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REVIEW

Thrombus heterogeneity in ischemic stroke

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Abstract

The structure of stroke thrombi has gained an increasing amount of interest in recent years. The advent of endovascular thrombectomy has offered the unique opportunity to provide and analyze thrombi removed from ischemic stroke patients. It has become clear that the composition of ischemic stroke thrombi is relatively heterogenous and various molecular and cellular patterns become apparent. Good understanding of the histopathologic characteristics of thrombi is important to lead future advancements in acute ischemic stroke treatment. In this review, we give a brief overview of the main stroke thrombus components that have been recently characterized in this rapidly evolving field. We also summarize how thrombus heterogeneity can affect stroke treatment.

KEYWORDS

Stroke, Thrombosis, Thrombectomy, Thrombus

History

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Introduction

The main cause of ischemic stroke is a thrombus in the blood that prevents sufficient blood supply to the brain. The mainstay of primary care is aimed at restoring the blood flow to the brain as fast as possible by recanalizing the occluded blood vessel. Recanalization can be achieved via pharmacological lysis of the occluding thrombus using recombinant tissue plasminogen activator (rt-PA) or via an endovascular thrombectomy intervention that physically removes the thrombus from the circulation. As central target, the thrombus itself is most likely a key factor dictating recanalization success rates in stroke patients. The composition and architecture of ischemic stroke thrombi has become a hot topic in stroke research, mainly instigated by the emergence of thrombectomy procedures that started to provide patient thrombus material for research [1]. Better understanding of thrombus organization and structure is not only necessary to increase our understanding of the pathophysiology that underlies the initial event of thrombus formation, but is also important to further improve thrombus-targeting therapies in ischemic stroke. Accumulating evidence points toward a complex and variable structure of ischemic stroke thrombi, with involvement of thrombotic factors such as platelets and fibrin, as well as inflammatory components such as leukocytes and neutrophil extracellular traps. In this review, we discuss the main thrombus components that have been recently characterized in ischemic stroke thrombi. We also consider how thrombus heterogeneity can impact stroke treatment.

Composition of Ischemic Stroke Thrombi

Red Blood Cells, Platelets and Fibrin

Prior to the availability of patient stroke thrombi, little information was available regarding their general composition and underlying architecture. However, with the implementation of thrombectomy in the stroke clinic, an increasing number of reports have started to describe various aspects of retrieved thrombus material. The first reports described the presence of mainly red blood cells (RBCs), fibrin and platelets, based on general hematoxylin and eosin stainings [2–4]. In the meantime, it has become clear that the rough layout of stroke thrombo-emboli indeed contains shared architectural features of fibrin/platelet deposits and confined erythrocyte-rich regions [2,5]. More detailed studies, using immunofluorescence, revealed that RBC-rich areas have a relatively simple structure of packed RBCs within a meshwork of thin fibrin [6]. In contrast, platelet-rich areas have a more complex internal architecture with much denser fibrin structures that demarcate platelet islands within the thrombus (Figure 1) [6]. These platelet-rich areas typically contain other structural elements, such as von Willebrand factor (VWF), leukocytes, extracellular DNA and neutrophil extracellular traps (NETs), as described further [6].

The relative contribution of RBCs and platelets is a commonly used parameter to describe a thrombus as RBC-dominant, platelet-dominant or mixed, as depicted in Figure 2 [6]. Most thrombi retrieved from patients have a mixed content of both RBC-rich and platelet-rich material, spanning a full range of relative amounts. At the respective ends of this spectrum, thrombi are either very platelet-rich and RBC-poor (typically referred to as "white" thrombi) or particularly RBC-rich and platelet-poor ("red" thrombi) (Figure 2). The reasons for this observed heterogeneity are not yet fully understood but most likely reflect the local environment in which thrombus formation occurred. Indeed, local hemodynamic forces, such as blood flow, shear and turbulence, in combination with the patients' hematological characteristics are known to regulate thrombotic pathways [7]. Platelet-rich thrombi are typically associated with arterial conditions of high-shear stress whereas venous, low shear stress is believed to promote the formation of platelet-poor thrombi that are composed of fibrin and high amounts of encapsulated RBCs[7].

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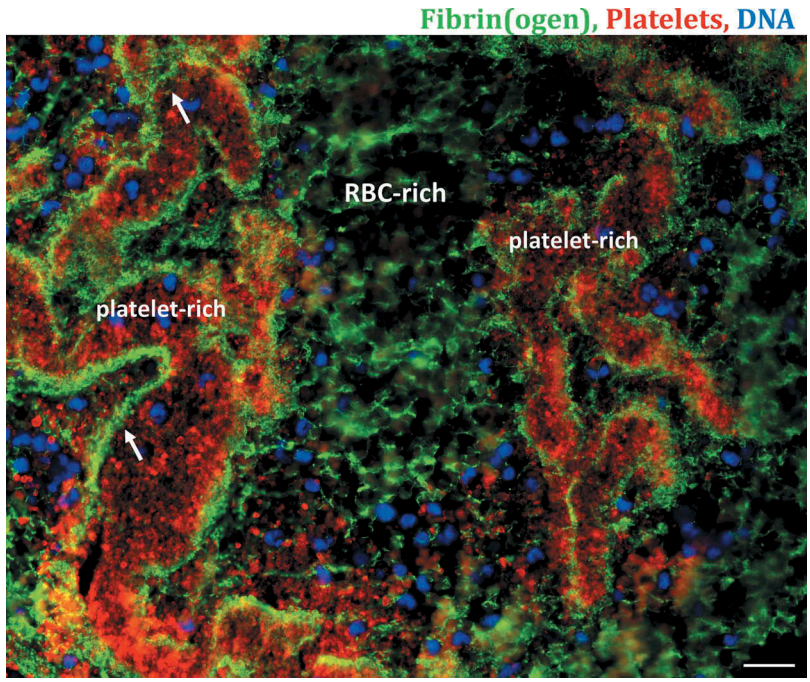

Figure 1. Structure of platelet-rich and RBC-rich areas in ischemic stroke thrombi.This picture shows a representative image of a stroke thrombus that was immunohistochemically stained for fibrin(ogen) (green), platelets (red) and DNA (blue). RBC-rich areas consist of thin fibrin(ogen) strands that surround packed RBCs. RBCs are not stained and their presence can be derived from the black gaps within the fibrin(ogen) network. Platelet-rich areas consist of dense fibrin structures (white arrows), that contain platelets (red). Scale bar = 20 μm. Figure adapted from Staessens et al. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. Haematologica. 2019 May 2:haematol.2019.219881, with permission.

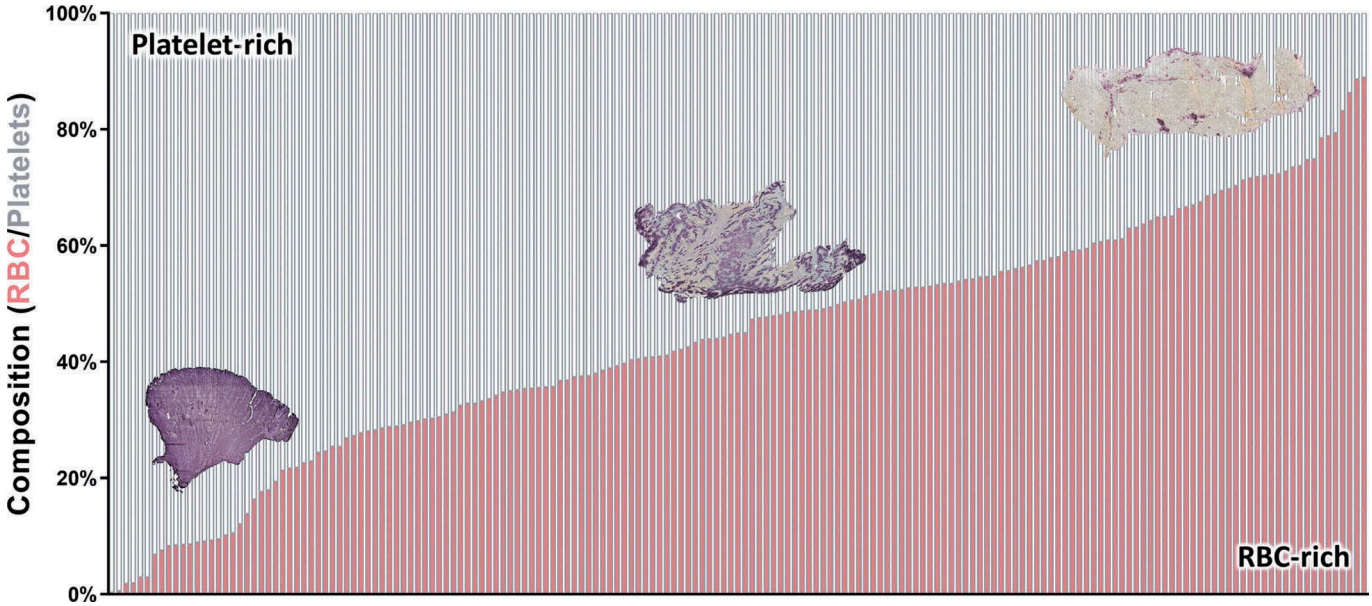

Figure 2. Relative content of platelet-rich and RBC-rich thrombus material in ischemic stroke.Stroke thrombi (n = 177, vertical bars) were quantitatively analyzed and the percentage of RBC-rich areas (red bars) and platelet-rich areas (white bars) were determined. Thrombus composition ranges from platelet-dominant (left) to RBC-dominant (right). Representative images of immunohistochemical platelet staining (purple) are shown of platelet-rich thrombi (left), mixed thrombi (middle) and platelet-poor thrombi (right). Figure adapted from Staessens et al. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. Haematologica. 2019 May 2:haematol.2019.219881, with permission.

Interestingly, thrombus contraction, mediated by contractile forces of platelets on fibrin, can result in the separation of RBCs and platelet aggregates in the thrombus [8]. Besides the redistribution of cells, clot contraction also results in the compression of RBCs to so-called polyhedrocytes [8,9]. Although not specifically studied, the typical irregular polyhedral structure of compressed RBCs seems to be present in stroke thrombi [6,10]. The process of thrombus contraction is still poorly understood and more studies are needed to better understand how platelet-mediated contraction and the formation of densely packed polyhedrocytes affect for example thrombus rigidity, thrombus permeability and thrombolysis in the setting of stroke.

Multiple studies attempted to link the relative content of RBCs and platelets to stroke etiology but inconsistent findings were reported [1,11]. These inconsistencies are most likely related to the low sample sizes and the use of aspecific stainings methods and semiquantitative analysis procedures [1,11]. Yet, two of the largest studies, each including more than 140 stroke thrombi, found that cardioembolic thrombi contain more fibrin/platelets and less RBCs compared to large artery atherosclerotic thrombi, which are more RBC dominant [12,13].

Additional studies, using large sample sizes and standardized histological procedures [14], are required to further clarify the potential link between stroke etiology and thrombus composition.

Von Willebrand Factor

Besides the typical components such as RBCs, platelets and fibrin, additional components have also been described in ischemic stroke thrombi. VWF is a large, multimeric glycoprotein that is synthesized in megakaryocytes and endothelial cells. VWF is crucial for normal hemostasis and absence or dysfunction of VWF leads to the bleeding disorder von Willebrand disease. By binding to platelets and the subendothelial matrix (collagen), VWF promotes the formation of a stable platelet plug at sites of vascular injury. Platelets have two different receptors that interact with VWF and both are important for thrombus formation: first, platelet glycoprotein (GP) Ibα binds to the A1 domain of immobilized VWF, mediating initial local adhesion of free-flowing platelets to VWF. Second, platelet integrin αIIbβ3 binds to the C4 domain of VWF. By binding to activated αIIbβ3, VWF, together with fibrinogen, serves as a molecular bridge that cross-links activated platelets, leading to stable platelet-platelet interactions and stabilization of the growing platelet thrombus. Although VWF is mainly known for its role in arterial thrombosis, current evidence shows that it is an important mediator of venous thrombosis as well [15,16].

Given its principal role in thrombus formation, VWF is of particular interest when studying the general composition and molecular architecture of ischemic stroke thrombi. Not surprisingly, different studies have identified VWF as an important constituent of stroke thrombi [6,10,17–19]. Whereas all thrombi retrieved from stroke patients contain VWF, the amount is variable and can range from as little as 1% to more than 90% (Figure 3) [17,19]. One study tried to link the amount of VWF with the suspected stroke etiology, but did not find a correlation [17]. As previously mentioned, low sample sizes most probably hampered good statistical analysis and future studies with increased sample sizes are needed to draw stronger conclusions.

In line with its function in thrombus formation, VWF is typically found in platelet-rich thrombi and is less abundant in RBC-rich thrombi [6,17,19]. Indeed, the presence of VWF was positively correlated with the amount of platelets and inversely correlated with the amount of RBCs [17,19]. Since VWF is also present in platelets, histology-based correlations between VWF and platelets may be expected, but detailed analysis showed the presence of extracellular VWF, accumulating specifically in platelet-rich thrombus areas [6]. Analysis of the microstructural organization of platelet-rich areas revealed the presence of VWF in dense structures that demarcate platelet-rich zones which stain positive for fibrin [6]. Together, fibrin and VWF delineate substructures that are packed with platelets. The association of VWF with fibrin in platelet-rich regions is intriguing and further supports a direct interaction between fibrin and VWF, which potentially is important for thrombus stabilization. For instance, VWF can be covalently linked to fibrin by FXIII [20] or can be incorporated during polymerization of fibrin [21]. Tight interactions between VWF and fibrin can have a direct impact on thrombolysis as plasmin-mediated fibrinolysis alone might not be sufficient to achieve effective thrombolysis of platelet-rich thrombus material. The presence of non-fibrin components, such as VWF, could impair efficient rt-PA-mediated thrombolysis. This concept is supported by studies showing that targeting VWF, for example using the VWF-cleaving enzyme ADAMTS13 or the reducing agent N-acetylcysteine, improves thrombolysis of rt-PA-resistant thrombi [17,22,23]. Increasing clinical evidence reveals that high levels of VWF and/or low levels of ADAMTS13 are associated with a higher risk of stroke, increased neurological worsening, poorer stroke outcome and even reduced rt-PA mediated recanalization rates [24,25]. Thus, it will be interesting to further consider VWF as a novel target in ischemic stroke thrombi.

Leukocytes

In recent years, the traditional view of the hemostatic system, consisting of platelet activation and the coagulation cascade, is changing due to the emerging evidence that also leukocytes strongly influence (pathological) thrombus formation. The interaction between thrombosis and inflammatory immune cells has led to the concept of thrombo-inflammation (or immunothrombosis). Leukocytes have been shown to promote venous and arterial thrombosis by localizing procoagulant factors (e.g. tissue factor and factor XII), releasing proteases/cytokines, interacting with platelets and releasing NETs [26]. Using general H&E analysis, various studies indeed confirmed the presence of leukocytes in ischemic stroke thrombi [5,12,13,27,28]. Immunostaining revealed that leukocytes typically accumulate on the interface between RBC-rich and platelet-rich areas, and also within platelet-rich thrombus material (Figure 4A) [6]. In contrast, leukocytes

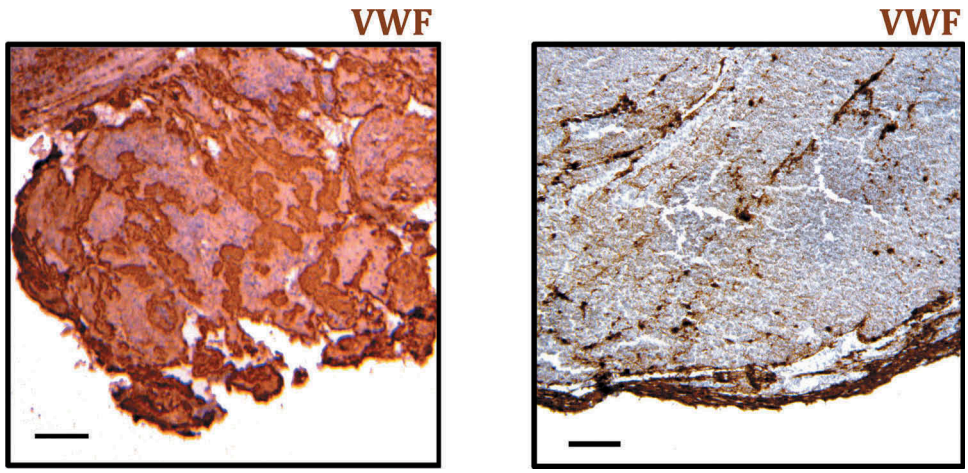

Figure 3. Stroke thrombi contain varying amounts of VWF.This figure shows immunohistochemical staining for VWF (brown). The two examples show a thrombus that is VWF-rich (left) and a thrombus that is VWF-poor (right). Scale bar = 50 μm. Figure adapted from Denorme et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. Blood. 2016;127(19):2337–2345, with permission.

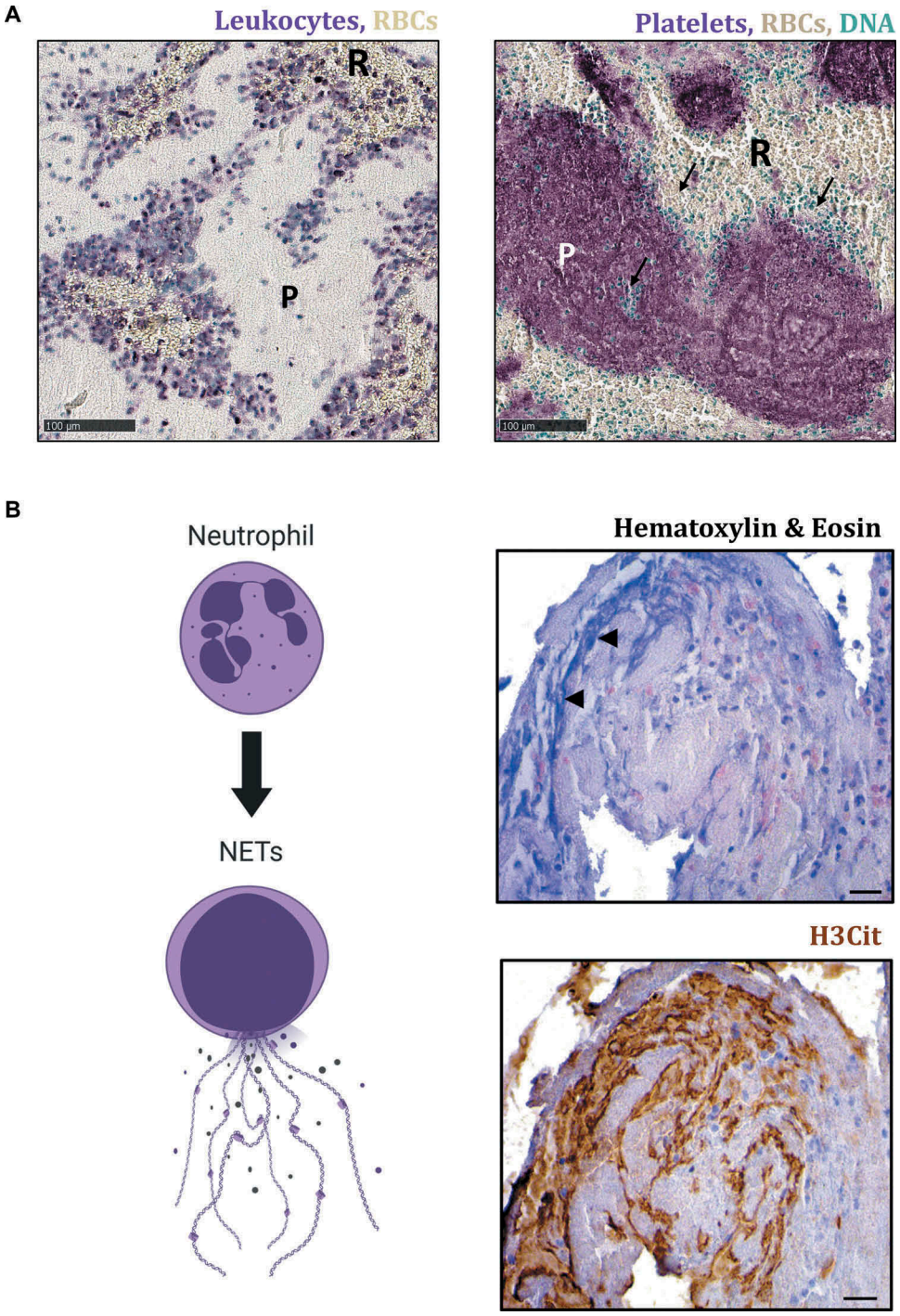

Figure 4. Leukocytes and NETs in ischemic stroke thrombi.Ischemic stroke thrombi have abundant amounts of leukocytes (A) and also contain NETs (B). Panel A, left shows the immunohistochemical staining of thrombi for the leukocyte marker CD45 (purple) and panel A, right shows the immunohistochemical staining of a platelet CD42b marker (purple) with a DNA counterstain (green, indicated by black arrows). Leukocytes, as indicated in purple (A, left) and in green by the black arrows (A, right), tend to accumulate in platelet-rich areas or at the boundary areas between platelet-rich and RBC-rich areas. Panel B show the process of NET formation, in which the nuclear chromatin of neutrophils is decondensed and subsequently released in the extracellular space, leading to the formation of a NET (left). Zones of extracellular nuclear material in ischemic stroke thrombi are shown on hematoxylin and eosin (H&E) staining (top, arrowheads). Zones of extracellular nuclear material stains positive for the NETs-marker H3Cit via immunohistochemistry using an antibody against citrullinated histone H3 (brown), indicating the presence of NETs (bottom). R: RBC-rich areas, P: platelet-rich areas. Scale bars = 100 µm (A) and 10 µm (B). Figure adapted from Staessens et al. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. Haematologica. 2019 May 2:haematol.2019.219881 and Laridan et al., Neutrophil extracellular traps in ischemic stroke thrombi. Ann. Neurol. 2017;82(2):223–232., with permission.

are not commonly found in RBC-rich areas, where, if present, they are homogenously distributed throughout the RBCs [6]. The majority of leukocytes in ischemic stroke thrombi are neutrophils [29]. However, other leukocytes such as T-cells, macrophages/monocytes and to a limited extent also B-cells have been identified in patient thrombi [12,30]. Although higher leukocyte numbers were reported to be associated with a cardioembolic origin [5], others studies could not establish a clear link between the origin of the thrombus and the specific presence of neutrophils, B-cells and macrophages/monocytes [12,18,30]. Higher

amounts $CD3^+$ T-cells were found in large artery atherosclerotic thrombi [30], but a recent report by Sporns et al. found no correlation between etiology and the presence of $CD3^+$ T-cells [12]. The amount of neutrophils was higher in more mature, older stroke thrombi compared to younger, fresh thrombi, indicating that neutrophils could also be involved in thrombus maturation [29]. Taken together, it is clear that leukocytes are important thrombus constituents. Whether the presence of leukocytes indicates a direct involvement of immune cells in the formation of thrombo-emboli is still an open question. Indeed, the biological involvement of leukocytes in thrombus formation, thrombus organization and thrombus stability remains elusive and more studies are needed to better understand the significance of different subtypes of leukocytes in thrombo-emboli retrieved from stroke patients.

NETs

As mentioned, neutrophils are the most abundant type of leukocyte in ischemic stroke thrombi. Neutrophil extracellular traps (NETs) have become a prime example of the intricate link between leukocytes and thrombosis. Generated by activated neutrophils that release their decondensed chromatin as a network of extracellular DNA fibers decorated with histones and granular proteins, NETs have been shown to serve as a scaffold for thrombus formation in both arterial and venous settings [31]. The fibrous network of NETs supports adhesion of platelets, red blood cells (RBCs), and various platelet adhesion molecules such as fibrinogen, VWF, and fibronectin, leading to activation of platelets and the coagulation cascade [31]. Interestingly, various studies have identified NETs as an important constituent of stroke thrombi [29,32,33]. By using immunostaining of neutrophil markers (CD66b, neutrophil elastase and myeloperoxidase) together with markers for NETs (citrullinated histones and extracellular DNA), neutrophil-derived extracellular DNA networks were indeed found to be a structural hallmark of all thrombi retrieved from ischemic stroke patients (Figure 4B), regardless of the underlying stroke pathology [29,32,33]. Although not consistent throughout different studies, some interesting findings were reported, such as a higher abundance of NETs in thrombi of cardioembolic origin compared to other etiologies [29], a higher presence of NETs in more mature, older stroke thrombi compared with younger, fresh thrombi [29], a specific accumulation of NETs in the outer layers of thrombi [32] and a lower amount of NETs in thrombi from patients who were previously treated with intravenous rt-PA [32]. Based on Feulgen's staining, which is highly sensitive for all DNA (thus not only NETs), we recently described abundant amounts of large DNA networks, appearing as extracellular smears throughout the stroke thrombus [6]. In analogy with the accumulation of neutrophils, these extracellular DNA networks were particularly present within platelet-rich areas and in the interface between platelet-rich and RBC-rich regions [6].

The presence of NETs and extracellular DNA networks could have major implications for the acute treatment of stroke thrombi. DNA and histones were shown to modify the structure of fibrin into thicker fibrin fibers, resulting in increased resistance to mechanical and enzymatic destruction [34]. Thus, NETs potentially contribute to overall thrombus stability, conferring resistance to thrombolysis and thrombectomy. The presence of NETs was positively correlated with the length of thrombectomy procedure and the number of device passes performed to achieve successful recanalization, indeed suggesting that NETs contribute to mechanical recanalization resistance [32]. Since NETs are composed of decondensed chromatin networks, they are vulnerable to nuclease activity, which led to the concept of using DNase1 to promote pharmacological breakdown of NETs to promote overall thrombus dissolution [29,32,35]. Proof-of-concept studies showed that ex vivo lysis of patient stroke thrombi was indeed more successful when DNase1 was added to standard rt-PA compared to rt-PA alone [29,32]. Hence, by targeting extracellular DNA, the use of DNase1 could become a promising novel prothrombolytic strategy in ischemic stroke.

Bacteria

Apart from leukocytes and NETs, bacteria also contribute to the concept of immunothrombosis. Through the activation of platelets and the initiation of the coagulation cascade, the process of immunothrombosis helps to localize the infection, preventing the systemic spread of pathogens and facilitating the immune system in the clearance of the infection [36]. Interestingly, infections have been shown to be a risk factor of ischemic stroke. Commonplace infections, such as periodontitis and urinary tract infections, have been shown to increase the risk threefold, for example [37]. As a consequence, recent studies started to focus on the presence of bacteria in ischemic stroke thrombi [38–41]. Using a Gram staining, two studies revealed the presence of Gram-positive bacteria, particularly Staphylococcus species, in patients suffering from infectious endocarditis [38,40]. Patrakka et al. identified an oral bacterial signature (e.g. Streptococcus viridans) in the majority of thrombi via 16S rDNA PCR [39], whereas a recent study was unable to find such evidence of bacterial 16S rDNA signature [41]. In sum, the presence of bacteria in ischemic stroke thrombi is intriguing, but more studies are needed to fully elucidate the role of bacteria in stroke thrombus formation and in the potential diagnosis of underlying infectious pathologies like infective endocarditis.

Coronary and Deep Vein Thrombi

In the last decade, understanding thrombus composition of various cardiovascular pathologies has become a major interest in the field of thrombosis and hemostasis. Hence, it is interesting to compare the increasing body of knowledge on ischemic stroke thrombi with other thrombotic diseases such as coronary heart disease and deep vein thrombosis.

Remarkably, various structural hallmarks found in ischemic stroke thrombi are comparable with observations in coronary and deep vein thrombi [42,43]. For example, Savchenko et al. found that deep vein thrombi consist of platelet and RBC-rich areas [42]. Similarly, coronary thrombi consist of platelet-rich areas that contain densely packed fibrin which colocalizes with VWF and tissue factor [43]. Moreover, leukocytes have been shown to be a key thrombus constituent in both coronary and DVT thrombi [42,44]. Neutrophils are the major leukocyte population in deep vein thrombi [42]. Coronary thrombi mainly contain of neutrophils in early, fresh thrombi whereas higher monocyte/macrophages counts are found in more organized, older thrombi [44]. NETs are also commonly found in coronary and DVT thrombi [33,42,45]. In deep vein thrombi, NETs are typically found in the vicinity of VWF-positive platelet-rich areas, corresponding to what we found in ischemic stroke thrombi [6,42]. Interestingly, in coronary thrombi, neutrophils are not the only inflammatory cell to release extracellular DNA traps, but also macrophages and to a lesser extent eosinophils and mast cells have been shown to form extracellular traps [46]. Hence, it is clear that although patient thrombi from different etiologies are quite heterogeneous, similar architectural features exist. More in-depth studies are needed to further reveal specific similarities and differences between thrombi from various types of thrombosis.

Thrombus formation in myocardial infarction, for example, is mostly secondary to a local rupture of a coronary plaque while stroke thrombi are most of the time embolic. Studying the differences of retrieved thrombi from intracranial arteries and coronary arteries could increase our understanding of underlying pathophysiological mechanisms and could help designing prevention strategies.

Therapeutic Implications for Treatment of Ischemic Stroke

In acute stroke treatment, the occluding thrombus is the primary target in both pharmacological and mechanical recanalization therapy. Hence, the growing body of knowledge on stroke thrombus characteristics could be of great value to overcome current treatment limitations and improve recanalization success rates.

At present, rt-PA is the only FDA-approved thrombolytic drug available. However, rt-PA can only be administered within a short time window of 4.5 hours after stroke onset due to the increased risk of hemorrhagic complications that outweighs the benefit of rt-PA administration beyond this therapeutic time frame. As a consequence, rt-PA is available to less than 15% of patients in most European countries [47]. Importantly, in more than half of patients who receive rt-PA, no successful recanalization can be achieved [48]. The reasons of this so-called rt-PA resistance are not well understood but thrombus composition is most likely part of the answer. Given the current limitations regarding rt-PA therapy, it is clear that additional pharmacological strategies are required.

There is strong evidence that RBC-dominant thrombi are more susceptible to rt-PA-mediated lysis than platelet-dominant thrombi [17,49–56]. rt-PA converts plasminogen to plasmin, which degrades fibrin in the thrombus. Plasmin-mediated fibrinolysis might be sufficient for lysis of RBC-rich thrombus material in which thin fibrin is the main extracellular scaffold. However, as described above, platelet-rich thrombi contain much denser fibrin structures that also include VWF and extracellular DNA or NETs. In addition, the structure of fibrin can be modified by various molecules, including NETs, VWF, platelet factor-4, ferric chloride and Factor XIII and such modifications potentially influence rt-PA resistance [20,21,34,57–59]. Hence, it seems reasonable to speculate that fibrinolysis alone is not adequate to dissolve platelet-rich thrombi in patients and that non-fibrin components contribute to rt-PA resistance. Instead of using the current "one size fits all" therapy aiming only at fibrinolysis via rt-PA for all patients, novel strategies combining rt-PA with novel drugs that target VWF and DNA could represent unique opportunities to improve pharmacological recanalization rates and reduce rt-PA-associated bleeding risks. Novel therapeutics could include ADAMTS13 [17] and N-acetylcysteine [22], which both degrade VWF, and the DNA-cleaving enzyme DNAse1 [29,32]. In addition, novel strategies that target direct inhibitors of fibrinolysis such as PAI-1 and TAFI could also help to further improve rt-PA resistance [60,61]. Of note, such "thrombolytic cocktails" would also be useful to break down the recently identified dense outer surface shell that encapsulates the stroke thrombus core and that is resistant to fibrinolysis due to the presence of high amounts of VWF, aggregated platelets, extracellular DNA and PAI-1 [10].

Mechanical thrombectomy has become frequently used after randomized trials have confirmed its efficacy and benefit for neurological outcome in patients with ischemic stroke [62–66]. Endovascular therapy can be used in selected patients with imaging-proven proximal large artery occlusion, regardless of whether they receive intravenous rt-PA. An important obstacle in endovascular therapy is that thrombi tend to differ in consistency and removability. This is important since higher amounts of thrombectomy attempts to achieve good recanalization significantly worsen clinical outcome [67,68]. In the worst case scenario, the thrombus remains totally irretrievable via thrombectomy, which is a problem in up to 20% of treated patients [69]. Why some thrombi are more difficult to remove than others is currently not entirely clear but increasing evidence indicates that RBC-rich thrombi are more easily retrieved via endovascular procedures in comparison to more complex fibrin/platelet-rich thrombi [70,71]. The composition of thrombi has been shown to influence the mechanical characteristics of thrombi [71–74]. For example, fibrin-rich thrombi have a higher coefficient of friction than RBC-rich thrombi [71,72]. Higher amounts of RBCs affect clot stiffness [73] and indentation [74]. Such differences in mechanical properties could, at least in part, account for differences in removalibity [74] and even explain why some thrombi are more prone to procedural defragmentation or pre-interventional clot migration [75,76].

Longer thrombectomy procedure time and less favorable recanalization rates have also been associated with a higher number of leukocytes in the thrombus [5], but such associations were absent in other studies [12,27,70,71]. Interestingly, Ducroux et al. found a positive correlation between the amount of NETs and the number of device passes needed to achieve successful recanalization [32]. Whether NETs directly affect the physical properties of thrombi remains to be investigated, but DNA is able to modify the structure of fibrin, rendering it more resistant to mechanical forces [34]. Future comprehensive studies are needed to better understand how the composition of thrombi affects mechanical properties. Such information could help to advance thrombectomy technology development, including new generations of stent retrievers that enhance therapeutic options. Novel developments are particularly needed for the platelet/fibrin-rich thrombi that are difficult to remove [77]. Of note, computed tomography (CT) and magnetic resonance imaging (MRI), performed upon patient arrival, can differentiate thrombi according to RBC content via the appearance of a hyperdense artery sign or blooming artifact, respectively [3,27,54,70,78–81]. Hence, radiological prediction of thrombus composition might inform pre-treatment decision-making on the ideal choice of thrombus retrieving technique. Finally, better understanding of the correlation between thrombus composition and etiology could guide secondary stroke prevention strategies, in particular in cases of cryptogenic stroke in which the exact etiology is not known.

Conclusion

Advances in endovascular thrombectomy and related imaging modalities have created a unique opportunity to analyze thrombi removed from cerebral arteries. Stroke thrombi are heterogenous in nature, but common structural features can be distinguished. Emerging evidence on thrombus composition indicates an interplay between thrombotic and inflammatory components, including RBCs, platelets, leukocytes, fibrin, VWF and NETs. Cellular and molecular components may be present in varying proportions between different thrombi. Platelet-rich thrombi are characterized by a complex structure that involves dense fibrin, VWF, leukocytes and NETs, which may, at least partly, explain why platelet-rich thrombi are difficult to remove via pharmacological lysis or mechanical thrombectomy. It must be noted that stroke thrombi retrieved via thrombectomy do not necessarily represent all thrombi that cause ischemic stroke. For instance, although little evidence is available so far, secondary thrombus formation at the site of occlusion can occur. In addition, only thrombi that cause large vessel occlusions, that did not dissolve spontaneously or after rt-PA administration and that can be successfully retrieved

via thrombectomy are available for study. This impedes the assessment of rt-PA susceptible and thrombectomy-resistant thrombi, but imaging-based assessments may provide useful surrogates for these thrombi in the future. Nevertheless, it is clear that our increasing understanding of the histological structure of stroke thrombi is crucial to better understand their pathogenesis, properties and clinical management.

Author contribution

The authors wrote the manuscript.

Disclosures statement

The authors declare no conflicts of interest.

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