. Platelets and fibrin bundles (thick fibrillar structures made up of several laterally aggregated fibers) are associated with a worse NIHSS at admission and 7 days after admission.
Novotny et al.[47]	A higher NET content is associated with a worse NIHSS score at discharge. Leukocytes, neutrophils, eosinophils, monocytes and T/B-cells are not associated with this parameter.
Association with favorable clinical outcome (modified Ranking Scale)	
Maekawa et al.[20]	Patients with RBC-dominant thrombi have a tendency of improved clinical outcome.
Sallustio et al.[49]	Total thrombus size is positively correlated with worse clinical outcome.
Khismatullin et al.[39]	Higher content of polyhedral RBCs correlates with a worse clinical outcome, while the presence of fibrin bundles (thick fibrillar structures made up of several laterally aggregated fibers) are associated with a more favorable clinical outcome. A higher leukocyte content tends to be associated with a worse clinical outcome.
Novotny et al.[47]	A higher NET content is associated with a worse mRS score. Leukocytes, neutrophils, eosinophils, monocytes and T/B-cells are not associated with this parameter.
No association with stroke severity, early neurological improvement or clinical outcome	

Douglas et al.[23]	RBCs, fibrin, leukocytes, platelets and VWF are not associated with NIHSS at admission.
Kim et al.[25]	RBCs, fibrin, platelets and leukocytes are not associated with NIHSS at admission.
Denorme et al.[8]	VWF is not correlated with NIHSS at admission.
Schuhmann et al.[52]	VWF, monocytes and CD4+ T-cells are not associated with clinical outcome (mRS) at discharge.
Ducroux et al.[11]	NETs content is not related to 3-month functional outcome.
Di Meglio et al.[40]	Fibrin shell thickness is not associated with 3-month functional outcome.
Singh et al.[50]	No association between thrombus histology (red, white and mixed) and 3-month functional outcome.

RBCs = red blood cells, VWF = von Willebrand factor, NIHSS = National Institute of Health Stroke Scale, mRS = modified Ranking Scale, NETs = Neutrophil extracellular traps