Stroke

TOPICAL REVIEW

Studying Stroke Thrombus Composition After Thrombectomy

What Can We Learn?

Senna Staessens, PhD; Olivier François, MD; Waleed Brinjikji, MD, PhD; Karen M. Doyle, PhD; Peter Vanacker, MD, PhD; Tommy Andersson, MD, PhD; Simon F. De Meyer, PhD

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 (eg, fibrin, von Willebrand Factor, and neutrophil extracellular traps) and cellular (eg, 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 patients with stroke. 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.

Key Words: fibrin ■ ischemic stroke ■ leukocytes ■ thrombectomy ■ von Willebrand Factor

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 thromboemboli. 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 (VWF), 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 patients with ischemic stroke. 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]

Correspondence to: Simon F. De Meyer, PhD, Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, E Sabbelaan 53, 8500 Kortrijk, Belgium. Email simon.demeyer@kuleuven.be

The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.121.034289.
For Sources of Funding and Disclosures, see page 3724.

Stroke is available at www.ahajournals.org/journal/str

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% to 75% of patients to achieve complete recanalization.[15,16] In 10% to 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 the Table and Tables I and II in the Data Supplement.

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[19] showed that the composition of thrombus material retrieved in the first 2 thrombectomy attempts contains significantly more RBCs and less fibrin compared with thrombus material retrieved in subsequent attempts, indicating that RBC-rich thrombus material is easier to remove than fibrin-rich thrombi. Several factors could contribute to this observation. RBC-rich thrombi have a lower coefficient of friction compared with fibrin-rich thrombi.[19,57] 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.[58,59] Of note, such increased deformability and reduced friction potentially also explains why RBC-dominant thrombi are more prone to preinterventional thrombus migration.[41,42] 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.[60]

Other thrombus components may also influence thrombus removability. Boeckh-Behrens et al[53] showed that higher amounts of leukocytes in the thrombus have a tendency to require more passes to be needed for removal of the thrombus. 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.[61] Similarly, bacteria increase thrombus stiffness by altering the fibrin microstructure,[62] which could explain why the presence of bacteria negatively affects septic thrombus removability.[55] Components of the vascular wall in a thrombus, such as a 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.[63]

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,[24–26,31,36,41,50,51] an increasing number of studies indicate that RBC-rich thrombi are associated with better recanalization outcomes (mTICI score >2b) in comparison to fibrin/platelet-rich thrombi.[19,21–23,63] Similarly, the presence of leukocytes and NETs was not associated with the mTICI score in various reports,[11,25,31,36,37,51–53] although some studies show that leukocytes, in particular neutrophil elastase-positive cells and NETs, contribute to lower rates of complete recanalization (mTICI score <2b).[47,53,46] 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.[55,63]

An important unwanted aspect of thrombectomy procedures is thrombus fragmentation, leading to a shower of small thromboemboli 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,[37] and higher amounts of neutrophil elastase-positive cells[46] have been suggested to be associated with the occurrence of secondary emboli.

In summary, 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 with 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

Table. 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[19,21,22] compared with fibrin/platelet-rich thrombi[19–23]	RBC-rich areas are more susceptible to thrombolysis compared with platelet-rich areas[24]	CE thrombi = higher RBC and lower fibrin/platelet content[21,25–27] vs LAA thrombi = higher RBC and lower fibrin/platelet content[20,28–35]	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.[20,21,24–26,28,34,36–38]	Higher NIHSS score at admission = higher RBC content,[24] higher platelet, or fibrin bundle content[39]
			Cryptogenic thrombi resemble CE[31,32,34,37] vs LAA thrombi[21]		Higher NIHSS score 7 d postadmission = higher polyhedral RBC, platelet, or fibrin bundle content[39]
	RBC-rich thrombi are prone to preprocedural thrombus migration[41,42]	Thrombi contain a dense outer shell of platelets that is resistant to fibrinolysis[40]	LAA thrombi = inner RBC core with platelets on the surface vs CE thrombi = platelets interspersed with RBCs throughout the entire thrombus.[31,43]	Increased thrombus perviousness is associated with RBC-rich thrombi[44] vs perviousness is associated with fibrin/platelet-rich thrombi[45]	Favorable clinical outcome = higher RBC content[20]
	Formation of secondary embolisms is more present in RBC-rich thrombi[46] vs secondary embolisms are more present in fibrin/platelet-rich thrombi[37]				Worse clinical outcome = higher polyhedral RBC content[39]
	No association[24–26,31,36,37,48,50,51]		No association[19,36,47–49]		No association[23,25,50]
VWF	Unknown	Higher VWF content = increased r-tPA resistance[8]	No association[8,52]	Unknown	Higher NIHSS score at admission = higher VWF content[52]
					No association[8]
Leukocytes	Higher leukocyte content = improved recanalization outcomes[53]	Unknown	CE thrombi = more leukocytes than LAA thrombi[32,33,53]	Unknown	Higher NIHSS score at admission/discharge = higher leukocyte content[53] or monocytes[52]
	Higher leukocyte content = more passes required[53]		No association[19,36]		No association[23,25]
	Higher neutrophil content = more secondary embolisms[46]		CE thrombi are associated with a higher neutrophil[28] and lower T-cell[54] content		
	No association[25,31,33,36,51,52]		No association (T/B cells, eosinophils, monocyte/macrophages, and neutrophils)[10,33,47]		
NETs	Higher NET content = increasing amount of thrombectomy attempts[11,47] and a worse recanalization outcome[47]	Higher NET content = increasing r-tPA resistance[10,11]	CE thrombi = higher NET content[10,28,47] and higher overall DNA content[35] than non-CE thrombi	Unknown	NETs are associated with a worse NIHSS score at discharge and a worse mRS score.[47]
			No association[11]		
Bacteria	Presence of bacteria = more thrombectomy attempts[55]	Unknown	Presence of bacteria = underlying infectious pathology (eg, infective endocarditis)[55,56]	Unknown	Unknown

CE indicates cardioembolic; HAS, hyperdense artery sign; LAA, large artery atherosclerosis; mRS, modified Rankin Scale; NETs, neutrophil extracellular traps; NIHSS, National Institutes of Health Stroke Scale; RBCs, red blood cells; r-tPA, recombinant tissue-type plasminogen activator; and VWF, von Willebrand Factor.

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[64] or to improve retrieval of difficult fibrin/platelet-dominant thrombi.[65]

THROMBUS COMPOSITION AND THROMBOLYSIS

At present, r-tPA (recombinant tissue-type plasminogen activator) is the only Food and Drug Administration-approved thrombolytic drug to pharmacologically dissolve the thrombotic cerebral occlusion. Use of r-tPA is, however, limited to <15% of patients due to the short therapeutic time window of 4.5 hours after stroke onset.[66] In addition, recanalization after r-tPA is only successful in less than half of patients with a proximal artery occlusion.[67] The reasons for the latter are not well understood, but it seems that thrombus length plays a major role as thrombi >8 mm respond poorly or not at all to intravenous thrombolysis.[68,69] Recent evidence indicates that thrombolysis reduces the size of a thrombus retrieved by thrombectomy, but this effect is not associated with recanalization outcome.[70] Most likely, also thrombus composition influences the response to intravenous thrombolysis and studies examining retrieved stroke thrombus material might shed some light on this so-called r-tPA resistance (summarized in the Table and Table III in the Data Supplement). The thrombolytic mechanism of r-tPA 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 r-tPA 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, VWF, extracellular DNA, and NETs.[8–11,52] Such nonfibrin components may contribute to r-tPA 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 r-tPA than platelet-rich thrombi.[8,24,71,72] Of note, Di Meglio et al[40] recently described a fibrinolysis-resistant outer thrombus shell composed of platelets, VWF, and extracellular DNA, forming a barrier that hampers r-tPA-mediated thrombolysis. Interestingly, this shell also contained inhibitors of fibrinolysis, such as PAI-1 (plasminogen activator inhibitor 1).[40] Thrombus contraction, a common phase of thrombus formation mediated by contractile forces of platelets on fibrin, might also influence thrombolytic success.[73] Thrombus contraction facilitates the redistribution of platelets and RBCs into separate areas and mediates the compression of RBCs into tightly packed polyhedrocytes,[73,74] which can reduce thrombus permeability and thus the degree of thrombolysis.[75] Intravital thrombus contraction was recently demonstrated in stroke thrombi, resulting in a compact structure with a limited porosity.[39]

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 preclinical studies.[8,10,11,76–78] 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 with alteplase.[79]

THROMBUS COMPOSITION AND STROKE ETIOLOGY

Due to the mainly embolic nature of acute ischemic stroke, thromboemboli can originate from different locations in the body.[80] 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.[81] 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 (eg, carotid dissection or paradoxical embolisms), or a cryptogenic origin.[82] 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 ≈25% of all patients with stroke within 5 years after the initial event, highlighting the need for improved primary and secondary prevention.[83] Cardioembolic strokes are commonly caused by atrial fibrillation and are mainly treated using anticoagulants, whereas LAA strokes are mostly treated using antiplatelet agents (eg, aspirin).[84] 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.[6,85] A summary of these studies is shown in the Table and Table IV in the Data Supplement.

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 with LAA thrombi,[21,25–27] other studies reported the opposite[20,28–34] or found no association at all.[19,36,47,48] 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,[30,31] whereas the opposite was reported by others.[35,51] Various studies showed no association between stroke etiology and platelet content.[25,27,34,47] Similarly, VWF has been shown to be present in all thrombi regardless of their origin, with amounts ranging from 0.1% to 95%.[8,23,52] Initial reports, using low sample sizes, showed no link between VWF content and stroke etiology.[8,52] Data on leukocytes also remain inconsistent. Various reports indicate that leukocyte content is not related to stroke etiology,[19,20,25,33,36,47] whereas several other studies did find an association between higher leukocyte content and cardioembolic origin.[32,33,53] 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 and Table IV in the Data Supplement).[10,28,33,47,52,54] NETs or extracellular DNA seems to be particularly present in cardioembolic thrombi.[10,28,35,47] 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.[86,87] 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,[88] 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,[48,49] 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.[31,43]

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, 2 of the largest patient thrombus cohorts indicate that cardioembolic thrombi contain higher amounts of fibrin/platelet aggregates and lower amounts of RBCs compared with other etiological subtypes.[32,33] Based on the histological analysis, these large studies suggest that the majority of cryptogenic thrombi most likely originate from a cardioembolic etiology.[32,33] 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.[7,89] 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 (atherosclerosis, small vessel disease, cardioembolism, other and dissections) criteria that assign a degree of likelihood to a patient-specific etiological classification.[90] Potentially, such improved etiological 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, 2 studies revealed the presence of Gram-positive bacteria in thrombi from patients with infective endocarditis or other infectious diseases.[55,56] 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 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 the Table and Table V in the Data Supplement.

At the site of the cerebral occlusion a hyperdense artery sign and a susceptible vessel sign is detected in ≈50% of patients with ischemic stroke using CT or magnetic resonance imaging, respectively.[91] The vast majority of studies indicate that both the presence and the density, typically measured in Hounsfield units, of hyperdense artery sign on CT is associated with RBC-dominant thrombi, while the absence of this radiological sign is indicative of fibrin/platelet-rich thrombi.[20,21,24–26,28,34,36–38,53] The correlation of hyperdense artery sign and susceptible vessel sign with the presence of RBCs can be explained by the concentration of hemoglobin in the thrombus.[92–95] 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[96] 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. Apart from CT and magnetic resonance imaging-based imaging, intravascular optical coherence tomography can also be used to determine thrombus composition. Intravascular optical coherence tomography 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.[97] While currently in use to evaluate the morphology of coronary plaques during coronary endovascular interventions, recent in vitro studies showed that optical coherence tomography can be used to discriminate between RBC-dominant, fibrin-dominant, and mixed-blood clots as well.[98,99]

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 noncontrast CT with that on CT angiography, thereby giving an idea about residual blood flow through the thrombus.[100] An increase in thrombus attenuation between the 2 respective imaging techniques implies a higher thrombus perviousness as contrast media enters the thrombus.[100] Higher thrombus perviousness is associated with better functional outcome, smaller infarct volumes, and improved recanalization outcomes with both thrombolytic and endovascular therapy.[100] Indeed, pervious thrombi have a porous structure that allows the passage of residual arterial flow or thrombolytics. Current reports with regard to the histology of the thrombus and thrombus perviousness are conflicting.[44,45] Benson et al[44] have shown that RBC-dominant, fibrin/platelet-poor thrombi are associated with more permeable thrombi on CT imaging. However, Berndt et al[45] have shown that higher amounts of fibrin/platelet conglomerates and lower amounts of RBCs are associated with more permeable thrombi. 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 pretreatment 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 Institutes of Health Stroke Scale score at admission,[101] diabetes,[102] multiple thrombectomy attempts,[15,16,101] higher neutrophil counts,[102] higher neutrophil/lymphocyte ratio,[103] and a higher VWF/ADAMTS13 ratio.[104] 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 (National Institutes of Health Stroke Scale) and clinical outcome (modified Rankin Scale; Table and Table VI in the Data Supplement). Whereas not all studies could confirm strong associations,[8,11,23,25,40,50,52] some interesting correlations have been reported. For example, stroke severity, based on admission National Institutes of Health Stroke Scale scores, is positively correlated with the content of RBC, platelets, fibrin, VWF, and monocytes in the occluding thrombus.[24,39,52] 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.[39] 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.[39,47,53]

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 before 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 r-tPA and that can be successfully retrieved via mechanical thrombectomy are available for study. Thus, a selection bias exists, excluding r-tPA-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.[96] Second, thrombus characteristics could be influenced by patient-specific variables, such as prestroke antithrombotic 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 (Embotrap Revascularization Device Registry; URL: https://www.clinicaltrials.gov; Unique identifier: NCT03685578),[105] STRIP (Stroke Thromboembolism Registry of Imaging and Pathology),[23,51] and the THRAPS (Thrombus Analysis in Intra-Arterial-Treated Patients With Acute Ischemic Stroke) (MR CLEAN [A Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands]) 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.

ARTICLE INFORMATION

Affiliations

Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, Belgium (S.S., S.F.D.M.). Department of Medical Imaging (O.F., T.A.) and Department of Neurology (P.V.), AZ Groeninge, Kortrijk, Belgium. Department of Radiology, Mayo Clinic, Rochester, MN (W.B.). CÚRAM-Centre for Research in Medical Devices, National University of Ireland Galway, Ireland (K.M.D.). Department of Neurology, University Hospitals Antwerp, Belgium (P.V.). Department of Translational Neuroscience, University of Antwerp, Belgium (P.V.). Department of Neuroradiology, Karolinska University Hospital and Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden (T.A.).

Acknowledgments

Data availability: The authors declare that all supporting data are available within this review article and in the Data Supplement.

Sources of Funding

This work was supported by research grants to Dr De Meyer from the Fonds voor Wetenschappelijk Onderzoek–Vlaanderen (FWO; research grants G0A8613, G078517, 1509216N, and G0E7620N), the KU Leuven (OT/14/099, ISP/14/02L2, and PDM/20/147), the Queen Elisabeth Medical Foundation and by the European Union's Horizon 2020 Research and Innovation Program INSIST under grant agreement no. 777072. Dr Brinjikji received funding from the National Institutes of Health Grant 1R01NS105853-01. Dr Doyle received funding from Science Foundation Ireland, funding from Cerenovus, and funding from Sensome.

Disclosures

Dr Andersson is a consultant for Anaconda, Amnis Therapeutics, Cerenovus – Neuravi, Rapid Medical, and Stryker. The other authors report no conflicts.

Supplemental Materials

Online Tables I–VI
References 106–108

REFERENCES

1. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, Schonewille WJ, Vos JA, Nederkoorn PJ, Wermer MJ, et al; MR CLEAN Investigators. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372:11–20. doi: 10.1056/NEJMoa1411587
2. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, Yan B, Dowling RJ, Parsons MW, Oxley TJ, et al; EXTEND-IA Investigators. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. 2015;372:1009–1018. doi: 10.1056/NEJMoa1414792
3. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, San Román L, Serena J, Abilleira S, Ribó M, et al; REVASCAT Trial Investigators. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. 2015;372:2296–2306. doi: 10.1056/NEJMoa1503780
4. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, Roy D, Jovin TG, Willinsky RA, Sapkota BL, et al; ESCAPE Trial Investigators. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med*. 2015;372:1019–1030. doi: 10.1056/NEJMoa1414905
5. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, Albers GW, Cognard C, Cohen DJ, Hacke W, et al; SWIFT PRIME Investigators. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med*. 2015;372:2285–2295. doi: 10.1056/NEJMoa1415061
6. Brinjikji W, Duffy S, Burrows A, Hacke W, Liebeskind D, Majoie CBLM, Dippel DWJ, Siddiqui AH, Khatri P, Baxter B, 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. doi: 10.1136/neurintsurg-2016-012391
7. De Meyer SF, Andersson T, Baxter B, Bendszus M, Brouwer P, Brinjikji W, Campbell BC, Costalat V, Dávalos A, Demchuk A, et al; Clot Summit Group. Analyses of thrombi in acute ischemic stroke: a consensus statement on current knowledge and future directions. *Int J Stroke*. 2017;12:606–614. doi: 10.1177/1747493017709671
8. Denorme F, Langhauser F, Desender L, Vandenbulcke A, Rottensteiner H, Plaimauer B, François O, Andersson T, Deckmyn H, Scheiflinger F, et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood*. 2016;127:2337–2345. doi: 10.1182/blood-2015-08-662650
9. Staessens S, Denorme F, Francois O, Desender L, Dewaele T, Vanacker P, Deckmyn H, Vanhoorelbeke K, Andersson T, De Meyer SF. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. *Haematologica*. 2020;105:498–507. doi: 10.3324/haematol.2019.219881
10. Laridan E, Denorme F, Desender L, François O, Andersson T, Deckmyn H, Vanhoorelbeke K, De Meyer SF. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann Neurol*. 2017;82:223–232. doi: 10.1002/ana.24993
11. Ducroux C, Di Meglio L, Loyau S, Delbosc S, Boisseau W, Deschildre C, Ben Maacha M, Blanc R, Redjem H, Ciccio G, et al. Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke. *Stroke*. 2018;49:754–757. doi: 10.1161/STROKEAHA.117.019896
12. Staessens S, De Meyer SF. Thrombus heterogeneity in ischemic stroke. *Platelets*. 2021;32:331–339. doi: 10.1080/09537104.2020.1748586
13. Fitzgerald S, Mereuta OM, Doyle KM, Dai D, Kadirvel R, Kallmes DF, Brinjikji W. Correlation of imaging and histopathology of thrombi in acute ischemic stroke with etiology and outcome. *J Neurosurg Sci*. 2019;63:292–300. doi: 10.23736/S0390-5616.18.04629-5
14. Yoo AJ, Andersson T. Thrombectomy in acute ischemic stroke: challenges to procedural success. *J Stroke*. 2017;19:121–130. doi: 10.5853/jos.2017.00752
15. García-Tornel Á, Requena M, Rubiera M, Muchada M, Pagola J, Rodriguez-Luna D, Deck M, Juega J, Rodríguez-Villatoro N, Boned S, et al. When to stop: detrimental effect of device passes in acute ischemic stroke secondary to large vessel occlusion. *Stroke*. 2019;50:1781–1788. doi: 10.1161/STROKEAHA.119.025088
16. Zaidat OO, Castonguay AC, Linfante I, Gupta R, Martin CO, Holloway WE, Mueller-Kronast N, English JD, Dabus G, Malisch TW, et al. First pass effect: a new measure for stroke thrombectomy devices. *Stroke*. 2018;49:660–666. doi: 10.1161/STROKEAHA.117.020315
17. Flottmann F, Leischner H, Broocks G, Nawabi J, Bernhardt M, Faizy TD, Deb-Chatterji M, Thomalla G, Fiehler J, Brekenfeld C. Recanalization rate per retrieval attempt in mechanical thrombectomy for acute ischemic stroke. *Stroke*. 2018;49:2523–2525. doi: 10.1161/STROKEAHA.118.022737
18. Seker F, Pfaff J, Wolf M, Ringleb PA, Nagel S, Schönenberger S, Herweh C, Möhlenbruch MA, Bendszus M, Pham M. Correlation of thrombectomy maneuver count with recanalization success and clinical outcome in patients with ischemic stroke. *AJNR Am J Neuroradiol*. 2017;38:1368–1371. doi: 10.3174/ajnr.A5212
19. Duffy S, McCarthy R, Farrell M, Thomas S, Brennan P, Power S, O'Hare A, Morris L, Rainsford E, MacCarthy E, et al. Per-pass analysis of thrombus composition in patients with acute ischemic stroke undergoing mechanical thrombectomy. *Stroke*. 2019;50:1156–1163. doi: 10.1161/STROKEAHA.118.023419
20. Maekawa K, Shibata M, Nakajima H, Mizutani A, Kitano Y, Seguchi M, Yamasaki M, Kobayashi K, Sano T, Mori G, 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. doi: 10.1159/000486042
21. 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. doi: 10.1371/journal.pone.0197492
22. Hashimoto T, Hayakawa M, Funatsu N, Yamagami H, Satow T, Takahashi JC, Nagatsuka K, Ishibashi-Ueda H, Kira JI, Toyoda K. Histopathologic analysis of retrieved thrombi associated with successful reperfusion after acute stroke thrombectomy. *Stroke*. 2016;47:3035–3037. doi: 10.1161/STROKEAHA.116.015228
23. Douglas A, Fitzgerald S, Mereuta OM, Rossi R, O'Leary S, Pandit A, McCarthy R, Gilvarry M, Holmegaard L, Abrahamsson M, et al. Platelet-rich emboli are associated with von Willebrand factor levels and have poorer revascularization outcomes. *J Neurointerv Surg*. 2020;12:557–562. doi: 10.1136/neurintsurg-2019-015410
24. Choi MH, Park GH, Lee JS, Lee SE, Lee SJ, Kim JH, Hong JM. Erythrocyte fraction within retrieved thrombi contributes to thrombolytic response in acute ischemic stroke. *Stroke*. 2018;49:652–659. doi: 10.1161/STROKEAHA.117.019138
25. 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. *AJNR Am J Neuroradiol*. 2015;36:1756–1762. doi: 10.3174/ajnr.A4402

26. Simons N, Mitchell P, Dowling R, Gonzales M, Yan B. Thrombus composition in acute ischemic stroke: a histopathological study of thrombus extracted by endovascular retrieval. *J Neuroradiol.* 2015;42:86–92. doi: 10.1016/j.neurad.2014.01.124
27. Sato Y, Ishibashi-Ueda H, Iwakiri T, Ikeda Y, Matsuyama T, Hatakeyama K, Asada Y. Thrombus components in cardioembolic and atherothrombotic strokes. *Thromb Res.* 2012;130:278–280. doi: 10.1016/j.thromres.2012.04.008
28. Essig F, Kollikowski AM, Pham M, Solymosi L, Stoll G, Haeusler KG, Kraft P, Schuhmann MK. Immunohistological analysis of neutrophils and neutrophil extracellular traps in human thrombemboli causing acute ischemic stroke. *Int J Mol Sci.* 2020;21:E7387. doi: 10.3390/ijms21197387
29. Liao Y, Guan M, Liang D, Shi Y, Liu J, Zeng X, Huang S, Xie X, Yuan D, Qiao H, 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. doi: 10.3389/fneur.2020.00811
30. 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. doi: 10.1186/s12883-020-01969-w
31. Ahn SH, Hong R, Choo IS, Heo JH, Nam HS, Kang HG, Kim HW, Kim JH. Histologic features of acute thrombi retrieved from stroke patients during mechanical reperfusion therapy. *Int J Stroke.* 2016;11:1036–1044. doi: 10.1177/1747493016641965
32. Boeckh-Behrens T, Kleine JF, Zimmer C, Neff F, Scheipl F, Pelisek J, Schirmer L, Nguyen K, Karatas D, Poppert H. Thrombus histology suggests cardioembolic cause in cryptogenic stroke. *Stroke.* 2016;47:1864–1871. doi: 10.1161/STROKEAHA.116.013105
33. Sporns PB, Hanning U, Schwindt W, Velasco A, Minnerup J, Zoubi T, Heindel W, Jeibmann A, Niederstadt TU. Ischemic stroke: what does the histological composition tell us about the origin of the thrombus? *Stroke.* 2017;48:2206–2210. doi: 10.1161/STROKEAHA.117.016590
34. Niesten JM, van der Schaaf IC, van Dam L, Vink A, Vos JA, Schonewille WJ, de Bruin PC, Mali WP, Velthuis BK. Histopathologic composition of cerebral thrombi of acute stroke patients is correlated with stroke subtype and thrombus attenuation. *PLoS One.* 2014;9:e88882. doi: 10.1371/journal.pone.0088882
35. Di Meglio L, Desilles JP, Solonomenjanahary M, Labreuche J, Ollivier V, Dupont S, Deschildre C, Maacha MB, Consoli A, Lapergue B, et al; compoCLOT study group†. DNA content in ischemic stroke thrombi can help identify cardioembolic strokes among strokes of undetermined cause. *Stroke.* 2020;51:2810–2816. doi: 10.1161/STROKEAHA.120.029134
36. Liebeskind DS, Sanossian N, Yong WH, Starkman S, Tsang MP, Moya AL, Zheng DD, Abolian AM, Kim D, Ali LK, et al. CT and MRI early vessel signs reflect clot composition in acute stroke. *Stroke.* 2011;42:1237–1243. doi: 10.1161/STROKEAHA.110.605576
37. Sporns PB, Hanning U, Schwindt W, Velasco A, Buerke B, Cnyrim C, Minnerup J, Heindel W, Jeibmann A, Niederstadt T. 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. doi: 10.1159/000481578
38. Fitzgerald ST, Wang S, Dai D, Douglas A, Kadirvel R, Gounis MJ, Chueh J, Puri AS, Layton KF, Thacker IC, et al. Platelet-rich clots as identified by Martius Scarlet Blue staining are isodense on NCCT. *J Neurointerv Surg.* 2019;11:1145–1149. doi: 10.1136/neurintsurg-2018-014637
39. Khismatullin RR, Nagaswami C, Shakirova AZ, Vrtková A, Procházka V, Gumulec J, Mačák J, Litvinov RI, Weisel JW. Quantitative morphology of cerebral thrombi related to intravital contraction and clinical features of ischemic stroke. *Stroke.* 2020;51:3640–3650. doi: 10.1161/STROKEAHA.120.031559
40. Di Meglio L, Desilles JP, Ollivier V, Nomenjanahary MS, Di Meglio S, Deschildre C, Loyau S, Olivot JM, Blanc R, Piotin M, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis. *Neurology.* 2019;93:e1686–e1698. doi: 10.1212/WNL.0000000000008395
41. Sporns PB, Jeibmann A, Minnerup J, Broocks G, Nawabi J, Schön G, Fiehler J, Wildgruber M, Heindel W, Kemmling A, et al. Histological clot composition is associated with preinterventional clot migration in acute stroke patients. *Stroke.* 2019;50:2065–2071. doi: 10.1161/STROKEAHA.118.023314
42. Maegerlein C, Friedrich B, Berndt M, Lucia KE, Schirmer L, Poppert H, Zimmer C, Pelisek J, Boeckh-Behrens T, Kaesmacher J. 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. doi: 10.1177/1591019917733733
43. Kim B, Kim YM, Jin SC, Lee JW, Lee BI, Kim SE, Shin KJ, Park J, Park KM, 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. doi: 10.1016/j.jocn.2020.01.089
44. Benson JC, Fitzgerald ST, Kadirvel R, Johnson C, Dai D, Karen D, Kallmes DF, Brinjikji W. Clot permeability and histopathology: is a clot's perviousness on CT imaging correlated with its histologic composition? *J Neurointerv Surg.* 2020;12:38–42. doi: 10.1136/neurintsurg-2019-014979
45. Berndt M, Friedrich B, Maegerlein C, Moench S, Hedderich D, Lehm M, Zimmer C, Straeter A, Poppert H, Wunderlich S, et al. Thrombus permeability in admission computed tomographic imaging indicates stroke pathogenesis based on thrombus histology. *Stroke.* 2018;49:2674–2682. doi: 10.1161/STROKEAHA.118.021873
46. Kaesmacher J, Boeckh-Behrens T, Simon S, Maegerlein C, Kleine JF, Zimmer C, Schirmer L, Poppert H, Huber T. Risk of thrombus fragmentation during endovascular stroke treatment. *AJNR Am J Neuroradiol.* 2017;38:991–998. doi: 10.3174/ajnr.A5105
47. Novotny J, Oberdieck P, Titova A, Pelisek J, Chandraratne S, Nicol P, Hapfelmeier A, Joner M, Maegdefessel L, Poppert H, et al. Thrombus NET content is associated with clinical outcome in stroke and myocardial infarction. *Neurology.* 2020;94:e2346–e2360. doi: 10.1212/WNL.0000000000009532
48. Marder VJ, Chute DJ, Starkman S, Abolian AM, Kidwell C, Liebeskind D, Ovbiagele B, Vinuela F, Duckwiler G, Jahan R, et al. Analysis of thrombi retrieved from cerebral arteries of patients with acute ischemic stroke. *Stroke.* 2006;37:2086–2093. doi: 10.1161/01.STR.0000230307.03438.94
49. Sallustio F, Arnò N, Di Legge S, Koc G, Martorana A, Rossi C, Diomedi M, Del Giudice C, Anemona L, Gandini R, 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.
50. 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. doi: 10.1161/STROKEAHA.113.000964
51. Fitzgerald S, Dai D, Wang S, Douglas A, Kadirvel R, Layton KF, Thacker IC, Gounis MJ, Chueh JY, Puri AS, et al. Platelet-rich emboli in cerebral large vessel occlusion are associated with a large artery atherosclerosis source. *Stroke.* 2019;50:1907–1910. doi: 10.1161/STROKEAHA.118.024543
52. 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. doi: 10.3390/ijms17030298
53. Boeckh-Behrens T, Schubert M, Förschler A, Prothmann S, Kreiser K, Zimmer C, Riegger J, Bauer J, Neff F, Kehl V, et al. The impact of histological clot composition in embolic stroke. *Clin Neuroradiol.* 2016;26:189–197. doi: 10.1007/s00062-014-0347-x
54. Dargazanli C, Rigau V, Eker O, Riquelme Bareiro C, Machi P, Gascou G, Arquizan C, Ayrignac X, Mourand I, Corlobé A, et al. High CD3+ cells in intracranial thrombi represent a biomarker of atherothrombotic stroke. *PLoS One.* 2016;11:e0154945. doi: 10.1371/journal.pone.0154945
55. Hernández-Fernández F, Rojas-Bartolomé L, García-García J, Ayo-Martín Ó, Molina-Nuevo JD, Barbella-Aponte RA, Serrano-Heras G, Juliá-Molla E, Pedrosa-Jiménez MJ, Segura T. Histopathological and bacteriological analysis of thrombus material extracted during mechanical thrombectomy in acute stroke patients. *Cardiovasc Intervent Radiol.* 2017;40:1851–1860. doi: 10.1007/s00270-017-1718-x
56. Bhaskar S, Saab J, Cappelen-Smith C, Killingsworth M, Wu XJ, Cheung A, Manning N, Aouad P, McDougall A, Hodgkinson S, et al. Clot histopathology in ischemic stroke with infective endocarditis. *Can J Neurol Sci.* 2019;46:331–336. doi: 10.1017/cjn.2019.8
57. Gunning GM, McArdle K, 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. doi: 10.1136/neurintsurg-2016-012721
58. Gersh KC, Nagaswami C, Weisel JW. Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes. *Thromb Haemost.* 2009;102:1169–1175. doi: 10.1160/TH09-03-0199
59. Weafer FM, Duffy S, Machado I, Gunning G, Mordasini P, Roche E, McHugh PE, Gilvarry M. Characterization of strut indentation during mechanical thrombectomy in acute ischemic stroke clot analogs. *J Neurointerv Surg.* 2019;11:891–897. doi: 10.1136/neurintsurg-2018-014601
60. Kaesmacher J, Maegerlein C, Kaesmacher M, Zimmer C, Poppert H, Friedrich B, Boeckh-Behrens T, Kleine JF. Thrombus migration in the middle cerebral artery: incidence, imaging signs, and impact on success of endovascular thrombectomy. *J Am Heart Assoc.* 2017;6:e005149. doi: 10.1161/JAHA.116.005149

61. Longstaff C, Varjú I, Sótonyi P, Szabó L, Krumrey M, Hoell A, Bóta A, Varga Z, Komorowicz E, Kolev K. Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, and histones. *J Biol Chem.* 2013;288:6946–6956. doi: 10.1074/jbc.M112.404301
62. Ma TM, VanEpps JS, Solomon MJ. Structure, mechanics, and instability of fibrin clot infected with Staphylococcus epidermidis. *Biophys J.* 2017;113:2100–2109. doi: 10.1016/j.bpj.2017.09.001
63. Funatsu N, Hayakawa M, Hashimoto T, Yamagami H, Satow T, Takahashi JC, Koga M, Nagatsuka K, Ishibashi-Ueda H, Iwama T, et al. Vascular wall components in thrombi obtained by acute stroke thrombectomy: clinical significance and related factors. *J Neurointerv Surg.* 2019;11:232–236. doi: 10.1136/neurintsurg-2018-014041
64. Chueh JY, Marosfoi MG, Brooks OW, King RM, Puri AS, Gounis MJ. Novel distal emboli protection technology: the EmboTrap. *Interv Neurol.* 2017;6:268–276. doi: 10.1159/000480668
65. Fennell VS, Setlur Nagesh SV, Meess KM, Gutierrez L, James RH, Springer ME, Siddiqui AH. 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.
66. Aguiar de Sousa D, von Martial R, Abilleira S, Gattringer T, Kobayashi A, Gallofré M, Fazekas F, Szikora I, Feigin V, Caso V, 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. doi: 10.1177/2396987318786023
67. Prabhakaran S, Ruff I, Bernstein RA. Acute stroke intervention: a systematic review. *JAMA.* 2015;313:1451–1462. doi: 10.1001/jama.2015.3058
68. Riedel CH, Zimmermann P, Jensen-Kondering U, 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. doi: 10.1161/STROKEAHA.110.609693
69. Rohan V, Baxa J, Tupy R, Cerna L, Sevcik P, Friesl M, Polivka J Jr, Polivka J, Ferda J. Length of occlusion predicts recanalization and outcome after intravenous thrombolysis in middle cerebral artery stroke. *Stroke.* 2014;45:2010–2017. doi: 10.1161/STROKEAHA.114.005731
70. Rossi R, Fitzgerald S, Molina S, Mereuta OM, Douglas A, Pandit A, Santos AMS, Murphy B, Alderson J, Brennan P, 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.* 2021;51:545–551. doi: 10.1007/s11239-020-02279-1
71. Jang IK, Gold HK, Ziskind AA, Fallon JT, Holt RE, Leinbach RC, May JW, Collen D. 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. doi: 10.1161/01.cir.79.4.920
72. Tomkins AJ, Schleicher N, Murtha L, Kaps M, Levi CR, Nedelmann M, Spratt NJ. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. *Exp Transl Stroke Med.* 2015;7:2. doi: 10.1186/s13231-014-0014-y
73. Cines DB, Lebedeva T, Nagaswami C, Hayes V, Massefski W, Litvinov RI, Rauova L, Lowery TJ, Weisel JW. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood.* 2014;123:1596–1603. doi: 10.1182/blood-2013-08-523860
74. Nechipurenko DY, Receveur N, Yakimenko AO, Shepelyuk TO, Yakusheva AA, Kerimov RR, Obydennyy SI, Eckly A, Léon C, Gachet C, et al. Clot contraction drives the translocation of procoagulant platelets to thrombus surface. *Arterioscler Thromb Vasc Biol.* 2019;39:37–47. doi: 10.1161/ATVBAHA.118.311390
75. Tutwiler V, Peshkova AD, Le Minh G, Zaitsev S, Litvinov RI, Cines DB, Weisel JW. Blood clot contraction differentially modulates internal and external fibrinolysis. *J Thromb Haemost.* 2019;17:361–370. doi: 10.1111/jth.14370
76. Martinez de Lizarrondo S, Gakuba C, Herbig BA, Repessé Y, Ali C, Denis CV, Lenting PJ, Touzé E, Diamond SL, Vivien D, et al. Potent thrombolytic effect of N-acetylcysteine on arterial thrombi. *Circulation.* 2017;136:646–660. doi: 10.1161/CIRCULATIONAHA.117.027290
77. Wyseure T, Rubio M, Denorme F, Martinez de Lizarrondo S, Peeters M, Gils A, De Meyer SF, Vivien D, Declerck PJ. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood.* 2015;125:1325–1332. doi: 10.1182/blood-2014-07-588319
78. Zhu Y, Carmeliet P, Fay WP. Plasminogen activator inhibitor-1 is a major determinant of arterial thrombolysis resistance. *Circulation.* 1999;99:3050–3055. doi: 10.1161/01.cir.99.23.3050
79. 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. doi: 10.2165/00003088-200241150-00001
80. Yaghi S, Bernstein RA, Passman R, Okin PM, Furie KL. Cryptogenic stroke: research and practice. *Circ Res.* 2017;120:527–540. doi: 10.1161/CIRCRESAHA.116.308447
81. Hathcock JJ. Flow effects on coagulation and thrombosis. *Arterioscler Thromb Vasc Biol.* 2006;26:1729–1737. doi: 10.1161/01.ATV.0000229658.76797.30
82. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* 1993;24:35–41. doi: 10.1161/01.str.24.1.35
83. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2017 update: a report from the American Heart Association. *Circulation.* 2017;135:e146–e603. doi: 10.1161/CIR.0000000000000485
84. Boehme AK, Esenwa C, Elkind MS. Stroke risk factors, genetics, and prevention. *Circ Res.* 2017;120:472–495. doi: 10.1161/CIRCRESAHA.116.308398
85. 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. doi: 10.1161/STROKEAHA.117.017630
86. Maurer CJ, Dobrocky T, Joachimski F, Neuberger U, Demerath T, Brehm A, Cianfoni A, Gory B, Berlis A, Gralla J, et al. Endovascular thrombectomy of calcified emboli in acute ischemic stroke: a multicenter study. *AJNR Am J Neuroradiol.* 2020;41:464–468. doi: 10.3174/ajnr.A6412
87. Bardon M, Hanson J, O'Brien B, Naeem A. Calcified cerebral emboli: incidence and implications. *J Med Imaging Radiat Oncol.* 2018;62:499–503. doi: 10.1111/1754-9485.12730
88. Almekhlafi MA, Hu WY, Hill MD, Auer RN. Calcification and endothelialization of thrombi in acute stroke. *Ann Neurol.* 2008;64:344–348. doi: 10.1002/ana.21404
89. Staessens S, Fitzgerald S, Andersson T, Clarençon F, Denorme F, Gounis MJ, Hacke W, Liebeskind DS, Szikora I, van Es A, et al. Histological stroke clot analysis after thrombectomy: technical aspects and recommendations. *Int J Stroke.* 2020;15:467–476. doi: 10.1177/1747493019884527
90. 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. doi: 10.1159/000352050
91. Mair G, Boyd EV, Chappell FM, von Kummer R, Lindley RI, Sandercock P, Wardlaw JM; IST-3 Collaborative Group. Sensitivity and specificity of the hyperdense artery sign for arterial obstruction in acute ischemic stroke. *Stroke.* 2015;46:102–107. doi: 10.1161/STROKEAHA.114.007036
92. 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. doi: 10.1148/radiol.2273020530
93. New PF, Aronow S. Attenuation measurements of whole blood and blood fractions in computed tomography. *Radiology.* 1976;121(3 Pt. 1):635–640. doi: 10.1148/121.3.635
94. Black DF, Rad AE, Gray LA, Campeau NG, Kallmes DF. Cerebral venous sinus density on noncontrast CT correlates with hematocrit. *AJNR Am J Neuroradiol.* 2011;32:1354–1357. doi: 10.3174/ajnr.A2504
95. 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. doi: 10.3349/ymj.2013.54.1.28
96. Brinjikji W, Michalak G, Kadirvel R, Dai D, Gilvarry M, Duffy S, Kallmes DF, McCollough C, Leng S. Utility of single-energy and dual-energy computed tomography in clot characterization: an in-vitro study. *Interv Neuroradiol.* 2017;23:279–284. doi: 10.1177/1591019917694479
97. 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. doi: 10.1007/s40119-020-00185-4
98. Ding Y, Abbasi M, Eltanahy AM, Jakaitis DR, Dai D, Kadirvel R, Kallmes DF, Brinjikji W. Assessment of blood clot composition by spectral optical coherence tomography: an in vitro study. *Neurointervention.* 2021;16:29–33. doi: 10.5469/neuroint.2020.00297
99. Liu HC, Abbasi M, Ding YH, Polley EC, Fitzgerald S, Kadirvel R, Kallmes DF, Brinjikji W, Urban MW. Characterizing thrombus with multiple red blood cell

compositions by optical coherence tomography attenuation coefficient. *J. Biophotonics*. 2021;14:e202000364. doi: 10.1002/jbio.202000364
100. Santos EM, Dankbaar JW, Treurniet KM, Horsch AD, Roos YB, Kappelle LJ, Niessen WJ, Majoie CB, Velthuis B, Marquering HA; DUST Investigators. 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. doi: 10.1161/STROKEAHA.116.013306
101. Linfante I, Starosciak AK, Walker GR, Dabus G, Castonguay AC, Gupta R, Sun CH, Martin C, Holloway WE, Mueller-Kronast N, et al. Predictors of poor outcome despite recanalization: a multiple regression analysis of the NASA registry. *J Neurointerv Surg.* 2016;8:224–229. doi: 10.1136/neurintsurg-2014-011525
102. Boisseau W, Desilles JP, Fahed R, Kyheng M, Zuber K, Sabben C, Taylor G, Ben Maacha M, Maier B, Botta D, et al. Neutrophil count predicts poor outcome despite recanalization after endovascular therapy. *Neurology*. 2019;93:e467–e475. doi: 10.1212/WNL.0000000000007859
103. Song SY, Zhao XX, Rajah G, Hua C, Kang RJ, Han YP, Ding YC, Meng R. 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. doi: 10.3389/fneur.2019.01032
104. 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. doi: 10.1182/bloodadvances.2019000979
105. ClinicalTrials.gov. EmboTrap® revascularization device registry [Internet]. Accessed September 1, 2020. https://clinicaltrials.gov/ct2/show/NCT03685578.
106. Krajíčková D, Krajina A, Šteiner I, Vyšata O, Herzig R, Lojík M, Chovanec V, Raupach J, Renc O, Waishaupt J, et al. Fibrin clot architecture in acute ischemic stroke treated with mechanical thrombectomy with stent-retrievers - cohort study. *Circ J.* 2018;82:866–873. doi: 10.1253/circj.CJ-17-0375
107. Hayosh ZI, Bandora EA, Shelestovich N, Nulman M, Bakon M, Yaniv G, Khaitovitch B, Balan S, Gerasimova A, Drori T, et al. In-thrombus thrombin secretion: a new diagnostic marker of atrial fibrillation in cryptogenic stroke. *J. Neurointerv Surg.* 2021;13:799–804. doi: 10.1136/neurintsurg-2020-016484
108. Boeckh-Behrens T, Kleine J, Kaesmacher J, Zimmer C, Schirmer L, Simon S, Poppert H. The CD31 molecule: a possible neuroprotective agent in acute ischemic stroke? *Thromb J.* 2017;15:11. doi: 10.1186/s12959-017-0134-4