Stroke

CLINICAL AND POPULATION SCIENCES

Quantitative Morphology of Cerebral Thrombi Related to Intravital Contraction and Clinical Features of Ischemic Stroke

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BACKGROUND AND PURPOSE: The purpose was to assess quantitatively and qualitatively the composition and structure of cerebral thrombi and correlate them with the signs of intravital clot contraction (retraction), as well as with etiology, severity, duration, and outcomes of acute ischemic stroke.

METHODS: We quantified high-resolution scanning electron micrographs of 41 cerebral thrombi for their detailed cellular and noncellular composition and analyzed histological images for the overall structure with the emphasis on red blood cell compression, fibrin age, and the signs of inflammation.

RESULTS: Cerebral thrombi were quite compact and had extremely low porosity. The prevailing cell type was polyhedral compressed erythrocytes (polyhedrocytes) in the core, and fibrin-platelet aggregates were concentrated at the periphery; both findings are indicative of intravital contraction of the thrombi. The content of polyhedrocytes directly correlated with the stroke severity. The prevalence of fibrin bundles was typical for more severe cases, while the content of fibrin sponge prevailed in cases with a more favorable course. The overall platelet content in cerebral thrombi was surprisingly small, while the higher content of platelet aggregates was a marker of stroke severity. Fibrillar types of fibrin prevailed in atherothrombogenic thrombi. Older fibrin prevailed in thrombi from the patients who received thrombolytics, and younger fibrin dominated in cardioembolic thrombi. Alternating layers of erythrocytes and fibrin mixed with platelets were common for thrombi from the patients with more favorable outcomes. Thrombi with a higher number of leukocytes were associated with fatal cases.

CONCLUSIONS: Most cerebral thrombi undergo intravital clot contraction (retraction) that may be of underestimated clinical importance. Despite the high variability of the composition and structure of cerebral thrombi, the content of certain types of blood cells and fibrin structures combined with the morphological signs of intravital contraction correlate with the clinical course and outcomes of acute ischemic stroke.

Key Words: blood platelets ■ erythrocytes ■ stroke ■ thrombosis

A big step toward studying composition and structure of cerebral thrombi and emboli has been made since the introduction of mechanical thrombectomy.[1–3] Knowledge of the composition of a cerebral thrombus can potentially help to improve and predict the effectiveness of thrombectomy or thrombolytic recanalization of an occluded vessel or to develop new methods of treatment.[1,4–7] However, given that there are many other factors that determine the success rate of any form of treatment,[8–10] it is still not clear how exactly the composition of the thrombus is related to the clinical course and outcomes of ischemic stroke.[11,12]

Cerebral thrombi consist of 3 major components: fibrin/platelet accumulations, red blood cells (RBCs), and leukocytes, while the content of these structures is quite variable.[2,13,14] RBCs are one of the most abundant components

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Nonstandard Abbreviations and Acronyms

mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
OR	odds ratio
RBC	red blood cell
VWF	von Willebrand factor

of cerebral thrombi,[11,13,15,16] so the classical definition of them as white is not always correct. Fibrin and platelets are the other dominant components of cerebral thrombi.[11,17] Thrombolysis was less effective in thrombi with a high fibrin content compared with RBC-rich thrombi, while thrombi with a high RBC count are associated with successful reperfusion.[16]

Despite many morphological studies on cerebral thrombi, the relation of their composition and structure to clinical features of stroke is still largely unknown, in part, due to the lack or insufficiency of systematic and quantitative morphological analysis. In this study, we assessed quantitatively and qualitatively the composition and structure of cerebral thrombi and correlated them with etiology, severity, duration, and outcomes of acute ischemic stroke. We put a special emphasis on the signs of intravital contraction (retraction) of a thrombus and association of this process with clinical characteristics of stroke. As a methodological advancement, we described and quantified subtypes of blood cells and fibrin structures to glean new information about correlations between the morphology of thrombi and manifestations of stroke.

METHODS

The authors declare that all supporting data are available within the article and its Data Supplement.

Arterial thrombi from 41 patients with acute ischemic stroke were analyzed. The design of this study was approved by the local ethical committee (Reference: 639/2017). All patients provided written informed consent to participate in the study. Following a mechanical thrombectomy procedure, all patients included in the study had confirmed large vessel thromboembolic occlusion. Clinical characteristics of the patients are presented in Table 1 and Table I in the Data Supplement.

Mechanical thrombectomy, electron and light microscopy, and statistical analyses are described in Materials in the Data Supplement. The cellular structures analyzed in this study are listed in Table 2 and shown in Figure 1 and Figure I [A] in the Data Supplement.

RESULTS

Overall Composition of Cerebral Thrombi

The overall composition of cerebral thrombi was analyzed from scanning electron micrographs by quantifying the volume fraction occupied by all types of cells, fibrin, and empty spaces (Figure 1; Figure I in the Data Supplement). As shown in Figure 2A, blood cells comprised on average about 73% (95% CI, 68–78) of the volume of thrombi and were the prevailing constituent over fibrin, which filled only about 20% (95% CI, 16–24) of the volume of thrombi. The empty spaces that characterized porosity occupied on average about 6% (95% CI, 5–9) of the volume of thrombi, indicating that the cerebral thrombi are relatively compact and hardly permeable structures. The volume fractions of blood cells, fibrin, and pores were statistically indistinguishable in thrombi that had atherothrombogenic or cardioembolic etiology.

Types of RBCs in Cerebral Thrombi

RBCs altogether comprised >80% (95% CI, 79–88) of all blood cells and were the prevailing constituent over platelets and leukocytes (Figure 2B; Table II in the Data Supplement). Deformed RBCs, such as polyhedrocytes (≈30%) and mainly polyhedral RBCs (>50%), originating from platelet-driven contraction and compression of a thrombus, comprised the vast majority of RBCs (on average >80% [95% CI, 81–86]) in all cerebral thrombi (Figure 2C). The content of mainly polyhedral RBCs was significantly higher (on average 58% [95% CI, 51–65]) in thrombi from the patients with an intermediate stroke severity (National Institutes of Health Stroke Scale [NIHSS] score, 11–20 points 24 hours after admission) than in the patients with a lower disease severity (NIHSS score, ≤10) that had an average content of 40% ([95% CI, 29–52] Figure 2D) with the difference between the medians of 21 ([95% CI for the difference, 6–32] P=0.041). The content of mainly polyhedral RBCs correlated inversely with the NIHSS 7 days after admission (r=–0.5, P<0.05). The content of mainly polyhedral RBCs correlated directly (r=0.37, P<0.05) with the stroke outcomes characterized by mRS values 3 months after admission. The difference in the content of the RBC types between thrombi from the patients with atherothrombogenic and cardioembolic stroke was statistically insignificant (Figure II in the Data Supplement). Graphical representation of the differences between medians (and 95% CI) for the volume fractions of various RBCs is shown in Figure III in the Data Supplement.

Platelets in Cerebral Thrombi

The content of platelet aggregates increased with an increase of stroke severity (Figure 3A). The volume fraction of platelet aggregates was significantly higher (on average ≈18% [95% CI, 14–22]) in thrombi from the patients with a higher NIHSS (21–30 points 24 hours after admission) than in the patients with a lower (≤10) NIHSS, in which they occupied only ≈9% volume (95% CI, 7–12) with the difference between the medians of 9 ([95% CI for the

Table 1. Clinical Characteristics of Patients With Acute Ischemic Stroke Enrolled in This Study (n=41)

Clinical data		Patients, n (%)
Average age, y		72.0±1.5
Sex (female)		17 (41%)
Etiology	Atherothrombogenic stroke	18 (44%)
	Cardioembolic stroke	23 (56%)
Stroke severity at the time of admission	NIHSS score, ≤10	2 (5%)
	NIHSS score, 11–20	30 (73%)
	NIHSS score, 21–30	9 (22%)
Stroke severity 24 h after admission	NIHSS score, ≤10	16 (42%)
	NIHSS score, 11–20	17 (45%)
	NIHSS score, 21–30	5 (13%)
Stroke severity 7 d after admission	NIHSS score, ≤10	19 (49%)
	NIHSS score, 11–20	12 (31%)
	NIHSS score, 21–30	8 (20%)
Stroke outcome 3 mo after admission	mRS score, ≤2	12 (30%)
	mRS score, 3	8 (20%)
	mRS score, 4–5	10 (25%)
	mRS score, 6	10 (25%)
Thrombolysis	Yes (Actilyse)	30 (73%)
	No	11 (27%)
Thrombosis duration	≥240 min	13 (34%)
	<240 min	25 (66%)
Treatment variables	Units	Median (IQR)*
Inception to intravenous thrombolysis	min	100.0 (80.0–123.0)
Inception to digital subtraction angiography	min	150.0 (120.0–202.5)
Arrival to intravenous thrombolysis	min	25.0 (20.0–30.0)
Arrival to digital subtraction angiography	min	60.0 (35.0–80.0)
Procedure duration	min	45.0 (30.0–65.0)
Recanalization	min	195.0 (170.0–257.5)

IQR indicates interquartile range; mRS, modified Rankin Scale; and NIHSS, National Institutes of Health Stroke Scale.
*Median with the interquartile range.

difference, 4–13] P=0.006). The same trend maintained 7 days after admission with the content of platelet aggregates prevailing significantly in thrombi from the patients with a higher NIHSS (21–30) than in the patients with an intermediate and lower (≤20) NIHSS (18% [95% CI, 14–22] and 10% [95% CI, 7–12], respectively). The difference between the medians was 9 ([95% CI for the difference, 4–14] P=0.012; Figure 3B). The content of balloon-like platelets identified as bloated cells with a size comparable with RBCs was significantly higher in the cardioembolic (≈3% [95% CI, 2–5]) than in atherothrombogenic thrombi (1% [95% CI, 0–3]) with the difference between the medians of 1 ([95% CI for the difference, 0–3] P=0.018; Figure 3C; Figure III in the Data Supplement).

Leukocytes in Cerebral Thrombi

Leukocytes were the least abundant cell type in cerebral thrombi and comprised on average ≈2% (95% CI, 1–2.5) of all blood cells. An important trend, although statistically insignificant due to a limited number of scattered variances, was that the content of leukocytes tended to prevail (≈2.5% [95% CI, 1–3]) in thrombi from the patients who had a worse outcome or died (the highest mRS score, 6 points) over the patients with an intermediate and lower mRS (≤3 points) 3 months after admission (≈1.5% [95% CI, 0.5–2]; Figure 3D).

Fibrin in Cerebral Thrombi

Fibrin was quantified as the relative content of the following morphological types: bundles, fibers, sponge, and debris (Figure 4A; Table 2). Fibrin bundles significantly prevailed over other types of fibrin in all thrombi and comprised on average about 40% ([95% CI, 31–47] Figure 4B), which was true for thrombi of both atherothrombogenic (Figure IV [A] in the Data Supplement) or cardioembolic (Figure IV [B] in the Data Supplement)

Table 2. Morphological Characteristics of the Structures Analyzed in This Study

Structures	Characteristics
Cell types	
Biconcave RBCs	4- to 5-μm disc-shaped flattened cells; both sides of the surface curve inward forming a dimple
Mainly biconcave intermediate-shaped RBCs	Similar to biconcave RBCs with a dimple in the middle, but the surface deviates from discoid and is partially rounded
Mainly polyhedral intermediate-shaped RBCs	Polyhedral-like RBCs with more or less flat sides except areas where the sides are distorted by either protrusions or indentations
Polyhedral RBCs	Polyhedral cells with surface made up of intersecting polygonal sides
Spherocytes	Convex-shaped RBCs ≈5 μm in diameter with a smooth round surface without protrusions, indentations, or dimples
Echinocytes	RBCs with multiple small, evenly spaced thorny projections
Balloon-like platelets	Bloated single or doubled platelets with a plicate surface of a size comparable or larger than RBCs (≥5–6 μm); multiple wrinkles and lines on the surface and no bumps, which makes them distinct from spherocytes and leukocytes
Platelet aggregates	Clusters of deformed irregular-shaped platelets with outgrowths and multiple filopodia
Leukocytes	Spherical or irregular-shaped cells varying from ≈5 up to ≈10–12 μm in diameter with much membrane folding and a tuberous rough surface with multiple short bumps
Fibrin types	
Fibrin bundles	Thick fibrillar structures made up of several laterally aggregated fibers
Fibrin fibers	Thin fibrillar structures single or usually arranged into a network
Fibrin sponge	Porous amorphous structure
Fibrin debris	Unstructured detritus and separate fibrin pieces

RBC indicates red blood cell.

origin. The overall content of fibrin was significantly higher in thrombi from the patients with cardioembolic than atherothrombogenic stroke, namely 23% (95% CI, 18–29) versus 13% (95% CI, 9–18), respectively, with the difference between the medians of 9 ([95% CI for the difference, 1–19] P=0.034; Figure 4C). The content of fibrin bundles prevailed significantly in thrombi from the patients with a higher stroke severity (NIHSS score, 21–30) at the time of admission (on average 59% [95% CI, 51–67]) than from the patients with less severe stroke, who had intermediate and lower (≤20) NIHSS (33% [95% CI, 24–42]), and the difference between the medians was 31 ([95% CI for the difference, 16–40] P=0.007; Figure 4D). The content of fibrin bundles remained the prevailing fibrin structure (on average about 60% [95% CI, 50–69]) in thrombi from the patients with a higher NIHSS (21–30 points) than from the patients with intermediate and lower NIHSS (0–20 points) 24 hours after admission (on average about 33% [95% CI, 25–41]; Figure IV [C] in the Data Supplement). The content of fibrin bundles correlated strongly and inversely with the stroke outcomes assessed by the mRS values 3 months after admission (r=–0.7, P<0.01). The content of fibrin sponge was somewhat higher (on average about 30% [95% CI, 21–39]) in thrombi from the patients with an intermediate and lower NIHSS (0–20 points at the time of admission) than from the patients with a higher NIHSS (21–30), who had on average only about 14% (95% CI, 0–29) fibrin sponge structures (Figure IV [D] in the Data Supplement). The relative content of fibrin bundles was significantly higher than the content of fibrin debris in the cardioembolic thrombi (Figure IV [B] in the Data Supplement). Remarkably, fibrin debris often contained free or barbed fiber ends, presumably originating from fibrinolytic cleavage.[18] Graphical representation of the differences between medians (and 95% CI) for the relative areas of various fibrin structures is shown in Figure V in the Data Supplement.

Composition and Structure of Cerebral Thrombi as Revealed by the Histological Examination

Polyhedrocytes (P) or polyhedral-like RBCs were the prevailing component of cerebral thrombi, located preferentially in the interior of thrombi (Figure VI [A] in the Data Supplement). We demonstrated earlier that in histological preparations, fibrin can be stained in variable colors depending on the age of the blood clot or thrombus.[19] Age-based segregation of fibrin stained with Picro-Mallory was the following: young (Y), up to 6 hours after formation, stained in red; mature (M), 6 to 18 hours after formation, stained in violet; old (O), over 24 hours after formation, stained in blue (Figure VI [B and C] in the Data Supplement). There were thrombi that contained only young fibrin almost completely located at the periphery and formed a massive and loose outer layer. Small isolated areas of young fibrin were diffusely located inside the same thrombus (Figure VI [B] in the Data Supplement).

The prevailing content of young fibrin was significantly higher in thrombi from the patients without thrombolytic therapy than in patients with thrombolysis (odds ratio [OR], 0.208 [95% CI, 0.043–1.001]; P=0.05), which suggests that thrombolysis dissolved preferentially young fibrin that was on the periphery of thrombi. Young fibrin

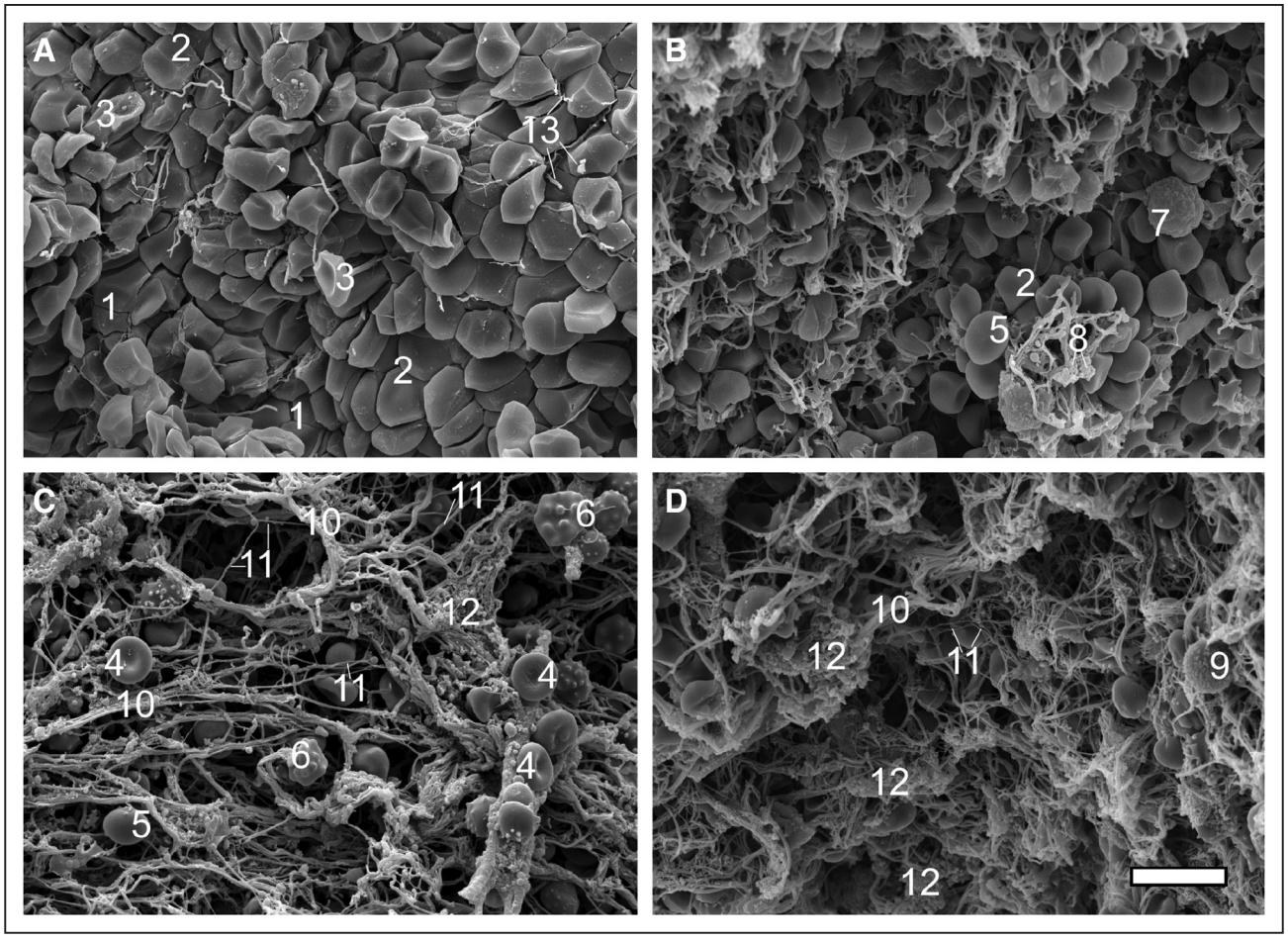

Figure 1. Representative scanning electron micrographs of cerebral thrombi, containing major structural elements. **A**, Prevalence of compressed red blood cells (RBCs) in the thrombus core. **B**, Partially deformed RBCs located closer to the thrombus periphery. **C** and **D**, Dominance of fibrin on the surface of thrombi: (1) polyhedral RBCs (polyhedrocytes), (2) mainly polyhedral intermediate-shaped RBC, (3) mainly biconcave intermediate-shaped RBC, (4) a biconcave RBC, (5) a spheroid RBC, (6) an echinocyte, (7) a balloon-like platelet, (8) a platelet aggregate, (9) a leukocyte, (10) fibrin bundles, (11) fibrin fibers, (12) fibrin sponge, and (13) fibrin debris. For detailed description of the cellular structures and fibrin types, see Table 2. Magnification bar=10 μm.

dominated significantly in thrombi from the patients with cardioembolic rather than atherothrombogenic thrombi (OR, 0.117 [95% CI, 0.013–1.056]; *P*=0.05). Within the same thrombus, fibrin of a different color and age could be often observed simultaneously.

Thrombi often contained alternating layers of erythrocytes and fibrin mixed with platelets (Figure VI [D] in the Data Supplement). These structures were similar to lines of Zahn that also have alternating layers (laminations) of platelet/fibrin mesh, which appear next to darker layers of RBCs. However, in our observations, these layers had some peculiarities, such that the fibrin/platelets layers were free of leukocytes, relatively thick, wavy, and randomly oriented, while in the typical lines of Zahn, fibrin layers form thin laminated regular patterns, often associated with leukocytes. The alternating layers prevailed significantly in patients with better stroke outcomes that had lower (0–2) and intermediate (3) mRS points 3 months after admission than in patients with worse outcomes and higher (4–6) mRS points (OR, 21.320 [95% CI, 1.103–411.943]; *P*=0.043). Fibrin formed layers that could be seen in any part of the thrombi. Remarkably, we found that compressed polyhedral erythrocytes were often colocalized with fibrin in the thrombi from patients with a high (21–30 points) and intermediate (11–20) NIHSS at the time of the admission compared with the patients with a lower (≤10) score (OR, 0.032 [95% CI, 0.001–0.942]; *P*=0.046; Figure VII in the Data Supplement). These compressed RBCs were stained atypically due to the overlap with fibrin staining.

Leukocytes (predominantly neutrophils) most often colocalized with fibrin, but they could also be found in all layers irrespective of fibrin location (Figure VIII [A] in the Data Supplement). Leukocytes could form sparse and randomly located sites with clusters (Figure VIII [B] in the Data Supplement), but in most cerebral thrombi, single leukocytes were distributed diffusely (Figure VIII [C] in the Data Supplement). The clustered leukocytes prevailed significantly (OR, 0.046 [95% CI, 0.003–0.866]; *P*=0.04) in thrombi from patients with worse outcomes

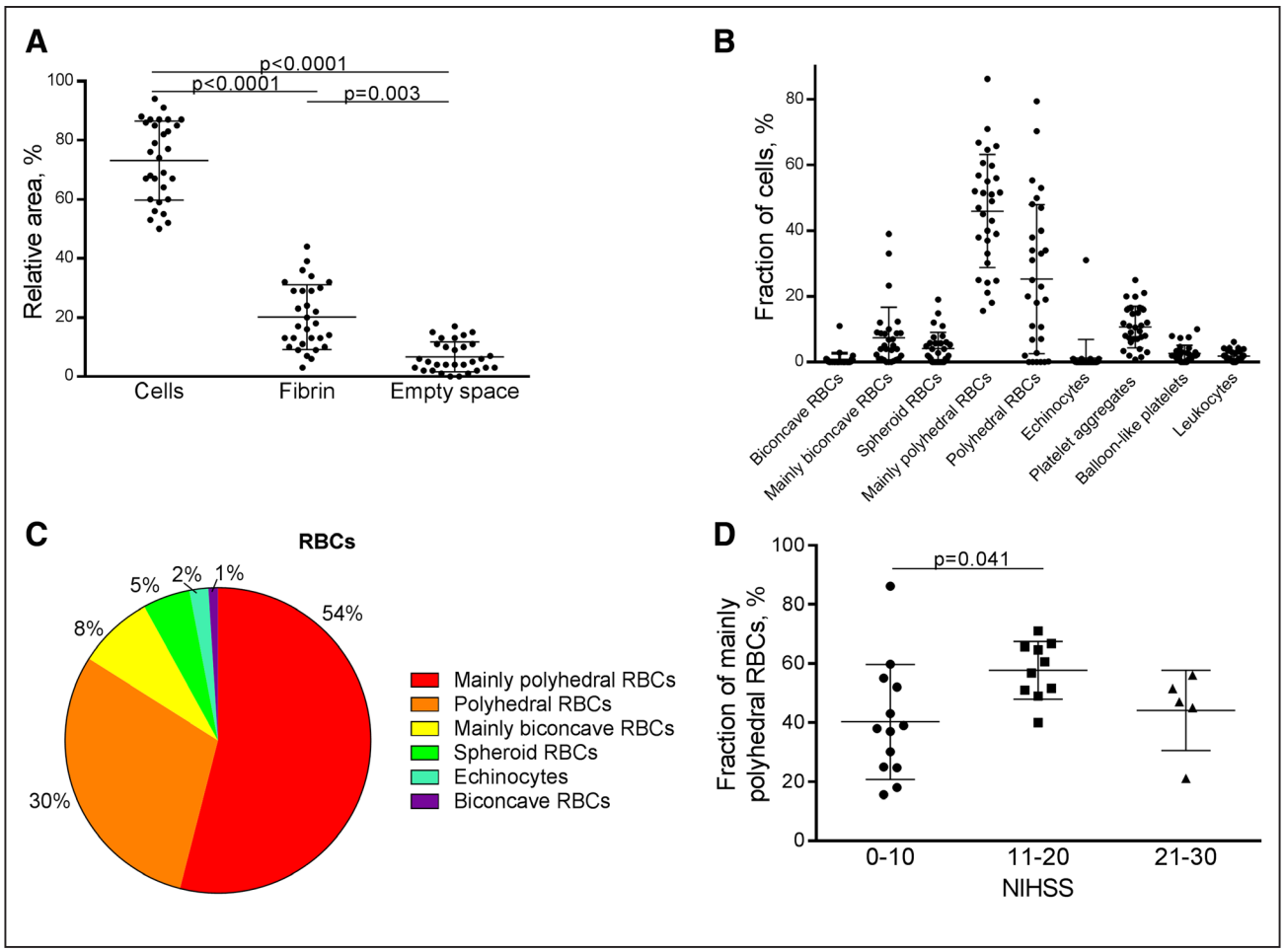

Figure 2. TThe overall composition of cerebral thrombi.
A, The relative area occupied by cells, fibrin, and empty space. **B**, The relative number (fraction) of various cell types. **C**, Distribution of various red blood cell (RBC) types in cerebral thrombi. **D**, The relative content of compressed mainly polyhedral RBCs in thrombi from patients with ischemic stroke of various severities based on National Institutes of Health Stroke Scale (NIHSS) 24 h after admission; Kruskal-Wallis test (**A**) and 1-way ANOVA test with Dunn post hoc test (**D**).

that had high (4–6) or intermediate (3) mRS points 3 months after admission value, compared with the patients with a lower (0–2 points) mRS.

DISCUSSION

Quantitative analysis of the overall structure and composition of cerebral thrombi showed that they are quite compact structures (Figures 1 and 2A; Figure I [B] in the Data Supplement). The high density indicates reduced accessibility of the interior space for thrombolytic agents and resistance to therapeutic thrombolysis, as found clinically.[20–22] There was also little porosity in both coronary artery and venous thrombi/emboli.[23] Surprisingly, in cerebral artery thrombi, the prevailing constituent was RBCs (Figure 2A), as in venous thrombi/emboli.

Of all blood cells that made up three-quarters of the volume of cerebral thrombi, >80% are represented by deformed polyhedral-like RBCs, indicating that thrombi undergo strong compaction that move RBCs toward the core of the thrombus, where they form tessellated arrays of polyhedrocytes. The platelet-driven compressive deformation of RBCs into polyhedral cells was first revealed in contracting blood clots formed in vitro[24] and later confirmed in thrombi extracted from coronary arteries of the patients with ST-segment–elevation myocardial infarction,[23–25] as well as in venous thrombi[23,26] and thrombotic emboli.[23,27] Now this observation has been extended to cerebral thrombi.

Another morphological sign of contraction is redistribution of the fibrin-platelet meshwork toward the periphery of a contracted blood clot or thrombus, which was found histologically in the vast majority of cerebral thrombi, regardless of the etiology, duration, and treatments of stroke (Figure VI [B] in the Data Supplement). Based on these findings, it can be inferred that cerebral thrombi undergo intravital contraction that can have substantial and multifunctional pathophysiological consequences, potentially influencing the course and outcome of acute ischemic stroke.

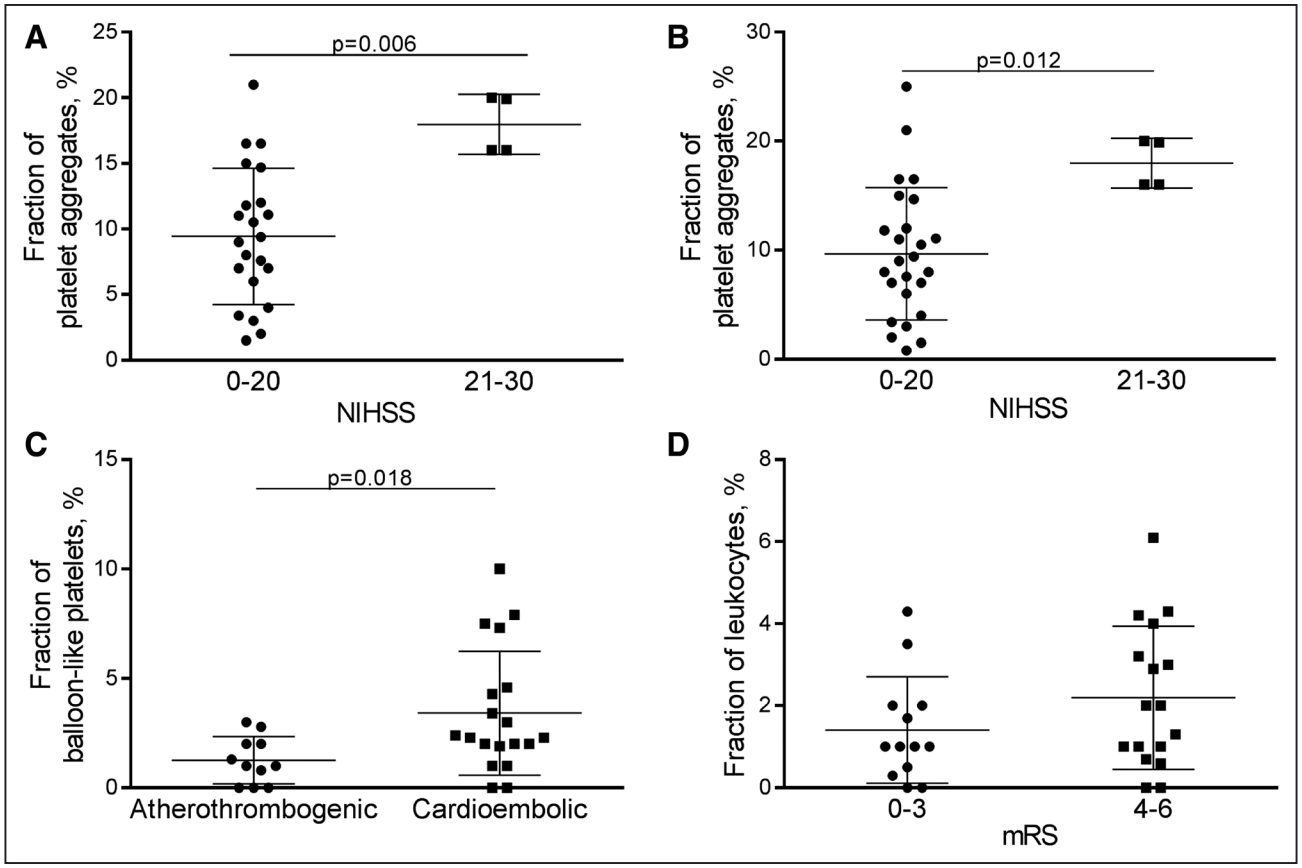

Figure 3. The relative content of platelets and leukocytes in cerebral thrombi extracted from patients with different etiology, severity, and outcomes of stroke.
A and **B**, The content of platelet aggregates in thrombi from patients with stroke of various severities (National Institutes of Health Stroke Scale [NIHSS]) estimated 24 h (**A**) and 7 d (**B**) after admission. **C**, The content of balloon-like platelets in the atherothrombogenic and cardioembolic thrombi. **D**, The leukocyte content in thrombi from patients with various stroke outcomes based on the modified Rankin Scale (mRS) disability scale 3 mo after admission (0, no residual symptoms; 6, died). Mann-Whitney *U* test (**A**, **B**, and **D**), unpaired 2-tailed Student *t* test (**C**).

First, the intravital contraction shrinks thrombi and can improve the local blood flow past otherwise obstructive thrombi by reducing vessel occlusion.[26,28] Second, compressed dense thrombi, unlike uncontracted loose blood clots, have a low susceptibility to external fibrinolysis induced by addition of exogenous plasminogen activator(s), implying that patients with contracted thrombi are resistant to therapeutic thrombolysis.[29] Third, the extent of compression can determine the mechanical stability of thrombi and their propensity to embolization, with less contracted clots more likely to embolize, which may be important for strokes arising from embolization.[26]

The thrombus structure, namely the content of polyhedrocytes, aggregated platelets, and fibrin bundles, was found to correlate directly with the NIHSS score at admission, which implies that the composition and physical properties of the thrombus have clinical consequences. Since polyhedrocytes are a morphological marker of clot contraction, a higher content of polyhedrocytes means that the thrombi are more contracted. In other words, the thrombi will be more compact, more stable, less deformable, and less permeable. Similarly, the prevalence of fibrin bundles and the higher content of platelet aggregates that were also typical for more severe cases also go along with more contracted and more dense thrombi. Moreover, this dense impermeable structure of thrombi/emboli must impair the efficacy of intravenous thrombolysis, and resistance to intravenous thrombolysis worsens the outcome of treatment. A combination of these and other conditions likely determines the importance of the composition of thrombi that might affect, directly or indirectly, the size of brain damage and the patient's neurological status (NIHSS) at admission.

The overall volume fraction of fibrin was found to be much higher in cardioembolic than in atherothrombogenic cerebral thrombi (Figure 4C), which is consistent with the earlier findings.[30] These observations suggest that fibrin is the major component of embologenic cardiac thrombi and that the likelihood of embolism is directly related to the content of fibrin. Although fibrin is supposed to provide integrity and mechanical stability of a thrombus, thrombotic embolization, in addition to mechanical rupture, may be triggered or reinforced by

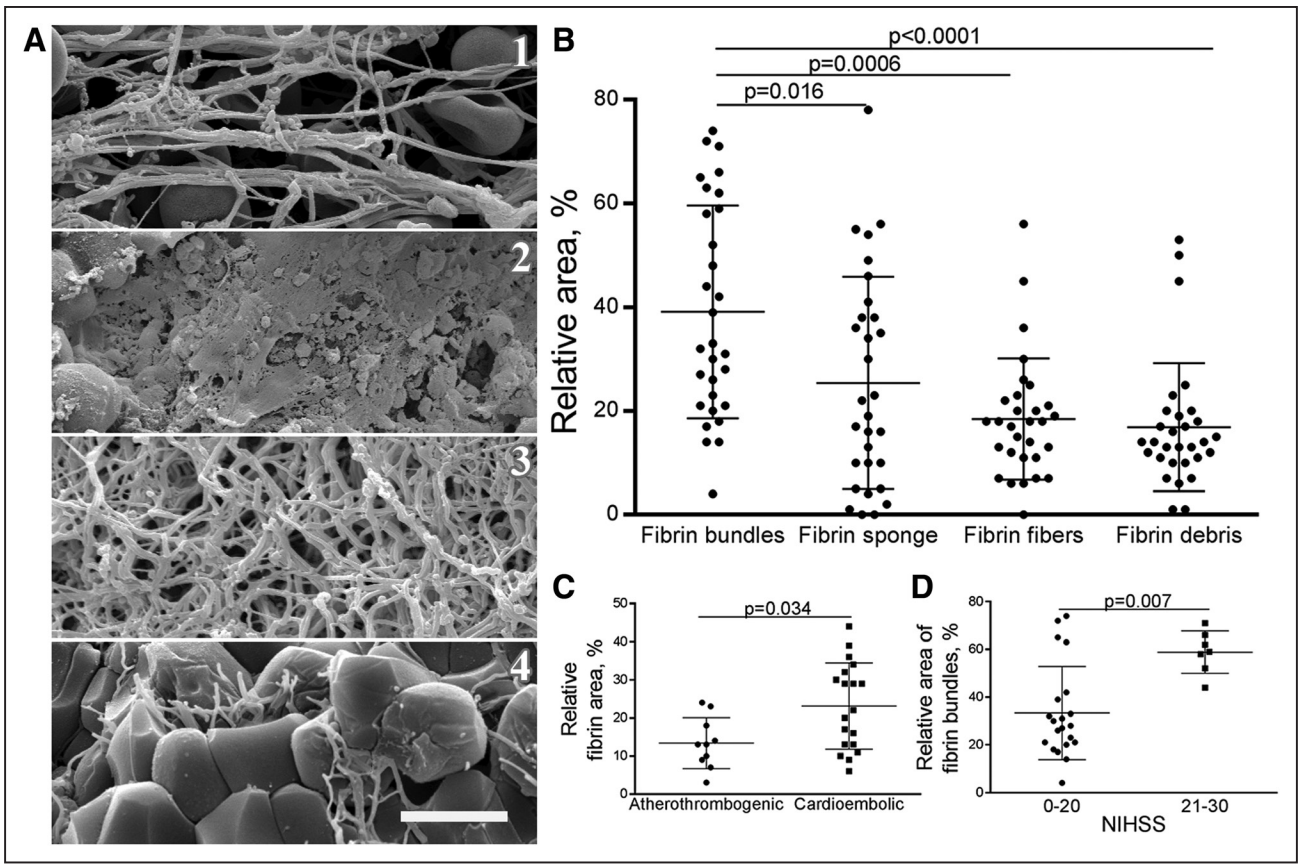

Figure 4. The structural variability of fibrin and the relative content of fibrin types in cerebral thrombi extracted from patients with stroke of different etiology and severity.

A, Characteristic colorized portions of scanning electron micrographs of cerebral thrombi, illustrating the types of fibrin structures (Table 2): fibrin bundles (1), fibrin sponge (2), fibrin fibers (3), and fibrin debris (4). Magnification bar=5 μm. **B**, The relative content of various fibrin types in cerebral thrombi. **C**, The content of fibrin in thrombi from the patients with cardioembolic and atherothrombogenic stroke. **D**, The content of fibrin bundles in cerebral thrombi from the patients with various severity of stroke based on National Institutes of Health Stroke Scale (NIHSS) at the time of admission. Kruskal-Wallis test with Dunn post hoc test (**B**) and Mann-Whitney *U* test (**C** and **D**).

fibrinolysis,[31,32] and higher fibrin content may increase susceptibility of a thrombus to proteolytic cleavage.

We have recently developed a semiquantitative color scale to determine the age of fibrin in histological preparations as young, mature, and old fibrin.[19] Using this scale, we have found that young fibrin prevailed in cardioembolic cerebral thrombi, while atherothrombogenic thrombi were composed mainly of older fibrin (Figure VI in the Data Supplement). This difference suggests that embolization of cardiac thrombi is more likely since these thrombi contain relatively immature fibrin that is less resistant to mechanical rupture and fibrinolysis.

A remarkable and unexplored type of fibrin revealed in cerebral thrombi is fibrin debris (Figure 4A; Table 2). Although it is hard to provide persuasive evidence for its origin, it has many free fiber ends, suggesting that fibrin debris may comprise remnants of partially lysed fibrin network.[18] In addition, fibrin debris could comprise a mixture of fibrin fibers and neutrophil extracellular traps because discrimination between fibrin and neutrophil extracellular traps by scanning electron microscopy is impossible.[33]

Fibrin sponge is another newly described form of fibrin of unclear origin (Figure 4A; Table 2). In spite of some apparent similarities, these spongy structures are distinct from both the fibrin film[34] and the fibrin shell,[35] both of which are found on the thrombus surface. The fibrin shell probably results from redistribution of components during clot contraction, as we described above.[23,24,27]

Arterial thrombi have been traditionally considered platelet rich with a relatively small content of RBCs and, therefore, called white thrombi.[14,36] The literature[15,16,37] and our data disprove this old notion because RBCs comprise a major component of various cerebral arterial thrombi (≈80% volume fraction), while the content of platelets is surprisingly small (≈10%; Figure 2B). In some studies, cerebral thrombi were found to contain more fibrin/platelets than RBCs, but these conclusions are based on histopathologic examination, while we used scanning electron microscopy. Although there are some scanning electron microscope images of cerebral thrombi in the literature, most of them are hardly comparable with our work. Autar et al[3] only studied interactions between stent retrievers and thrombus. Mehta et

al[38] show one image without quantification. Di Meglio et al[35] studied the outer shell of fibrin and platelets, which is qualitatively consistent with our results on redistribution of thrombus components indicative of intravital contraction. To the best of our knowledge, there are no other studies using electron microscopy to quantify cerebral thrombus structure.

There are numerous publications using standard histopathologic methods to quantify cerebral thrombus structure. The Sporns et al[2] and Liebeskind et al[15] studies both show considerably higher and similar fibrin content than RBCs. On the other hand, the Boeckh-Behrens et al[11] study shows only slightly higher fibrin content. By contrast, in our study using scanning electron microscopy, we found a higher RBC content than fibrin. There are several potential reasons for the discrepancies between histology and scanning electron microscopy because of the differences in methodology. The primary reason is the considerably lower resolution of the histological images, in which large areas will stain but may have quite low fibrin content. By scanning electron microscopy, we visualize individual fibrin fibers, so we are only counting those areas and not all the space between fibers or areas with sparse fibrin fibers or fibrin sponge. Another reason is that hematoxylin and eosin and other staining do not allow adequate distinction between fibrin and platelets. Since platelets are included in the fibrin total, fibrin is overestimated. Last but not the least, in our study, unlike others, the samples were cut open so that the interior of the thrombi could be viewed, while in many other studies, the surface or the exterior parts of thrombi, containing accumulations of fibrin and platelets, were analyzed. Many other methodological distinctions between histopathologic examination and scanning electron microscopy may explain why the results on morphology of cerebral thrombi are not always consistent.

Similarly, we found that the volume fractions of blood cells and fibrin were statistically indistinguishable in thrombi that had atherothrombogenic versus cardioembolic etiology. Although some histological studies found distinctive differences with respect to etiology, other studies have reported just the opposite findings. Thus, our results are not out of line with the literature overall.

The content of balloon-like platelets prevailed significantly in the cardioembolic more than in atherothrombogenic thrombi (Figure 3C), which can be an indicator of platelet activation. The balloon-like platelets provide a surface for thrombin generation and formation of fibrin.[39,40] Balloon-like platelets are bloated single or double platelets with a plicate surface and a size comparable to or larger than RBCs (≥5–6 μm); they have multiple wrinkles and lines on their surface and no bumps.[41,42] These structural features make them distinct from leukocytes, which are spherical or irregular-shaped cells varying from ≈5 up to ≈10 to 12 μm in diameter with pronounced membrane folding and a tuberous rough surface with multiple short bumps.[43]

The relationships between thrombus structure and 3-month mRS are probably both direct and indirect. The high content of mainly polyhedral RBCs (a signature of intravital contraction) prevailed in thrombi/emboli from patients with more severe stroke outcomes characterized by mRS. As described for patients at entry, this means that the thrombi are more contracted, so that they are more compact, more stable, and less permeable. As a result, the 3-month mRS is also affected in addition to the severity at intake. More generally, the fact that a higher volume fraction of compressed polyhedral-like RBCs correlates with worse long-term outcomes most likely due to persistent thrombinemia and continuous platelet activation that also sustain thrombosis.[44] The abundance of neutrophils prevailed significantly in thrombi from patients with worse long-term outcomes. The accumulation of leukocytes in the thrombus represents the conditions for the development of neutrophil extracellular traps that enhance fibrin deposition, platelet aggregation, disturb the local blood flow around a thrombus, and reduce the susceptibility of thrombi to fibrinolysis and to therapeutic thrombolysis.[45–47] Indirect associations between the composition of thrombi/emboli and long-term stroke outcomes could be related to the delayed alterations in the cerebral arteries and microcirculation at the site of occlusion after removal of the thrombus. These alterations include chronic endothelial activation at the site of occlusion and in the distal parts of cerebral vessels, followed by disturbed microcirculation, a high level of VWF (von Willebrand factor) expression, and a higher probability of reocclusion, leukocyte extravasation, and inflammatory damage to surrounding tissue. All these processes worsen the course and long-term outcomes of stroke.

CONCLUSIONS

Cerebral thrombi were made of blood cells and fibrin and had low porosity. The prevailing cell type was compressed polyhedral erythrocytes (polyhedrocytes), indicative of intravital contraction, and the content of polyhedrocytes directly correlated with the stroke severity. The presence of fibrin bundles over single fibers was typical for more severe cases, while the content of fibrin sponge prevailed in cases with a more favorable course of stroke. The higher content of platelet aggregates was another marker of stroke severity, although the overall platelet content in these arterial thrombi was unexpectedly small. Fibrillar types of fibrin dominated over fibrin sponge in atherothrombogenic thrombi, whereas the content of total fibrin of any type was characteristic of cardioembolic thrombi. Cerebral thrombi with a substantial number of leukocytes, often arranged into clusters, were usually associated with fatal cases. Histologically, the majority of thrombi also had pronounced morphological signs of contraction, including polyhedrocytes in the core and

redistribution of fibrin to the periphery. Thrombus age assessed by histological staining of fibrin revealed that younger fibrin prevailed in cardioembolic thrombi. Alternating layers of erythrocytes and fibrin/platelets were typical for thrombi from the patients with more favorable outcomes. Thus, despite the high variability of the composition and structure of cerebral thrombi, the content of certain types of blood cells and fibrin structures combined with the morphological signs of intravital contraction correlate with the clinical course and outcomes of acute ischemic stroke.

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Disclosures

None.

Supplemental Materials

Expanded Materials & Methods
Tables I and II
Figures I–VIII

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